National Research Council

3RD BIENNIAL CONFERENCE

BIOMATERIALS AND NOVEL TECHNOLOGIES FOR HEALTHCARE

a cura di
Julietta V. Rau, Franca Rossi e Marco Ortenzi

PROCEEDINGS

BIOMATERIALS AND NOVEL TECHNOLOGIES FOR HEALTHCARE

3rd biennial International Conference BIOMAH

CONFERENCE PROCEEDINGS

A cura di

Julietta V. Rau, Franca Rossi e Marco Ortenzi

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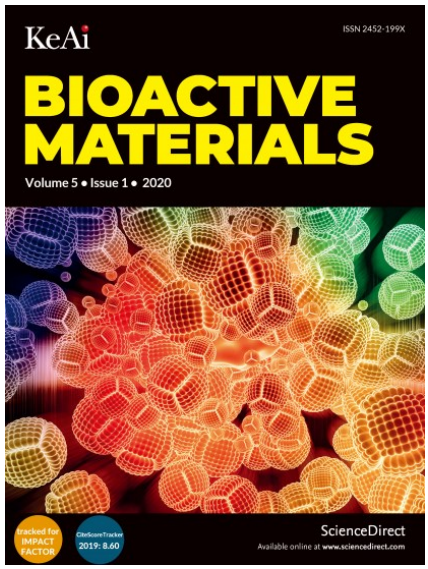

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Keynote Speakers

Prof. Iulian Antoniac

(Romania)

Materials Science and Engineering Faculty, University Politehnica of Bucharest

Prof. Iulian Antoniac received the M.E., Ph.D. and Postdoc degrees in Materials Science at University Politehnica of Bucharest. Since 2002, he has been associated with the Medical Engineering program in the Faculty Materials Science and Engineering, University Politehnica of Bucharest, which is focused on biomaterials obtaining and characterization, medical image processing and the development of new implants for medical applications. Dr. Iulian Antoniac is the leader of the Biomaterials Group, head of the Biomaterials & Interface Phenomenon Laboratory, full professor at Faculty Materials Science and Engineering. He was appointed Vice Dean of Faculty Materials Science and Engineering and member of the Senate of University Politehnica of Bucharest in 2016. Professor Antoniac has published widely, with over 200 papers published in peer-reviewed journals and conference proceedings, 9 patents, several books (like *Handbook of Bioceramics and Biocomposites*) and over 50 invited lectures at conferences focused on biomaterials, bioceramics and materials science. He is currently President and Council Member of the Romanian Society for Biomaterials (SRB), Former President and permanent Member of Executive Committee of the International Society for Ceramics in Medicine (ISCM). Research interests include metallic biomaterials, bioceramics, coatings, biocomposites, polymers, retrieval analysis of explants, microscopy techniques for materials characterization, surface modification, interaction tissue-biomaterials, bone regeneration, retrieval and failure analysis of implants.

Prof. Aldo R. Boccaccini

(Germany)

Head, Institute of Biomaterials
Chair, Department of Materials Science and Engineering University of Erlangen-Nuremberg

Aldo R. Boccaccini is Professor of Biomaterials and Head of the Institute of Biomaterials at the University of Erlangen-Nuremberg, Germany. He is also visiting Professor at Imperial College London, UK. He holds an engineering degree from Instituto Balseiro (Argentina), Dr-Ing. from RWTH Aachen University (Germany) and Habilitation from TU Ilmenau (Germany). The research activities of Prof. Boccaccini are in the field of glasses, ceramics and composites for biomedical, functional and/or structural applications. He is the author or co-author of more than 600 scientific papers and 15 book chapters. His work has been cited more than 14,000 times and he was named in the 2014 Thomson Reuters Highly Cited Researcher list. Boccaccini has been a visiting professor at different universities around the world, including Japan, Italy, Spain, Slovenia, Netherlands, Singapore, Germany, Argentina and Poland. His achievements have been recognized with several awards including, most recently, the Materials Prize of the German Materials Society (DGM) in 2015. Boccaccini is the editor-in-chief of the journal Materials Letters and serves in the editorial board of more than 10 international journals. In 2015 he was elected member of the Council of the European Society for Biomaterials (ESB). He also serves in the Review Panel of the German Science Foundation (DFG) and is an international advisor to the Ministry of Science and Technology of Argentina.

Prof. Dr. Ilaria Cacciotti

(Italy)

Department of Engineering, INSTM RU, University of Rome Niccolò Cusano

Prof. Dr. Ilaria Cacciotti is Full Professor of Biomaterials & Tissue Engineering and Materials Science and Technology at University of Rome "Niccolò Cusano", with consolidated expertise in the synthesis and characterization of (bio)materials and eco-sustainable systems. She graduated in Medical Engineering at the University of Rome “Tor Vergata” (Master of Science Award ‘Fondazione Raeli’). Afterwards, she completed the Ph.D in Materials Engineering (Ph.D Thesis Award ‘Marco Ramoni 2011, Ph.D Thesis AIMAT Award 2012) and she obtained the II Level Master degrees in “Forensic Genetics” and in "Protection against CBRNe events". She is expert in the synthesis, processing and characterisation of biocompatible nanostructured materials, particularly for applications in the biomedical, environmental and agri-food sectors, including bioceramics (undoped and doped calcium phosphates, bioactive glasses), biopolymers and composites. She is reviewer for more than 100 peer-review journals, e.g. Acta Biomaterialia,Biofabrication, Chemical Engineering Journal, has been/is Guest Editor of several Special issues, and is a member of the Editorial Board of several international journals, including Applied Science-MDPI, Applied Surface Science Advances-Elsevier, Frontiers in Biomaterials, Open Journal of Materials Science-Bentham Science, Recent Patents on Materials Science-Bentham Science, The Open Materials Science Journal-Bentham Open, Reviews on Advanced Materials Science-DE GRUYTER OPEN, and serves on various grant review committees, such as National Science Foundation (NSF), Canada Foundation for Innovation (CFI) and National Science Center (NCN, NARODOWE CENTRUM NAUKI).

For her research activity, she achieved several awards, including 8th CCT Award “Best Oral Presentation for Young Researchers 2011”, 10th International Award "Giuseppe Sciacca" for Young Students, the European Biomaterials and Tissue Engineering Doctoral Award (EDA-ESB (European Society of Biomaterials)) Award 2011, "Top Cited Author 2011-2012 ChemEngJ", “L’ORÉAL-UNESCO Italy for Women and Science 2011”, “Under 35 Talents in Lazio 2012”, “Featured Articles and Cover Pages for the following International Journals Chem Eng J, Nanotechnology, Advanced Materials Interfaces”, ”Young Researcher Award Elsevier" Materials Science and Engineering C" 2014, “Good Energy Award 2016”, “SRB Excellence Award 2018”, “Best Poster Award for 18th International Conference ”Life Sciences for Sustainable Development” 2019”, “BIOREMED Excellence Award 2019”, “Polymers 2020 Outstanding Reviewer Award”, “IOP trusted reviewer 2021”, “Inspirational Scientist Award in the International Scientist Award on Engineering, Science, and Medicine 2021”, “Women Researcher Award" in the International Scientist Awards on Engineering, Science and Medicine 2021”, ”International Research Awards on Science, Technology and Management (INSO) 2022”.

Prof. Dante Dallari

(Italy)

Director of the “Reconstructive Orthopaedic Surgery Innovative Techniques – Musculoskeletal Tissue Bank”, Rizzoli Orthopaedic Institute

Director of the “Reconstructive Orthopaedic Surgery and Innovative Techniques –Musculoskeletal Tissues Bank” Rizzoli Orthopaedic Institute, Bologna – Italy

Professional Experience

Nov 2017 – to present - Director of the “Reconstructive Orthopaedic Surgery and Innovative Techniques – Musculoskeletal Tissues Bank”; Rizzoli Orthopaedic Institute, Bologna – Italy

Aug 2016 – Nov 2017 - Acting Director of the “Reconstructive Orthopaedic Surgery and Innovative Techniques – Musculoskeletal Tissues Bank”; Rizzoli Orthopaedic Institute, Bologna – Italy

Nov 2011 – Nov 2017 - Director of the “Conservative Orthopaedic Surgery and Innovative Techniques”; Rizzoli Orthopaedic Institute, Bologna – Italy

Nov 2008 – Oct 2011 - Head of the Specialist Center "Clinical Applications of Regenerative Medicine in orthopedics” at the 1st Clinic of Orthopaedic and Traumatology; Rizzoli Orthopaedic Institute, Bologna – Italy

Jan 2001 – Nov 2008 - Head of the Operative Unit "Prosthetic Surgery in Secondary Coxarthrosis " at the1st Clinic of Orthopaedic and Traumatology, Rizzoli Orthopaedic Institute, Bologna – Italy

Apr 1992 – to present - Orthopaedic Surgeon, Rizzoli Orthopaedic Institute, Bologna – Italy

Jun 1990 – Apr 1992 - Orthopaedic Assistant USL n. 35, Ravenna (RA) – Italy

Jul 1989 – Oct 1989 - Orthopaedic Assistant USL n. 41, Riccione (RN) – Italy

Oct 1987 – Oct 1990 - Fellowship Granted by the Rizzoli Orthopaedic Institute Research Project: “Neoplastic and systemic diseases

Memberships

Member of the: American Academy of Orthopaedic Surgery; International Cartilage Repair Society; International Society of Hip Arthroscopy; Italian Society of Orthopaedics and Traumatology; Italian Society of Arthroscopy;Italian Hip Society.

Teaching Activity

2013 to present; Intensive courses of Hip surgery: conservative, prosthetic and revision ; Specialization School of Orthopaedics and Traumatology.University of Bologna – Italy

2008 to present; Clinic and operating room training to resident and fellow students; Rizzoli Orthopaedic Institute, Bologna – Italy

2012; Founding Member of the School of Hip Arthroscopy

2010 - 2016; Consulting activities in Orthopaedics and Traumatology Fondazione Policlinico “San Marco” Zingonia (BS) – Italy

1992 - 1995; Intensive courses of Orthopaedics and Traumatology School of Professional Nurses USL n. 29, Bologna (BO) – Italy

1991 - 1992; Intensive courses of Orthopaedics and Traumatology School of Professional Nurses USL n. 35, Ravenna (RA) – Italy

Scientific Interest and Activity

Author of 116 scientific publications and speaker at 124 scientific conferences in the field of prosthetic and revision surgery of hip and knee, hip arthroscopy, arthroplasty in severe secondary osteonecrosis of the hip, treatment of nonunion of long bones and treatment of osteochondral injury.

Prof. Neil Davies

(South Africa)

Cardiovascular Research Unit at the University of Cape Town

Assoc. Prof. Davies obtained his academic qualifications from the University of Cape Town (South Africa). After a 3-year fellowship in the Chemical Pathology Department at the University of Oxford (United Kingdom), he then joined the *Cardiovascular Research Unit* at the University of Cape Town in 1999.

His main research focus is on regenerative medicine with particular reference to delivery of biomaterials and stem cells to infarcted hearts.

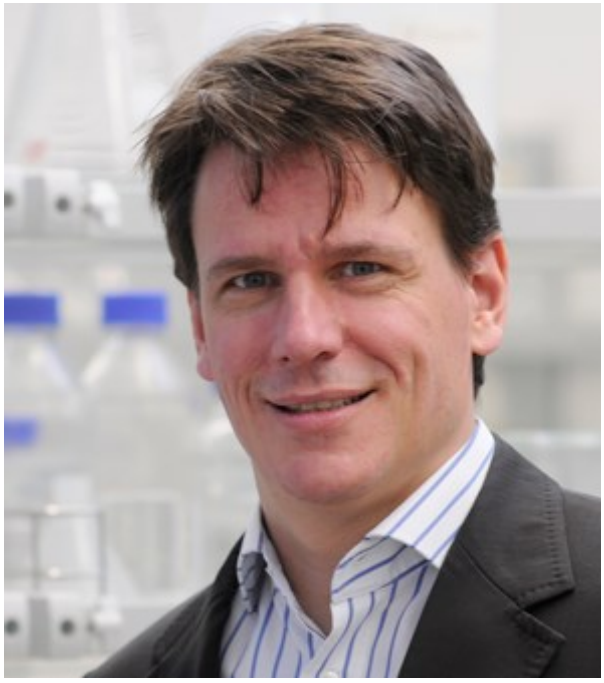
Integral to this focus is the development of novel therapeutic angiogenesis strategies and investigations into the manipulation of cellular invasion through the engineering of bioactive smart hydrogels. His laboratory was the first to show that reduction in pathological remodelling of an infarcted heart could be achieved through the cardiac injection of synthetic biomaterials. Another first was the recent demonstration that cell type specific invasion of hydrogels could be realised through manipulation of protease specific hydrogel crosslinkers. His laboratory is internationally recognised for its work on controlling biomaterial degradation after implantation. Other notable findings have been the discernment of an optimal dosage of VEGF, a key angiogenesis regulator, for sustained vascular ingrowth and cell specific control of migration speed via utilisation of combinations of cell adhesive peptides.

Assoc. Prof. Davies is author of ***50*** *peer reviewed full papers* that have been cited more than 2000 times with a total *of 189 impact points and an H-factor of 21*. He has obtained international academic and industry grants of more than *40 million Rand*. He is a regular reviewer of the 12 top journals in his field and is on the editorial board of Journal of Biomaterials and Tissue Engineering. He is currently funded by the National Research Foundation (South Africa), Medical Research Council (South Africa) and the International Centre For Genetic Engineering and Biotechnology.

Prof. Georg Duda

(Germany)

Director of the Julius Wolff Institute for Biomechanics and Musculoskeletal Regeneration, Charité – Universitätsmedizin Berlin



Prof. Dr. Georg Duda is interested in the tension between the fields of Biology and Mechnics. He is involved in investigating the interaction between bone and muscles as well as the biomechnical influences and its impacts in both the intact and injured musculoskeletal system (e.g. loading of joints and bones).

Prof. Duda focuses in particular on the interaction between the physical and mechanical conditions, and the biological regeneration of the musculoskeletal system. By using examples of bone and cartilage healing, he demonstrated the importance of biomechanical characteristics for cells and tissues of the human musculoskeletal system. He showed that a certain amount of mechanical stimulation is helpful to healing, while too much could result in a delay to the healing processes.

Prof. Duda is also involved in the research on regenerative medicine. With his work he aims to understand the body's own processes and, where necessary, to stimulate them, so as to reproduce natural regeneration of the musculoskeletal system.

The basic understanding of mechanical stimulation helps activation of the endogenous healing processes to be targeted, understood and guided.

.

Prof. Silvia Farè

(Italy)

Department of Chemistry, Materials and Chemical Engineering G. Natta, Politecnico di Milano, Milan, Italy

Silvia Farè obtained her Master of Science degree in Management and Industrial Engineering, Politecnico di Milano, in July 1994.
In May 1998, she obtained her PhD degree in Biomaterials (X^ cycle), Politecnico di Milano, with a thesis on the investigation of the mechanisms related to the *in vitro* oxidative degradation of medical-grade polyurethanes.
Silvia Farè obtained a Post-doc research Fellowship (February 1998 – February 1999) within the project "Third generation artificial heart" and she was Research Assistant from March 1999 to March 2003, at the Bioengineering Department, Politecnico di Milano, Milan, Italy.
From January 2005 to November 2014, she was Assistant Professor at the Bioengineering Department (2005-2012), and at the Department of Chemistry, Materials, and Chemical Engineering "G: Natta" (2013-2014), Politecnico di Milano.
From December 2014 to March 2021, she was Associate Professor in Industrial Bioengineering at the Chemistry, Materials and Chemical Engineering Department, Politecnico di Milano.
From April 2021, she is Full Professor in Industrial Bioengineering at the Chemistry, Materials and Chemical Engineering Department, Politecnico di Milano.
From February 1993 to February 1996, she was visiting Master and PhD student at the Joint Research Centre, Institute for Advanced Materials, Ispra (VA), Italy.
From March 1996 to September 1996 and from January 1997 to March 1997, she was visiting PhD student at the University of Laval and Quebec Biomaterials Institute, Quebec, Canada under the supervision of Gaetan Laroche and Diego Mantovani.
She was Member of Scientific or Organizing Committee at National and International Congresses.
From January 2018 she is Editor in Chief for the Journal of Applied Biomaterials and Functional Materials (Sage Ed.).
In 2017 she was elected as President of the Italian Society for Biomaterials (SIB).
From 2017 she is member of the Council of the European Society for Biomaterials; from 2021 she is Treasurer of ESB.
Teaching activity
From 2001 and up to the current academic year Silvia Farè has been in charge of different courses in biomaterials and regenerative medicine fields at Master and Bachelor in Biomedical Engineering, Politecnico di Milano, Milan, Italy.

Scientific activity
The scientific activity of Silvia Farè has been devoted to different areas related to the bioengineering applications and Research activity is carried out in collaboration with National and International Universities and Research Centers, and National and International industries.
Her main research interests: characterization of design, synthesis and characterization of synthetic and natural polymerbased scaffolds for tissue engineering (vascular tissue, adipose tissue, skeletal and cardiac muscle, cartilage) and drug release applications; development of polymer matrices for biomimetic composites (bone substitutes); design and characterization of shape memory polymer device and scaffolds for a mini-invasive surgical approach; elastomers for cardio-vascular applications (vascular prosthesis, heart valves, artificial ventricles).
Silvia Farè is author and co-authors of one hundred international and national scientific publications related to the biomaterial and regenerative medicine fields.
The H index of Silvia Farè Scientific Production calculated by Scopus in April 2022 is ranking 33.

Prof. Rainer Gadow

(Germany)

Managing director, Institute for Manufacturing Technologies of Ceramic Components and Composites (IFKB), University of Stuttgart
Director materials and process engineering, Graduate school of excellence for Advanced Manufacturing Engineering GSaME, University of Stuttgart

Prof. Gadow has completed his M.Sc. and PhD both in chemistry at the University of Karlsruhe (T. H.), Germany, now KIT. As assistant professor he was head of the ceramics and composites group at ICT, University of Karlsruhe. In his industrial career he was head R&D and general manager in world leading companies in technical ceramics, surface technologies and advanced mechanical engineering. Since 1995 he is full professor and managing director of IFKB, the institute of manufacturing technologies of ceramic components and composites at the University of Stuttgart, Germany. He managed various national and international projects in advanced cermics, surface and nano technologies. He is the acting dean of the faculty of mechanical, automotive and production engineering with 17 research institutes in Stuttgart. Furthermore he is managing director of New Materials Technologies at TTI GmbH, Stuttgart. He has published more than 700 papers in reviewed journals and more than 60 patents in the field of product development with new materials and manufacturing processes. He is serving as an editorial board member and peer reviewer of various scientific journals and visiting professor worldwide.

Current Research Interest

Materials engineering and product development;
Processing and manufacturing of ceramic components;
Processing and manufacturing of nano structured materials and components;
High energetic surface technologies;
Coatings and layer composites;

Biomaterials and biomedical technologies;
Technology of composite materials;
Modelling and Simulation of Manufacturing Processes;
Total quality management in industrial manufacturing and management.

Prof. Dr. Gultekin Goller

(Turkey)

Professor in Materials Science in Istanbul Technical University, Department of Metallurgical and Materials Engineering, Maslak, Istanbul

Prof. Dr. Gultekin Goller is a materials science professor who graduated from Istanbul Technical University in 1989 with a B.S. in Metallurgical Engineering. In 1997, he received his Ph.D. in the field of Metallurgical and Materials Engineering from Istanbul Technical University. He attended to the Tribology Group of Cleveland State University in 1995 as a UNIDO fellow for the PhD level studies. He joined to the Metallurgical and Materials Engineering Department of ITU in 1999 as an assistant professor.

Professor Goller was promoted to associate professor in 2005 and became a full professor in 2010. His research fields include Modern Armour Sytstems, High Entropy Alloys, Refractory Metals and Alloys (TZM alloys), Ceramic and Composite Materials (ultra-high temperature ceramics and composites, bioactive and bioinert ceramics and composites, polymeric matrix composites), Glass and Glass Ceramics, Biomaterials, Synthesis of High Technology Ceramic Powders, Plasma Spray Coating Process (thermal barrier coatings) and Spark Plasma Sintering (SPS) Technique (Boride, carbide, nitride based monolithic and composite materials), Characterization of Materials (X-Ray and Electron Microscopy).

Prof. Dr. Gultekin Goller is the founder of Biomaterials Research and Characterization Laboratory, Laser Cutting and Welding Laboratory, Composite Material Production Laboratory and Spark Plasma Sintering Laboratories in Istanbul Technical University Metallurgy and Materials Engineering Department. Prof. Dr. Gultekin Goller has supervised 7 completed and 6 ongoing PhD studies; 32 completed, 4 ongoing graduate studies. As a date of August 2021, his professional and scientific activity comprises: papers, which are cited over 1896 times (h index = 25; As of April 2022), published in science citation index journals (119); papers published in international peer-review periodicals (7); the proceedings of international or national conferences (130); participating in different international or national research projects (47); author of 2 international book chapter; member of the scientific committee of different meetings; head of the organizing committee for different international conferences; member of the International Editorial Board of some journals; and reviewer for different journals. Prof. Dr. Gultekin Goller served as a chair of Metallurgical and Materials Engineering Department between 2010-2013, and contributed to the Ministry of Defence R&D Technologies Division, Material Processes and Technologies Panels as a university representative on 2010-2016. In 2019, Prof. Goller established a company called "GG Materials Techologies" at ITU ARI Teknokent operating in the field of production and characterization of modern armour systems and advanced materials. Prof. Goller has been acted as an international expert in the accreditation studies of the Materials Science doctoral program of the Romanian Agency for Quality Assurance in Higher Education (ARACIS) since 2021. Prof. Goller has been included in the list of the most influential scientists (top 2%) carried out by Stanford University created according to the calculation of the citation numbers, H-index values, co-authorships and career-long impact factors of the scientists.

Prof. Carlos Roberto Grandini

(Brazil)

UNESP – Univ. Estadual Paulista, Laboratório de Anelasticidades e Biomateriais, Bauru, São Paulo

Carlos Roberto Grandini earned his B.S. degree at Physics from Universidade Federal de São Carlos (Brazil) in 1985, his Master's at Basic Physics from Universidade de São Paulo (Brazil) in 1988 and his Ph.D. at Basic Physics from Universidade de São Paulo (Brazil) in 1993. He joined the Physics Department at the Universidade Estadual Paulista (UNESP) at Bauru (Brazil) as an Assistant Professor in 1988 and since 1995 is leader of Anelasticity and Biomaterials Laboratory. Beto Grandini compled post-doctoral research at Università Degli Studi di Roma "La Sapienza" as a FAPESP fellow in 1998 and obtained the position of Associate Professor in 2003.He was Head of Physics Department (1998-2000) and since 2009, is Full Professor at UNESP, in Bauru. Since 2014 is member of Institute of Biomaterials, Tribocorrosion and Nanomedicine (IBTN). He is member of several physics and materials societies and, has been Vice-President (2010-2012 and 2012-2014) and President (2016-2018) of the Latin American Society for Biomaterials and Artificial Organs. He is currently Latin-American Delegate in the International Union of Societies for Biomaterials Science and Engineering since 2016. He was elected a Fellow Biomaterials Science and Engineering in 2016. In his more than 30 years in academics, Prof. Grandini has graduated/supervised over 120 visiting faculty, post-doctoral students, and thesis completing B.S., M.S., and Ph.D. students. He published more than 120 paper in refereed journals. The focus of Grandini's group is the development and characterization of new metastable titanium alloys with low elastic modulus and no elementsthat can cause cytotoxicity, aiming orthopedic and dental applications.

We have developed binary and ternary systems containing molybdenum, tantalum, niobium and zirconium. Such titanium alloys have the predominance of beta phase (in some situations have the coexistence of up to three metastable phases) and have elastic modulus between 60-80 GPa, reducing the stress-shielding effect. We also have acted in the functionalization of titanium alloys surfaces, with the aim of favoring osseointegration and antimicrobial action, improving corrosion and wear resistance, besides the tribocorrosion.

Dr. Michele Iafisco

(Italy)

Institute of Science and Technology for Ceramics (ISTEC), National Research Council (CNR)

Michele Iafisco is senior researcher at Institute of Science and Technology for Ceramics (ISTEC) of the National Research Council (CNR) in Faenza (RA), Italy. He is co-founder and CTO of the CNR spin-off Recover Ingredients S.p.A.

He is the principal investigator and responsible of research unit of several national and international projects National Project PRIN 2020 "AppliCare". (WP leader, Responsible of CNR research unit); EU project "UNAT" (WP leader, Responsible of CNR Research Unit). Bilateral project CNR/MoST (Taiwan). (Principal Investigator))

He has authored more than 130 papers published in international peer-reviewed journals, 18 book chapters, 2 books and 7 patents. H-index=39, total citation: more than 4400, Source: Scholar (March 2022).

He is member of the editorial board of several international journals (i.e. Scientific Reports, Colloids and Surfaces B: Biointerfaces, Frontiers in Bioengineering and Biotechnology) and expert evaluator for the European Commission and other European research agencies. In 2015 he was recipient of the prize "Ricercatamente 2014" for the

best research activity of most promising young researcher "under 35" at the National Research Council of Italy in the field "Chemical Sciences and Materials Technology.

He graduated in Chemistry in 2005 from the Alma Mater Studiorum, University of Bologna, Italy in 2005. From the same university he obtained the PhD degree in Chemical Science in 2009.

His research interests include biomaterials, biomineralization, nanotechnology, calcium phosphates, and bioceramics.

Prof. Gigliola Lusvardi

(Italy)

Department of Chemical and Geological Sciences, University of Modena and Reggio Emilia,

Associate Professor in General and Inorganic Chemistry at the Department of Chemical and Geological Sciences of the University of Modena and Reggio Emilia since 1 November 2005.

Previous positions and felloships::

From May 1996 to November 2001 Technical collaborator at the Department of Chemistry of University of Modena and Reggio Emilia

From November 2001 to November 2005 Associate Researcher (General and Inorganic Chemistry, CHIM03), at the Department of Chemistry of University of Modena and Reggio Emilia

From November 2005 to present, Associate Professor (General and Inorganic Chemistry, CHIM03), at Department of Chemical and Geological Sciences, University of Modena and Reggio Emilia.

From 2017 qualified as Full Professor by ASN.

Affiliations:

Member of the “Società Chimica Italiana (SCI)”

Member of the Division of Inorganic Chemistry of the Italian Chemical Society (SCI)

Member of the Doctorate School "Modellistica, Simulazione Computazionale e Caratterizzazione Multiscala per le Scienze dei Materiali e della Vita " dell'Università di Modena e Reggio Emilia., renamed from 2013 "Models and Methods for Material and Environmental Sciences";

Member of the Interuniversity Consortium INSTM

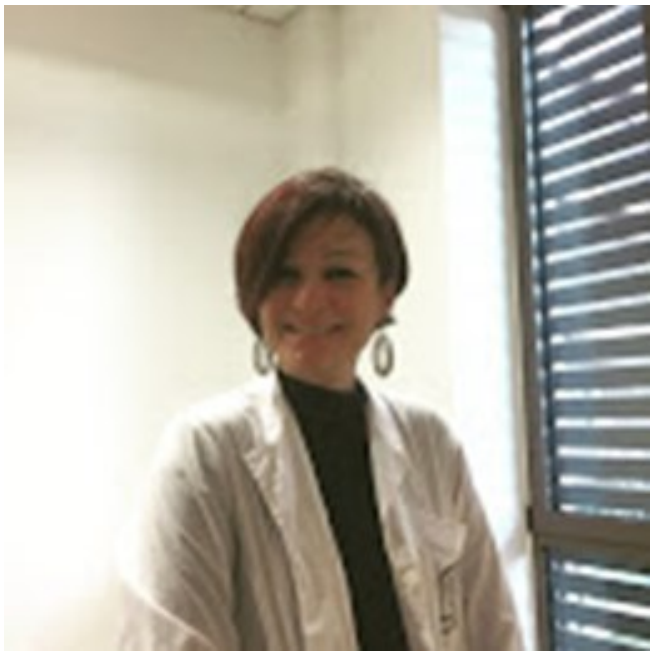
Member of Spanish and European Network of Excellence for the Prevention and Treatment of Osteoporotic Fractures, “Ageing” (www.agening.net).

Member of Centro di Ricerca Interdipartimentale sulla Sicurezza e Prevenzione dei Rischi” (CRIS) dell’Università degli studi di Modena e Reggio Emilia.

Research:

Author and co-author of over 100 publications on international and national journals

Referee of international journal of inorganic and material chemistry: Material Chemistry, Journal of American Ceramics Society, Acta Biomaterialia., Journal of Colloid and Interface Science, Journal of Non Crystalline Solids., Materials Sciences



&Engineering, Langmuir, Construction and Building Materials Scientific Reports

Guest Editor, Editorial Boards for Materials

Talent research reviewer.

Brief description of the research activity:

The research activity is mainly carried out in the field of inorganic materials; it concerns their synthesis and characterization from quali/quantitative point of view. In particular, it deals with the study of the properties of biomaterials, specifically bioactive glasses (silicate and phosphosilicate) in order to define the best conditions of synthesis, modulating also their dimensions for specific applications (scaffolds, drug delivery systems, self-assembled monolayers, coatings). Moreover, new formulations of bioactive glasses are studied through the addition of therapeutic inorganic ions (TTIs) and biomolecules (both of natural and artificial origin) with the aim of improving their properties, adding new ones for targeted applications of the new materials obtained. The characterization involves the use of specific analytical techniques and studies both *in vitro* and *in vivo* were performed to verify their applications for both hard and soft tissues.

Prof. Dr. Horia Manolea

(Romania)

Department of Dental Materials, Vice-Dean Faculty of Dentistry, University of Medicine and Pharmacy

Horia Manolea is Professor at the Prostheses Technology and Dental Materials Department and currently Vice-Dean of the Faculty of Dentistry from the University of Medicine and Pharmacy, Craiova, Romania. He graduated as valedictorian the Faculty of Dentistry in 2001, holds a PhD in Medicine and a Master degree in implant supported prosthesis. Research interests include resin restorative materials research, implant supported prosthesis technologies, the bioactive materials study, development of ceramic and metal-ceramic technology, dental and periodontal structures morphology study. His professional and scientific activity comprises 10 textbooks, more than 50 papers published in scientific journals of which 27 were published in ISI journals with impact factor, more than 100 papers published in abstract in the proceedings of international or national conferences, invited speaker at several national and international scientific meetings, reviewer for several specialty scientific journals.

Prof. Giovanni Marletta

(Italy)

Laboratory for Molecular Surfaces and Nanotechnology, Department of Chemical Sciences, University of Catania and CSGI

Full Professor of Physical Chemistry since 1994, currently at University of Catania.

The research interests include Physical Chemistry of Surfaces and Interfaces, Physical Chemistry of Microelectronics materials, Nanotechnology and Biosurfaces, Surface Engineering of Functional Materials, Radiation-Surfaces Interactions (particle and cold plasmas), Surface analysis techniques, including XPS, REELS, SIMS, Spectroscopic Ellipsometry, Near Field Microscopies (AFM, Force Microscopy, Kelvin Probes, etc…), Techniques to study adsorption processes at L/S interfaces, including QCM-D, SPR, L-SPR.

Author or co-author of more than 200 papers published in international Journals (with more than 4,000 citations, Hindex= 33), has given about 100 Invited Lectures at International Conferences and Workshops in the last 15 years and is Coeditor of 11 books with contributed papers, mostly dealing with Nanoscience and Nanotechnology and Radiation Effects in Solids, for World Publishing Co. (1992), Elsevier (1996, 2000, 2001, 2002, 2003, 2004, 2005, 2006,2007), Materials Research Society (2011), etc…..

G.Marletta has been Member of several International Conferences Committees in the areas of Biological Surfaces and Nanotechnology, Radiation effect in Matter and Member of the Editorial Boards or the Advisory Board of Journal of Applied Biomaterials & Functional Materials (Wichtig, Italy). Past–member: J. of Physical Chemistry Chemical Physics (PCCP) (RSC, UK); J.of Applied Biomaterials and Biomechanics (JABB, Switzerland); J. Applied Physics/Applied Physics Letters (American Physical Society, USA), J. Adhesion Science and Technology (JAST); Advanced Biomaterial in Advanced Engineering Materials (Wiley).

G.Marletta is, since 2003, Fellow of the Royal Society of Chemistry (UK); he is currently Past-President of the Physical Chemistry Division of Italian Society of Chemistry (SCI), Vice-Chair of the Physical Chemistry Division of the European Chemical Society (EuChemS), Member and President of the Senate of the European Materials Research Society (E-MRS), President of the National Interuniversity Consortium for the Study of Large Interface Systems (CSGI), Coordinator of the Laboratory for Molecular Surfaces and Nanotechnology (2002-present, Catania, Italy) and Coordinator (as rector delegate) of the Nanotechnology Laboratory of the University of Catania (at BRIT Center).

G.M. has been President of the European Materials Research Society (E-MRS, from 2000 until 2003), and then member of the Executive Committee and Senate of the European Materials Research Society (2004-today); Director of the

Targeted Program “Special Materials for Advanced Technologies II” (CNR) (1999-2003); Member of the UE Expert Advisory Group for “Nanotechnology, Knowledge-based materials, Innovative Processes” for the 6th Framework Plan (2002-2006), President of the “Sicilian District of Micro and Nanosystems” (2008-2013).

Coordinator or Partner of several Italian National Programs as well as European Research Projects in the fields of Biosurfaces and Surface Engineering, he also served in many International Peer Review Boards for Research Program evaluation, including the National Committee for Chemical

Sciences of CNR, Italy, the European Committee for COST-Chemistry, D-5 Action: “Surface and Interface Chemistry”; the Scientific Board of GANIL, Section of “Physique de la Matière Condensée”; the Evaluation Panels of SRSIM-CEA – Saclay, France and LSI-CEA-CNRS – Palaiseau, France; the Monitoring panel for MELARI-NID Program (IST Program, EU, Bruxelles); the Peer Review Panel of the Engineering Physical Sciences Research Council (EPSRC, UK,); the Review Panel for the NEST Program (UE, IST priority, 6th FP. Several PRIN (Italian) Programs. He has also been member of the Scientific Board of about 15 Italian and foreign Research Institutes.

Prof. Horatiu Moldovan

(Romania)

"Carol Davila" University of Medicine and Pharmacy, Bucharest

Habilitated Professor of Cardiovascular Surgery - "Carol Davila" University of Medicine and Pharmacy, PhD and residents coordinator, Associate Professor at the Polytechnic University of Bucharest - Faculty of Materials Science and Engineering, Doctor of Medical Sciences, Head of Cardiovascular Surgery Department and coordinator of the Heart Transplant Program at the Clinical Hospital Emergency Bucharest, coordinator of the Clinical Department of Pediatric Cardiac Surgery within the Emergency Clinical Hospital for Children "Grigore Alexandrescu".

He is part of the group of specialists who introduced coronary surgery and aortic dissection surgery in Romania, as well as the implantation of the first mechanical assistance devices of circulation: intraaortic counterpulsation balloon, partial artificial heart type "Novacor" and more recently Heart Mate 3; within all cardiovascular surgery departments he worked in, he implemented new technologies related to minimally invasive cardiac surgery, endovascular surgery (EVAR, TEVAR) and transcateter valve prosthesis (TA).

Full Member of the Academy of Medical Sciences, an Associate Member of the Romanian Academy of Scientists, a Fellow of the American College of Cardiology, a Full Member of the European Association for Cardio-Thoracic Surgery and a member of the Board of the Romanian Society of Cardiovascular Surgery. as well as a member of 10 other national and international scientific societies.

Published as main author 7 specialized books, participated with chapters in other 20 books and manuals, out of which 4 in prestigious international publishers. He is the author / co-author for a number of over 120 published scientific papers: 60 ISI indexed publications, 36 ISI indexed extenso articles, of which 26 as lead author, FCIAP 55.181, Hirsch Index 10; Key note speaker in over 200 scientific national and international scientific events.

Areas of excellence for his practice and publications are major vascular surgery - aortic, minimally invasive cardiac surgery, science and engineering of biomaterials and cardiovascular devices (local surgical and hemostatic adhesives, valve and vascular prostheses), and mechanical ventricular assistance and transplantation.

Prof. Dr. Lorenzo Moroni

(Netherlands)

Director MERLN Institute for Technology-Inspired Regenerative Medicine
Chair of Complex Tissue Regeneration DepartmentProfessor in Biofabrication for Regenerative Medicine

Prof. Dr. Lorenzo Moroni received his Ph.D. cum laude in 2006 at University of Twente on 3D scaffolds for osteochondral regeneration, for which he was awarded the European doctorate award in Biomaterials and Tissue Engineering from the European Society of Biomaterials (ESB).

Since 2014 he works at Maastricht University, where he is a founding member of the MERLN Institute for Technology-Inspired Regenerative Medicine. In 2016, he became full professor in biofabrication for regenerative medicine. Since 2019, he is chair of the Complex Tissue Regeneration department. He was vice-director of MERLN from 2019 till 2022. Since 2022, he is director of MERLN.

In 2014, he received the Jean Leray award from the ESB and an ERC starting grant. In 2016, he also received the Robert Brown Award from TERMIS. In 2017, he was elected as faculty of the Young Academy of Europe and in the top 100 Italian scientists within 40 worldwide by the European Institute of Italian Culture.

His research group interests aim at developing biofabrication technologies to generate libraries of 3D scaffolds able to control cell fate, with applications spanning from skeletal to vascular, neural, and organ regeneration. From his research efforts, 3 products have already reached the market.

Prof. J. Miguel Oliveira

(Portugal)

3B's research group, University of Minho, Portugal

J. Miguel Oliveira BSc, Ph.D. (Portuguese, M, 44 years old) is a Principal Investigator with Habilitation at the PT Government Associate Laboratory ICVS/3B's (https://3bs.uminho.pt/people/94). He is the Vice President of I3Bs - Institute 3B's (Univ. Minho) and Director of Pre-Clinical Research at the FIFA MEDICAL CENTER, Estádio do Dragão, Porto, PT since Feb. 2013. Currently, he is a Lecturer in Doctoral Program in Tissue Engineering, Regenerative Medicine and Stem Cells (TERM&SC) at UMinho, PT (since Dec. 2013). He was also an invited lecturer at the Faculty of Medicine, U. Porto (2013-2019). Over the years he has focused his work on the field of biomaterials for tissue engineering, nanomedicine, stem cells, and cell/drug delivery. More recently, he set up a new research line within the ICVS/3B's on 3D in vitro models for cancer research.

As a result of his proficiency, he has published so far more than 400 scientific contributions in scientific journals with the referee, being 4 of those review papers produced under invitation. Miguel Oliveira has approved 22 patents (filled & submitted), published 7 books (plus 4 in preparation), 11 special issues/topic collections in scientific journals, and more than 100 book chapters in books with international circulation. He has participated in more than 500 communications in national/international conferences and has been invited/keynote speaker in more than 70 plenary sessions. He has an h-index of 59, i10 of 212 and received more than 13000 citations. He has been awarded several prizes including the prestigious "Investigador FCT 2012 and Investigador FCT 2015" attributed by FCT (Portugal), and Jean Leray Award 2015 from European Society for Biomaterials for Young Scientists for Outstanding Contributions within the field of Biomaterials. He is very active on the elaboration and scientific coordination of several PT and international funded projects. In addition, he is member of the National Ethics Committee for Clinical Trials from Serviço Nacional de Saúde (SNS) (PORTUGAL). He is the Editor-in-chief of the In vitro Models Journal (Springer Nature). He also integrates the advisory /editorial board of the Journal Bio-Design and Manufacturing, Journal of Materials Science: Materials in Medicine, Materials (MDPI), International Journal of Tissue Engineering, Journal of Composites and Biodegradable Polymers, Journal ISRN Biomaterials, The Journal of Experimental Orthopaedics, Journal "Recent Patents on Corrosion Science", and has several editorial roles in Frontiers. He is referee in more than 50 international journals. In 2020-21, he was listed among the TOP50 worldwide experts in tissue engineering and cartilage by EXPERTSCAPE (https://expertscape.com/ex/tissue+engineering), and has been listed in the World TOP2% most cited researchers (2021).

Prof. Ketul C. Popat

(USA)

Biomaterials and Surface Micro/Nano-engineering Laboratory
Department of Mechanical Engineering/School of Biomedical Engineering/School of Advances Materials Discovery
Colorado State University, Fort Collins CO

Dr. Popat is a Professor in the Department of Mechanical Engineering/School of Biomedical Engineering/School of Advanced Materials Discovery at Colorado State University. He is also the Director of Undergraduate Programs in Biomedical Engineering. Prior to that, he was working as a Research Specialist in the Department of Physiology at University of California, San Francisco. His research is in the areas of biomaterial surface modification, nanoscale surfaces for cellular manipulation and tissue engineering.

He has authored over 140 peer-reviewed publications in journals such as Langmuir, Biomaterials, Journal of Orthopedic Research, Journal of Biomedical Materials Research, etc. and has and h-index of 44.

He has also presented his work at numerous national and international level conferences. He received his Ph.D. in Bioengineering from University of Illinois at Chicago in 2003, M.S. in Chemical Engineering from Illinois Institute of Technology, Chicago in 2000 and B.E. in Chemical Engineering from M. S. University in India in 1998.

Prof. Dr. Vicentiu Saceleanu

(Romania)

Associate Professor of Neurosurgery,
Lucian Blaga University of Sibiu, Head of Neurosurgery Department County Hospital Sibiu

Dr. Vicentiu Saceleanu was born in 1968 in Sibiu - Romania, graduated from the Faculty of Medicine and Pharmacy, Iuliu Haţieganu,, Cluj-Napoca, Romania in 1994, received his Ph.D. from Lucian Blaga University (Sibiu-2011) and since 2011 he is the head of the Neurosurgical Department (County Hospital - Sibiu). Since 2000 he is a member of the Romanian Society of Neurosurgery, AOS International, Medical College of Sibiu and since 2016 he became a member of the Romanian Society of Biomaterials. He was a Associate Professor at Lucian Blaga University and since 2017 he joined the University of Sibiu as a full Professor, directing the Neurosurgical Department. In 2011 dr. Saceleanu founded the Neurosurgical Circle from Sibiu with 50 active students, participating in over 15 international and national congresses and coordinated over 20 presentations. Dr. Saceleanu is the author of 3 neurosurgical books, coauthor in 2 books and he published 194 articles in national and international journals.

Scientific Activity:

- Participation in numerous congresses and courses of Continuing Medical Education in Neurosurgery starting from 1995; participation in the national and international conferences as chairman and speaker: Romanian-German Courses of Neuro-traumatology; Sibiu Medical Days Conference, Chairman at Sibiu Medical Days 2011, 2013; International Seminar Paulo Freire "For a pedagy of the dialogue"; Stroke Conferences with international participation and symposia of Neurology and Psychiatry, Neurophysiology Electrodiagnostic Conference, Congresses and conferences on Neuroprotection and Neuroplasticity, Neurooncology Conference, Symposium "Il metodo Feuerstein", National Symposium of corticosteroids, the Conferences of the Society of Surgery;
- Participation to specialized courses in the field of neurosurgery: Skull Base Surgery Course; Bucharest; Vertebroplasty course Bucharest;
- Participation to international course Hands on: "Microsurgical and Endovascular Technoques", decembrie 2018, Salzburg;
- Local coordinator of the International Grant: Comparison of Cerebrolysin and Standard Treatment;
- Coordinator of project Brain Revealed: Innovative Technologies in Neurosurgery Study–2018-1-RO01-KA203-049317, 01.09.2018 – 31.08.2021;
- Patent: cranial implant with osseointegration stryctyres and functional coatings, international exhibition of scientific research, innovation and invention, 2018.

Publishing Activity:

Editor of the book of Clinical Neurosurgery, 2014; 3 specialty book in Romanian language, 2014; 1 specialty book in English language, 2014; 194 articles.

Research Activity:

- Professional training of residents (Neurology, Surgery, Orthopedics, Ophthalmology, Forensic Medicine), since 2011 till present;
- Founder and coordinator of the Neurosurgery Circle, LBUS, 2013,
- Development of Syllabus and course description of Neurosurgery discipline within the II Department of V. Papilian Faculty of Medicine Sibiu;
- Local organizer of 2 Neurosurgery Conferences, 2002, 2009 and one Conference of Neuro-rehabilitation at Ocna Sibiului, 2007;
- Organiser of Sibiu Medical Days, 2014;
- MAIN organizer of the Conference: "Treatment options and methods of rehabilitation in stroke and spinal pathology ", Sibiu, 2014, December 5.
- Member of the Scientific Commission of the County Clinical Emergency Hospital Sibiu, decision of Sibiu County Council no. 111/23.01.2015.
- Local main coordinator at the International Grant: Comparison of Cerebrolysin and Standard Treatment Evaluating the Neuroprotective and Neurorehabilitation Outcome in a Randomized, Double –Blind, Controlled Trial on Efficacy and Safety in Patients with Moderate to Severe Traumatic Brain Injury.2014, no.3455/27.02.2014
- Development of the research plan for the Neurosurgery discipline for the years 2012-2014 & 2014-2020 "V. Papilian" Faculty of Medicine Sibiu;
- Member of the Research Group of Ocular Surface, since 2014;
- Chairman of the Organizing Committee of the 45th edition of the Congress of the Romanian Society of Neurosurgery, Sibiu, October 2019;
- manager of the interntional project on Erasmsus Programme - Brain Revealed: Innovative Technologies in Neurosurgery Study – Universitatea Lucian Blaga din Sibiu;

Dr. Simone Sprio

(Italy)

Institute of Science and Technology for Ceramics, ISTEC-CNR,

Simone Sprio, Senior Researcher at the Institute of Science and Technology for Ceramics of the Italian National Research Council. Scientific Responsible of the research group on Bioceramics for Regenerative Medicine at ISTEC-CNR.
Main fields of investigation are: biomimetic calcium phosphates, bone cements and 3D scaffolds with cell-instructive ability for hard tissue regeneration; intrinsic antibacterial biomaterials; biomorphic transformation processes; drug delivery systems. Author of >120 peer-reviewed papers and book chapters. Inventor of 9 funded international patents. More than 100 communications at International Conferences including 25 Invited Lectures. Scientific Responsible or WP Leader in several national and EC-funded projects. Organizer of International Congresses, Symposia and Workshops. Supervisor of various M.Sc. and Ph.D students and young fellow researchers.

MD, PhD Gianluca Vadalà

(Italy)

[a] Laboratory of Regenerative Orthopaedics, Reaserch Unit of Orthopaedics, Department of Medicine and Surgery, Università Campus Bio-Medico di Roma.
[b] Operative Research Unit of Orthopaedic and Traumatology, Fondazione Policlinico Universitario Campus Bio-Meidco

Gianluca Vadalà, MD, PhD, is Associate Professor of Orthopaedic at Università Campus Bio-Medico di Roma (UCBM), Rome, Italy. He is currently employed at the Operative Unit of Ortopaedic of Fondazione Ppoliclinico Universitario Campus Bio-Medico. His activity is both focused on clinic - spine and orthopaedic surgery (80% of his time) - and research (20% of his time). He performs over 250 surgical casis each year (60% spine andm40% orthopaedic). He is the Director of the Laboratory for Regenerative Orthopaedics at UCBM focused on developing and translating new biological therapies included stem cell-based therapies in Orthopaedic and Spine Surgery. The Lab is composed by two full-time biologists with expertise on cell and molecular biology as well as on histology and one PhD student.

Over the his past 20 years of education and scientific production, Prof. Gianluca Vadalà devoted his career to acquire a multidisciplinary knowledge and to organize a multitask network of collaborators to develop new biological therapies for the treatment of intervertebral disc degeneration (IDD) aiming to move toward human clinical trials.

In 2013 and 2018 he received the *AOSpine Europe Young Research Award*, the most prestigious European award for research in the Spine Surgery field, for his enthusiastic research on IVD regeneration. In 2016, he received the Best Paper Award at the annual meeting of the International Society for the Study of the Lumbar Spine (ISSLS), the most prestigious and scientifically rigorous society in the spine field.

He has been ISSLS European Representative (2016-19). He is currently the immidiate Past-President of the European Orthopaedic Research Society (EORS) and Vice-President of the Italian Orthopaedic Research Society (IORS).

He works in close collaboration with the Musculoskeletal Regeneration Program of the AO Research Institute of Davos, Switzerland, coordinated by Prof. Mauro Alini. In 2005 he performed a 2 years fellowship at Ferguson Laboratory for Orthopaedic and Spine Research at University of Pittsburgh Medical Center (UPMC) under the supervision of Dr. James Kang.

He published over 100 paper in major international peer-reviewed scientific journals, including multidisciplinary scientific journals. He co-edited 2 peer-reviewed special issues in peer-reviewed scientific journals. He has an H index of 29 with 2400 citations (according to scopus) with a total impact factor of 350,512 with a mean of 7,544.

He received several national and international research grants in the field of regenerative orthopaedics and spine surgery as PI. He is the coordinator UCBM unit in the RESPINE and iPSpine research consortia of the Horizon 2020 European Research Great Program.

He performed over 50 invited lectures in the research proposal field in well-established international conferences and University and Research Institutes worldwilde (Europe, Japan, South Korea, Argentina, USA, etc);

Dr. Luuk van Dijk, PhD

(Netherlands)

Scientific Affairs Manager of Kuros Biosciences

Luuk van Dijk is a biomedical scientist from the Netherlands. He did a Master's programme in Regenerative Medicine and Technology, a collaborative programme between Utrecht University, the University Medical Center Utrecht (UMCU) and the Faculty of Biomedical Engineering at Eindhoven University of Technology (TU/e). During this programme, Luuk performed research focusing on tissue engineering of pre-vascularized bone tissue analogs, and a project on stem cell encapsulation in hydrogels for potential new therapeutic applications. After obtaining his postgraduate, he started his PhD project to investigate osteoinductive calcium phosphate ceramics for bone regeneration at the University Medical Center of Utrecht. Luuk's research focused on pre-clinical and clinical evaluation of osteoinductive calcium phosphates, which included investigation of bone grafts in in vitro studies, in clinically relevant animal models, and a clinical trial at the University Medical Center Utrecht. Since January 2021, Luuk works as Scientific Affairs Manager at Kuros Biosciences. In this role, he is responsible for medical education, scientific communications and scientific support for the various departments within the company.

About Kuros Biosciences

Kuros Biosciences is a fast-growing leader in the development of spinal fusion biologics that ease the. burden of back pain. With locations in the United States, Switzerland and the Netherlands, the company is listed on the SIX Swiss Exchange

The company's first commercial product, MagnetOs, is a unique synthetic bone graft that has already been used successfully across three continents and in over 10,000 spinal fusion surgeries.

The next candidate in the Kuros pipeline is Fibrin-PTH – the first drug-biologic combination for interbody spinal fusions, currently undergoing a Phase 2 clinical trial in the U.S. For more information on the company, its products and pipeline, visit kurosbio.com.

Prof. Franz E. Weber

(Switzerland)

University Zurich, Center for Dental Medicine, Oral Biotechnology & Bioengineering

Franz E. Weber graduated from the University Konstanz (Germany) with a PhD in Biology/Muscle Biochemistry.
He completed a 3-year postdoctoral training on muscle biology at Cornell University Medical College in New York City and spent two years at the ETH Zurich (École polytechnique fédérale) working on the lipid uptake from the small intestine. In 1995, he joined the Department of Cranio-Maxillofacial and Oral Surgery at the University (Hospital) Zurich. His research encompasses additive manufacturing, bone substitutes, osteoconduction, bone morphogenetic proteins, delivery systems, epigenetics, and *in vitro* bone and pulp tissue engineering. Franz E. Weber has authored 143 peer-reviewed research articles published in international journals amounting to more than 6655 citations, 16 patents, and an h-index of 38.
He is currently appointed as Professor of Craniofacial and Oral Biotechnology and member of the Medical and the Science Faculty at the University of Zurich and as visiting Professor in the Dental School at the University of Hong Kong.

Prof. Yufeng Zheng

(China)

School of Materials Science and Engineering, Peking University

Harbin Engineering University, China; Materials Science; B.S., 1993.
Harbin Institute of Technology, China; Materials Science; Ph. D., 1998
Appointments
2004.09-Present, Professor, Peking University, China
1998.07-2004.08, Professor (2003-2004), Associate Professor (2000-2003), Assistant Professor (1998-2000), School of Materials Science and Engineering, Harbin Institute of Technology, China
2003.12-2004.05, Research Fellow, School of Mechanical Engineering, The University of Western Australia, Australia
1999.10-2000.06, Senior Research Associate, Department of Physics and Materials Science, City University of Hong Kong, China
Research activities
Principal investigators of over 50 projects, supported by MOST (973, 863, international collaboration projects), NSFC, MOE, etc..
Development of new kinds of biomedical metallic materials (biodegradable Mg, Fe and Zn alloys, Ti alloys with low elastic modulus, Bulk metallic glass, nanocrystalline metals and alloys, etc)
Publications and Patents
Over 700 peer-reviewed journal articles (with the citation of over 28, 000 times, and H-index of 83 (https://publons.com/researcher/4236574/yf-zheng/) and 18 book/book chapters published.
Owners of 50 Chinese invention patents being issued.
Synergistic Activities
Founding Editor-in-Chief of “Bioactive Materials”(KeAi)

Editor of “Materials Letters”(Elsevier)
Vice Editor-in-Chief of “Journal of Materials Science & Technology” (Elsevier).
Honors
2020, Fellow of Biomaterials Science and Engineering (FBSE) by the International Union of Societies for Biomaterials Science and Engineering (IUSBSE)
2019, American Institute for Medical and Biological Engineering (AIMBE) College of Fellows

Session 1 – Advanced Materials in Orthopaedics

Immuno-Mechanics in Bone Defect Healing: Drive Regeneration by Employing the Immune-Structure Interface

Georg N. Duda[1,2], Christian H. Bucher[1,2], Sven Geissler[1,2], Simon Reinke[1,2], Hans-Dieter Volk[3], Katharina Schmidt-Bleek[1,2]

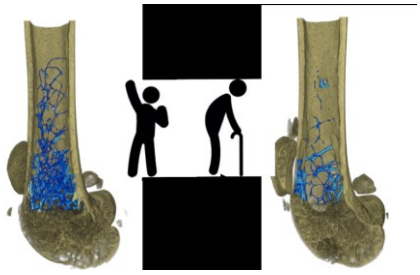
[1]Julius Wolff Institute, Berlin Institute of Health @ Charité – Universitätsmedizin Berlin, DE
[2]Berlin Center for Regenerative Therapies, Berlin Institute of Health @ Charité – Universitätsmedizin Berlin, DE[3]Institute for Medical Immunology, Charité - Universitätsmedizin, Berlin, DE

Introduction

Research on regeneration frequently uses young pre-clinical model systems. However, challenges to regenerate e.g. after an injury are found in compromised patient settings, usually different from commonly used model systems. In order to steer regeneration in such compromised patient settings, it is essential to understand how systemic challenges like increased age, metabolic challenges or chronic inflammation alter endogenous regenerative cascades. With increased age, the mechanical competence of bones, the number of available stem cells as well as the overall bone turnover is reduced. Thus the regenerative capacity is also reduced, however little is known on its details. Aim of our work is to analyse the alterations in bone regeneration and adaptation with increasing age and how these are driven by the interdependency of the immune and the bone systems.

Material & Methods

The immune status of 3, 12 and 24 months old C57BL / 6 mice, kept under pathogen-free or under environmentally exposed housing, was determined by flow cytometry. Splenocytes and bone marrow cells were isolated, stained and analyzed on a LSR Fortessa with 12-color panels. The bone structure of mice was analyzed by X-ray microcomputer tomography on a SkyScan 1172 high resolution desktop micro-CT and biomechanical competence was assessed using torsional testing. To investigate the interaction of the bone and immune systems, in vitro differentiation studies were performed using conditioned medium from immune cells on mesenchymal stromal / stem cells (MSCs). Specifically, more or less immune-experienced human donor cell compartments were compared in their osteogenic differentiation capacity and compared to bone regeneration in a more or less immune experienced mouse model system. Finally, a mechano-therapeutic experiment was performed to rescue an experienced immune system's lack of bone adaptation.



Results

As expected, the femurs of 24-months-old mice with a more naive immune system were stiffer and broke at lesser deformation (more brittle) when compared to a young control. The microarchitecture was characterized by a reduced mineralization and dwindling trabecular density. However, if these aged mice had the chance to gain animmulogical memory and to develop effector memory T cells, the bone was even less elastic and even more brittle. Most structural parameters describing trabecular as well as cortical bone properties substantially changed with a more experienced immune system developing with age that included highly active effector/ effector memory cells of the adaptive immune system. In such settings, the osteogenic differentiation of MSC was severely impaired under a cytokine milieu of these effector immune cells. Under limb loading, simulating a mechanically active moving behaviour, a more naïve phenotype was induced and thus the immune system's aged negative effects were partially rescued.

Discussion & Conclusions

Our findings indicate that, in old age, there is a pro-inflammatory milieu in the bone which further reduces the chronologically caused decrease of the regenerative ability. This effect is even more pronounced in a "humanized" mouse model with a more experienced (high effector / effector memory percentage) immune system. We found a combination of weakened bone structure and increased inflammatory reactivity that substantially affected bone's osteogenic capacity. The findings explain why not only bone structure but – specifically in regenerative situations – the immune systems inflammatory capacity and experience is a key aspect to be addressed if regeneration is to be reached. So far, the interaction of an experienced immune system with the cascades of endogenous regeneration have been widely neglected in clinical practice. Including a better understanding on the interaction of the immune and skeletal system and eventually re-balancing a mismatch of the two opens new avenues in treatment of patients with compromised healing situations.

Allogeneic grafts as biomimetic materials for clinical application

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Keywords: biomimetic materials; autograft alternatives; commercial bone allografts; tissue bank products

Introduction

Bone tissue regeneration remains an important challenge in the field of orthopaedic surgery. Traumatic injuries and acute/chronic diseases can impair normal bone functions and lead to bone fractures, non-unions, immobility, severe pain, and deformity [1]. Furthermore, the ageing population, poised to become one of the most significant social transformations, explains the increasing number of bone graft procedures performed every year.
As such, the demand for bone grafts is considerable with over 2.2 million bone graft procedures conducted worldwide annually [2]. Although autogenous bone remains the gold standard graft material, it is associated with an unacceptably high incidence of morbidity since operative time, blood loss, and length of stay in hospital are often increased. Therefore, alternative strategies are needed. In this respect, the scaffolding for osteoconduction and the presence of growth factors for osteoinduction, are essential elements which must be considered to replicate the optimal bone healing properties of autogenous grafts. However, although current clinical treatments aim at overcoming the limitations of conventional bone grafting approaches, relatively few innovative orthopaedic biomaterial grafts designed with biomimetic and bioresponsive characteristics have been translated into clinical solutions [3].

Critical issues, such as high costs, regulatory issues, or severe complications are potential drawbacks that may reduce the emphasis related to new bone biomimetic substitutes reaching the market. Nevertheless, according to the EU-Report on orthobiologics, this market in Europe reached USD 1.35 billion in 2022 and is estimated to rise to USD 1.75 billion by 2027 [4]. Besides companies which invest in orthobiologics, bone tissue banks−i.e., accredited non-profit organizations−play a pivotal role in the production and distribution of bone grafts. In this regard, Musculoskeletal Tissue Bank (BTM) of IRCCS Istituto Ortopedico Rizzoli (Bologna, Italy) provides biological allografts for a series of orthopaedic procedures, therefore, it is claimed as a link between the donor and the recipient. Among numerous allogeneic bone grafts prepared by BTM, some of them are featured by biomimetic osteoinductive and osteoconductive properties suitable for several orthopaedic applications, such as bone filling defects, arthrodesis, or revision surgery, demonstrating their safety and efficacy in promoting bone regeneration and repair.

Results and Discussion

By evaluating the commercially available allogeneic products claimed as an alternative to autografts for orthopaedic indications, several distinct commercial products were identified on the global market. Based on their characteristics (i.e., presence of competent cells or growth factors), they were divided into two main categories: cellular bone matrices and growth-factor-enhanced bone grafts. However, although an accurate characterisation of each product has shown many advantages, also many potential drawbacks were highlighted, thus reducing the emphasis related to these bone substitutes [5]. On the other hand, the retrospective evaluation of BTM bone biomimetic products have shown good results in terms of osteointegration and bone remodelling, and low immunogenicity. Moreover, they are easily handled, and to date, no severe side effects have been reported. However, the main limitation concerning the use of commercial, as well as tissue bank products, is lack of large randomised clinical trials and prospective cohort studies with high-quality design and execution, which are necessary to better enhance treatments for patients undergoing bone grafting.

Conclusions and Outlook

Modern bone grafts and bone substitutes attempt to enhance the natural healing process via osteoinductive, osteoconductive and/or osteogenic mechanisms. However, when evaluating the complex landscape of grafting materials, it is difficult to guide clinicians in selecting the products which best fit the clinical indications and relative expected outcomes due to several existing formulations and related different mechanism of action, and the lack of supportive clinical evidence in the scientific literature.

Hence, further scientific efforts should be encouraged to promote a translational approach that bridges research and clinical setting, intending to minimise potential biases concerning the efficacy and safety of both innovative and outdated products.

Among new approaches in the field of bone regeneration, peptides can trigger some specific signalling pathways that control osteogenic-related cellular functions. Nevertheless, up to the present, in commercially available products, bioactive peptides are exclusively used in combination with xenogeneic bone sources, while based on the biomimetic principle (i.e., a material as similar as possible to the host bone is recommended to allow for the best biological behaviour [6]), it could be interesting to investigate the synergistic effect exerted by peptides and allogeneic bone tissues. To this aim, R&D efforts driven by tissue banks together with the R&D expertise of ortho-biological companies could make the difference in terms of innovation and distribution of new products in the field of bone grafting.

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Biomimetic 3D scaffold-based in vitro models for bone pathology investigation

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Keywords: 3D porous structures, CaPs, polyurethanes, PLLA, cancer models

Introduction

The in vitro mimicking of bone tissue for the study of pathologies is a challenging field that requires the design of scaffolds with suitable morphological, structural and cytocompatible properties [1]. Traditional fabrication technologies allow obtaining random pore size distribution with low reproducibility in the porous structure morphology, while 3D printing allows achieving defined and reproducible pore sizes and geometries [2].

In this work, polyurethane foams and 3D printed thermoplastic poly-ether-urethane (TPU) designed with different geometrical patterns are proposed as scaffolds for in vitro model of bone tissue. Surface coatings for a biomimetic behaviour of the 3D scaffold models were also investigated. After evaluation of morphological, chemico-physical, and mechanical properties, scaffolds were investigated in terms of biological in vitro interaction different cell phenotypes.

Results and Discussion

Polyurethane foams for metastases investigation. The suitability of a poly-ether-urethane (PU) foam as 3D in vitro model to study the interactions between BC tumor initiating cells and the bone microenvironment was investigated. PU foam open porosity (>70%) appeared suitable to mimic trabecular bone structure. The PU foam showed good mechanical properties under cyclic compression (E = 69–109 kPa), even if lower than human trabecular bone. The scaffold supported osteoblast SAOS-2 cell line proliferation, with no cytotoxic effects. Human adipose derived stem cells (ADSC) were cultured and differentiated into osteoblast lineage on the PU foam, as shown by alizarin red staining and RT-PCR, thus offering a bone biomimetic microenvironment to the further co-culture with BC derived tumor-initiating cells (MCFS). Tumor aggregates were observed after three weeks of co-culture by e-cadherin staining and SEM; modification in CaP distribution was identified by SEM-EDX and associated to the presence of tumor cells. In conclusion, we demonstrated the suitability of the PU foam to reproduce a bone biomimetic microenvironment, useful for the co-culture of human osteoblasts/BC tumor-initiating cells and to investigate their interaction.

3D printed scaffold as tumor bone model. The morphology of the printed scaffolds matched the one of the CAD-designed models, after printing process optimization. 3D printed scaffolds have pores with a precise and regular geometry; particularly, 0°-90° scaffolds have quadrangular pores, 0°-45°-90°-135° scaffolds have complex polygonal pores and 0°-60°-120° scaffolds have hexagonal pores when the pore size is 1 mm, and triangular for 0.7 mm pore size. Porosity can be considered opened, due to the fact that morphological analysis shows open pores, indeed 3D printing techniques allow to obtain 100% interconnected structures. PU scaffold porosity evidenced values from 55 to 67%, values that belong to the porosity range of the trabecular bone tissue (30-90%). Scaffolds with 1 mm pore size have higher porosity compared to those with 0.7 mm pore size. The stability test demonstrated that all scaffolds are stable in culture medium up to 21 days, indeed there is no weight reduction during the test. The compressive modulus varied between 2 and 4 MPa, depending on the printed pattern. Moreover, stress-strain curves show that scaffolds with 0.7 mm pore size are stiffer than those with 1 mm pore size. *In vitro* viability was higher than 70%, proving the absence of cytotoxic effects. An increase in cell metabolic activity was detected from 1 to 14 days of culture on the printed scaffolds.

Conclusions and Outlook

Based on the obtained results, scaffolds with different geometries have been successfully produced. Morphological and structural properties of the scaffolds here presented are suitable for mimicking the bone tissue, in order to produce a 3D in vitro model useful for bone pathologies research.

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Towards deciphering neutrophils role in the immune response to biomaterials

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Keywords: Biomaterials; Neutrophils; inflammation; hyaluronan; Collagen

Introduction
Neutrophils are the most prevalent immune cell in the human body and first line responders to injury, infection or material implantation. As such, they have a leading role in determining the fate of immune response upon implantation of biomaterials, and the onset and resolution of chronic inflammation, and macrophage polarization [1]. Type and intensity of this response depend on the physical and chemical properties of the biomaterials, as well as boundary conditions such as the injury severity at the site of implantation [2] or general health condition of the patient. Despite the central role played, the neutrophil response to biomaterials and the consequent impact on tissue engineering approaches is still scarcely understood. Investigating and understanding the interactions between biomaterials and neutrophils is key to establish design principles for biomaterials to provide an immune response fostering tissue repair and regeneration. In this study we report an in vitro culture model to describe the most important neutrophil functions in relation to tissue repair. Additionally, we present the effect of material stiffness, coating and culture conditions in the measured neutrophils response.

Methods
Human primary neutrophils were isolated from peripheral blood after informed consent using EasySep™ Direct Human Neutrophil Isolation Kit (STEMCELL Technologies, Vancouver, Canada) according to the manufacturer's instructions. Purity was determined by flow cytometry using CD66b, CD16 (positive) and CD14 (negative marker). Tyramine-modified hyaluronic acid (THA) was synthesized according to a previously reported procedure [3], and hydrogels were prepared via enzymatic crosslinking using horseradish peroxidase (HRP) and H2O2 [4]. The THA hydrogels were casted into 48-well plates and crosslinked with 0.65 mM (≈0.5kPa), 1.3 mM (≈1kPa), and 2.6 mM (≈4kPa) hydrogen peroxide to determine the effect of storage modulus on metabolic activity of neutrophils. Further gels were prepared from gelatin methacryloyl (GelMA), collagen, THA/collagen and polyvinyl alcohol (PVA) [5]. Polycaprolactone (PCL, MW:45000 Da) scaffolds were fabricated with topography consisting of parallel lines by an extrusion-based bioprinter (3D Discovery; RegenHU, Switzerland). 3-D printed PCL constructs with linear or criss-cross printing paths were incubated in phosphate buffer saline solution containing increasing concentrations of Foetal Bovine Serum (FBS) overnight to examine the influence of FBS proteins on the viability of neutrophils. Cells were seeded on biomaterials and tissue culture plastic (TCP) with a density of 300'000 cells/scaffold, then the metabolic activity and percent cytotoxicity were determined (1, 3, 5, 7 and 24 hours) via CellTiter-Blue® Cell Viability Assay (CTB, Promega, Dübendorf, Switzerland) and Cytotoxicity Detection KitPLUS LDH (Roche, Sigma-Aldrich) according to the manufacturer's protocols. Reactive oxygen species were determined from the rate of cytochrome C reduction, measured using the Cytochrome C Assay Kit (Abcam, Cambridge, United Kingdom) according to the manufacturer's instructions. A live and dead assay with ethidium homodimer and calcein AM was carried out imaging with confocal microscopy. Myeloperoxidase (MPO), Matrix Metalloproteinase 9 (MMP9) and neutrophil elastase were quantified via ELISA; a panel of 92 inflammation-related cytokines were measured in the media supernatant using the Olink® inflammation multiplex immunoassay (OlinkLINK® Proteomics, Uppsala, Sweden).

Results and Discussion
Based on metabolic activity and live and dead assay, neutrophil survival was higher on the hydrogels prepared from hyaluronan, gelatin and collagen, decreasing to 60-70% on PVA and TCP, and lower than 50% on PCL (**Figure 1**). Metabolic activity after 1, 3, 5, and 7 hrs was 2-3-fold higher on TCP and PCL than on soft materials. Although metabolic activity is indicative of cell survival, neutrophils activation might also increase it, and therefore a correct interpretation of these results needs further experiments to independently verify the inflammatory activity. MPO and elastase are

markers of Neutrophil Extracellular Traps (NETs) formation. Their levels were 3-10-fold higher on TCP, PCL, and PVA surfaces than on hyaluronan-and collagen-containing hydrogels. In contrast, ROS production was higher on soft hydrogels than on TCP and PCL. VEGFA, HGF, EN-RAGE OSM and MMP9 secretion was significantly higher on TCP, PVA and PCL surfaces than on hyaluronan- and collagen-containing hydrogels. Metabolic activity was higher for neutrophils cultured on FBS-free PCL compared to FBS-treated at all time points. THA storage modulus variation between 0.5 and 2.6 kPa did not induce major variations of metabolic activity. Neutrophils incubated on THA hydrogels released less LDH compared to PCL up to 24 h (Fig. 1B). LDH released from the neutrophils was higher on FBS treated PCL scaffolds than the FBS-free PCL scaffolds between 1-7h of incubation.

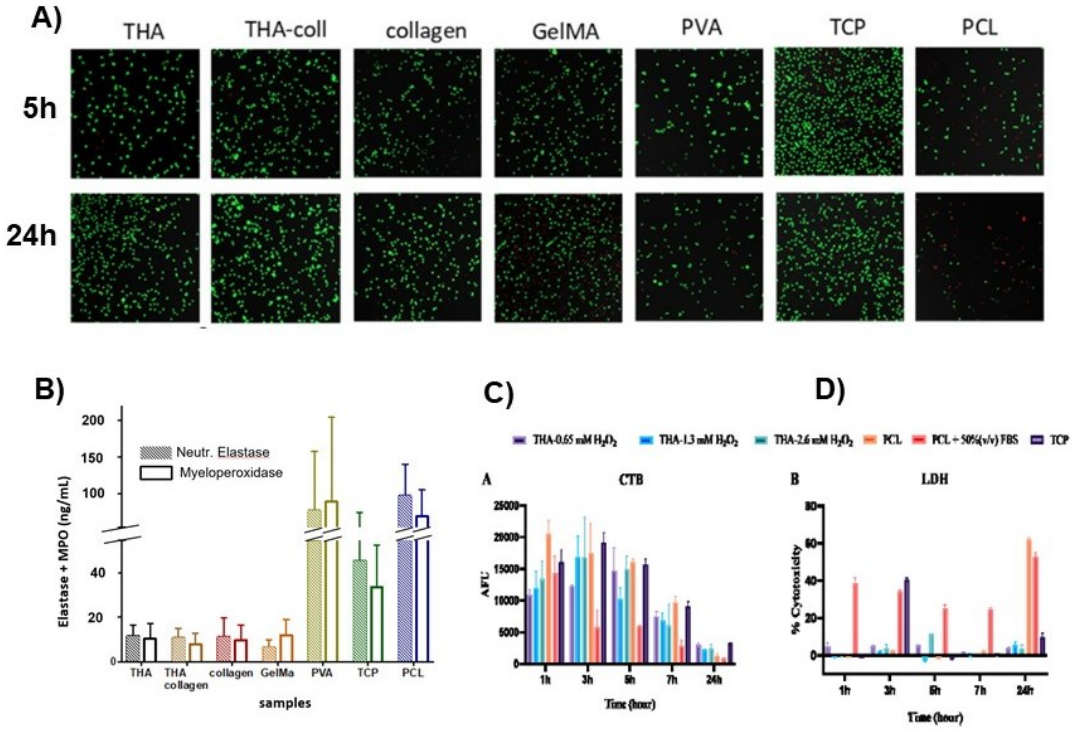

Figure 1: A) Representative images of live/dead staining after 5 and 24 hours showing living cells in green with dead cells in red. B) Release of NET components on different materials C) Metabolic activity of neutrophils represented as arbitrary fluorescence units over time on different material surfaces, D) LDH release by neutrophils cultured on different material surfaces represented as percent cytotoxicity. More details on reference [5].

Conclusions and/or Outlook

The array of assays and tests introduced was suitable to identify clear differences in the response by neutrophils that were cultured on materials with a different chemical composition. Overall, substrates from biopolymers of natural origin resulted in increased survival, less NET formation, and more ROS production than synthetic polymers. Within the range of mechanical properties explored, storage modulus of THA did not significantly alter neutrophils response. Further studies are needed to correlate the neutrophil response to tissue healing and to better understand how to use material chemistry and properties for immune modulation.

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A new Gellan Gum/Lignin bioink: a promising route for cartilage repair

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Keywords: gellan gum; lignin; hydrogel; bioink; cartilage; bioprinting

Introduction

Recent developments in tissue engineering have been addressed to the development of cartilage-replacinghydrogels based on gellan gum, a bacterial exopolysaccharide, in combination with different polymers and/or fibers to enhance its mechanical and biological performances [1, 2]. In this respect, our research group focused theattention on gellan gum-based hydrogels reinforced with different inorganic clays as cartilage substitutes [3, 4]. Inthis study, a composite hydrogel, based on a blend of two gellan gums with different acyl content embedding different amounts of lignin (up to 0.4% w/v), was developed for the regeneration of cartilage tissue. Indeed, the combination of the two gellan gums with lignin, led to the obtainment of an easily bioprintable and eco-friendly hydrogel, overcoming the issue of the low printability of bare gellan gum related to its high viscosity and sharp gelling temperature, Hydrogels were physically crosslinked with magnesium ions and underwent an accurate chemical-physical (FT-IR, XPS, SS-NMR), mechanical (static compression tests) and rheological (viscosity measurements) characterization.
The cytocompatibility of the developed hydrogels and the chondrogenic activity of seeded human mesenchymal stem cells (hMSCs) was assessed *in vitro*. Moreover, the bacteriostatic potential of the hydrogels conferred by lignin was studied *in vitro* towards the joint pathogens *Staphylococcus aureus* and *Staphylococcus epidermidis*. Finally, the optimized hydrogel encapsulating hMSC, as bioink, was bioprinted with an extrusion-based bioprinter, in order to manufacture three-dimensional porous constructs for cartilage regeneration.

Results and Discussion

The formulation of the hydrogel composites has been optimized in terms of GG high acyl (GG_H) and GG low acyl (GG_L) ratios, obtaining the best performances using GG_H/GG_L 3:2. Based on the lignin content, the obtained gellangum hydrogels were coded as GG_{HL}Lign0.1, GG_{HL}Lign0.2 and GG_{HL}Lign0.4. Gellan gums hydrogels without lignin (GG_{HL}) have been prepared as controls.

The XPS, FT-IR and SS-NMR analyses of the novel hydrogel composites established that no chemical interaction between lignin and the polysaccharides occurred. Moreover, the mechanical tests evidenced that the formulated hydrogels were able to recover their original shape after compression.

Lignin exploitation resulted cytocompatible, since the viability of hMSC 3D-seeded onto scaffolds resulted comparable to the lignin-free control hydrogel (>70% viable cells, $p>0.05$, **Figure 1a**). Moreover, the 0.4% lignin composites significantly improved the hMSC 3D-constructs chondrogenesis bringing to a significant ($p<0.05$) up-regulation of the collagen type II, aggrecan and SOX 9 chondrogenic genes (**Figure 1b**).

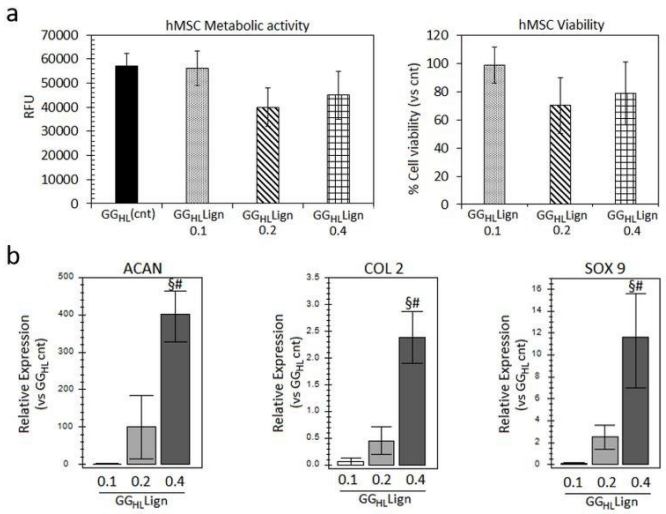

Figure 1: Cytocompatibility assessment (a) and chondrogenic differentiation (b) of the GG_{HL}Lign hydrogels.

The presence of lignin conferred bacteriostatic properties to the hydrogel too, thus reducing the proliferation of the joint pathogens *Staphylococcus aureus* and *Staphylococcus epidermidis* of >2 logs in comparison to the lignin-free control hydrogel.

Finally, cellularized 3D constructs were manufactured via 3D bioprinting (**Figure 2**), without affecting cells viability, confirming the processability of the hydrogel formulation as a bioink and its unique biological features for creating a physiological milieu for cell growth.

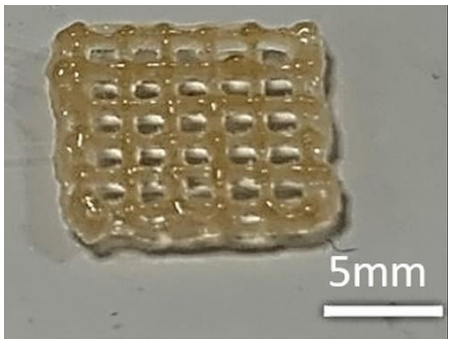

Figure 2: Grid structure obtained using GG_{HL}Lign0.4.

Conclusions

The developed composite formulation fulfilled all the conditions for being considered as suitable bioink for bioprinting 3D-constructs with different geometry and resolution, thanks to the favorable viscosity due to the incorporation of lignin. The bioprinted hydrogels did not adversely affect the encapsulated hMSC, allowing cell proliferation. Considering its processability and biocompatibility, this first printable gellan gum/lignin hydrogel will have potential in different applications in tissue engineering, beyond the regeneration of cartilage.

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Early Mineral Ion Release and *In-vivo* Bio-integration of Glass Fiber-Reinforced Orthopedic Implants

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Keywords: Bio-integrative, Orthopaedic implants, mineral fiber-reinforced, ICP-OES, Degradation, Bioglass

Introduction

Mechanical strength and durability are key factors for achieving successful bone osteosynthesis when using bone fixation implants[1]. Therefore, traditionally most of the implants were made of metals. However, metal introduces inherent risks such as infections, acute or chronic inflammation, implant migration and bone tissue loss, all of which pose the frequent need for hardware removal[2]. The use of biomaterials for orthopedic fixation can provide a solution to these limitations[3]. The new class of mineral fiber-reinforced implants used in this study offer strong bone fixation while bio-integrative in nature, enabling complete restoration to native anatomy. The implants are composed of continuous reinforcing mineral fibers, comprised of elements found in native bone (SiO_2, Na_2O, CaO, MgO, B_2O_3, and P_2O_5; approximately 50% w/w), and bound together by a degradable polymer resin [poly (L-lactide-co- D,L-lactide), PLDLA](**Figure 1A&B)**[4,5]. The mineral fiber component is similar in composition to resorbable bone fillers, such as bioactive glasses that are known for their osteoconductive properties. Bioglass is the most widely known bioactive material, made typically from different ratios of Na_2O-CaO-MgO-P_2O_5-SiO_2 elements. These bioactive materials are known for their unique ability to form a mechanically strong bond with bone[6,7]. Furthermore, unlike other biocomposites and bioresorbable orthopedic implants, which rely solely on the polymer content for mechanical performance, the fiber-reinforced implants leverage both the biological, as well as the biomechanical advantage of the natural mineral fiber matrix. The bone-healing benefit of these fiber- reinforced implants has been demonstrated in preclinical studies and is thought to be related to the high mineral content of these implants. However, the release of these mineral ions has not previously been demonstrated.

The purpose of this study was to evaluate the early release profile of the major 4 elements of the mineral component in these bio-integrative implants. ICP-OES analysis was performed on the degradation media, to evaluate the amount of minerals (Silicone (Si), Calcium (Ca), Magnesium (Mg) and Phosphate (P)) released at several predefined timepoints throughout a 15-day period.

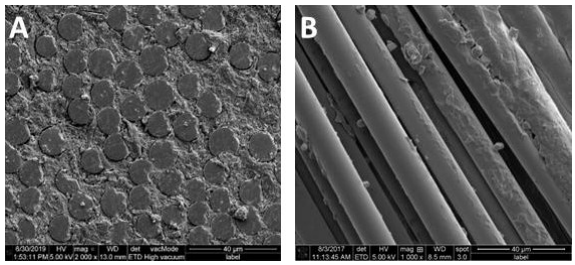

Figure 1: Scanning Electron Microscopy (SEM). Continuous mineral fibers surrounded by polymeric material, in cross section (A), and longitudinal section (B).

Materials and Methods

Fixation nail implants (3mm x 50mm, OSSIO*fiber*®, Ossio Ltd.) were incubated in saline at physiological conditions (0.9% NaCl, 37 °C) for 3h, 12h, 1, 4, 7, 11 and 15 days. Saline samples served as negative control (blank). The pH level was measured at each time point. Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) analysis was performed on the degradation media to evaluate the amount of fiber mineral ions - Si, Ca, Mg and P - released into the media at the different timepoints throughout the 15-day period.

Results and Discussion

The implants were incubated in 0.9% saline solution, simulating physiological conditions with slightly acidic pHlevel of 5.5. Results demonstrate an increase of the test group media pH level throughout the 15-day period from 5.5 to 8.87 (**Figure 2)**. ICP-OES analysis results indicate an increase in all the 4 tested ions concentrations in the degradation media. The detected rate and initiation time point of ion release is different between the ions.While Si started its release in the media as soon as after 3 hrs of incubation, P ions were evident at day 4. The media concentration of Si remained the highest at the end of the study (the 15th day, 64.3 mg/L) (**Figure 3A)**, while P

concentration reached the value of 2 mg/L (**Figure 3D)**. Ca and Mg ion release demonstrated anintermediate trend of initiation point and rate (**Figure 3B&C)**. These results indicate the alkaline pH impact of ion release, which may play a role in balancing the pH effect of the polymer component degradation,
the degradation products of which are acidic by its nature.

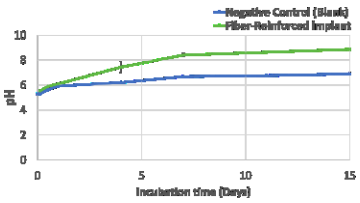

Figure 2: Degradation media pH

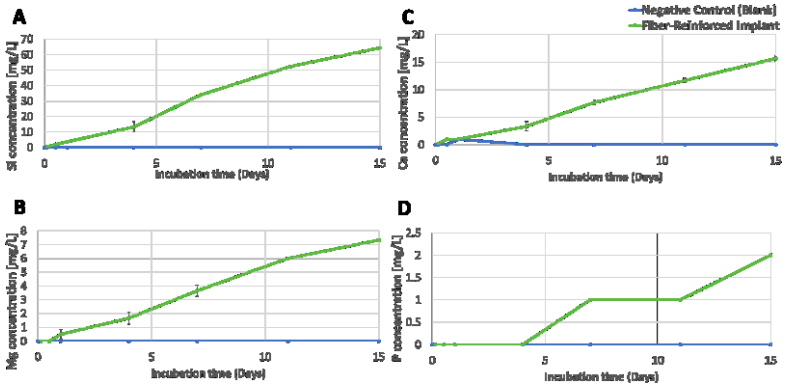

Figure 3: Degradation media ions concentration: Si (A), Mg (B), Ca (C) and P (D)

Conclusions and Outlook

The excellent bio-integration of glass fiber-reinforced implants have been previously demonstrated in preclinical studies (**Figure 4A-D**), where extensive mesenchymal cell ingrowth and bone attachment to the implant have been observed following implantation[4].

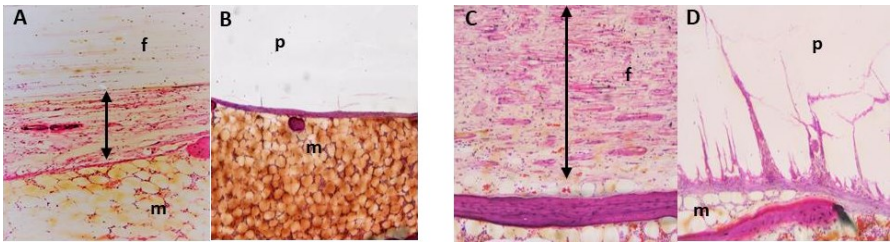

Figure 4: Histology view of fiber-reinforced vs. polymer control in a rabbit femur model, H&E stain. Fiber-reinforced: 26- week (A) and 78-week (C) implantation. Polymer–control: 26-week (B) and 78-week (D). Double arrows indicate extent of mesenchymal tissue infiltration into implant wall, m= medullary cavity; f= bioactive glass fibers; p= polymer implant.

This study demonstrates, for the first time, that mineral fiber-reinforced implants provide their local environmentwith a steady supply of key mineral ions in the early days following simulated implantation in the body.
Minerals have a significant role in biocomposite implants, as they balance the decreasing pH level caused by the polymer component as its being degraded. The alphahydroxy acidic degradation products of the polymer are balanced by the alkaline degradation products of the mineral fibers. Within the implant matrix, two bio-integrative mechanisms are occurring concurrently: polymer resorption and mineral fiber replacement by bone. This hybrid material degradation mechanism ensures that the resorption of the fiber-reinforced implants is pH balanced. In contrast to existing minerals compounds, such as hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP)[8], fiber-reinforced implants are comprised of thousands of continuous mineral fibers, built into an organized layered structure, oriented to provide desired biomechanical properties. Since the mineral components in the fiber matrix are similar to those found in native bone and have the ability to create strong bond with bone, these implants, in addition to their mechanical properties, can potentially contribute to an efficient and rapidbone healing process. The release of the major 4 elements of the mineral component in these bio-integrative implants starts early upon exposure to physiological conditions and remains steady for many days thereafter. This level of mineral ion availability is consistent with the hypothesis that the mineral content of the fiber- reinforced implants can benefit the local bone healing environment.

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Session 2 –Advanced Functional Materials for Orthopaedic and Spine Surgery

MSC based Intervertebral disc regeneration: from bench to bedside

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Keywords: Mesenchymal Stem Cells, Intervertebral disc degeneration, Spine

Low back pain caused by intervertebral disc disease (IDD) presents a large, unmet medical need which results in a disabling loss of mechanical function. Today, no efficient therapy is available. Chronic cases often receive surgery, which may lead to biomechanical problems and accelerated degeneration of adjacent segments.
Bone marrow mesenchymal stem cell (MSC) based regenerative therapies showed encouraging results in in vitro, in animal model and in phase 1 and 2 clinical trials.
Our group devoted 15 years of research moving from in vitro cells studies and organ culture studies to preclinical studies and randomized clinical trials.
The lecture aims to present the state of the art of bone marrow MSC based Intervertebral disc regeneration focusing on our contribution in this field of research.

Autologous mesenchymal stem cells in the treatment of spinal aneurysmal bone cyst

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Keywords: mesenchymal stem cells, bone regeneration, spinal diseases

Introduction

Aneurysmal bone cyst (ABC) of the spine is a locally aggressive benign lesion which can be treated by en bloc resection with wide margin to reduce the risk of local recurrence. To avoid morbidity associated with surgery, selective arterial embolization (SAE) can be considered the first-line treatment for ABCs of the spine. Other emerging treatments for ABCs include bisphosphonates, percutaneous doxycycline, sclerotherapy and Denosumab. In addition, we previously introduced the use of autologous bone marrow concentrate (BMC) injection therapy to stimulate bone healing and regeneration in ABC of the spine. One of the potential advantages of such a method is that surgical treatments are not necessary, thus allowing for both a minimally invasive approach and the treatment of poorly accessible lesions.

In this prospective study we described the clinical and radiological outcomes of percutaneous injection of autologous BMC in a series of patients affected by ABCs of the spine and followed for at least one year.

Fourteen patients (6 male, 8 female) were treated between June 2014 to December 2019 with BMC injection for ABC of the spine. The mean age was 17.85 years. The mean follow up was 37.4 months (range 12- 60 months). The dimension of the cyst and the degree of ossification were measured by Computed Tomography (CT) scans before the treatment and during follow-up visits [1].

Results and Discussion

Six patients received a single dose of BMC, five patients received two doses and in three patients three doses of BMC were administered. The mean ossification of the cyst (expressed in Hounsfield units) increased statistically from 43.48±2.36 HU to 161.71±23.48 HU during follow-up time and the ossification was associated to an improvement of the clinical outcomes. The mean ossification over time was significantly higher in patients treated with a single injection compared to patients treated with multiple injections. No significant difference in ossification was found between cervical and non-cervical localization of the cyst. Moreover, the initial size of the cyst was not statistically associated with the degree of ossification during follow-up.

We also observed that five out of six female patients (83.3%) were less than sixteen years old and four of these (66.7%) were managed with a single dose of BMC injection, while a higher percentage of male patients (6/8, 75%) were more than sixteen years old and more than one injection was administered to them [1].

In **Figure 1** we report the pre-operative and follow up imaging of a patient affected by spinal ABC treated with autologous bone marrow concentrate injection.

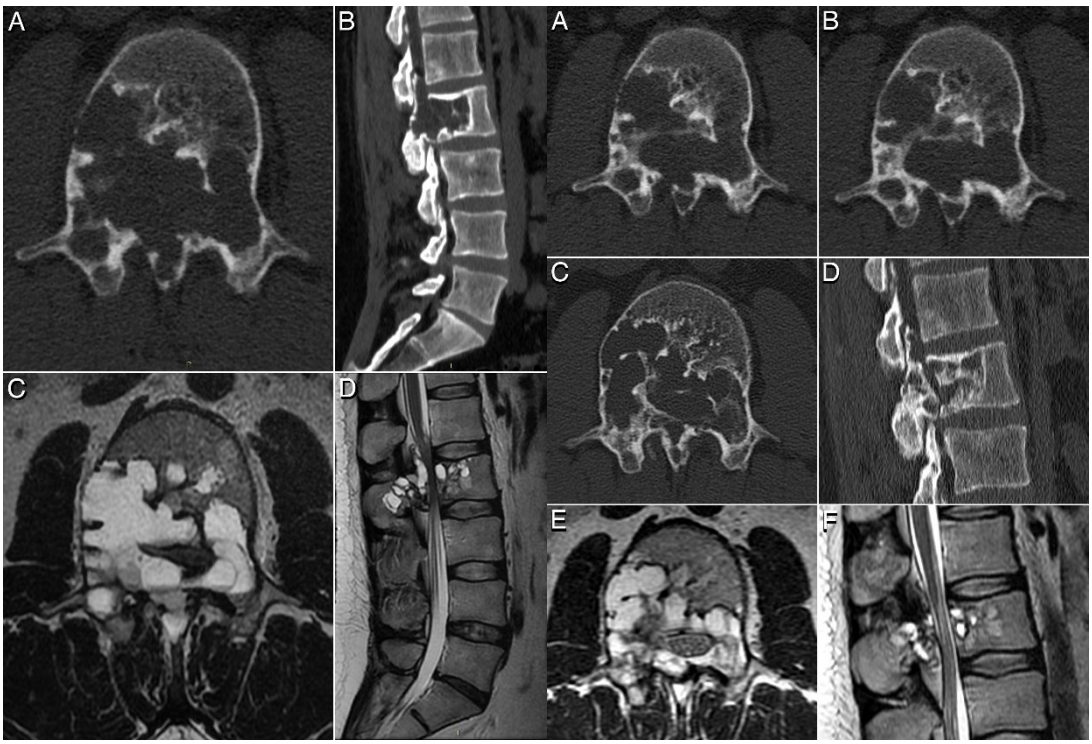

Figure 1: Left panel: Pre-operative imaging of a patient showing the ABC at L2 level before the treatment with concentrated bone marrow. A, B: Axial and sagittal CT scan; C, D: axial and sagittal MRI.

Right panel: Follow up imaging of the same patient showing the ABC at L2 level after the treatment with concentrated bone marrow. A: axial CT scan at 2 months FU; B: axial CT scan at 4 months FU; C, D: axial and sagittal CT scan at 18 months FU; E, F: axial and sagittal MRI at 18 months FU.

Conclusions and/or Outlook

The results of this study reinforce our previous evidence on the use of BMC as a valid alternative for spinal ABC management when SAE is contraindicated or ineffective.

The initial size of the cyst and its localization does not influence the efficacy of the treatment. However, BMC injection could be indicated as treatment of choice for spinal ABC in young adolescent women.

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Carbon Fiber Implants for Orthopaedic Oncology Surgery

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Keywords: Carbon-fiber implant, orthopedic oncology, metastasis

Introduction

Orthopaedic oncology represents one of the most complex branches in orthopaedic surgery. In case of malignant bone tumors, surgeons' main goal is to resect the whole neoplasm including the bone segment from which it originates and the soft tissues that surround the lesion. The aim of the demolitive phase is to achieve the complete removal of all neoplastic cells and tissues from the involved site, but at the same time preserve the health tissues as much as possible. A correct resection, carried out with wide margins, is necessary in order to minimize the risk of local recurrence in the months and the years that follow the intervention. Once resection is over, bone length and continuity could be restored using prostheses or bone grafts depending on the width of the bone loss, patient's necessities and site's characteristics.

In some other cases with systemic diseases with multiple metastases or a short life expectancy attributable to any other clinical condition, surgical approach could be performed with palliative aims rather than eradicative purposes. In such situations, the stabilization of the affected bone segment might be effective in reducing patient's pain and minimize the risk of pathological fractures.

In recent times, improvements in surgical techniques, material knowledge and implant engineering led the way to the invention of many implants, different if shape, design and materials.

Among the others, carbon fiber alloy represents one of the most promising materials for oncologic use and has been drawing increasing interest in the orthopedic community in light of its peculiar characteristics.

Results and Discussion

Modern days' therapeutical approach to malignant bone tumors is often multidisciplinary, involving orthopedic surgeons alongside with medical oncologists, radiant therapists and other allied medical specialists. In this context, carbon fiber devices represent one of the most intriguing and innovative solutions for stabilization and occasionally reconstruction of bone segment involved by malignant neoplasms. In order to increase their biomechanical qualities, carbon fibers are generally wound in a double helix on a plastic support. This gives rise to a new composite material named Carbon Fiber-Reinforced Poly-Ether-Ether-Ketone (CFRP), which is the main component of modern generation carbon-made implants. The clinical applications of CFRP described so far in orthopedic literature have highlighted good mechanical properties, with strength and elasticity that can compare with the ones of the most popular metallic alloys.

Beyond their good physical properties, carbon fibers implants drew the interest of surgeons and physicians mainly due to two different properties that find no match in the alternative materials.

The first fundamental property of carbon alloys stands in the fact that they are not responsible for imaging artifacts in CT scans nor in MRI images. It has been shown that the use of intramedullary nails, plates, rods and screws made of carbon alloy permits to enhance imaging, to reduce artifacts and plan an optimal radiation strategy if necessary. Carbon response to radiations represents the second prominent reason of its success and spreading use among the oncological clinics of the whole world.

Carbon's responses to radiations are similar to those of biological fibers. Unlike metal implants, carbon does not heat significantly during radiation therapy and does not cause reflected thermic lesions on the nearby tissues. Therefore it is more suitable for patients who have to undergo radiotherapy, or rather in patients with bone metastases for whom surgery only has a mechanical intent while radiotherapy is mandatory to improve local disease control.

Conclusion

Carbon fiber implants represent a promising and intriguing option for the stabilization and occasionally the reconstruction of neoplastic bone lesions due to their good mechanical properties, their low impact on imaging procedures and their being inert towards radiations used in common radiant therapies for bone sarcomas or metastases.

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The vertebral bone marrow clot as new and advanced autologous cell therapy in spinal surgical procedures

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Keywords: bone marrow clot, spinal surgery, bone regeneration, cell therapy

Introduction

Due to the presence of megakaryocytes, platelets and clotting factors, bone marrow aspirate (BMA) tends to coagulate. For the first time, starting from our previous studies on mesenchymal vertebral stem cells, it has been hypothesized that coagulated BMA represents a safe and effective autologous biological scaffold for bone regeneration in spinal surgery. The present research involved advanced preclinical in vitro models and the execution of a pilot clinical study.

Evaluation of cell morphology, growth kinetics, immunophenotyping, clonogenicity, trilineage-differentiation, growth-factors and HOX and TALE gene expression were analyzed on clotted- and un-clotted human V-BMA [1]. In parallel, a pilot clinical study on ten patients with degenerative spinal diseases submitted to instrumented posterior arthrodesis is ongoing to assess the ability of clotted-V-BMA to improve spinal fusion at 6- and 12-months follow-up.

Results and Discussion

Results demonstrated that clotted-V-BMA have significantly higher growth-factor expression and mesenchymal stem cell (MSCs) viability, homogeneity, clonogenicity, and ability to differentiate towards the osteogenic phenotype than un-clotted-V-BMA. Clotted-V-BMA also highlighted significant reduced expression of PBX1 and of MEIS3 genes negatively involved in osteoblast maturation and differentiation (**Figure 1**). From December 2020, nine patients have already been enrolled with first promising results concerning spinal fusion and clinical outcomes.

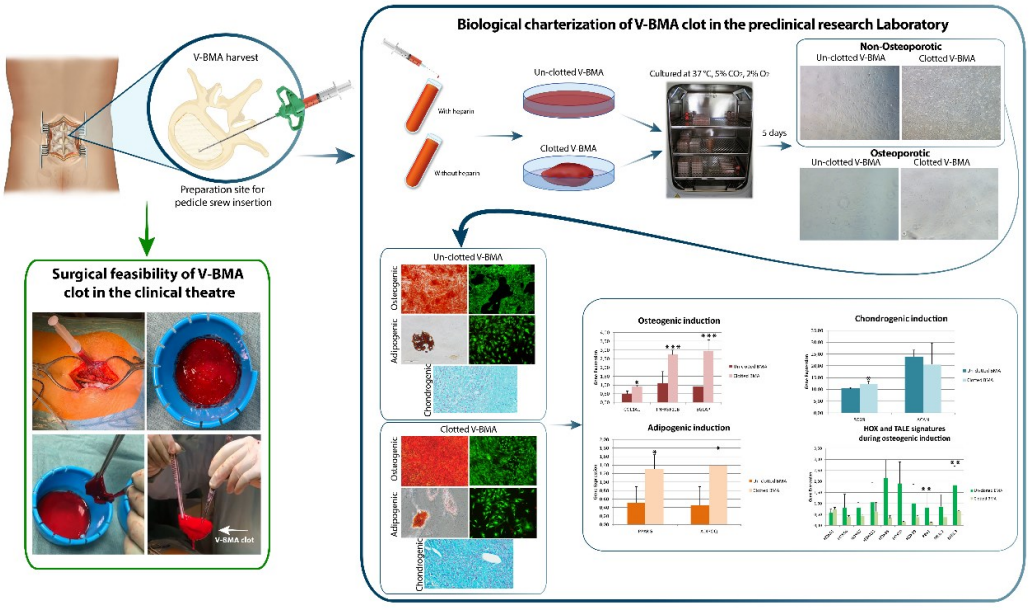

Figure 1: Schematic representation of the preclinical biological characterization of V-BMA clot.

Conclusions and/or Outlook

The application of V-BMA-clot as carrier of progenitors and cytokines and as natural scaffold with a structural texture represents a point-of-care orthobiologic product to improve spinal fusion. Clinical application seems to be efficacy, and we will confirm and strengthen these data with the final results of the pilot clinical study.

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Biomimetic 3D-printed custom-made prosthesis for anterior column reconstruction after en bloc resection for spinal tumors

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Keywords: spinal tumours, en bloc resection, anterior column reconstruction, 3D printed titanium implants

Introduction

Reconstruction of the segmental defect after en bloc resection for spinal tumours aims at immediate stability and secondary solid fusion. The present communication provides an update on the results of an ongoing study concerning the use of 3D printed custom made implants for anterior column reconstruction following en bloc resection.

In 24 patients submitted to en bloc resection for spinal tumour between November 2015 and November 2019 at the same Institution, anterior column reconstruction was performed using 3D printed custom made implants. Resection was planned according to Enneking and Weinstein-Boriani-Biagini staging systems. Implants were designed according to the preoperative planning of the resection on CT-scan.

Results and Discussion

We analysed data concerning clinical and radiographical follow up of 24 patients: in 21 patients the thoracolumbar spine was reconstructed, while in the remaining 3 cases, 2 involved the upper cervical spine (C2) and 1 case sacrum and lumbo-sacral junction. All patients had a long follow up period (range 29-77 months). Intra-operative and post-operative complications were collected, as well as mechanical complications during the follow up period.

One major mechanical complication occurred requiring the implant removal and one implant was replaced due to recurrence of the disease. Mechanical complication consisted in a massive subsidence of the prosthesis into the adjacent vertebral body and occurred with development of progressive distal junctional kyphosis (Figure 1). Critical analysis of the construct revealed insufficient posterior instrumentation, but custom made implants itself did not show post-operative mechanical complications (breakage or migration of the implant). However, because of the necessity of a surgical revision of the construct, it was considered a major mechanical complication. The removed implant was processed and sectioned for histological analysis that revealed the presence of new bone formation into the implant.

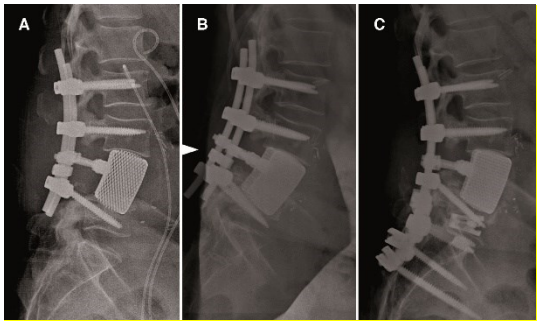

Figure 1. Lateral X-ray showing the anterior column reconstruction after L4 en bloc resection with a custom made 3D printed titanium implant: post-operative image (A), complication at 18 months FU (B), revision surgery (C).

Conclusions

Custom made 3D printed titanium implants seems to be a viable option for restoration of the anterior column after en bloc resection for spinal tumor.

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In vivo PET imaging of implant-associated inflammation and bone regeneration

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Keywords: PET, biodegradable magnesium, implants

Introduction

Tissue inflammation is a known post-operative process in implantology, directly related to wound healing, implant stability and patient quality of life. For bone implants, bone regrowth, remodelling and mineralization are equally important to guarantee implant stability that is necessary for healing. With the expanding use of biodegradable implants, it is necessary to understand the interaction between implant material and tissues that becomes increasingly complex, involving long-term interaction of metabolites and structural changes. Until now, PET has mostly been used to study infections and the effect of the implant presence on the image quality, but not the actual effect of implants on local metabolism. In this study, we used a small animal model to compare the traditional permanent implants made of titanium with novel biodegradable magnesium implants, using ^{18}F-FDG and ^{18}F-NaF tracers to analyse their effects on inflammatory response and bone growth.

Transcortical holes with the diameter of 1.55 mm were drilled into both femurs of 10-13 weeks old female Wistar rats and press fitted with 1.6×8 mm titanium (Medical University of Graz, Graz, Austria) or WE43 magnesium pins (Syntellix AG, Hannover, Germany), or left empty as sham group. Animals were imaged on days 1, 3, 7, 14 and 45 after surgery. Thirty-five (35) animals were imaged with ^{18}F-FDG tracer to track the inflammatory response and 16 animals with ^{18}F-NaF to follow the mineralization changes in bone. The injected tracer activity was around 14 MBq, and 60 minutes after injection PET imaging was performed with IRIS PET/CT scanner (Inviscan Imaging System, Strasbourg, France), acquiring a set of 3 static acquisitions over 15 minutes (5 minutes each). CT images were acquired for corrections and anatomical reference. Images were reconstructed with all corrections and saved in DICOM format.

Images were analysed in A Medical Image Data Examiner (AMIDE), where CT acquisition was used to locate the implantation site. For ^{18}F-FDG, 4.8×8 mm volume of interest (VOI) was centred over the implant or in the case of sham group, over the wound channel. For ^{18}F-NaF, 4.8×4 mm VOI was drawn axially centred on implant and transaxially covering the bone. PET VOI data were exported into Excel document. The concentration values were all corrected to represent the tracer activity concentration at 60 min after injection and standardized uptake values (SUVs) were calculated. The presence of metal implants was taken into account by phantom-based quantitative correction.

Results and Discussion

For ^{18}F-FDG (**Figure** 1), both implant materials show higher SUVs compared to sham, with similar SUVs on day 1 and day 3, which is the time point when all ^{18}F-FDG uptake peaked. On day 7 the SUV of Mg group persisted higher than in Ti, before reaching similar levels again at days 14 and 28.

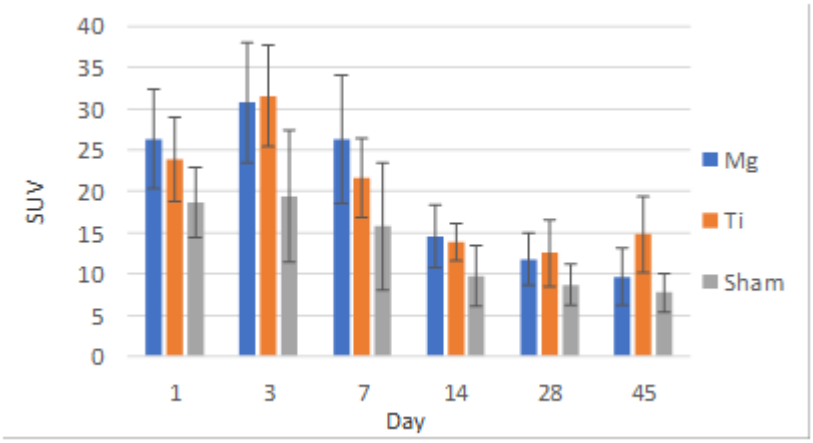

Figure 1: Standardized uptake values for ^{18}F-FDG

The use of ^{18}F-FDG revealed the possible difference in the dynamics of the inflammation between magnesium and titanium implants. It is probable that Mg reaction in tissues extended the inflammatory reaction, or the inflammation at days 1 and 3 was more intense but was obscured by Mg-caused gas accumulation that could interfere with tracer presence within the volume of interest. Possible solution could be extraction of gas using syringe or cannula before imaging. It is worth noting the increased uptake of ^{18}F-FDG for titanium samples at day 45, compared to magnesium or sham that show similar values. Due to the implant placement causing a large part of it interfering with the soft tissues, the microtrauma with titanium may provoke chronic inflammatory reaction, which appears to be noticeably less prominent in the cases with magnesium.

For ^{18}F-NaF (**Figure** 2), the uptake peak for all groups is at day 7, when also Mg has high standard deviation that lasts to day 28. From day 3 to day 14, magnesium group has distinctly higher uptake, while titanium and sham have little difference.

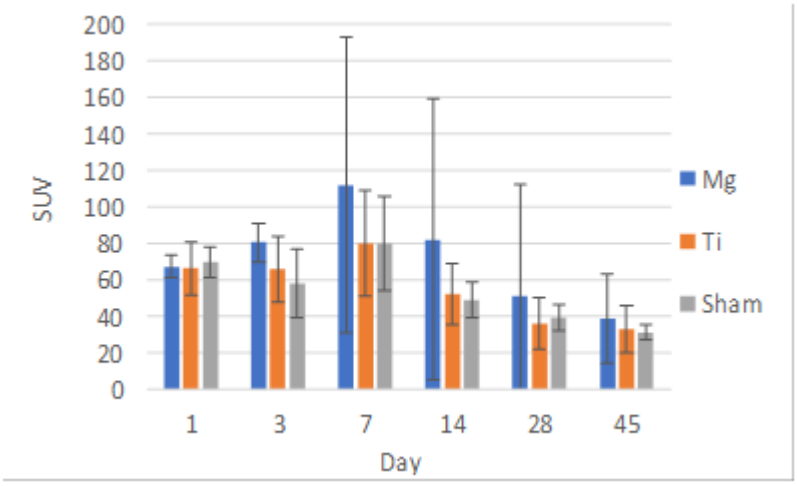

Figure 2: Standardized uptake values for ^{18}F-NaF

Besides the possibility of biochemical causes, differences in magnesium samples may have been caused by increased pressure during implantation due to increased friction from the magnesium surface reacting with tissues at initial contact. The possible resulting damage to the bone could be higher than in cases of titanium and sham, and that would cause larger extent of bone healing corresponding to the increase in tracer uptake for some samples, leading to larger standard deviation. Alternatively, the difference could be caused by increased mobility of the animals soon after surgery, causing additional damage to the bone, as that would explain the lack of such difference before day 7. It is unknown if that could be related to reduced pain perception or something else.

Conclusions

PET was shown to be a viable tool to detect the effect of implants on local metabolism. Simultaneously, new questions were raised that have potential for future research. Currently an additional group of animals is being studied. Extra samples will hopefully provide additional data for statistical analysis that would allow to reach a more accurate conclusion soon.

Acknowledgements

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A poly(SBMA) zwitterionic hydrogel coating of polyimide surfaces for reducing the foreign body reaction to implanted neural electrodes

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Keywords: Intraneural electrodes; Polyimide; Zwitterionic hydrogel; Surface coating; Foreign body reaction.

Introduction
Invasive intraneural electrodes can control advanced neural-interfaced prostheses in human amputees [1]. Nevertheless, in chronic implants the gradual loss of functionality due to the progressive formation of a fibrotic capsule can ultimately isolate the electrode surface from the surrounding tissue. This is due to a nonspecific inflammatory response called foreign-body reaction (FBR) [2,3]. Poly(ethylene glycol) (PEG) is one of the most widely used low-fouling coatings of implantable devices. However, PEG can be easily encapsulated and susceptible to oxidative damage in long-term *in vivo* applications [4]. Recently, sulfobetaine-based zwitterionic hydrogels have emerged as an important class of robust ultra-low fouling biomaterials that ameliorate the performance of metal electrodes, holding great potential to reduce the FBR [5].

Results and Discussion
In this *in vitro* work, we compared the organic zwitterionic coating – poly(sulfobetaine methacrylate) [poly(SBMA)] hydrogel – with the synthetic coating – PEG – in reducing adhesion and activation of inflammatory and pro-fibrotic cells, early hallmarks of the FBR, to Polyimide surfaces. Firstly, the hydrogel was synthesized and characterized **Figure 1**. Adhesion, viability and morphology of human myofibroblasts on PEG-, SBMA hydrogel-coated and polystyrene surfaces were evaluated *via* high-content analysis (HCA). Activation of M_1 pro-inflammatory macrophages cultured on hydrogels was assessed through HCA. We performed a coating of Polyimide surfaces with a thin film of the hydrogel through covalent bond and analyzed the long-term release of an anti-fibrotic drug from the hydrogel.

Conclusions and Outlook
Because of the high hydration, biocompatibility, low stiffness, and ultra-low fouling characteristics, the hydrogel allowed lower adhesion and activation of pro-inflammatory and pro-fibrotic cells *vs.* polystyrene controls. Our soft zwitterionic hydrogel could potentially outperform PEG as more suitable dressing of intraneural electrodes for reducing the FBR and could be envisioned as long-term delivery system for gradual and controlled anti-inflammatory and anti-fibrotic drug release *in vivo.*

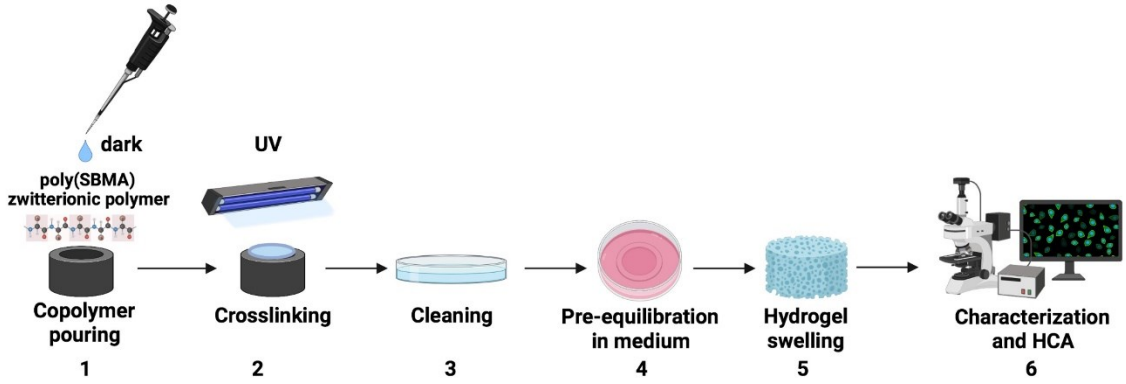

Figure 1: synthesis of the soft poly(SBMA) zwitterionic hydrogel through sequential steps followed by mechanical characterization and confocal microscopy-based high-content analysis (HCA). Created with BioRender.com

Acknowledgments

The authors acknowledge the Istituto Nazionale per l'Assicurazione contro gli Infortuni sul Lavoro (INAIL) for the financial support.

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Session 3 - Materials and Novel Technologies for Biomedical Implants and Implant Coatings

Suspension Flame Sprayed Metal Doped Calcium Phosphate Coatings with Antibacterial Properties for Infection Prophylaxis

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Abstract

High Velocity Suspension Flame Spraying (HVSFS) has been successfully employed to produce a wide variety of calcium phosphate and bioglass based coatings for metal bone implants. The HVSFS process has proven to be capable to process all kinds of these biomaterials resulting in dense and well adherent coatings on all types of metal substrates. In recent years the focus has gradually shifted to a group of degradable bioceramic coatings, as they offer a faster osseointegration of the endoprosthetic structure. A common problem that occurs after the implantation of coated implants is the risk of infection due to the presence of bacteria which can result in severe post operative inflammation reactions associated with a high risk to lose the implant. In a novel approach, metal ions with known antibacterial properties, such as silver (Ag) or copper (Cu), are incorporated into the coating as a metal dopant to reduce the risk of inflammation.

In this study, metal doped coatings based on HA, tricalcium phosphate, bioglass and a calcium potassium sodium phosphate were suspension flame sprayed using modified suspensions containing additional metals or metal salt based precursors. Metal doped coatings were evaluated regarding their microstructure and phase composition, as well as their in-vitro behavior. The presence of metal and metal oxide particles in the coating were detected using micro-Raman and scanning electron microscopy. To evaluate the biocompatibility, a live/dead-assay viability / cytotoxicity study based on MG-63 cells was performed. Results did not give evidence for any cytotoxic reaction.

Advances in biodegradable metals for potential usage within bone

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Keywords: Biodegradable metals, Mg alloys, Zn alloys, bone implants

Introduction

After decades of developing strategies to minimize the corrosion of metallic biomaterials, there is now increasing interest in using intentionally corrodible metals in a number of critical medical device applications. A term "biodegradable metal"(BM) had been used internationally to describe these new kinds of degradable metallic biomaterials for medical application. In this paper, Mg-based BMs and Zn-based BMs were studied as bone implant materials with emphases on their microstructures, mechanical properties and degradation behaviors, in vitro and in vivo biocompatibility evaluations.

Results and Discussion

In this study, we developed a novel Mg-Si-Ca alloy with increased mechanical strength and bioadaptability. We demonstrated that the addition of Ca and Si to Mg matrix accelerated the formation of a self-assembled, multilayered implant–tissue interface, which coordinated its biodegradation with the bone healing process. At the initial stage of implantation, a burst release of Mg2+ from Mg-Si-Ca alloy activated the monocyte-macrophage lineage, leading to an immune microenvironment favoring the recruitment of MSCs and initiation of osteogenic differentiation. With the formation of the biomimicking calcified matrix at the degrading bone–implant interface, the ion release kinetics of the Mg-Si-Ca alloy was turned down, leading to a new peri-implant microenvironment for osseointegration and osteogenesis by targeting the integrin signaling pathways in MSCs. Our study revealed that the spontaneously formed degradation layers at the bone–implant interface are the key to the superior performance of Mg-Si-Ca alloy, as they regulate the degradation behaviors of the implant to elicit an appropriate host response at different stages of bone healing.

A novel biodegradable metal system, ZnLiCa ternary alloys, were systematically investigated both in vitro and in vivo. The ultimate tensile strength (UTS) of Zn0.8Li0.1Ca alloy reached 567.60 ± 9.56 MPa, which is comparable to pure Ti, one of the most common material used in orthopedics. The elongation of Zn0.8Li0.1Ca is 27.82 ± 18.35%, which is the highest among the ZnLiCa alloys. The in vitro degradation rate of Zn0.8Li0.1Ca alloy in simulated body fluid (SBF) showed significant acceleration than that of pure Zn. CCK-8 tests and hemocompatibility tests manifested that ZnLiCa alloys exhibit good biocompatibility. Real-time PCR showed that Zn0.8Li0.1Ca alloy successfully stimulated the expressions of osteogenesis-related genes (ALP, COL-1, OCN and Runx-2), especially the OCN. An in vivo implantation was conducted in the radius of New Zealand rabbits for 24 weeks, aiming to treat the bone defects. The Micro-CT and histological evaluations proved that the regeneration of bone defect was faster within the Zn0.8Li0.1Ca alloy scaffold than the pure Ti scaffold. Zn0.8Li0.1Ca alloy showed great potential to be applied in orthopedics, especially in the load-bearing sites.

Outlook

Mg-based biodegradable metals, exhibit 200-350MPa ultimate tensile strength, relatively fast degradation rate, acceptable new bone forming ability, which is suitable for non-load-bearing applications within bone, whereas Zn-based biodegradable metals, exhibit 500-650MPa ultimate tensile strength, relatively fast degradation rate, acceptable new bone forming ability, which is suitable for load-bearing applications within bone.

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The Best Way to Advance Biomedical Implants: Biomaterials or Clinical Needs

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Biomaterials offer the surgeon a powerful set of clinical tools for patient treatment and are found in virtually every instrument, device, implant, or piece of equipment in the operating room. Material science has been described as part of a health care system that will be driven by clinical need, with the ultimate goal of restoring or replacing function in part of a failed organism through biomaterial science. A significant number of materials including metals, ceramics, polymers, composites, and some nanomaterials exist and are used in medicine for current and potential orthopedic applications.
Metallic biomaterials are engineered systems designed to provide internal support to biological tissues and they are being used largely in joint replacements, dental implants, orthopedic fixations and stents. Higher biomaterial usage is associated with an increased incidence of implant-related complications due to poor implant integration, inflammation, mechanical instability, necrosis, and infections, and associated prolonged patient care, pain, and loss of function. In this paper, we will briefly explore major representatives of biomaterials along with the key existing and emerging strategies for surface and bulk modification used to improve biointegration, mechanical strength, and flexibility of biometals, and discuss their compatibility with the concept of 3D printing.
New trends in metallic biomaterials, surface modification, and characterization techniques will be reviewed and discussed with particular reference to their relevance in biomaterials-tissue interactions phenomena. Experimental results performed on different new metallic biomaterials like biodegradable metals will be shown. The surface design of Mg-based materials should base on their application situations, such as implantation sites, surrounding biological environment, and required service duration. After the proper surface design and bioceramic coatings, magnesium alloys could be considered a very promising candidate for biodegradable implants used in orthopedics and neurosurgery. In consideration of the strengths and weaknesses of biodegradable metals, some innovative systems like magnesium/titanium (Mg/Ti) hybrid fixation systems for long bone fracture fixation or magnesium/hydroxyapatite systems for bone substitutes could be developed.
Current knowledge of biomaterials focusing on design and surface helps us to understand which implant surfaces have more predictable clinical outcomes. The clinicians at the interface of biomaterial applications and patient care can drive that development and innovation, taking a critical view of the currently available medical devices and defining future needs in clinical practice.
In conclusion, future research and studies on some promising metallic biomaterials are essential in terms of biocompatibility, structure, and properties to make them clinically viable.

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Research Studies on Biocomposites

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Keywords: *Sintering, spark plasma sintering, hydroxyapatite, alumina, bioinert, bioactivity, composite, processing*

Abstract

This presentation will provide a detailed overview of the processing techniques and properties of bioceramics and related research studies on bioceramic based composites.

The entry mainly covers the types of bioceramic implant-tissue attachments, powder synthesis, processing methods for porous and dense bulk bioceramics, and bioceramic based composites production using conventional pressureless sintering and pressure-assisted sintering, mainly focusing on spark plasma sintering. Effects of reinforcement materials such as GNP, CNT and BN on the properties of bioactive hydroxyapatite based composites and zirconia toughened alumina composites are discussed. Further physical, microstructural, mechanical characterization techniques and biological behavior and bioactivity measurements of these composites in in-vitro environments such as cell viability assays are also investigated.

Surface modifications on novel beta Ti-based alloys for biomedicalapplications

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Keywords: Ti alloys, Surface, Biomaterials

Introduction

Titanium is a transition metal that has an allotropic transformation around 883 °C. Below this temperature, its crystalline structure is a hexagonal compact (α phase). Above this temperature, it has a body-centered cubic crystalline structure (β phase). Titanium and its alloys have favorable properties such as high specific mechanical strength, low elastic modulus, fatigue resistance, anti-corrosion, and biocompatibility. Due to these good properties, some titanium alloys (including pure titanium) have been successfully applied as implants in thehuman body [1].

The α + β-type Ti-6Al-4V alloy has been widely used in orthopedic implants, particularly for total hip arthroplasties. However, the alloying elements present in this alloy raise some clinical concerns, such as neurological diseases and cytotoxic effects [2].

The difference between Young's moduli of the implant and bone leads to bone resorption and eventually implant loosening, so-called stress shielding. Long-term titanium alloys as implant materials indicated some issues, such as stress shielding, biotoxicity from some alloying elements, surface abrasion, and corrosion in body fluids. To weaken even solve those issues, researchers have been devoted to developing new low Young's modulus titanium alloys and/or making porous scaffold structures to reduce their Young's modulus [3].

However, they are still insufficient for long-term clinical usage as they are bio-inert and cannot bond to living bone directly at the early stage after implantation into a human body. Their surfaces play an essential role in response to the artificial devices in a biological environment; for these materials to meet the clinical demands, they must modify their surface. The corrosion resistance and biological properties of titanium and its alloys can be improved selectively by using the appropriate surface modification techniques while the desirable bulk attributes of the materials are retained. The proper surface treatment expands the use of these materials in the biomedical field [4].

The development of new titanium alloys with low elastic modulus and toxic element free and its surface modification is the fundamental, efficient, and commonly used method for weakening even solving the issues mentioned above. This study developed titanium-based alloys with body-centered cubic crystalline structure (β phase) using appropriate surface modification, aiming for orthopedic applications.

Results and Discussion

Zirconium, molybdenum, and silver were used as alloy elements. The alloys were prepared in an arc-melting furnace and submitted to a homogenization heat treatment (1273 K, 43.2 ks, slow cooling), hot-rolling (1273 K, air cooling), and solution treatment (1123 K, 21.6 ks, water quenching). The bulk microstructural analysis was performed by x-ray diffraction (XRD) and optical (OM), scanning (SEM), and transmission (TEM) electron microscopy. The cytotoxicity of the alloys was checked using MTT colorimetric test.

Micro arc oxidation (MAO) treatments were performed in a DC power source at 300 V, 2.5 A, room temperature, for 60 s. The electrolyte was composed of 0.35M calcium acetate monohydrate, 0.02 M β-glycerol phosphate, and 0.1 M magnesium acetate tetrahydrate.

Surface chemical analysis was evaluated by x-ray photoelectron spectroscopy (XPS). The contact angle was evaluated in a Ramé-Hart 100–00 goniometer. Structural analysis was conducted at a small angle, and OM and SEM performed microstructural analysis. Roughness (Ra) was measured by confocal optical microscopy (COM).

For Ti-15Zr-xMo alloys (Figure 1), before anodic treatment, the substrates in as-cast condition exhibited structure of α' phase (Ti–15Zr), α" + β phases (Ti-15Zr-5Mo), and only β phase for Ti-15Zr-10Mo and Ti-15Zr- 15Mo alloys, due to the strong β-stabilizing action of Mo. This increase in the Mo content was accompanied by modifications from lamellar to granular microstructure in bulk. Submitted to energetic sparks and micro-arcs during MAO, all films showed a typical porous morphology, with most of the pores having an almost circular shape. However, smaller pores were observed over the surface for alloys containing Mo, indicating changes in the dielectric breakdown by forming the β phase in bulk. Suppression of lamellar structures may also influence film thickness. Only small contributions of MoO_3 were detected in the coatings, mainly composed of other oxides (TiO_2 and ZrO_2). Regardless of the amount of Mo in bulk, all coatings were successfully doped with bioactive ions, as they also showed Ca-containing compounds ($CaCO_3$ and calcium phosphate) [5].

For Ti-15Zr-15Mo pure and modified by Ag alloying and surface MAO treatment (Figure 2), phase composition and microstructure were composed of the β phase as equiaxial grains. MAO treatment produced a porous oxide layer, formed mainly through TiO_2 (anatase form). The surfaces were enriched with bioactive ions, having microscale roughness and good contact angle values, with a significant apatite forming ability [6].

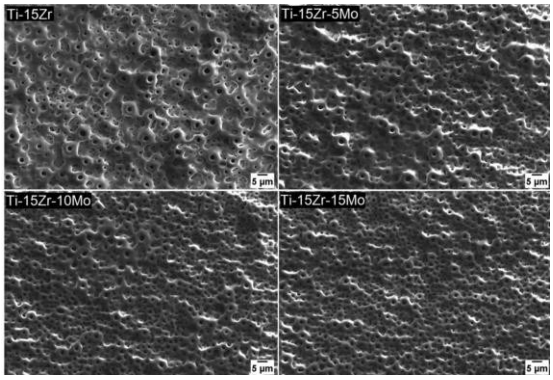

Figure 1: Morphology of the MAO-treated Ti-15Zr-xMoalloys.

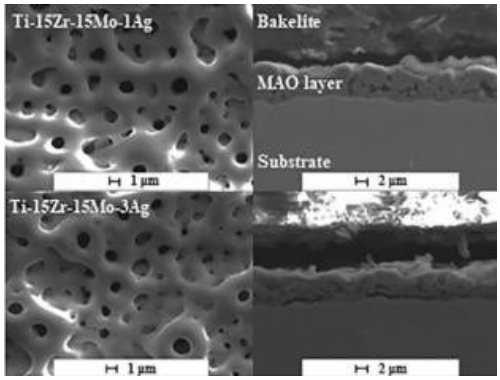

Figure 2: Topographic and cross-section view of the MAO-treated Ti-15Zr-15Mo-xAg alloys.

Conclusions

In all studied alloys, an improvement in surface properties was observed by using the appropriate surface modification techniques. At the same time, the desirable bulk attributes of the materials are retained. The proper surface treatment expands the use of these materials in the biomedical field.

Aknowledgements

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Nanostructured metal-based biomimetic and antibacterial coatings for custom-made orthopaedic devices

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Keywords: Nanostructured coatings, infection, antimicrobial, osseointegration, orthopaedics

Introduction

Orthopaedic infections are among the main complications in orthopaedics, hence new solutions are needed that can exert suitable efficacy while not causing toxicity and/or development of resistant bacterial strains [1,2]. The development of highly porous and/or rough bone substitutes and prostheses (including custom-made 3D printed and/or patterned implants) permits to better address osseointegration, but does also negatively impact on infection. In fact, all the features that increase cells adhesion, also tend to increase the adhesion of bacteria, that is the first stage in biofilm formation and infection.
Here, antibacterial nanostructured metal-based thin films are proposed, obtained by Ionized Jet Deposition (IJD), a plasma-assisted technique that can be applied to either metallic or heat sensitive substrates [3,4]. Metallic (Ag, Zn, Cu) and metal-doped β-TCP coatings are manufactured to address different microbial contaminations and obtain bioactive and antibacterial films. For their validation, antibacterial efficacy is validated *in vitro* in terms of reduction of viability and of adhesion of relevant Gram-positive and -negative pathogenic bacteria (E. coli, S. aureus, P. aeruginosa, E. faecalis) and by FEG-SEM. In addition, advanced microbiological characterization is performed by the Calgary Biofilm Device (CBD), a high-throughput technology optimized for simultaneously evaluating both the microbial biofilm formation capacity and the growth in planktonic form. The use of the CBD for biomaterials and coatings validation was proposed, for the first time, by the research group.
All metal and metal-TCP coatings are deposited onto titanium alloy disks. Deposition of metal doped-TCP is particularly challenging as it is a metastable and ion-doped phase. Different thicknesses are examined, corresponding to different release profiles. Composition (FT-IR, EDS, GI-XRD), morphology and topography (FEG-SEM, AFM) and degradation profiles of the coatings are investigated (FEG-SEM, ICP), then, their biocompatibility is shown (adipose tissue-derived mesenchymal stem). Experiments are carried out in triplicate and statistics is performed using one-way Anova test (significance for $p < 0.05$).

Results and Discussion

All films are nanostructured: Ag and Cu films are composed of spherical aggregates of 10-20 nm diameter and have a 150 nm thickness, while ceramic coatings show coarser aggregates (50nm-2 microns) and higher thickness (in the range of 500 nm). Zinc films show intermediate characteristics.

A good transfer is found in composition from the deposition target to the coatings, also for metal doped-βTCP, where both the CaP phase and Ag, Cu and/or Zn doping are conserved. All films show good stability (>14 days in medium). Excellent biocompatibility (no reduction in cell viability and differentiation) is achieved, as well as good antibacterial activity and a significant reduction of bacterial adhesion. Experiments with the CBD show efficacy against the planktonic and biofilm growth of the bacteria, in a concentration-dependent fashion (in all cases >70% and up to 100%). Different metals have different effects against bacteria. Gram negative strains (E. coli and P. aeruginosa) are more sensitive to silver and copper. Gram positive strains (S. aureus and E. faecalis) are more sensitive to zinc. In addition, different microbial response to the metal is found according with the growth behavior (planktonic / biofilm).

Conclusions and/or Outlook

Nanostructured antibacterial thin films are successfully deposited by IJD, that show high antibacterial and antibiofilm activity against all the tested bacterial strains. The results obtained here also prove that the CBD can be successfully applied for evaluation of coatings for biomedical applications. In vivo validation of the coatings in an infection model (already performed for silver films) is needed for further validation of the obtained results.

Acknowledgements

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Injury, 48 (2017), pp. 599-607

Tailoring the surface properties of anodically grown Ti and Nb oxide layersto promote osteointegration

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Keywords: niobium; titanium; ASD; etching; SEM; AFM; XPS; EIS; Ca/P; osteoblast

Introduction

The first interaction between an implant and the hosting tissue occurs at the surface of the implant. For this reason, large attention has been focussed on the surface modification of implant materials in order to improve their biocompatibility. As long as orthopaedic implants are considered, transition metals are the most widely used materials [1] since, together with their good mechanical properties, the presence of a spontaneous oxide layer on their surface favours their biocompatibility. Biocompatibility can be improved by the growth of thicker oxide layers which, increasing the chemical stability and the corrosion resistance of the material, reduce ion release into the host body. Moreover, the development of a bioactive surface helps the fast fixation to the bone, avoiding the encapsulation of the implant in fibrous tissue. Anodic oxidation is a versatile surface modification method that allows to tailor the surface morphology, the thickness and the chemical composition of the oxide layer [2]. By changing the anodizing parameters (such as applied potential, anodic current, electrolyte, anodizingtime), the process can be tuned and the so-called Anodic Spark Deposition (ASD) regime can be achieved, with the formation of pores and the inclusion of chemical species from the electrolyte into the oxide layer.

We investigate the anodic oxidation of titanium, niobium and titanium/niobium alloys to grow oxide layers with tailored morphological and compositional properties. Moreover, while the pore size of anodic oxide can be tunedby changing the applied anodizing conditions, it is difficult to tailor both the micro and the nanoscale roughness simultaneously. Indeed, when anodizing produces micropores, their borders usually present rather flat areas. Aiming to control both the micro and nano roughness, we propose a two-step surface modification process, combining anodizing with chemical etching. Anodizing is firstly employed to obtain oxide layers with micro- sized pores and chemical etching is subsequently used to add nano roughness to the porous surface.

Results and Discussion

Niobium, titanium, and niobium/titanium alloys were anodically oxidized in a solution containing calcium phosphate, calcium acetate and EDTA, a chelant for Ca ions. The negative charge of Ca-EDTA complexes at basic pH favours the incorporation of Ca into the oxide matrix during anodic growth. We compare the anodic oxidation in the not-ASD regime with that in the ASD regime, i.e. studying the oxide growth below and above the breakdown potential, and correlate the potential-dependent corrosion behaviour of the oxide to its morphology and chemical composition [3-5].

The changes in surface morphology, from the polished substrate to the oxide layers grown at increasing anodizing potentials, have been studied by Atomic Force Microscopy (AFM), obtaining a quantitative characterization of surface roughness, pores diameter and pores depth. AFM data have been correlated with a Scanning Electron Microscopy (SEM) cross section investigation to evaluate the thickness of the oxide layers. Energy Dispersion X-ray spectroscopy (EDX) and high-resolution X-ray Photoelectron Spectroscopy (XPS) analysis allowed to evaluate the Ca and P content within the oxide layer and on the outer oxide surface respectively, as a function of the anodizing conditions. Electrochemical tests were carried out by monitoring the sample Open Circuit Potential (OCP) and analysing the electrochemical behaviour through Electrochemical Impedance Spectroscopy (EIS) and anodic polarization. The use of electrochemical analysis, in terms of AC and low voltage DC methods, provides a not perturbative characterization of the material corrosion properties thus allowing to model its service lifetime.

Below the breakdown potential, thin (few hundreds of nanometers) and uniform oxide layers are formed, with negligible inclusions of electrolyte ions. Conversely, in the ASD regime, thicker and microporous oxide layers are observed, with a significant enrichment of the matrix with Ca and P. Increasing the maximum potential attained during oxidation leads to an increase of the oxide thickness and pore size accompanied by a noticeable

surface enrichment with calcium and phosphorus, reaching Ca/P ratios close to the 1.67 value of hydroxyapatite. At potentials around 250 V the structure evolves towards a highly inhomogeneous and porous layer with the presence of some fractures, potentially affecting the oxide passivation properties. Anodizing in mixed galvanostatic/potentiostatic regimes, limiting the potential of about 200 V and increasing the anodizing time upto 30 min represents a good trade-off between morphological and compositional properties and resistance to corrosion, which are important issues in view of osteoconductive properties for orthopaedic implant applications.

Post-anodizing etching has been further employed to add nano roughness to the micro-sized pores of the oxide surface. As shown in **Figure 1a**, acid etching leads to the formation of nano-sized pits (see inset) on the anodically grown oxide surface.

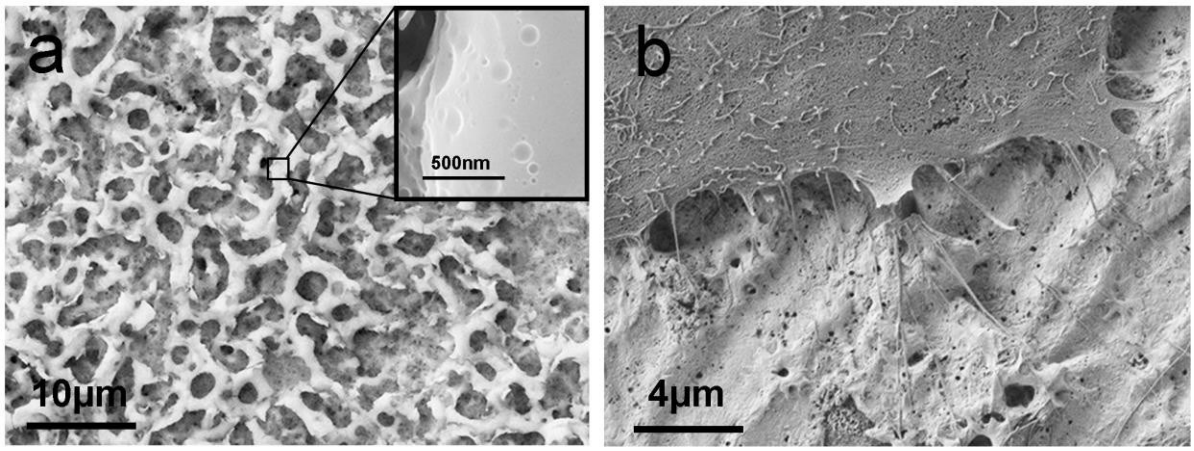

Figure 1: SEM images of oxidized and etched Nb, before (a) and after (b) 48h incubation with MG-63 osteosarcoma cells

To assess the influence of surface nanomorphology on cell adhesion and spreading, we performed in vitro tests using a MG-63 osteosarcoma cell line. SEM micrographs show that cells incubated on anodized and etched Nb (**Figure 1b**) exhibit well pronounced cytoplasmatic projections and well developed filopodia, almost absent in the case of cells incubated on anodized niobium.

Conclusions

We investigated the anodic oxidation of Nb, Ti and Nb/Ti. Through a multi-technique approach, we evaluated the changes in oxide surface morphology, thickness, chemical composition and passivation properties as a function of the anodizing potential and anodizing time. Post-anodizing etching was successfully employed to addnano roughness to the micro-sized pores of the oxide surface. In vitro tests show that the etching-induced nanostructure effectively promotes cell adhesion and spreading onto the niobium oxide surface, confirming the importance of nano/micro roughness in promoting osteointegration.

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RF and DC Magnetron Sputtering Methods for Deposition of Biocoatings

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Keywords: magnetron sputtering methods, deposition, biocoatings, structure. properties

Introduction

Surface modification of medical implants is a rapidly developing multidisciplinary field that combines expertise of materials science and healthcare professionals. Improvement of medical implants in terms of their biocompatibility, hemocompatibility, antibacterial properties, osteointegration and osteoinduction are the main topics of modern research. These properties are crucial to ensure desirable biological response to the newly implanted material, in the manner that the cells, which are adhered to the surface of such scaffolds can function in a way that is similar to physiological conditions or help to prevent possible complications. To improve the above listed biological properties the different methods are used for modification of implants and scaffolds surface. Physical vapor deposition (PVD) of thin films, allowing the deposition of multicomponent coatings, has been available for some years. One of PVD methods is magnetron sputtering of ceramic, metallic, composite targets to form the thin dense coatings of different element and phase compositions realised complex of biological. mechanical and physical properties. The magnetron sputtering method as a part of PVD has been available for some years for deposition of biocoating. The aim of this work is to demonstrate the real applications of magnetron coatings on metallic implants and bioinert polymers scaffolds.

Results and Discussion

An emerging method for bioactive coating deposition in the field of PVD is radiofrequency (RF) magnetron sputtering method [1]. Magnetron sputtering is widely used in the formation of coatings for various applications. The continuous interest of scientists for this method is due to the possibility

of modifying the coating structure and its physicochemical properties by variation of the deposition parameters. There is a significant interest in radiofrequency (RF) magnetron sputtering of bioactive calcium phosphate (CaP) thin films. This method allows deposition of CaP coatings with a high level of adhesion to substrate. In the light of demand for antibacterial coatings, hydroxyapatite is frequently substituted by ions of Zn, Cu, and Ag. Zinc ions, for example, are well-known not only for its antibacterial properties, but it also acts as an inhibitor of crystal growth. It is essential trace element for tissue regeneration. An RF magnetron sputtering of Zn- or Cu-substituted hydroxyapatite allow to deposit antibacterial calcium phosphate coatings that not only improve osteointegration, but act as a protecting layer that could alter the degradation rate of the substrate and induce local antibacterial effect. For example, it is possible to deposit an antibacterial Cu-containing CaP comprised of crystalline Cu-substituted hydroxyapatite, amorphous CaP (ACP) interlayer on the biodegradable Mg-based substrate by RF magnetron sputtering of Cu-substituted hydroxyapatite target and subsequent coating annealing. The structure of such layered coating is visualized by a transmission electron microscopy (TEM) (**Figure 1**).

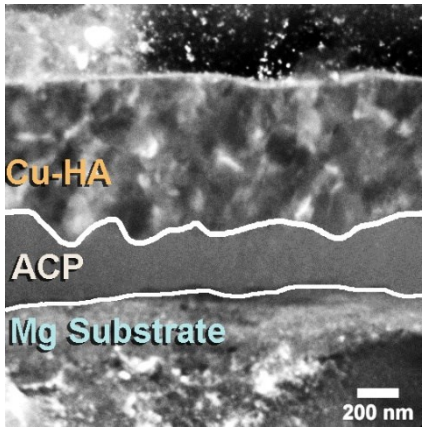

Figure 1. Dark-field TEM image of layered Cu-CaP based coating

On the other hand, binary and ternary titanium compounds (oxides and oxynitrides) standalone among other coatings due to their high bio- and hemocompatibility. This is especially true for cardiovascular implants. The titanium oxynitride film, deposited using reactive magnetron sputtering, combines the properties of two components: titanium oxide and nitrogen oxide (NO). Modification of deposition parameters such as substrate bias, working gas, deposition pressure, allow to change the structure and properties of deposited layers.

The magnetron sputtering method has been used to modify scaffolds from bioinert polymers, ceramics and metallic alloys. The magnetron co-sputtering method with using of copper and titanium targets in an argon atmosphere is applied to modify the PCL scaffolds surface for improvement of osseointegration and antibacterial properties. PCL scaffolds were plasma modified by magnetron co-sputtering method with using of copper (Cu, 99.95 %) and titanium targets (Ti,

99.95 %) in an argon (Ar, 99.99 %) atmosphere. Two power supplies APEL-M-5PDC (Applied Electronics, Tomsk, Russia) with a pulse frequency of 100 kHz and a duty cycle of 70 % were used to power two magnetrons with titanium and copper target. The modification was provided in ion-plasma setup equipped with magnetron sputtering system [2]. The setup was developed at the B.P. Weinberg Research and Education Center at Tomsk Polytechnic University. Modes of PLGA scaffolds plasma modification are presented in **Table 1**.

Table 1. Modes of PLGA scaffolds modification by magnetron co-sputtering

Sample (modification mode)	Discharge power, W		Voltage, V		Current, A		Modification time, min
	Cu	Ti	Cu	Ti	Cu	Ti	
1	65	750	430	500	0.15	1.5	26.6
2	129	500	430	450	0.30	1.1	29.3
3	194	500	430	450	0.45	1.1	19.5

In further, the unmodified PCL scaffold will be marked with the letter «u», the Cu-Ti modified PCL scaffolds will be marked as: «1», «2» and «3».

A scanning electron microscope (SEM) JCM-6000 Plus (Jeol, Akishima, Japan) equipped with energy dispersive X-ray spectroscope (EDS) was used to investigate the morphology and elemental composition of the PCL scaffolds surfaces. The SEM images, fiber diameter histograms, and EDS spectra of unmodified and Cu-Ti modified PCL scaffolds are presented in **Figure 2**.

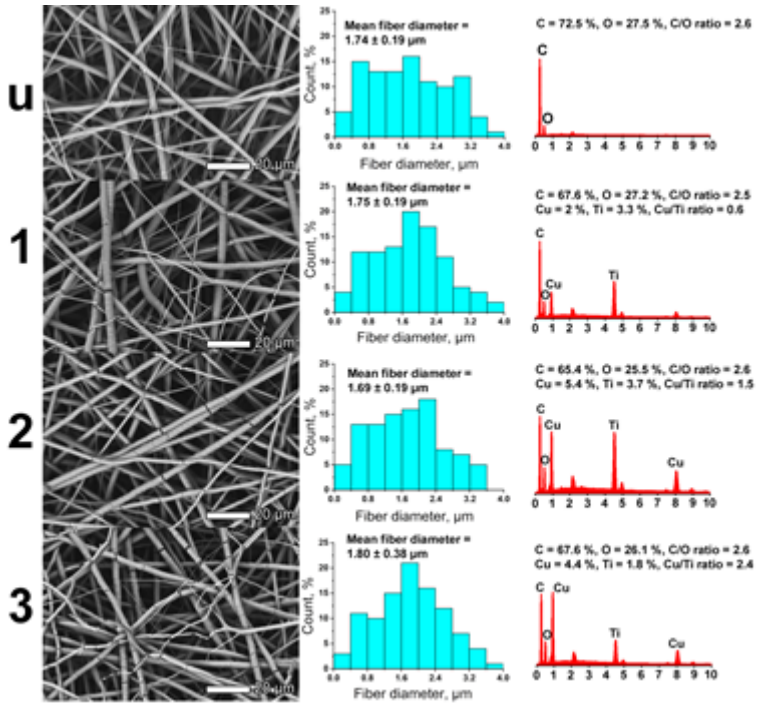

Figure 2. SEM images at 1000× magnification, fiber diameter histograms and EDS spectra of unmodified (u) and Cu-Ti modified (1,2,3) PCL scaffolds.

From SEM images it can be concluded that PCL scaffolds consist of chaotically interlaced fibers with different diameter. The mean fiber diameter of unmodified PCL is 1.74 ± 0.19 µm and the mean fiber diameters of Cu-Ti modified PCL are in the range of $(1.69 - 1.8) \pm 0.38$ µm. The Cu-Ti modification didn't change the surface morphology of PCL scaffolds. This is confirmed by the absence of a reliable change in the mean fiber diameters after plasma modification of PCL scaffolds. After analysis of obtained EDS spectra, it can be concluded that the ratio of carbon and oxygen doesn't change, but as the power on the magnetron with a Cu target increases, the Cu/Ti ratio increases too.

Conclusions

Magnetron Sputtering Methods for Deposition of Bioinert and Bioactive Coatings on surface of different implants and scaffolds prepared from metals, ceramics and polymers are effective for medical applications.

Acknowledgments

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Surface modification of a bioresorbable Mg alloy using a bioactive coating based on diatomite

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Keywords: Micro-arc oxidation, bioactive coating, diatomite, magnesium implants

Introduction

Recently, there has been a growing interest in magnesium being used as the material for biomedical applications (e.g. orthopaedic implants, blood vessel stents, etc.). Magnesium is a biodegradable material, which means that it has the ability to interact with the liquid media of a human body, dissolving gradually and forming the nontoxic corrosion products [1]. However, the dissolution rate of pure magnesium is very high, which can potentially lead to inflammation at the implantation site. One of the ways to prevent this from occurring is to modify the surface of an Mg implant with a protective biocompatible coating, which would not only slow the corrosion rate of Mg, but also stimulate the processes of osteogenesis (i.e. formation of new bone tissue). Such surface modification can be achieved via the method of micro-arc oxidation (MAO).

The MAO process is characterized by a large number of micro-arc discharges on the surface of the alloy, which occur in a strong electric field. It is considered to be the simple yet very effective, when compared to other methods of coating deposition [2,3]. Furthermore, both the physicochemical and biological properties of such a coating can be improved by the addition of various oxide microparticles to the electrolyte solution [4,5]. The main purpose of this work is to study the influence of adding particles of diatomite – a biogenic silicon oxide, on the physical, mechanical and biological properties of the resulting coating.

Results and Discussion

This research work presents the results of a study of a diatomite coating deposited on a high-purity Mg alloy by the micro-arc oxidation method (MAO). The coating was deposited at four different voltages – 350, 400, 450 and 500 V. The structure and morphology of the resulting coatings were examined by a number of investigation methods. The strength characteristics of the coatings were assessed by the scratch test method. The potentiodynamic polarization test was used to determine the resistance of the coatings to corrosion processes. The *in vitro* assessment of cytotoxic properties of diatomite coatings was carried out via the MTT assay.

It has been established that the coatings grow thicker at higher applied voltages and reaches approximately 150 µm at the highest voltage (Figure 1a). This was found to be the reason for the considerable increase in adhesion strength of the coatings, synthesized at higher voltages. The level of the coating adhesion to the surface of the substrate was ≈ 67 % higher compared to the coating generated at the lowest MAO voltage (Figure 1b).

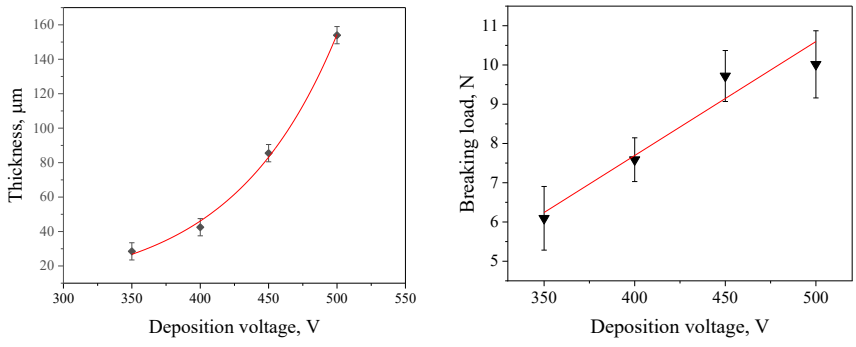

Figure 1. Dependence of the coating thickness (a) and breaking load (b) on the MAO process voltage

Figure 2 a,b presents the SEM micrographs of the synthesized micro-arc coatings with the inclusions of diatomite particles. It can be seen that the structure of the surface layers of the coatings is porous. The porosity, however, gradually decreases at higher deposition voltages. It can be justified by the more intense micro-arc discharge processes occurring during the deposition, which leads to melting of the porous structures of diatoms, as well as to the overall compaction of

the material. The SEM investigation revealed that the coatings inherited a specific type of pore structure. Such structure was formed as a result of the fragments of diatomeae melting into the surface layers of the coating during the micro-arc oxidation process.

The 3T3 mouse fibroblast cells were used to assess the cytotoxicity of the diatomite coatings using the MTT assay. It was found that the extract of the coating generated at 500 V had a high number (≈ 95 %) of viable cells remaining when it was added to the cell culture. An adhesion of large quantities of 3T3 cells to the coating surface layer was observed (Figure 2 c,d). Some of them have long membrane pseudopodia.

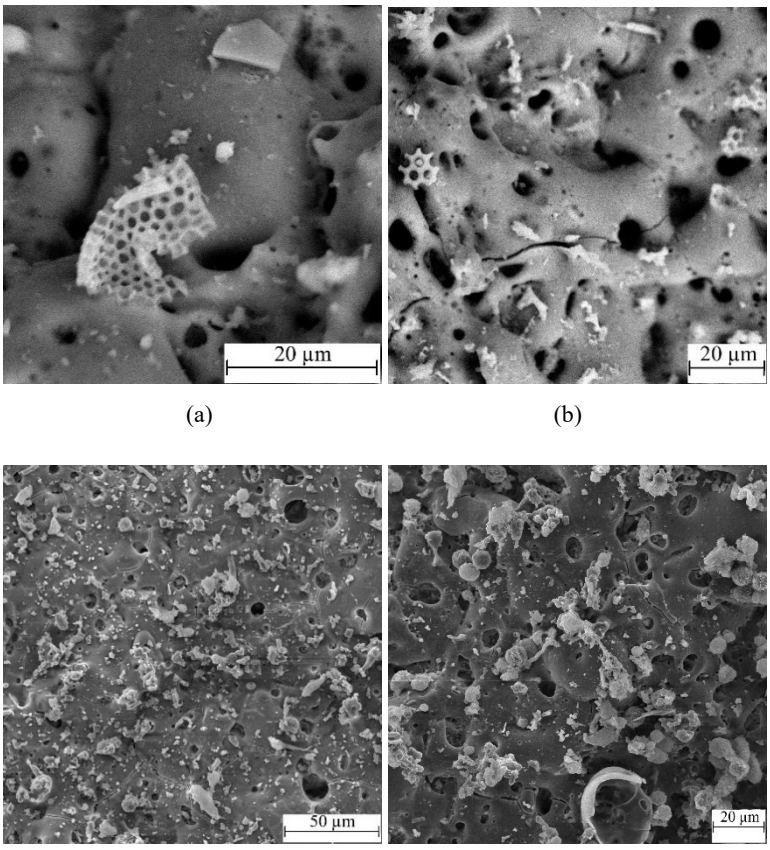

Figure 2. SEM images of coatings with the inclusions of diatomeae fragments (a,b) and of 3T3 cells cultivated on the surface of the coatings for 24 hours (c,d)

The diffraction analysis has revealed the formation of a new phase during the MAO process – forsterite (magnesium orthosilicate). This has likely occurred due to the intensive interaction of the substrate and the coating materials during MAO at the sites of plasma discharges.

The corrosion resistance of the coatings was revealed to be two to three orders of magnitude higher in comparison with the initial magnesium alloy. Coatings synthesized at 400 and 500 V have shown the highest level of resistance to corrosive processes, owing to the formation of the highly crystalline phase of forsterite in first case and to the thickest coating in the second case.

Conclusions and/or Outlook

A new biologically active diatomite-based coating for magnesium implants has been developed using the micro-arc oxidation method. The coatings have shown good strength of adhesion to the substrate surface, especially at higher voltages. The coatings have shown to be 2 to 3 orders of magnitude less corrodible in relation to Mg. It has been revealed that diatomite coatings significantly lessen the cytotoxicity of an Mg alloy towards 3T3 fibroblast cells, making them an excellent biogenic mediator between a magnesium implant and liquid physiological human media.

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In Vitro Corrosion Behavior of Mg-Nd-Y-Zn-Zr Alloys Type

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Keywords: Mg-alloys; fluoride treatment; corrosion behaviour; in vitro testing

Introduction

Magnesium (Mg) is a unique material, which presents physical and mechanical biocompatibility to human bone. It's Young's modulus and density have similar values to those of bone [1,2,3]. Mg bone implants could be classified as bone fixation devices (bone screws, pins, and plates) and bone tissue engineering. The main drawback of the innovative Mg implants is their higher corrosion rate in physiological media. Due to the increased value of this factor, a high degradation rate of the implant's mechanical properties can be noticed, followed by a premature failure of the device, which takes place before the patient tissues healing. Thermo-mechanical processing of the biodegradable Mg alloy surfaces [4,5] is used for improving the mechanical properties and adapting the corrosion process to clinical needs.

The purpose of this work is to study the *in vitro* corrosion behaviour of some Mg-Nd-Y-Zn-Zr alloy types before and after a combination of surface modification: sandblasting and/or fluoride treatment. The novelty of this manuscript is related not just to the corrosion evaluation of the novel biodegradable magnesium alloys type Mg-Nd-Y-Zn-Zr, but also to the clarification on the effect of sandblasting before fluoride treatment in the case of these biodegradable magnesium alloys.

The surface morphology and elemental composition of the samples before and after immersion testing were examined under a scanning electron microscope (SEM, Philips XL 30 ESEM TMP) coupled with energy X-ray dispersive spectroscopy system (EDS, EDAX Sapphire Spectrometer). The structural aspects of the experimental samples were determined by using a Panalytical X-Pert PRO Diffractometer (Malvern, UK), which works in Bragg-Brentano symmetric geometry. The contact angle measurements (CA, KRÜSS DSA30 Drop Shape Analysis System was used) were made in order to determine the wettability properties of the surface which is very important in the case of implantable biomaterials. Corrosion resistance was investigated using polarization resistance technique at 37 ± 0.5 ◦C in NaCl (Sodium chloride purchased from Sigma-Aldrich, Darmstadt, Germany) using a PARSTAT 4000 Potentiostat/Galvanostat (Princeton Applied Research, Oak Ridge, TN, USA). The immersion test consisted of the immersion of the experimental samples, at 37 ± 0.5 °C, in sodium chloride with a pH value of 7.0. The corrosion behaviour of the experimental samples was observed by weighing them after removing them from NaCl solution and subsequent drying.

Results and Discussion

The results revealed that the untreated sample suffered considerably from the environment while the fluoride-treated sample showed significantly better performance for longer exposure time in corrosive solution. The surface treatment of Mg-alloys by immersion in HF solution ensures a uniform, smoother surface without changing the morphology of the substrate, which results in better protection of the substrate. We also investigate the effect of sandblasting treatment of Mg-alloys using particles of alumina on *in vitro* degradation behavior. As a general remark after the corrosion experiments, it can be highlighted that for both investigated magnesium alloys the sandblasting treatment leads to a worsening of corrosion resistance, while after chemical treatment with HF both investigated magnesium alloys exhibit the lowest corrosion current density and highest polarization resistance.

Conclusions

We can conclude that, for both magnesium alloys investigated, HF treatment of magnesium alloy surfaces reduces the corrosion process, while sandblasting is not an efficient method of surface modification to achieve this goal. The chemical composition of investigated magnesium alloys does not significantly influence the degradation process; regardless of the type of treatment applied the evolution is similar. In the case of all samples, a more accelerated increase in weight loss is observed in the first 5 days, after which the intensity of the degradation process decreases. In conclusion, our study reveals that HF treatment is a beneficial way to improve the biofunctional properties required for magnesium alloys type Mg-Nd-Y-Zn-Zr to be used as biomaterials for manufacturing orthopaedic implants. For obtaining a better interaction between the magnesium alloys and surrounding tissue, we propose sandblasting followed by HF treatment as a biodegradable magnesium alloy processing protocol that allow obtaining optimal surface properties.

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Session 4 - Materials for Dental Applications

Biocompatibility assessment of bone augmentation materials

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A sufficient bone volume is the main condition for the stability and long-term osseointegration of dental implants. Thanks to numerous surgical procedures and the latest research, the possibility of reconstructing bone tissue is now much more predictable than in the past. Bone replacement materials play an important role in oral rehabilitation. A wide range of bone augmentation materials have been so far approved for clinical use , but even this wide variability makes it difficult to choose the ideal material for each case.

Biocompatibility assessment of bone augmentation materials can be done by several methods, but histological examination remains the main tool that can provide data on the integration of these materials in bone tissue. Studies can be performed on laboratory animals or clinical studies on human subjects.

Our studies were performed on Wistar rats and aimed to evaluate the integration mode of some commercial bone augmentation materials, but also some experimental materials.

Well-designed observational studies can play a key role in providing a powerful database for the use of bone augmentation materials in a well-documented medical act. As we have also noticed in our study, a simple examination and clinical observation may provide important data related to the integration of a bone augmentation material.

Optical coherence tomography has the ability to monitor noninvasively the healing process of bone tissue both ex-vivo and in-vivo, as opposed to the invasive histological examination which is the gold standard, allowing the differentiation between soft and hard tissues, similar to CBCT and with a higher resolution than radiography, even if it has a lower depth of penetration and a more limited field of view.

In the histological evaluation of the augmented cavities with hydroxyapatite-based materials, it was always noticed the presence of residual particles of synthetic material surrounded by a connective tissue or a young bone tissue with many osteoblasts depending on the moment of integration of the studied material. No necrosis or encapsulation and rejection areas of the applied material were noticed on all the studied preparations, the degree of resorption of the synthetic material being related to the size of the particles and to the time elapsed from the moment of their insertion.

3D printed ceramic lightweight bone substitutes with periodic minimal surface microarchitectures are highly osteoconductive

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Keywords: TPMS; osteoconduction; microarchitecture; bone substitute; 3D printing; ceramics

Introduction

Autologous bone is still the gold standard bone substitute in the clinic. Therefore, the microarchitecture of newly developed synthetic bone substitutes, which reflects the distribution of material in the scaffold often, aims to recapitulate the microarchitecture of natural bone. The microarchitecture of natural bone, however, is optimized towards a mechanically stable lightweight structure. In context of synthetic bone substitutes, the application of triply periodic minimal surface (TPMS) algorithm could yield in stable lightweight microarchitectures, which, despite their demanding architectural complexity, can be produced by additive manufacturing. In this study, we produce scaffolds from hydroxyapatite (HA) by a lithography-based layer-by-layer methodology and characterized them for compression strength, osteoconductivity (1), and bony regeneration (2).

Results and Discussion

The in vivo results, based on a rabbit calvarial defect model showed that bony ingrowth into periodic minimal surface microarchitectures, as a measure of osteoconduction was as good as in pore based or lattice derived microarchitectures. The compression strength and the microarchitectural integrity in vivo, however, were significantly higher for scaffolds with their microarchitecture based on periodic minimal surface microarchitecture algorithms. Therefore, high osteoconductivity needed for an accelerated bony bridging of the defect can be realized with curvy and strait pore systems resembling natural or technical microarchitectures.

Conclusions and/or Outlook

Since TPMS and other periodic minimal surface microarchitectures are true lightweight structures, optimized for high mechanical stability with a minimal amount of material, such microarchitectures appear most suitable for bone substitutes used in the clinic to treat bone defects in weight-bearing and non-weight-bearing sites.

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Challenges regarding the use of bone regeneration materials in implant surgery

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Keywords: implant, alveolar bone, bone grafts, regeneration, complications

Implant-prosthetic therapy represents the optimal treatment solution from a biological, functional and biomechanical point of view. The interdisciplinary management of implant-prosthetic therapy frequently involves the reconstruction of the alveolar ridges in the pro-implant stage. Specialists in implantology and oral surgery must collaborate to face the issue of selecting grafting materials and techniques in relation to systemic, loco-regional, local factors and the planned prosthetic solution. Various complications can be associated with alveolar bone grafting (infection, membrane exposure, incision line opening, perforation of sinus mucosa, partial graft failure, total graft failure, implant failure). In this context, a practical guide regarding ways to optimize alveolar bone reconstruction techniques and to manage possible post-operatory complications following regeneration techniques of the alveolar bone can be useful in order to significantly reduce the risk of implant failure and to ensure long-term success.

Biocompatibility of dental materials in contemporary pediatric dentistry

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Keywords: Biocompatibility, toxicity, dental materials, pediatric dentistry.

Biocompatibility is defined as the ability of a biomaterial to perform its desired function, without eliciting any undesirable local or systemic effects.

In this presentation, the biocompatibility of various commonly-used materials used in contemporary pediatric dentistry will be discussed.

This presentation will also focus on the local and systemic potential toxicity of materials used in pediatric dentistry, toxicity test methods, and the mechanism of the cytotoxicity in living tissues.

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Imagistic explorations in paediatric dentistry

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Keywords: dental digital radiography, paediatric dentistry, lanthanides sensor, irradiation

Introduction

In pediatric imaging, in order to reduce radiation exposure to the lowest possible levels, clinicians need to be prudent when determining the need for radiographs and adhere to the "as low as reasonably achievable" (ALARA) principle. There exists a large difference between the patient exposures among different dental facilities. The disparity may arise from the different X-ray units used, exposure techniques, film speed or even inadequate exposure settings. Although radiation exposure arising from dental radiology is considered to be low, a child may undergo repeated dental radiological procedures during childhood and adolescence [1,2]. Therefore, the accumulated effect of the radiation exposure should be taken into consideration. Salivary gland and the thyroid gland are among the organs at risk in dental radiology. Digital radiography is a type of X-ray imaging that uses digital X-ray sensors to replace traditional photographic X-ray film, producing enhanced computer images of teeth, gums, and other oral structures and conditions. Digital radiography requires 90% lesser dose compared to E-speed film. In digital imaging, image quality may be interactively manipulated after image acquisition, i.e., contrast, blur and noise may be altered digitally. Digital radiography sensors are divided into: storage phosphor plates (SPP) called photostimulable phosphor plates (PSP), silicon devices such as charge-coupled devices (CCD) or complementary metal oxide semiconductors (CMOS).

The present paper is intended to review all types of imagistic explorations in paediatric dentistry, including sensor selection and image acquisition techniques for intraoral radiological evaluation.

Results and Discussion

The key clinical feature of the sensors used for intraoral exploration is the rapid availability of the image after exposure. For this purpose, lanthanide-based luminescent probes are particularly attractive for their long luminescence lifetimes (Eu(III), Tb(III), Ln(III), Gd(III) etc.). The general design and photophysical properties of lanthanides and how these parameters are tuned to develop bioresponsive probes for optical imaging are the main concern of manufacturers of optical imaging systems, high resolution and sensitive techniques with fast response times [3]. The most important role of the sensor is that the sensor is photostimulabile and it is reusable by thausand and thausand of times, taking into consideration the financial aspects of working with sensors [4]. We present in this study different clinical situations in pediatric dentistry using multiple imagistic exploration techniques such as: retrolaveolar dental radiographies, bite-wing radiographies, occlusive radiography, orthopantomography (OPT), lateral cephalometrics and con beam computer tomography (CBCT). Among these techniques, the bite-wing radiographies are the most common used in paediatric dentistry, for small children. The indications of retroalveolar dental radiographies are reduced in this case, OPT being preferred for orthodontic patients, because it offers a panoramic view [5].The occlusive X-rays of 5/8 cm are used in orthodontic purpose, after disjunction therapy with RPE (rapid palatinal expander), to visualize the breaking of the inter-maxillary suture [2]. The lateral cephalometric radiographies are, immediately after OPT, the most common used radiological investigations in orthodontics [6]. The CBCTs are indicated in orthodontics for the cases of supranumerary teeth or impacted maxillary canine [7]. While CBCT offers imaging of tissues in three dimensions (axial, sagital and coronal planes), it also results in higher radiation dose than other radiographic modalities. Thus, the indications for CBCT — especially in pediatric patients — should be carefully weighed against potential risks. [8].

Conclusions:

The use of digital imagistics is nowadays indispensable for the clinician. The radiological examination indication should be based on the clinical examination of the patient. The risks of radiation exposure, however, must be balanced against the clinical knowledge that radiography provides. Proper technique and the implementation of strict safety protocols are critical steps during pediatric imaging. Clinicians need to be prudent when prescribing radiographs for children, and adhere to the "as low as reasonably achievable" (ALARA) principle in order to reduce radiation exposure to the lowest possible levels while still attaining diagnostic-quality images. The indication of con beam computer tomograph (CBCT)

in the child patient must be very well justified from a professional point of view and balance the benefits and risks of irradiation exposure.

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Einstein's Splendid Light: Clinical Applications of Lasers in Dentistry

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Keywords: laser, wavelength, diode, frenectomy, gingivectomy

Introduction

*The word **'LASER'** conjures in the mind's eye many aspects of what might be described as 'modern' life. The words 'powerful', 'precise' and 'innovative' complement our conception of the word in terms of technology whereas patients often associate the terms 'magical' and 'lightening quick' with the use of lasers.*[1, 2, 3]

The word 'Laser' is an acronym for **Light amplification by stimulated emission of radiation**. It is a technology that can amplify and produce a highly directional, intense, monochromatic and coherent beam. [4]

LASER is the mightier new *"avatar"* of light. The wonder beam has penetrated into normal life, more than any other form of concentrated energy. [4]

Today, laser is familiar to everyone because of its multifarious nature - be it in medicine, communication, industry/defense etc. ***almost anything and everything***.[4]

Lasers were first developed in the 1960's, and research into their applications in dentistry began soon thereafter. Early lasers were continuous wave devices with non contact delivery that were found to be too hot for practical dental use. In the early 1980's, short- pulsed, fiber optic contact delivery laser technology was developed. Further technologic advances have led to smaller laser units such as Nd: YAG and diode lasers.[5] In the past 15 years, dental lasers have had a huge growth in practical dental applications. Currently there are 20 specific indications, both soft tissue and hard tissue for use of a variety of dental lasers. However, no one device can accomplish all the practical dental uses. There has been continued growth in this new and maturing field of dentistry.[5]

Research is still ongoing in areas of caries prevention and areas of optical coherence tomography for un-destructive imaging of enamel and dentin to determine lesion progression over time.[5]

The decision to include laser in everyday dental care will depend not only on financial consideration, as to how their use enhances practice profitability, the greatest factor in making that decision will be an understanding of now laser wavelength interact with oral tissue, together with a appreciation of now their use can improve patient management.[6]

Lasers have a widespread applications in dentistry. An essential condition for the proper and successful use of lasers in ant specialty is an understanding of the characteristics and limitations of wavelengths, interaction with tissues, mode of transmission, delivery systems and settings.

The presentation aims to introduces lasers through a clinicians point of view and further augments the information by citing the studies done in different aspects of dentistry. From diagnosing a simple caries to the treatment of the most complex lesions or pathologies, lasers are by far the best as compared to conventional techniques.

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Variants of Use of Zirconia in Dentistry

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Keywords: dental prostheses, zirconia, implants, fracture, translucency

Introduction

As an alternative to metal-ceramic dental restorations, zirconia has been brought into dental practice. Zirconia restorations have superior aesthetics compared to conventional porcelain fused to metal restorations because of their great optical qualities and, more importantly, since they do not have the black line, that conventional restorations created by metal in the cervical region do. Also, zirconia implants are more and more frequently used. Nowadays, stabilized zirconia with various Y2O3 concentrations that can also be doped with additional elements is used in dentistry. In addition to improving the physical properties of zirconium, the inclusion of yttria is intended to stabilize the transition of the crystalline structure under conditions of higher temperature. The main issues with zirconia-supported restorations that are reported in clinical research are more connected to failure than to the framework's integrity. From this point of view monolithic zirconia restorations are a promising option in high-stress-bearing areas. A question of special interest is the wear behaviour of monolithic zirconia restorations comparing to the wear of the enamel of natural antagonists opposed to the extremely hard zirconia material.

Results and Discussion

Zirconia has several benefits, however a curious phenomenon known as low temperature deterioration (LTD) happens in vivo. Studies were carried out to discover a scientific explanation for this phenomenon. According to published accounts, LTD is a zirconia ageing process that results in surface degradation with grain pull-out and subsequent microcracking of the structure, which is mostly caused by the presence of water. The studied zirconia samples in our biocompatibility study have been shown to be biocompatible, even though compositional variations may affect how it interacts with soft tissues. Some samples did, however, exhibit a phase transformation after 6 weeks in the oral mucosa, which may have an impact on their mechanical properties. Despite slight compositional changes, the local tissues react well to zirconia materials, but the material itself may not, after prolonged maintenance in the oral tissues.

Conclusions and/or Outlook

The future perfection of technology and materials must be considered. To improve the wear behaviour and optical properties of zirconia prosthetic devices and reduce the risk of low temperature degradation in vivo, additional research and study are required.

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Zirconia-based Biomaterials: from Composition to Structural Stability

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Keywords: zirconia; sol-gel; crystalline structure; doping; ageing

Introduction

Nowadays, there is a wide range of biomaterials available to patients for hard tissue fixation and reconstruction therapies [1]. Previous studies evidenced that zirconia implants can be alternative candidates to titanium due to their superior biocompatibility, soft-tissue response and aesthetics [2]. There is a need of long-term clinical trials on zirconia based implants as most of current clinical studies are short-terms. The most important aspect related to orthopaedic implant failure proved to be its ageing in humid atmosphere determined by the slow phase transformation of metastable tetragonal zirconia to monoclinic phase [3]. The studies revealed a stronger need to correlate the microstructure and LTD resistance *in vivo*. While orthopaedic implants are under consideration due to ageing phenomenon, the dental applications are encouraged by the aesthetical and mechanical properties of zirconia, its market increasing with more than 12% each year. Alloying zirconia with appropriate cations leads to stabilization of tetragonal or cubic phases at body temperature, depending on cation type, concentration and manufacturing technology [4]. The present work aims to evaluate the contribution of cerium and titanium cations to the morphology, thermal behaviour and structural stability of yttria-stabilized zirconia.

Results and Discussion

Yttria-stabilized zirconia (YSZ) (3 mole %) samples - pure and co-doped with ceria or titania - have been synthesized using the Pechini method, with citric acid as complexation agent and ethylene glycol as copolymerization agent. The as-obtained polycrystalline powders have been characterized using X-ray diffraction (XRD), Raman spectroscopy, and scanning electron microscopy (SEM). The presence of the broad characteristic peaks of tetragonal phase in XRD patterns of the powders indicate their nanocrystalline nature, in agreement with SEM images (Fig. 1a). The dilatometry measurements allowed us to establish the optimal sintering procedure in order to get dense samples.

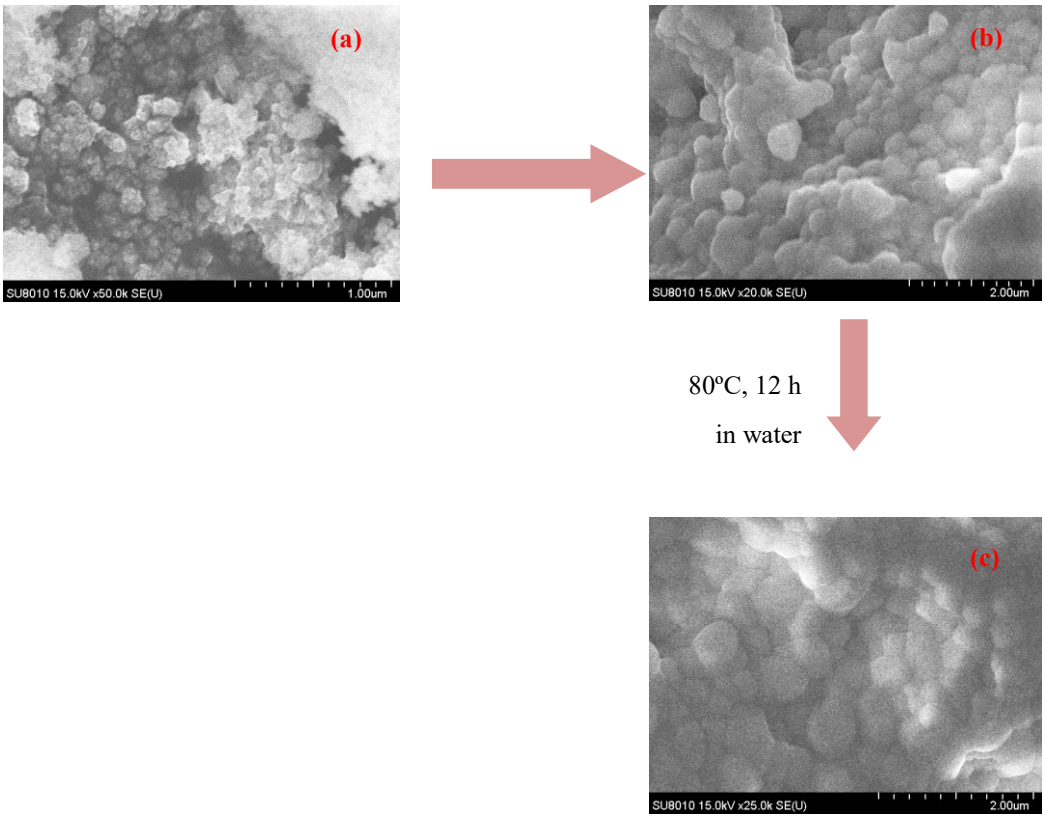

Figure 1: SEM images of (a) calcined powders; (b) sintered pellets; (c) aged pellets

XRD analysis of the sintered pellets revealed the sharpening of the characteristic peaks, indicating an increase in crystallite sizes after thermal treatment. Raman spectroscopy confirmed the presence of tetragonal phase in the sinters, while SEM images shown grain sizes of several hundreds of nm (Fig. 1b). The sinters were further aged in water under pressure at 80ºC for 12 h. XRD patterns and Raman spectra of aged samples shown the partial transformation of tetragonal to monoclinic phase in YSZ and cerium-codoped YSZ samples. However, titanium-codoped YSZ proved to be very stable. In this case, only the presence of tetragonal phase was evidenced.

Conclusions

Nanocrystalline pure and cerium or titanium co-doped yttria-stabilized zirconia powders have been synthesized using a Pechini method. XRD analysis and Raman spectroscopy evidenced the presence of tetragonal phase, while SEM images shown agglomerated nanosized particles. The most appropriate sintering procedure for the synthesized was established using horizontal dilatometry measurements. The sinters also exhibited the presence of tetragonal phase, with an increase in crystallite sizes compared to powders. However, ageing led to the formation of monoclinic secondary phase in YSZ and Ce-co-doped YSZ, while Ti-co-doped sample proved to be stable against phase transformation.

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Microporous Metal-Organic Frameworks (MOFs) in the Control of Extracellular Matrix Degradation and Improvement of Dental Material's Therapeutic Capabilities

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Keywords: MOFs, collagen degradation, extracellular matrix, biomimetic mineralization, dentin, drug delivery

Introduction

This contribution identifies potential biomedical applications of Metal-Organic Frameworks (MOFs) in the dental field, envisaging the improvement of dental material's therapeutic capabilities and the stabilization of tissues critical to restorative therapies. Traditional MOF synthetic routes provide bulk particles that can serve as storage, delivery, or sensing platforms. On the other hand, some unique MOFs – e.g., zeolitic imidazolate framework-8 (ZIF-8) – can also combine with biomolecules through "one-pot" methods to generate biomolecule-embedded MOFs. The biomimetic synthesis of MOFs is a relatively new concept in the family of bioinspired materials and shows great potential for engineering biocomposites and protecting vital biological entities. Here, microporous MOFs have been synthesized in situ as protective coatings for native collagen fibrils or as crystalline shells to immobilize collagen-degrading enzymes. Protecting the fibrillar collagen structure and restraining enzyme catalysis are strategies that ultimately envisage the prevention of collagen degradation at the tooth–restoration interface.

Results and Discussion

Collagen fibrils (from dentin extracellular matrix/ ECM) can induce the biomimetic growth of MOF crystals as coatings that strengthen and stabilize the fibrils. ZIF-8, a zinc-based microporous MOF, was used to fabricate the MOF composites via a "one-pot " reaction in water. The ZIF-modified dentin ECM presented superior mechanical strength and resistance to proteolysis, which can positively affect the longevity of collagen as an anchoring substrate. Cell viability results suggest that ZIF-8, as a crystalline coating, can support cellular behavior and essential mechanical parameters (e.g., stiffness) of native organic matrices. Another core problem in restorative dentistry has been addressed by fabricating MOF-encapsulated proteases: the degradation of dental tissues by endogenous endopeptidases. The *in-situ* encapsulation of collagenases in microporous MOFs is a novel concept that could be helpful in the control of collagen catabolism. This physiological process, when unbalanced, is implicated in numeral pathological conditions, *e.g.*, metastasis, neurodegenerative disorders, osteoporosis, periodontal disease, etc. Besides the biomimetic approach, water-based synthesis was utilized to produce an erodible MOF system for the on-demand release of flavonoids with therapeutic potentials relevant to the oral environment. Quercetin (QCT) was encapsulated in the frameworks in neat water through a one-step synthetic process. The resulting QCT@ZIF-8 microparticles were analyzed concerning chemical composition, crystal structure, thermal behavior, micromorphology, and release profile under acidic and physiological conditions. Different amounts of microparticles were incorporated into a model dental adhesive; both the degree of conversion (DC) of methacrylic double bonds and the polymer thermal behavior were accounted for. Crystalline QCT@ZIF-8 microparticles with attractive loading capacities (up to 10.4%), submicron sizes, high thermal stability, and responsiveness to environmental pH change were successfully manufactured. Under a pH condition representative of the carious process, a complete (100%) release of QCT was rapidly induced. The concentration of QCT@ZIF-8 in the adhesive resin was a key factor in maintaining an optimal DC plateau and rate of polymerization. One-step encapsulation of QCT in pH-sensitive ZIF-8 matrices can be easily achieved, and QCT@ZIF-8 microparticles proved to be functional platforms to carry bioactive compounds with potential to prevent microbial/ enzymatic activity degradation of hard tissues and native ECM components.

Conclusions and/or Outlook

We demonstrate that collagen fibrils (from dentin extracellular matrix/ ECM) can induce the biomimetic growth of MOF crystals as coatings that strengthen and stabilize the fibrils. ZIF-8, a zinc-based microporous MOF, was used to fabricate the MOF composites via a "one-pot " reaction in water. This work summarizes our group's efforts in identifying potential biomedical applications of microporous MOFs in the fields of dentistry and biomimetics.

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Ion-doped, citrate stabilized amorphous calcium phosphate: a multifunctional material for preventive dentistry

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Keywords: dental remineralization, amorphous calcium phosphate, ion doping, antibacterial activity

Introduction

Dental caries is the most worldwide widespread noncommunicable disease, which results from a prevalence of acidogenic bacteria, such as *Streptococcus mutans*, in the biofilms that permanently colonize any oral surface. These species produce organic acids that lower the pH of the microenvironment on teeth surfaces (enamel and dentine) dissolving their mineral component, hydroxyapatite (HA). HA dissolution is referred to as the demineralization process, and leads to the formation of dental cavities. Modern dentistry is oriented towards early prevention of tooth decay, aiming to stop the demineralization by promoting the remineralization of teeth surfaces and by preventing the formation of cariogenic bacterial biofilms [1]. The first goal can be achieved with remineralizing agents, while the second is achieved with use of antimicrobial agents. Therefore, an ideal material for preventive caries treatment should show remineralizing capabilities and express antimicrobial activity. Remineralizing agents restore the structure and mechanical properties of dental hard tissues by quickly delivering a significant amount of HA constituent ions in the oral environment, in order to stimulate epitaxial growth of new HA crystal into the acid-eroded sites [2].

In this regard, we have recently developed an amorphous calcium phosphate (ACP) stabilized by citrate ions as innovative remineralizing agent [3]. ACP is the most soluble phase among calcium phosphates, so its ion release capability would give rise to a strong remineralizing activity. In addition, in presence of water ACP crystallizes into HA, so it can form new HA crystals onto enamel as well as occluding dentinal tubules responsible of dentinal hypersensitivity. To improve its potential for preventive dentistry, we have also doped our citrate-stabilized ACP with fluoride ions (F-ACP) as well as strontium ions (Sr-ACP) as F^- and Sr^{2+} ions were proved to inhibit cariogenic bacterial metabolic activity [4].

Therefore, the aim of this work was to prepare multifunctional ACP materials able to remineralize the damaged mineral phase and at the same time to show antimicrobial activity against cariogenic bacteria [5]. We have i) synthesized citrate-stabilized ACPs doped with of F^- and Sr^{2+}, ii) evaluated their remineralization ability as well as dentin desensitization effect, and iii) evaluated their direct antibacterial activity as well as the resistance of ACP-remineralized enamel surfaces on biofilm formation.

Results and Discussion

ACP samples doped with Sr^{2+} and F^- ions with several doping levels were prepared and thoroughly characterized. X-ray diffraction and infrared spectroscopy confirmed that all ACP materials were amorphous independently from the presence of doping ions. The analysis of ACP samples chemical composition shown that F-ACP and Sr-ACP samples were successfully doped with F^- and Sr^{2+}, achieving doping levels up to 2 wt.% (F) and 12 wt.% (Sr). Stability analysis of the ACP powders proved that these materials remained stable as amorphous phase up to several years during storage at room temperature.

Afterward we have evaluated ACPs ion release *in vitro* in artificial saliva in order to assess their potential as remineralizing agents. All samples show a gradual and progressive ion release that is superior to HA materials currently used for dental remineralization.

In vitro enamel remineralization tests proved that all ACP materials induce the growth of new HA crystals onto the ordered microcrystals of enamel prisms. Therefore, our ACPs are suitable for enamel remineralization since were able to restore enamel in its native structure. In addition, all ACP samples showed good levels of occlusion of dentinal tubules, indicating a promising dentin desensitization activity.

Subsequently we have evaluated the antibacterial activity of ACP samples. The antimicrobial effect was tested against *S. mutans* biofilms, evaluating ACP direct inhibitory effect as well as the inhibitory effect induced on demineralized human enamel remineralized with these compounds. The antibacterial activity was tested under normal or enhanced cariogenic conditions, which were controlled by sucrose addition. Both F-ACP and Sr-ACP samples showed a significant direct inhibitory effect against the biofilm both with normal and enhanced conditions. In addition, also human enamel remineralized with F-ACP showed a significant antibacterial effect both in normal and enhanced cariogenic conditions.

Conclusions

Innovative citrate-stabilized ACP doped with Sr^{2+} and F^- ions have been produced and were proved to be promising dentin desensitizers and remineralizing agents for preventive dentistry. Ion doping did not alter ACP physicalchemical properties but allowed to incorporate up to 2 wt. % F^- and 12 wt.% of Sr^{2+}. Our investigation confirmed that all ACP samples are stable for several years when stored at room temperature, and release ions and crystallize into HA when in contact with water. All the samples were able to restore enamel in its native structure and showed good ability to occlude dentinal tubules. F-ACP and Sr-ACP were proved to have good antibacterial activity against *S. Mutans* biofilm, with ≈ 50% inhibition of the viable biomass on contact. In addition, enamel remineralized with F-ACP had a significant antibacterial activity, probably because the deposition of a fluoride-rich layer onto enamel imparted an antibacterial/bacteriostatic effect. From these results, we proved that citrate-stabilized ACP doped with fluoride or strontium ions are effective remineralizing materials with good antibacterial properties. These ACPs express great potential as advanced materials for oral healthcare as can be able to treat dental caries by restoring demineralized enamel as well as by inhibiting cariogenic bacteria.

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A evaluation of a new Ti-Nb orthodontic wires using a Typodontlaboratory device Based On A Clinical Case

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Keywords: orthodontic wire, Typodont, occlusal analysis, Ti-Nb

The aim of the study was to evaluate new materials available based on Ti-Nb comparative to the wildly use wire sequence Ni-Ti, avoiding the variables associated with patients anatomical variables and treatment methods. It also analyses the differences between clinical assessment and objective means of evaluation of occlusion and orthodontic movements.

Material and methods: For this study we used ten typodonts (Savaria- Dent, Hungary), which is a laboratory device which simulates the orthodontic movements. The typodonts were created by setting the teeth in the same silicone mould, five for each material studied Ni-Ti vs Ti-Nb. They were treated using the same orthodontic technique in the typodonts. Afterwards, the used wires to complete each stage were analysed using electronic microscope and spectroscope in order to discover indentations and material stress at brackets contact during orthodontic movement in both groups treated. An occlusal analysis was also performed before and after orthodontic treatment.

Results: The number of occlusal contact areas after the treatment was significantly larger than before treatment, for both study groups, with a $p<0.0001$. The force distribution before and after the treatment revealed a significantly equilibration of occlusal contacts after the treatment. However, to obtain a functional occlusion and enhancing stability the finishing is mandatory which is not possible using Typodonts as chain elastics and class II elastics could destroy the Typodont due to high force. Indentation and bending deformation were more often found in Ni-Ti group at bracket slots, as all brackets were fixed by metal ligature not with an elastic module. Also time spent to obtain levelling and alignment was favourable to Ti-Nb group.

Conclusions: Simulated orthodontic treatment lead to a significantly increase in occlusal contact area, in both control groups, occlusal distribution being favourably changed, even if we cannot describe a balanced occlusion yet. Variability of the results obtained after treatment is explained by factors related to typodonts.

Peri-implantitis' signature on failed dental implants

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Keywords: Dental implants, Peri-implantitis, Bio-rejection, Ti-6Al-4V, Passivation layer, Biocompatibility.

Introduction

Dental implants are fabricated from Titanium and its alloys, with the most common being Ti-6Al-4V. These metals are considered biocompatible due to their passive oxide layer, which is widely viewed as an inert layer extremely stable that rebuilt itself even under oxygen scarce condition and that promotes the osseointegration process [1-2].
Peri-implantitis is a common disease in implant dentistry, believed to be caused by bacterial biofilm, in which osseointegration is progressively lost resulting in the dental implant failure [3].
The titanium oxide layer, which is in direct contact with the bacterial biofilm and the inflammatory process during disease progression, is probably affected by the environmental changes [1][4]. This degradation, might modify the surface properties, rendering it from bio-inert to bio-reject.

Methods

The study systematically collected retrieved Ti-6Al-4V dental implants which were explanted due to peri-implant disease. A thorough investigation of the implants' surface was carried out using high resolution scanning methods, i.e. Scanning Electron Microscopy (SEM), Time of Flight Secondary Ion Mass Spectrometry (ToF-SIMS), Focused Ion Beam (FIB) and Scanning/Transmission Electron Microscopy (S/TEM), on representative locations along the retrieved implants. The oxide layer's nature (thickness, coherency, and integrity) was determined together with a detailed characterization of the implant's surface composition. The analysis included a comparison with reference ("out of the box") unused implants with an intact native titanium oxide layer.

Results and Discussion

The analysis has shown a clear change in the surface composition along the implant's length. Areas which experienced extensive inflammatory process and completely lost their bone support showed inconsistencies in the surface oxide layer's thickness, continuity, and composition with an appearance of a new organic, yet unidentified, carbon-nitrogen species. Areas which were exposed to minimal or no inflammation showed much less modifications compared to the reference unused implants.

Conclusions

The study identified alterations of the titanium surface oxide layer properties, thickness, and continuity; and identified the impurity's element content and distribution, all of which can be tentatively correlated with the disease progression and may possibly contribute to its catalysis.

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Preclinical and histological study of boron-containing compounds hydrogels on experimental model of periodontal disease

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Keywords: experimental model, inflammation, periodontal disease, ligature, Calcium Fructoborate, Boric Acid

Introduction

Periodontitis is a disease that has shown a high prevalence, which has led to an increased interest in the research field on the development of new therapies. Animal models have proved to be an important factor in helping the research of the pathogenesis of periodontal disease and of new therapeutic materials. Studies have found that Calcium Fructoborate has an important role in the decrease of intracellular reactive oxygen species induced by exposure to oxidative stress and can stimulate the differentiation of osteoblasts from bone marrow, as well as to restrict superoxide formation within cells.

Results and Discussion

In this study, we have tested two boron-containing compounds (BCC) hydrogels with Calcium Fructoborate (CaFB) and Boric Acid (BA) on the periodontal level on Wistar rats on an induced periodontal defect. Firstly, we induced the periodontal disease at the lower incisors level, we locally applied the hydrogels and after a week, the rats were euthanized and the probes were collected. Next, the oral soft tissue reaction was clinically and then histologically investigated.

Our study has shown good clinical response of the oral tissue, and we have noticed lower levels of inflammation on the experimental groups treated with the BCCs hydrogels. The histological study revealed the reduction of inflammatory response when using the hydrogels, compared to the traditional methods.

Conclusions and/or Outlook

The effect of BCC hydrogels on periodontal level has shown promising results. Since BCCs have shown its efficacy, more scientifically proved information is needed to adapt the manufacturing technology of the compounds and to extend the research in other areas in the field of oral health.

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Session 5 – Bioactive Glasses

Ion releasing biomaterials: overview of well-known and less common ions enhancing the biological activity and functionalities of bioactive glasses

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Bioactive glasses (BGs) continue to attract interest for tissue engineering (TE) applications, including both hard (bone) TE and soft tissue repair. Such applications are possible due to the biochemical reactivity of BG surfaces leading to bioreactions at the interface between BG surfaces and biological media. In this context the controlled release of biologically active ions, which are in fact the dissolution products of BG being in contact with body fluids, is key to stimulate cellular responses involved in new tissue growth [1]. In addition, an emerging number of studies investigates the immunomodulatory effects of BGs in the framework of bone regeneration and wound healing [2]. In the first part of the lecture the general field of BGs in TE approaches will be discussed in particular considering the interaction of biologically active ions (released from BGs) and stem cells leading to osteogenic and angiogenic effects.

Numerous metal ions released from BGs have been shown to induce an angiogenic effect, e.g. in specific concentrations they enhance the secretion of vascular endothelial growth factor (VEGF) from stem cells. The effect of BGs on angiogenesis will be discussed in detail, showing results on different scaffold types and BG compositions.

The second part of the talk will focus on less common ions (or even exotic ions) which are being investigated to enhance the biological activity of BGs. Indeed, in addition to well-recognized biologically active ions that provide osteogenic, angiogenic, anti-inflammatory and antibacterial effects, such as zinc (Zn), magnesium (Mg), silver (Ag), strontium (Sr), gallium (Ga), fluorine (F), iron (Fe), cobalt (Co), boron (B), lithium (Li), titanium (Ti), and copper (Cu), a range of less common ions is being considered by researchers worldwide. For example, barium (Ba), bismuth (Bi), chromium (Cr), dysprosium (Dy), europium (Eu), gadolinium (Gd), ytterbium (Yb), thulium (Tm), germanium (Ge), holmium (Ho), iodine (I), lanthanum (La), manganese (Mn), molybdenum (Mo), nickel (Ni), niobium (Nb), nitrogen (N), palladium (Pd), rubidium (Rb), samarium (Sm), selenium (Se), tantalum (Ta), tellurium (Te), terbium (Tb), erbium (Er), tin (Sn), tungsten (W), vanadium (V), yttrium (Y) and zirconium (Zr) are being investigated [3]. The effect of incorporating such less-common elements in BGs will be covered with focus on tissue engineering applications.

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Functionalization of glasses for the design of biomaterials

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Keywords: bioactive glasses, cerium, antioxidant, antibacterial, regeneration

Introduction

Bioactive glasses (BGs) are well-established biomaterials traditionally used as hard tissue regeneration in e.g. traumatology, orthopaedics, and oral surgery, and are emerging as promising scaffolds for soft tissue applications such as muscle regeneration and wound dressing [1].

A frequent complication following the insertion of a biomaterial (e.g. BG) is a post-surgery inflammation, which involves harmful reactive oxygen species (ROS) and can significantly delay recovery. To overcome these issues [2,3], the BG must act as an antioxidant, converting ROS into non-harmful species; this can be achieved by doping with redox therapeutic inorganic ions (TIIs). Among the TIIs, cerium is known to add relevant antioxidant and anti-inflammatory properties to the BG due to its ability to quench ROS.

An alternative strategy to the same scope is loading the BG with antioxidant organic molecules, this approach is dependant by the ability of the BG to bind and release the organic guest, acting as a drug delivery system (DDS).

Results and Discussion

We evaluated the antioxidant properties of a series of cerium doped BGs (Ce-BGs) [4,5] by assessing their ability to scavenge common ROS, hydrogen peroxide, acting as the enzyme catalase, and superoxide radical anion, acting as the enzyme Superoxide dismutase (SOD) by tailored enzymatic assays. We found that the addition of cerium to conventional Hench-type BGs (of low specific surface area, SSA) confers them significant catalase-like properties (CAT-like activity) and only limited superoxide dismutase properties (SOD-like activity). We then explored the use of high SSA cerium-doped mesoporous bioactive glasses (Ce-MBGs) as DDS by loading them with an antioxidant mixture of polyphenols extracted from chestnut flour, POLY.

Interestingly, (**Figure 1**), CAT-like activity is strongly correlated with cerium doping, whereas POLY loading alone does not confer significant CAT-like properties to undoped MBGs.

In contrast, undoped MBGs show little SOD-like activity even at the highest implementable cerium content of 5.3 mol%, which is instead dramatically increased by POLY loading, with 98% scavenging of the superoxide anion produced by the assay. We were then able to modulate the Ce-MBGs ability to scavenge different ROS species as required to achieve a complete detoxification process. *In vitro* (SBF) studies also indicate that bioactivity is preserved after POLY loading.

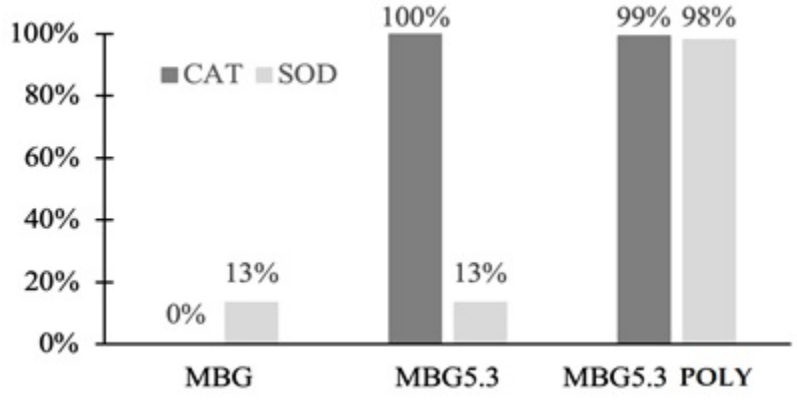

Figure 1: Modulation of the antioxidant activity of Ce-MBGs at 37°C. Dark columns (CAT-activity) represent the % of a 50 micromolar solution of hydrogen peroxide dismutated after 30 minutes of incubation with Ce-MBGs; light columns (SOD-activity) represent the percent of superoxide anion produced by the assay scavenged after 20 minutes of incubation with Ce-MBGs

We also loaded Ce-MBGs with gentamicin to assess their ability to act as DDSs, reaching a 40% of loading efficiency, regardless of the presence and amount of cerium. Furthermore, loading with gentamicin does not affect bioactivity or

CAT activity. An antibacterial assay optimized to monitor antimicrobial activity using E.coli (ATCC 11229.2) indicates controlled antibiotic release for up to 10 days. Finally, given that the presence of POLY confers antioxidant properties and that of gentamicin antibacterial ones, we studied a mixture of POLY-loaded Ce-MBG and gentamicin-loaded Ce-MBG. The mixture retained significant antioxidant and antibacterial properties, demonstrating that we can manufacture MBGs combining broad antioxidant activity with other biological properties. In summary, Ce-MBGs are excellent DDS that also present bone regeneration capacity and antioxidant properties

Conclusions and/or Outlook

Loading with organic molecules of Ce-BGs has been studied to propose materials suitable for both hard and soft tissue applications. The new hybrid materials obtained combine the bioactivity and biocompatibility of BGs with modulable antioxidant properties and with the ability to function as DDS.

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Development of bioactive glass scaffolds by vat photopolymerization

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Keywords: Bioactive glass; Additive manufacturing; Porosity; Scaffold; Tissue engineering.

Introduction

The application of additive manufacturing technologies (AMTs) to biomaterials has opened new horizons in the field of scaffold fabrication allowing a great control on pore/strut architecture to be achieved. In general, AMTs build porous scaffolds following a layer-wise approach in which a computer-aided design (CAD) model is reproduced. AM of bioactive ceramic and glass scaffolds is typically more challenging as compared to the processing of polymeric materials [1]. In this regard, vat photopolymerization allow obtaining high-quality ceramic products with the best spatial resolution among the currently-available rapid-prototyping techniques (few tens of micrometres). Digital Light Processing (DLP), which relies on a dynamic mask, allows promoting the polymerisation of a complete layer of photocurable resin gluing the ceramic particles per irradiation cycle [2]. Like most AMTs applied to bone scaffolds, DLP-based vat photopolymerization aims at layer-wise reproducing a virtual model which is typically derived from computer-built geometries based on repetitive geometrical elements such as unit cells (e.g. grid-like structures) that, however, are unable to properly replicate the trabecular architecture of cancellous bone. In order to overcome this limitation, the present study reports, for the first time, the use of a tomographic reconstruction of an open-cell polymeric foam as an input file to the DLP-based vat photopolymerization system for the fabrication of bioactive glass scaffolds. Polymeric sponges are indeed highly suitable templates for scaffolding since they have been used for many years in the production of bone-like glass and ceramic scaffolds by the conventional sponge replica method owing to their similarity with the trabecular architecture of cancellous bone [3].

Materials and methods

Melt-derived silicate bioactive glass particles (<32 μm) with 47.5B composition (47.5SiO_2-10Na_2O-10K_2O-10MgO-20CaO-2.5P_2O_5 mol.%) were used to prepare the photocurable slurry, which was processed by using a CeraFab 7500 stereolithographic system according to a procedure described in a previous work [4]. Porous cylindrical scaffolds were obtained by applying a multistep thermal treatment to the printed "green" bodies, which were eventually sintered at 650 °C for 1 h.

The sintered scaffolds were analysed by wide-angle X-ray diffraction to assess the presence of crystalline phases. Scaffold morphology was investigated by scanning electron microscopy and micro-computed tomography. Mechanical characterization of scaffolds included the assessment of compressive strength through crushing tests and the quantification of the mechanical reliability by determining the Weibull modulus in compressive loading mode, according to the procedure described in ASTM C1239-07. The elastic modulus was determined from the linear region of the stress-strain curve. Values of porosity, compressive strength and elastic modulus are expressed as average ± standard deviation.

In vitro bioactivity of scaffolds was assessed in terms of apatite-forming ability by immersion in Kokubo's simulated body fluid (SBF) [4] for up to 1 month. A mass-to-volume ratio (scaffold/solution) of 1.5 mg/mL was used, according to previous works [5]. The samples were kept at 37 °C in an orbital incubator with a constant shaking speed of 100 rpm over the whole immersion period.

Results and Discussion

After sintering, the scaffolds material was found to be predominantly amorphous with negligible devitrification. DLP-based vat photopolymerization allowed achieving a good reproduction of the starting foam, which was used as a virtual template, both in as-printed samples (**Figure 1a**) and in sintered scaffolds (**Figure 1b**). The total porosity of scaffolds was of 34.5 ± 5.6 vol.% which, however, is lower than the typical threshold value recommended for an ideal bone tissue-engineering scaffold (50 vol.%) [6]. On the other hand, pore size is within of 150-600 μm (**Figure 1c**), which perfectly fits the optimal size range for vascularisation, mineralization and formation of new bone tissue.

The scaffold compressive strength was 21.9 ± 6.2 MPa, which is even higher than the standard reference for trabecular bone (0.1-16 MPa) [7]. The scaffold elastic modulus was 4.79 ± 0.1 GPa, which is comparable to that of cortical bone. A Weibull modulus equal to 3.9 was assessed, which is interestingly higher than that obtained for hydroxyapatite scaffold produced by the same method [4].

The pH value of SBF in contact with the samples increased towards a moderate alkalinity (around 7.70), which is known

to be beneficial for the osteoblast activity and the overall osteogenic process [8].

The in vitro bioactivity of the scaffolds was confirmed after the immersion tests in SBF, which revealed the formation of an apatite-like layer on the surface at two days.

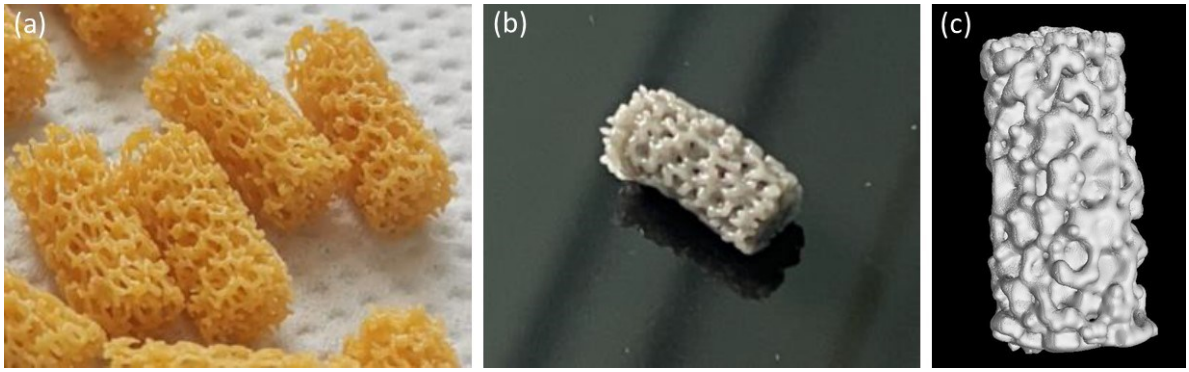

Figure 1: Scaffolds produced by DLP-based vat photopolymerization in the present work: (a) "green" bodies, (b) sintered scaffold, (c) micro-CT reconstruction.

Conclusions and/or Outlook

It has been shown, for the first time, that porous bioactive glass can be produced by DLP-based vat photopolymerization using a micro-CT reconstruction of an open-cell polymeric foam as a virtual template (CAD model). As a result, it was possible to fabricate 47.5B scaffolds with 3D porous architecture, pore size, elastic modulus and compressive strength comparable to those of human bone. This novel approach opens new horizons in the functional design and manufacturing of bone-like scaffolds that could be highly beneficial in bone replacement and bone tissue engineering strategies.

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Tantalum Doped Bioactive Glass: Towards an Anti-Inflammatory and Antibacterial Response

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Keywords: bioactive glass; tantalum pentoxide; cytotoxicity; antibacterial activity; bone regeneration

Introduction

The development and study of new synthetic materials, used in medicine to restore lost bone tissue and improve implant integration, is one of the major focuses in biomaterials science and tissue engineering. For this purpose, Bioactive Glasses (BGs) are attracting considerable attention, due to their excellent features in terms of bioactivity [1], biodegradability [1], [2], osteogenesis [2], [3] and angiogenesis [4]. However, this material does not have an effective antibacterial activity capable of maintaining an infection-free wound for long periods, which is the main problem in bone restoration implants, since this medical procedure is associated with high risk of bacterial colonization, resulting in implant failure. [5]

One of the strategies to prevent biofilm formation is doping BGs with therapeutic inorganic ions (TIIs). [6], [7] Among the TIIs, tantalum (Ta) has shown considerable potential as a dopant since it is already used in medicalfields, such as orthopaedic and dental implant applications, due to Ta implants having excellent mechanicalproperties, biocompatibility, osseointegration ability, haemostatic capacity and antibacterial properties. [8]–[11] In consequence of these properties several types of Ta doped BG have been manufactured and tested,demonstrating improved biocompatibility, haemostatic capacity and antibacterial abilities compared to theundoped BG. [12]–[14] However, there is no biological analysis of this BGs.

So, this work aims to improve the performance of BG-coated implants by doping BG with Ta, produced by the melt quenching technique, and then characterize them both physically and biologically. The biological analysis focused on both cell responses and microbiological assays. Cell responses were analyzed by cytotoxicity assays, using both osteoblasts and macrophages, and then macrophage polarization. The microbiological assays were used to determinate minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

Results and Discussion

The physical caracterization shows that incorporation of Ta ions in BG does not alter the glass structure at low concentrations. However, for the highest Ta concentration, Ta-doped BG presented characteristic peaks of the Ta_2O_5 crystal. The FTIR analysis presented the characteristic BG bands, with five silica absorption bands.

The results of the synthesized glass cytotoxicity assay with osteoblast-like cells (Saos2) and macrophages (THP- 1 derived) are presented in Figure 1. These graphs show an increase in cell viability with increasing doping concentration. Furthermore, the passivation decreased extracts cytotoxicity in all tested materials, allowing for a viable response at much higher concentration. These results could be due to the decrease in Si release and pH control after passivation.

The preliminary results of macrophage polarization indicates that 45S5 has an inflamatory response and4 mol% Ta leads the macrophages towards an anti-inflamatory response.

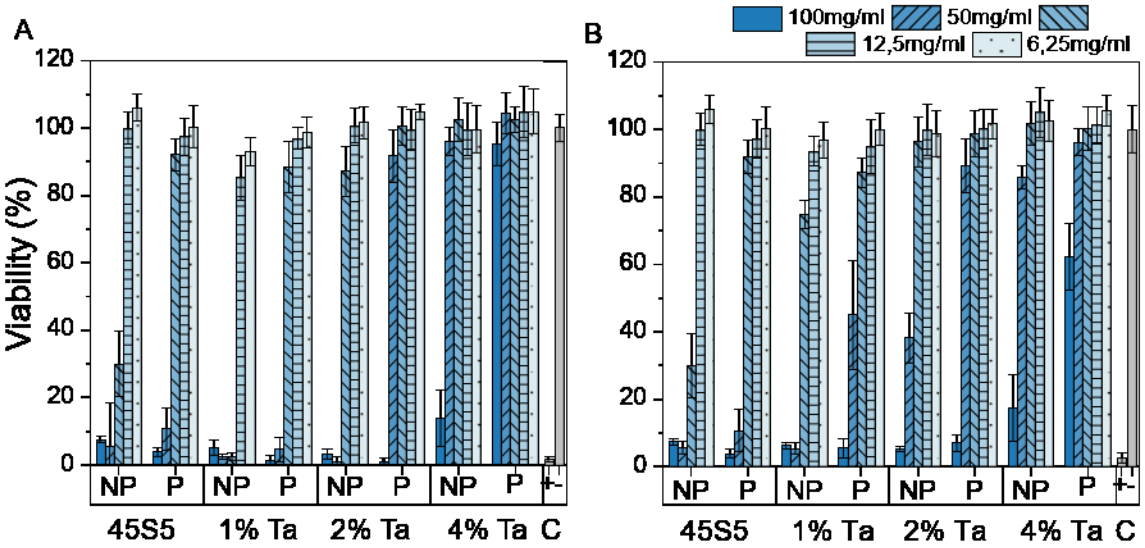

Figure 1: Cytotoxicity results with Osteoblast-like cells (A) and Macrophages (B).

The microbiological assays with Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram- positive bacteria (*methicillin-resistant Staphylococcus aureus*) showes that by incorporating 4 mol% Ta in BGled to an effective bactericidal activity with non-cytotoxic (towards human cells) concentrations.

Conclusions and/or Outlook

This work shows that Ta-doped BG is a promising material capable of reducing inflammatory response, thatresults in an accelerated and better tissue regeneration, while simultaneously preventing bacterial infections.

Acknowledgements

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Bioactive and Antibacterial Glass Doped with Ionic Copper by Ion-Exchange in Aqueous Solution

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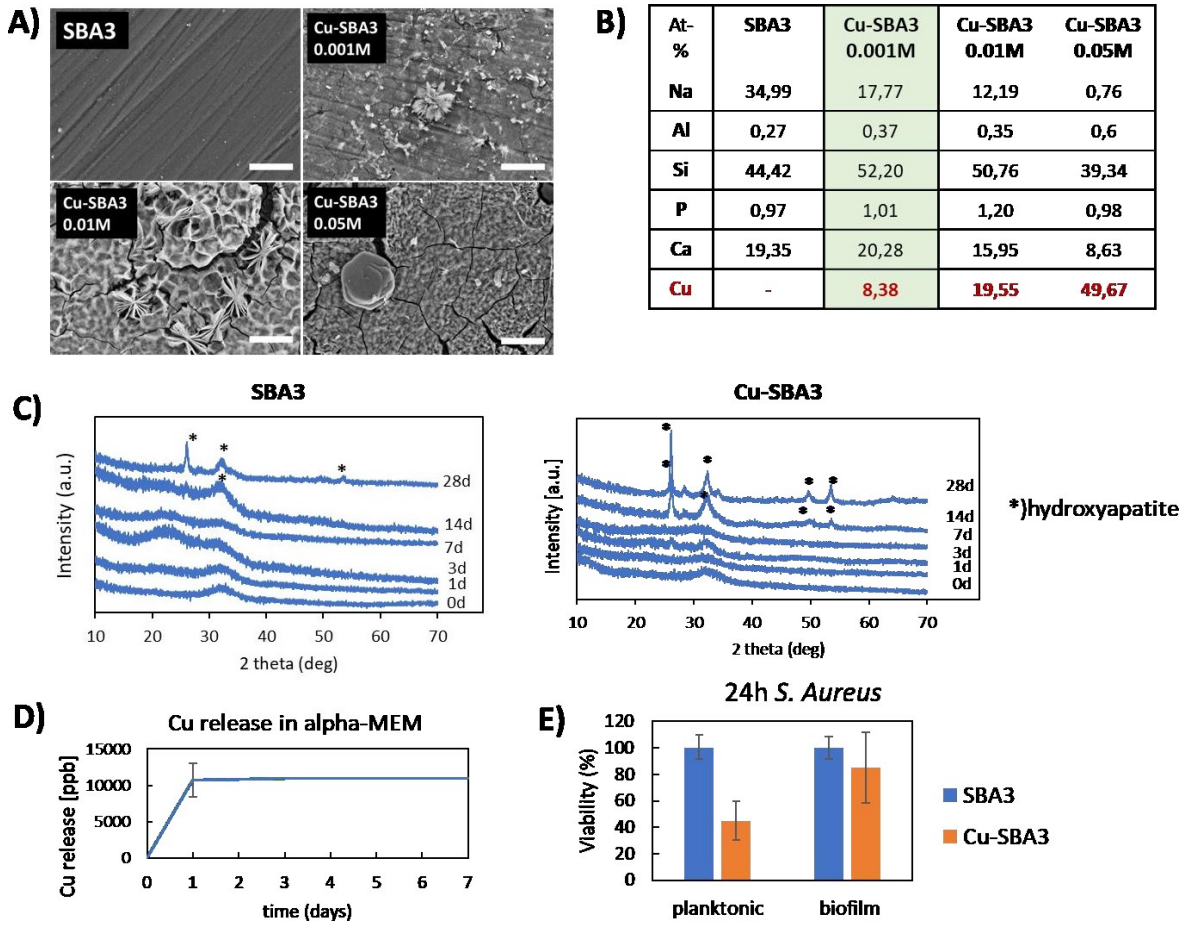
Keywords: bioactive glass, ion-exchange, copper, in vitro bioactivity, antibacterial activity

Introduction

Fighting the formation of bacterial biofilm and simultaneously providing a bioactive environment for bone regeneration during treatment of orthopaedic infections is one of the greatest challenges in surgery. Moreover,the major global threat of rapidly increasing antimicrobial resistance (AMR) calls for non-antibiotic alternatives to tackle bacterial infections. Bioactive glass doped with inherently antibacterial metal ions, such as copper (Cu),present one viable solution to the dilemma. One interesting approach to introduce Cu-ions on the surface of the glass without modifying its bulk properties is ion-exchange process in aqueous solution with copper (II)acetate. The aim of this work was to optimize the previously published Cu ion-exchange protocol [1] to a bulk silicate- based melt-derived bioactive glass (SBA3), to obtain Cu-doped glass Cu-SBA3. Cu-SBA3 was characterized in terms of its morphology, chemical composition, *in vitro* bioactivity, and the release kinetics of the introduced ionlayer. In addition, the antibacterial performance was evaluated against pathogenic drug-resistant *Staphylococcus aureus* (*S. aureus*) with a method designed to force a separation between adherent biofilm forming bacteria and non-adherent floating planktonic bacteria [2].

Results and Discussion

The optimal concentration for ion-exchange was found to be 0.001M, where the unwanted crystallization phenomenon was minimized, evidenced by field emission scanning electron microscopy (FESEM) (**Figure 1A**), energy dispersion spectrometry (EDS) (**Figure 1B**), and X-ray diffraction (XRD). For the chosen condition *in vitro* bioactivity test was performed both in simulated body fluid (SBF) and in sodium acetate buffer with pH 5to mimic inflammatory conditions. XRD spectra from SBF-soaked samples (**Figure 1C**) confirmed that the introduction of the ion-exchanged Cu-layer does not prevent the nucleation and growth of hydroxyapatite, and thus the bioactivity of the glass. In addition, in order to assess the release kinetics of the Cu-layer, the Cu ion leaching was evaluated in cell culture medium without added serum by ICP-OES analysis (**Figure 1D**). The release of antibacterial Cu-ions was found to be rapid, as the majority of the incorporated ions were released in 24-hour soaking. Finally, the Cu-doped glass was found to elicit antibacterial action towards *S. aureus*. Cu- SBA3 was found to be especially effective against planktonic bacteria thanks to its rapid release of antibacterial Cu-ions in aqueous environment (**Figure 1E**).



At-%	SBA3	Cu-SBA3 0.001M	Cu-SBA3 0.01M	Cu-SBA3 0.05M
Na	34,99	17,77	12,19	0,76
Al	0,27	0,37	0,35	0,6
Si	44,42	52,20	50,76	39,34
P	0,97	1,01	1,20	0,98
Ca	19,35	20,28	15,95	8,63
Cu	-	8,38	19,55	49,67

Figure 1: Characterization of Cu-doped bulk bioactive glass (Cu-SBA3) in terms of A) morphology, B) composition, C) *In vitro* bioactivity, D) release kinetics of the Cu-layer obtained by ion-exchange, and E) its antibacterial performance

Conclusions and/or Outlook

In this work the ion-exchange process to dope bulk bioactive glass surface with copper was evaluated by morphological, compositional, and structural viewpoints. The optimal concentration of the ion-exchange solution was set to 0.001M, which was used for further analyses concerning *in vitro* bioactivity and antibacterial performance. Cu-SBA3 glass was found to be bioactive and antibacterial, and therefore, it has potential to be used to treat early infection due to the rapid release of antibacterial Cu-ions without compromising long-term bioactivity. However, further evaluation of the cytocompatibility of the doped glass is needed to verify its suitability for use in bone infection treatments.

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Cu-doped bioactive glass nanoparticles as icariin carrier: non-convergent osteogenic and osteoimmunogenic performance

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Keywords: Copper; Bioactive glass; Icariin; Osteogenesis; Osteoimmunogenesis.

Introduction

Bioactive glass nanoparticles (BGNPs) are progressively gaining attention in orthopedic and orthodontal applications. These amorphous biomaterials can interact with cells and the surrounding tissue by releasing bioactive ions such as calcium, zinc, magnesium, strontium and copper. BGNPs attach to every soft and hard tissue as soon as body fluids wet the surface and initiate hydroxycarbonate apatite (HCA) formation [1]. Besides,a variety of biomaterials have been developed incorporating BGNPs as the bioactive and reinforcing agent. Among all the elements, Copper (Cu) as osteogenic, angiogenic, and antibacterial element is favourable in bone regeneration and repair [2]. Regarding the very early interaction, it is believed that Cu ions are the main reasonof inflammation due to the increase of serum copper levels in rheumatoid arthritis patients [3]. Moreover, it is well known that immune reactions play a key role in bone regeneration. During the first phase (inflammation phase) of bone healing, innate and adaptive cells including macrophages migrate to the bone defect site and develop an inflammatory environment. The released inflammatory cytokines such as TNF-α, IL-6, IL-8, and IL- 17 recruit and differentiate MSCs to the osteoblasts. In the second phase (repair phase), IL-10 and IL-4 rich anti- inflammatory environment inhibits osteoclastogenesis and leads to higher bone density. Icariin (Ic) as the bioactive flavonoid compound extracted from the chines herb epimedium is an ancient drug known for modulating MSCs osteogenesis, angiogenesis and anti-inflammatory properties [4]. However, the osteogenic properties of different concentrations of this drug have been studied through MSCs-based in vitro models which are not translatable to in vivo models [5]. In this study, mesoporous Cu-doped BGNPs (containing 3 and 5% copper) were synthesized using microemulsion modified sol-gel method. In the following, the mesopores were loaded with icariin to provide a local delivery platform. To see the indirect effect of Cu and icariin on osteoimmunogenic performance, an in-vitro model including THP-1 derived macrophage cell line and hMSCs was used. Cytotoxicity and alkaline phosphatase (ALP) activity of loaded and non-loaded nanoparticles in different concentrations was studied to explain the immunomodulatory effect of this novel biomaterial. Cu-free bioactive glass, Cu ions, Cu complex with icariin and hydroxyapatite (HA) nanoparticles were also studied as the controls.

Results and Discussion

For the whole in-vitro study, the released supernatant of the nanoparticles in osteogenic media (complete mem-alpha containing 1 mg/ml dexamethasone) was collected and used. After indirect incubation of THP-1 derived macrophages, 0.1 mg/ml of loaded and non-loaded nanoparticles was determined as the non-toxic concentration of the nanoparticles. The released icariin also showed a slight improvement in the metabolic activity determined by Alamar blue assay. **Figure 1c,d** shows the metabolic activity of the cells after 72 hours of incubation. To compare the osteogenic and osteoimmunogenic properties of the released ions and drugs, hMSCs were incubated without and with immune stimulation. **Figure 1a,b** illustrates the two mentioned methods. Bare nanoparticles could increase the osteoimmunogenesis (ALP activity) due to the released ions such as Ca and Cu (**Figure 1f).** Adding icariin as the drug, the osteogenic properties of the biomaterial increased. However, the osteoimmunogenesis decreased (**Figure 1g)**. This effect can be explained based on the role of inflammation in bone formation. Metal ions can develop the beginning inflammation phase as it was mentioned before [3]. Nevertheless, Icariin as an anti-inflammatory drug inhibits the inflammation phase which can damp the ALP activity.

Conclusions

Macrophage containing model used in this study showed that in-vitro models based on MSCs are not able to predict the bioactivity of biomaterials used for bone formation. Although icariin is known as an osteogenic drug, the anti-inflammatory properties of this drug could interrupt this property

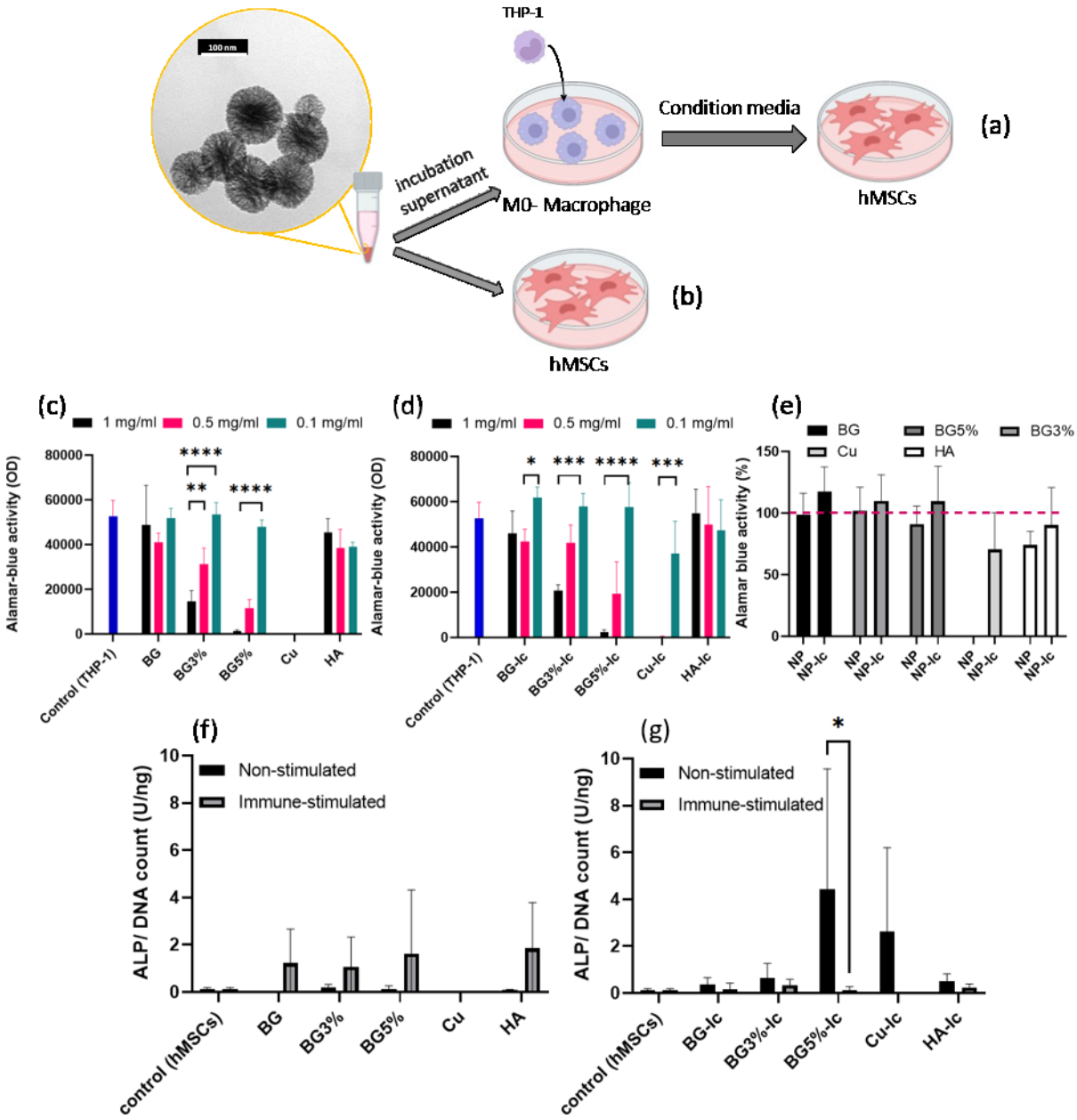

Figure 1: The schematic explaining two approaches of immune stimulated setup (a) and non-stimulated setup used for the ALP study. The metabolic activity of THP-1 derived M0-macrophages after 72 hours of incubation with non-loaded (c) and icariin loaded (d) nanoparticles provided by Alamar blue assay. Effect of icariin on metabolic activity normalized to THP-1 control after indirect incubation with 0.1 mg/ml of nanoparticles (e). ALP activity after 7 days incubation in non-stimulated and immune-stimulated setups for non-loaded (f) and icariin-loaded (g) nanoparticles

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In Vivo Implantation of Aluminum-Containing Silicophosphate Glass

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Keywords: glass, implantation, bioactivity, new bone tissue

Introduction

Prospects for the development of bioactive osteoplastic materials are primarily due to the lack of the required level of reproducibility of the results of osteoplastic operations associated with the use of inert alloplastic grafts for lateral and vertical augmentation of resorbed bone areas.In this regard, of particular interest are studies aimed at studying the regenerative properties of various types of bioglass, which represent a new generation of materials capable of biodegrading and stimulating the growth of their own tissue at the same time [1].

Moreover, according to some researchers, a characteristic feature of this group of synthetic allografts is their potential ability to integrate with both hard and soft tissues without signs of encapsulation and the formation of granulation tissue around the implanted material [2].

The properties of any bioactive glass depend on many factors, among which the main ones are considered to be the chemical composition, the percentage of trace elements, the degree of amorphism and crystallization of the substance. At the same time, the conclusions of most of the studies carried out are based on data obtained using materials related to the Na_2O-CaO-SiO_2-P_2O_5 system [3].

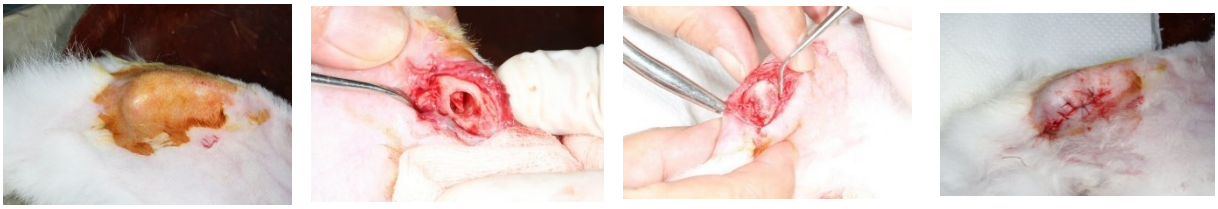
However, at the moment there is a sufficient amount of data indicating the possibility, as well as the need to correct or modify the properties of bioactive glass by introducing various microelements into the main composition. In the future, such studies will help in the development of alternative treatment algorithms depending on the characteristics of the clinical situation [4-7].

The most important requirement for the use of biocompatible glasses in medicine is their biological safety, which is determined by biochemical and toxicological studies in the course of in vivo experiments.

The aim of this study was to assess the effectiveness of the use of a prototype osteoplastic material based on bioglass in an *in vivo* experiment.

In the study was used a sample of C-2 glass composition, obtained by traditional glass technology. C-2 glass composition had the following physical and chemical properties: coefficient of thermal expansion - $80x10^{-7}$ $°C^{-1}$; density - 2.74 g/cm^3; water resistance - 1/98; acid resistance – 1st class; alkali resistance – 1st class. The material was utilised in granular form with a particle size of 1-0.5 mm^3. The biocompatibility of the synthetic allograft was studied in outbred rabbits using a tibia model.This animal had already been under observation for 6 weeks, as it had two implants of a cylindrical shape (by one implant of an equal size (Ø5/6 mm) in the right and left tibia). After the implants were removed, the resulting bone defects were filled with an excess of experimental osteoplastic material, and the wounds were sutured with resorbable sutures without the use of barrier membranes (Fig. 1).

Intravenous anesthesia with 1% sodium ethaminal solution and local infiltration anesthesia with 2% lidocaine hydrochloride solution were used as anesthetics.



a b c d

Figure 1: Stages of osteoplastic surgery: a) preparation of the surgical field; b) defect on the inner surface of the tibia after removal of the cylindrical implant; c) filling the defect with bioactive glass morsels; d) wound closure.

Results and discussion

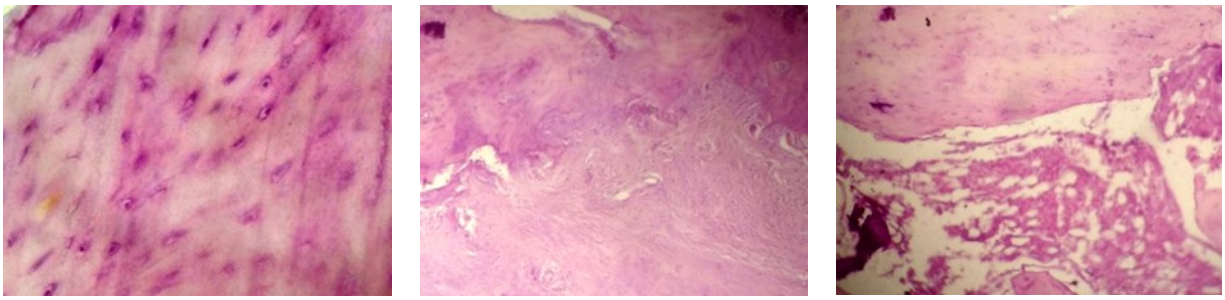
Reentry surgery was performed after 6 weeks using a similar technique. The condition of the skin was preliminarily visually assessed and the absence of hyperemia and swelling was detected, as well as the absence of fistulous tracts was noted. On palpation, a dense formation was determined under the skin, resembling bone tissue in consistency.

After the revision of areas of surgical intervention it was found that the C-2 bioglass composition contributed not only to osteoreparation, but also to a significant volumetric increase in bone tissue (Fig. 2). It was also noted that the newly formed bone tissue resembled a vitreous mass in appearance, which was firmly integrated with the rabbit tibia bones.

a **b** **c**

Figure 2: The result of implantation of C-2glass composition: a) zone of bone tissue growth; b) sampling of a bone block from the newly formed tissue; c) bone sample for histological analysis.

The results of histological studies of the newly formed bone tissue are shown in Figure 3 using several slices for examples.



a **b** **c**

Figure 3: histological slices of a newly formed bone (staining with hematoxylin and eosin).

Figure 1a (1x40) shows a uniform distribution of osteocytes in a separate area of the newly formed bone. At the same time, the formation of a young tissue of a fibrous structure (Fig. 1b, 1x20) is noted, which borders on elements of the bone marrow (Fig. 1c, 1x20), among which weakly calcified bone trabeculae are determined.

Conclusion

Thus, according to the results of a single experiment aimed on studying the biocompatibility of a prototype osteoplastic material based on C-2 bioactive glass, it was found that the allograft obtained by traditional glass technology resorbed in the animal body and contributed to the formation of newly formed strong bone tissue.

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Session 6 – Cranioplasty materials and tumourmodels

Reconstruction of skull defects using different cranioplasty materials

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Keywords: head injury, titanium mesh, cranioplasty, reconstruction

Introduction

Background: The surgical procedure of choice for repairing cranial defects is cranioplasty. This procedure has been practiced for decades and reconstruction techniques are constantly evolving. There are currently a variety of options for filling cranial defects, the most discussed of which is cranioplasty using allogenic materials, more specifically, titanium mesh; which opens the door to the most advanced techniques as well as the best materials on the market. This reconstruction presents few complications, but is closely linked to the doctor's experience in restoring good functionality to the area concerned.

Materials and methods: Cranioplasty by application of titanium mesh helps to correct the disfigurement, protects the intracranial contents from compression or exposure and is at the same time a way of reducing the aesthetic defect. The intervention is based on the principles of repairing the injury as early as possible and in a single stage. We compared three groups of patients according to the materials used for cranioplasty: autologous bone and titanium mesh, PMMA and titanium mesh alone. We analyzed clinical outcome, postoperative complications, aesthetics and 6 months follow up.

Results and Discussion

Although autologous bone grafting is the best therapeutic option from a biological point of view, it has narrow indications and requires a clinically healthy patient with viable donor sites and no more than medium-sized defects. Titanium mesh and autologous bone had the best clinical outcome whereas PMMA was more associated with postsurgical infections and instability of skull reconstruction.

Conclusions and/or Outlook

Progress and results in the field of alloplastic materials used in cranioplasty are still developing and bring significant results in terms of healing and aesthetics, but also on the patient's psyche. Neurosurgeons should be taking into consideration age, associated diseases and different postoperative complications in choosing the best cranioplasty material for each patient.

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Innovative 3D printed scaffolds and implantsfor the cranio-maxillofacial sector: a functionalised and multidisciplinary approach

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Recently, the printing of custom made implants by additive manufacturing has been proposed as a promising alternative to the traditional cranioplastic approach, based on the use of hand malleable pastes to be intra-operatively shaped to fit the patient's cranial defect contours [1]. Indeed, the custom-made strategy presents several advantages, e.g., reduced morbidity, lower infection/inflammation risk, shorter surgery times, higher aesthetical results, less blood loss, better correspondence between the custom made implant and the defect contours [2]. Up to date, no one material has satisfied all the necessary requirements for an optimal cranial implant [3] and all the tested materials lead to several criticisms, i.e. induction of inflammation, lack of osteointegration, low mechanical properties. In this context, the design and printing of graded scaffolds, as well as of implant prototypes, by fused deposition modelling (FDM) technique were performed, using different biopolymers (e.g., polylactid acid (PLA), polymethylmethacrylate (PMMA), polycaprolactone (PCL)), and different pattern depositions [4-6]. Specific solutions to the identified criticisms were proposed, including the functionalization of the produced graded structures with calcium phosphates and suitable antibiotic/anti-inflammatory, to promotethe osteointegration and to avoid the post surgery inflammation occurrence, respectively. The functionalised supports were fully characterised by observation at scanning electron microscopy (SEM), infrared spectroscopy (FTIR/ATR), X-ray diffraction (XRD), differential scanning calorimetry (DSC), tensile and compression tests. and release kinetics tests. The cytotoxicity was investigated by MTT assay, the osteoblastic differentiation capability by *in vitro* cell tests with preosteoblasts.

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3D *in vitro* models of hard and soft tumours

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Keywords: biomaterial, scaffold, cancer cells, 3D models, sinonasal cancer.

Introduction

Cancer tissue engineering is an emerging field of research aimed at generating biomaterial-assisted (three-dimensional) 3D *in vitro* models of tumours. By acting as an artificial extracellular matrix, biomaterial scaffold can mimic a tissue-specific microenvironment, thus allowing cancer cells to regenerate some distinctive traits of the original tumours, which are not obtained in 2D cultures and are often hardly maintained in 3D cellular aggregates, such as spheroids and organoids. Therefore, mastering Biomaterials Engineering could enable the generation of scaffolds tailored to specific cancers, including sarcomas and carcinomas. To this purpose sinonasal cancers, due to their variegated nature, offer a challenging case study. They are rare tumours that account for 3% of all cancers of head and neck district, with prevalence in some geographical areas [e.g., China, Japan, Italy (Tuscany and Eastern Piedmont)], and are associated to specific job categories, like carpentry and tannery. Diverse tissues can be affected, including epithelia, glands, bone, cartilage and olfactory nerve, as the anatomy of nasal cavities and sinuses is very complex. Being rare and diversified in hundreds of subtypes, much is still to be understood to treat those tumors in a personalized manner. The goal of this work is to create a platform of scaffolds able to reproduce 3D models of selected sinonasal cancers, with the future aim to make this platform available for new drug design and personalised screening. Among the different polymers available for biomaterial scaffolding, polyhydroxyalkanoates (PHAs) were chosen as a very versatile class of polymers, according to their physico-mechanical properties [1]. In addition, polyvinyl alcohol (PVA) was investigated due to its higher hydrophilicity with respect to PHAs and its ability to be produced as sponges with very suitable characteristics for nasal tissues [2].

Results and Discussion

We developed different scaffold types, based on biocompatible polymers, i.e., polyhydroxybutyrate (PHB) and polyhydroxybutyrate-co-hydroxyvalerate (PHBV) or PVA, to mimic sinonasal tissues affected by sinonasal cancers, i.e., epithelium (mucous melanoma; MM, and intestinal-type adenocarcinoma; ITAC) and bone (osteosarcoma). By studying quaternary polymer-solvent systems, we obtained and characterized collagen/PHBV (50/50 w/w%) and chitosan/PHBV (5/95 w/w%) electrospun scaffolds to mimic epithelial tissues, and nano-$BaTiO_3$/PHB (5/95 w/w%) 3D printed scaffold for bone tissue. In addition, we developed PVA/gelatine (PVA/G 90/10 and 70/30 w/w%) sponges by emulsion and freeze drying to mimic specifically the mucosal epithelium. The scaffolds were sterilized using absolute ethanol overnight. Cancer cell lines (saos-2 for osteosarcoma, FaDu for epithelial tissue) representative of the selected tumours and their normal counterparts (human mesenchymal stem cells and HaCaT keratinocytes) were cultured on PHB-based scaffolds and the obtained 3D models characterized according to cell viability and morphology via metabolic tests and fluorescence microscopy (**Figure** 1).

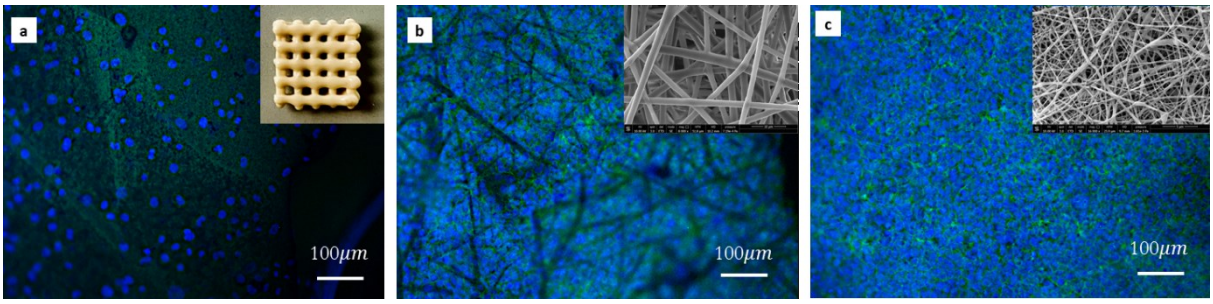

Figure 1: Fluorescence micrographs of tumour cell lines cultured for 1 week on PHB-based scaffolds: (a) Saos-2 cells cultured on 3D printed nano-$BaTiO_3$/PHB (5/95 w/w%); (b, c) Fadu cells cultured on: (b) collagen/PHBV (50/50 w/w%), and (c) chitosan/PHBV (5/95 w/w%) electrospun scaffolds. Lens show images of the different scaffolds.

We also isolated 10 cell lines derived from patients affected by sinonasal tumors, including ITAC, and MM, which

were tested on our PVA/G scaffold platform. The results analysed via histology and metabolic activity tests (AlamarBlue assay), showed that these sponges can replicate the morphologic features of ITAC and MM sinonasal tumors (**Figure** 2). In particular, PVA/G 90/10 appeared to be a promising candidate for ITAC.

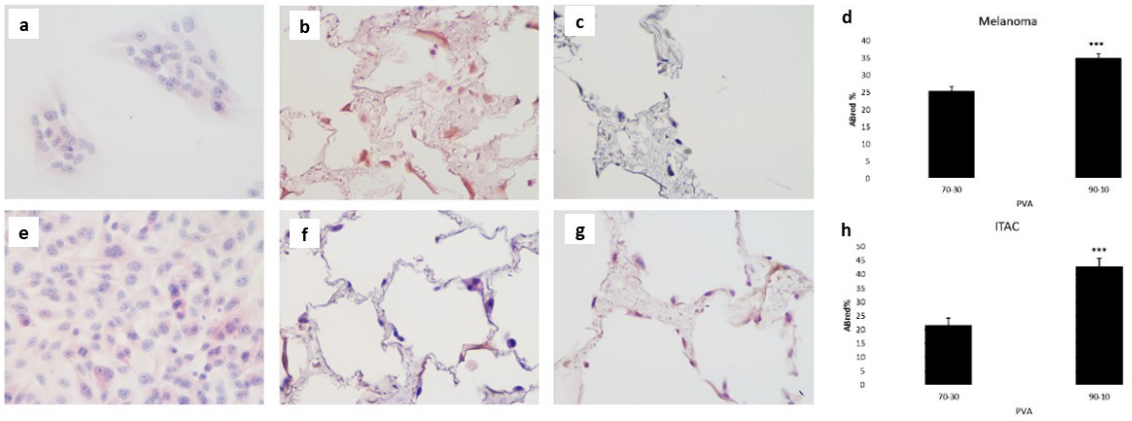

Figure 2: Hematoxylin-eosin staining on: (a) Monolayer cells isolated from mucous melanoma (MM); (b, c) MM cells cultured for 1 week on: PVA/G 70/30, and (c) PVA/G 90/10 w/w% scaffolds. (d) Bar graph showing the results of AlamarBlue test of MM cells in PVA/G 70/30 and PVA/G 90/10 w/w% scaffolds after 1 week. (e) Monolayer cells isolated from intestinal-type adenocarcinoma (ITAC); (f, g) ITAC cells cultured for 1 week on: PVA/G 70/30, and (c) PVA/G 90/10 w/w% scaffolds. (h) Bar graph showing the results of AlamarBlue test of ITAC cells in PVA/G 70/30 and PVA/G 90/10 w/w% scaffolds after 1 week. Magnification 40×; *** $p < 0.0001$.

Conclusions and/or Outlook

By studying the interactions between cancer cells and specifically patient-derived cancer cells with correctly designed scaffolds, it will be possible to replicate *in vitro* 3D tumours useful for improving the understanding on carcinogenesis mechanisms and setting up personalised therapies [3]. Sinonasal cancers offer a variegated set of neoplasms of hard and soft tissues. In our study, we demonstrated the exceptional processability of PHA into ultrafine fibres (useful to mimic the extracellular matrix of connective tissues, thus soft cancers) if blended with a biopolymer (i.e., collagen or chitosan), and 3D printed filament structures, useful to mimic hard tissue cancers using nanoceramic as fillers. Finally, nasal mucosal tissues can be mimicked by other more hydrophilic scaffold types, such as PVA/G sponges.

Acknowledgements

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Multivariate Statistics, Machine Learning and Cancer grading by Raman Spectroscopy

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Keywords: Multivariate Statistics, Machine Learning, Raman Spectroscopy, Cancer grading, Chondrosarcoma.

Introduction

In the last decade, Raman Spectroscopy (RS) has demonstrated to be a label-free and non-destructive optical spectroscopy able to improve diagnostic accuracy in cancer diagnosis [1]. RS exploits inelastic scattering of light with tissues which Raman signals are correlate with the molecular vibrations of biomolecules belonging to tissues under investigation. The positions and relative magnitudes of spectral peaks correspond to the vibrational energies associated with specific chemical bonds. Based on such scattering processes, RS is capable of detecting molecular and/or biochemical changes linked with diseases progression and pathology. This ability is principally based on the great amount of biochemical information dealt out by the Raman scattering with biological tissues under investigation. However, to achieve clinical requirements, the spectroscopic analysis and its ability of grading cancer tissues require sophisticated multivariate statistics.

RS analysis of cancer tissues generate a great number of datasets where the pathologists have to discriminate between tissues, their malignant degree, and between biocomponents inside any tissues, such as cells, extracellular matrix (ECM) and others. This differentiation must rely on RS signals that includes single spectra, figure 1, or images, figure 2. Multivariate analysis based on sophisticated statistical methods is, hence, mandatory to grade the tissues and perform a diagnosis with highest accuracy. Analogously, Machine Learning (ML) is an emerging intersection approach that involves several disciplines, which is good at associating multiple discrete variables and accurately predicting outcomes. ML algorithms and artificial neural networks gain more interest for extracting Raman spectral features and classifying specimen. In this paper, we critically review multivariate statistics methods analyzed at the light of their ability to process datasets generated by RS on chondrogenic tumors where distinguishing between enchondroma and first grade of malignancy is a critical problem for pathologists, and, in addition, we show the classification accuracy obtained by applying machine learning to improve multivariate statistics of RS data when applied to chondrosarcoma, a cartilaginous cancer among most frequent primary bone tumors, and melanoma.

Results and Discussion

RS analysis was carried out on two different cancers, chondrosarcoma and melanoma, with two different and independent Raman imaging microscopes, respectively, i) on chondrosarcoma tissues from Thermo Fisher (DXR2) on 10 patients under clinical treatments from the Azienda Ospedaliera Universtaria Pisa, Pisa University, Italy; ii) on melanoma cells and tissues from Horiba Xplora Plus.

Spectra correspondent to 4 different chondrosarcoma grades for the 10 patients under investigation are showed in **Figure 1**.

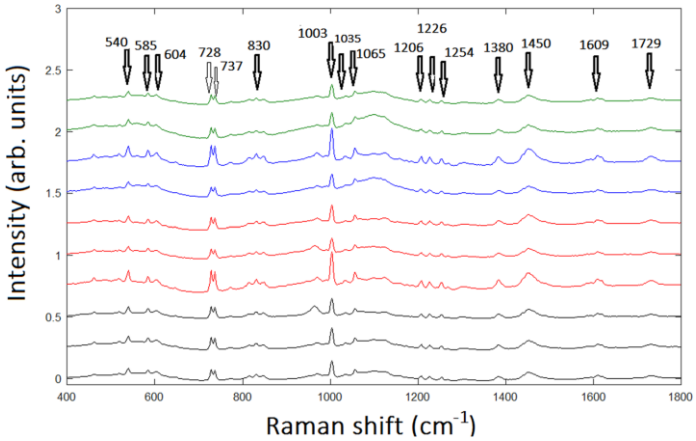

Figure **1**: Averaged Raman spectra of 10 patients under investigation, EC, black line, CS GI, red line, Grade II chondrosarcoma, CS GII, blue line, Grade II chondrosarcoma, CS GIII, green line, respectively. The arrows and the correspondent Raman shift value remark the most significant bands.

Principal Componenta Analysis (PCA) and Linear Discriminant Analysis (LDA) are the first two methods used to differentiate between tissues, and between cells and extracellular matrix (ECM). PCA (unsupervised method) consists in the projection of features into an hyperplane with most of the data variance (approximately 95%) and its orthogonal complement. LDA (supervised method) finds the feature subspace that optimizes the separability of cartilaginous tissues analyzed. When applied to 400-1800cm^{-1} spectra the sequence PCA+LDA accomplishes best diagnosis performances, based exclusively on RS signals, reproducing the diagnosis as made by pathologists [1]. In turn, a question arises from these excellent and encouraging results: by selecting ranges of the spectrum around only significant peaks is it possible to improve these results? We can affirm that by selecting suitable Raman bands the LDA analysis can be improved, as shown in **Figure 2**, where the 4 different grades are well separated.

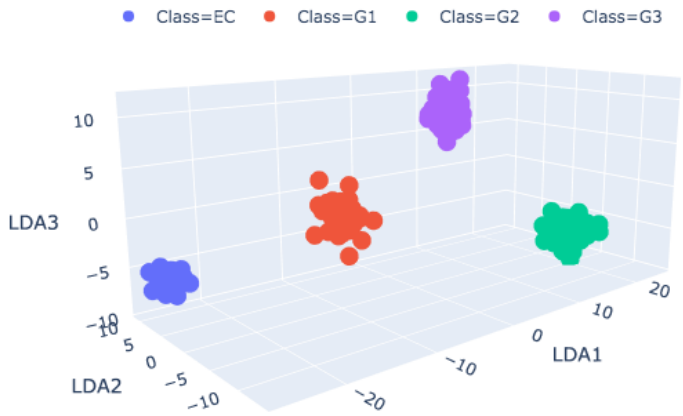

Figure **2**: 3D representation of LDA component for the four CS malignancy groups of Chondrosarcoma. Mentioned from [4] with permission.

We are currently applying multivariate statistical methods improved by ML techniques to melanoma cells and melanoma tissues via Horiba Xplora plus, of which we hope to provide the results, at the moment preliminary, in an accomplished form such as those described relating to chondrosarcoma.

Conclusions and Outlook

Currently, one limitation of our study to chondrosracoma is given by narrow number of patients which frames our study as a pilot study. However, the supervised method based on PCA+LDA algorithms is highly scalable and not only can be applied to more large datasets, but also to any type of cancer tissue. Our interest indeed is to replicate this study for melanoma. Skin cancer is one of the most common cancers in humans and its incidence is increasing dramatically [2-3]. Diagnosing malignant skin tumors is difficult as many common benign skin lesions resemble them visually, e.g., pigmented nevi and seborrheic keratosis. Currently, trained dermatologists clinically diagnose melanoma 63% to 75% correctly. We are confident that we can improve these results using of our multivariate analysis.

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Conflicts of Interest: Authors declare no conflict of interest.

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Ratiometric Sensing Materials for Intracellular and Extracellular pH Mapping at Single Cell Level in *in vitro* Tumour Models

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Keywords: in vitro tumour models; silica microparticles; hydrogels; pH sensing; live cell imaging; cell tracking; automated computational analysis; image segmentation.

Introduction

The tumour microenvironment is characterized by an elevated hydrogen ion concentration, which is the result of increased cellular metabolic demand and altered perfusion, e.g., oxygen availability or acidic metabolic waste products.[1] The acidity of the tumour microenvironment, which is spatially and temporally heterogeneous,[2] affects cancer initiation and progression, but also the efficacy of anti-cancer drugs treatments.[3] Therefore, monitoring the local pH metabolic fluctuations is critical in understanding the basic biology of the tumour, and can also be used as a valid metabolic readout for cancer diagnosis and treatment. Importantly, the complexity of the tumour microenvironment, which includes cell-cell interactions and extracellular matrix composition, coupled with the fast diffusion and mobility of extracellular protons, makes the extracellular pH mapping extremely challenging.[4]

Herein, the synthesis and application of nanostructured micro- and nanomaterials will be described with a special focus on the properties they could offer for sensing appli-cations (**Figure 1**). Examples will be given in which particles-based sensors are applied **(i)** for automated and precise quantification of organelle acidification in single tumour cells,[5] **(ii)** for spatio-temporal mapping the extracellular pH metabolic variations in the local environment of 3D cellularized scaffolds.[6]

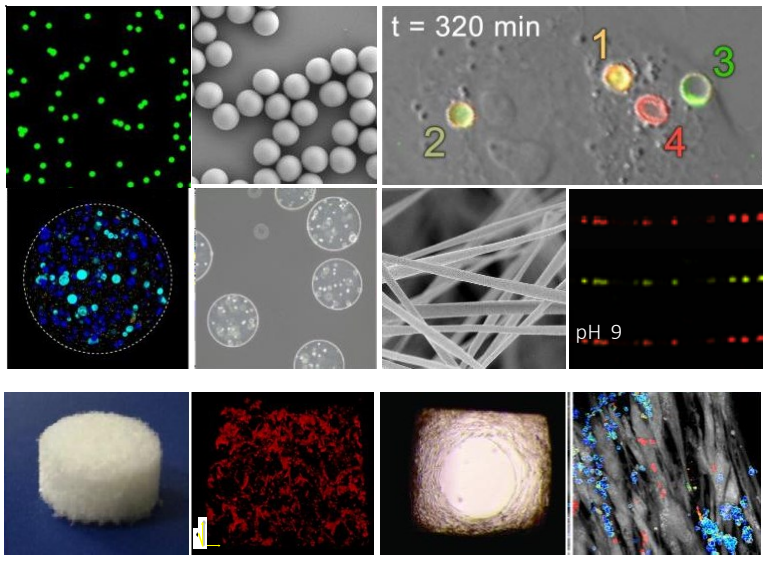

Figure 1: Optical sensing systems for pH sensing in tumor cell cultures, based on either fluorescent ratiometric microparticles, hydrogels, electrospun nanofibers and additive manufactured scaffolds.

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Session 7 – Materials in Cardiovascular Surgery

Biomaterials as Haemostatic Agents in Cardiovascular Surgery: Review of Current Situation and Future Trends

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Abstract

Intraoperative haemostasis is of paramount importance in the practice of cardiovascular surgery. Over the past 70 years, topical haemostatic methods have advanced significantly and today we deal with various haemostatic agents with different properties and different mechanisms of action. The particularity of coagulation mechanisms after extracorporeal circulation, has encouraged the introduction of new types of topic agents to achieve haemostasis, where conventional methods prove their limits. These products have an important role in cardiac, as well as in vascular, surgery, mainly in major vascular procedures, like aortic dissections and aortic aneurysms. This presentations reviews those agents used for topical application and the mechanism of haemostasis and offers general recommendations for their use in the operating room.

Hydrogels as engineerable systems for cardiovascular regenerative medicine

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Heart failure is a major complication for up to one third of those patients that suffer myocardial infarction. Considering that even with optimal modern therapy the annual mortality rate after diagnosis for heart failure is around 10%, it is clear alternative therapies are urgently needed. One such approach is the delivery of injectable hydrogels to the infarcted region of the heart with the aim of biomechanically supporting the heart and limiting infarction generated wall stress that drives pathological remodeling. These hydrogels can also serve as delivery scaffolds for potentially therapeutic cells or as controlled release depots for other bioactive agents. Extracellular matrix (ECM) mimics derived from multi-armed polyethylene glycol (PEG) hydrogels have significant promise in regenerative medicine approaches. We investigate their utility as an injectable myocardial infarction therapy.

The ECM mimics are formed by polymerisation with enzymatically degradable peptides via thiol linkages and cell adhesion peptides can be appended. Thus, cell invasion is dependent on cellular proteolytic activity. Through manipulation of the protease specificity of the crosslinkers and titration with hydrolytically cleavable PEG monomers, both cell specific invasion in vitro and tissue ingrowth in vivo can be regulated. Tissue invasion rate control increases their functionality as controlled release depots for growth factors and cell delivery vehicles.

The ECM mimicry of these hydrogels has been further increased by the covalent linkage of the growth factor binding glycosaminoglycan, heparin. This has been shown to control the release of a range of growth factors and to allow for substantial stimulation of vessel ingrowth into hydrogels releasing VEGF and bFGF.

The PEG ECM mimics have been used to study the influence of hydrogel degradability and timing of injection on therapeutic outcome in a rat myocardial infarction model. Most recently, the heparinised form has been shown to increase the efficacy of stem cell therapy for myocardial infarction.

Most recently, we have begun to explore the utility of fibrin-based hydrogels to deliver RNAi nanoparticle and exosomes.

Design and Material Considerations for Transcatheter Heart Valve Tissue Engineering

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Keywords: Heart valves, Electrospinning, TAVR, Tissue engineering, Scaffolds**Introduction**

Surgical aortic valve replacements (SAVR) offer excellent outcomes but have drawbacks related to their application procedure (need for open-heart surgery) and their properties: mechanical valves are durable but need life-long anticoagulation; bioprosthetic (BP) valves do not require blood thinning but have limited durability. Transcatheter heart valve replacements (TAVRs) obviate the trauma associated with open-heart replacement but still have unknown long-term durability, especially in younger patients, as they contain leaflets similar to those used in BP SAVRs. Much progress has been made on the development of polymeric SAVR and TAVR valves that combine the positive aspects of Mech and BP valves, and these have promise to be the next generation of clinical prostheses [1]. The holy grail of heart valve therapy is, however, replacement of the damaged or diseased structure with living, viable tissue that will have lifelong durability and function, and may have the potential to grow with the patient. This fuels research into the development of tissue engineer (TE) valves [2], and this paper discusses various approaches to the development of TE-TAVR in particular.

Methods

Electrospinning was used to produce leaflet scaffolds from degradable and non-degradable polyurethanes, as well as combinations of the two types. Porosities were manipulated by selection of spinning parameters and by inclusion/extraction of sacrificial co-spun fibres and beads. Drugs were included by coaxial spinning, admixture into the fibres or by surface modification to influence the response towards the scaffolds (subcutaneous implants). Planar and 3D scaffolds were investigated, as well as single and multilayer scaffolds with different orientations. Scaffolds were evaluated for degradability, morphological, mechanical and drug-eluting properties. Balloon-expandable Co-Cr stents with continuous attachment scallops containing suture holes and self-actuating anchoring arms were used (Strait Access Technologies, Cape Town, South Africa). Skirts were electrospun fromnon-degradable polyurethane and several designs attached to the abluminal surface of the stents using heat bonding. Leaflets were punched from spun samples and sutured to stents, after which completed valves were tested for hydrodynamic function (effective orifice area, pressure gradient and leakage fraction) and for durability using accelerated fatigue testing.

Results and Discussion

Tissue ingrowth into the scaffolds was significantly improved by increasing porosity by incorporation and extraction of sacrificial fibres or beads, while vascularisation was significantly increased by incorporation in and release of heparin from the fibres. Valves showed excellent hydrodynamics (within ISO standards) under aortic (non-degradable material) and pulmonary (degradable material) conditions, with non-degradable valves lasting up to 260 million cycles in accelerated testing. Co-spinning degradable fibres with non-degradable materials resulted in reinforcement and strengthening of the scaffolds, while materials of various degradability could be used to control degradation rate. Three-dimensional spinning and multi-layering of leaflets offered additional methods to influence and optimise the valve properties.

Conclusions and Outlook

There are many levels and approaches to the design and materials used for heart valve tissue engineering, ranging from modification of materials for improved biocompatibilty to full remodelling to create living constructs with the potential to grow with the host. The techniques and findings presented in this paper are useful tools towards the attainment of this lofty goal.

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TAVI with three-dimensional multi-layered electro-spun leaflets for tissue engineering application

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Keywords: TAVI, tissue engineering, electrospinning, 3D printing, multi-layer

Introduction

Progressively, transcatheter aortic valve implantation (TAVI) overtakes conventional surgical aortic valve replacement (biological and mechanical valves). Despite the benefits of a far less traumatic procedure and the expendability of anticoagulation, it can be estimated that their life span will not exceed that of biological ones. Tissue engineering (TE) aims to provide solutions through in-vitro or in-vivo remodelling, often using polymeric scaffolds[1]. Thus heart valves developed in this study mimic the natural layers of human heart valves by the use of three-dimensional, triple-layered, electro-spun scaffolds for the use as leaflets that possess a fibrous structure to allow the migration of cells in a TE-TAVI approach.

Methods

Scaffolds were electro-spun using conductive 3D printed PLA targets. Layers were deposited successively in different orientations by manipulating the target orientation and rotational speed. After spinning non-orientated and fully aligned polyurethane samples three-layered scaffolds were produced by electrospinning orthogonally orientated aligned fibre layers sandwiching a non-orientated middle layer[2]. Morphological (SEM, fluorescence microscopy) and mechanical (tensile extension test, suture pull-out test, porosity measurement) assessment was performed. Analysis of SEM micrographs were used to determine fibre diameter, fibre orientation and pore size. Cell culture was performed followed by histological analysis (H.E., DAPI, TE-7, Collagen I).
Three-dimensional cusp-shaped leaflets of similar construction were subsequently made by spinning onto rotating targets containing 3D printed conductive inserts and manipulating spinning parameters.
Valves for TE-TAVI application were made by suturing the planar and three-dimensional leaflets to Cobalt-Chromium (Co-Cr) balloon-expandable stents. The valves were evaluated for hydrodynamic properties and durability using pulse duplication and accelerated fatigue testing, respectively.

Results and Discussion

The average fibre diameter was 4.1 ± 1.6 µm. In circumferential direction fully aligned scaffolds were clearly stronger than random samples (UTS = 12.26 ± 2.59 N/mm^2 vs. UTS = 7.54 ± 1.59 N/mm^2), showing that overall strength in multi-layered scaffolds can be increased when aligning fibres.

Fluorescence microscopy images show the three-layered structure and the histogram of fibre orientations (Figure1). Red and blue fibres create orthogonally orientated outer layers, with randomly distributed green fibres in between.

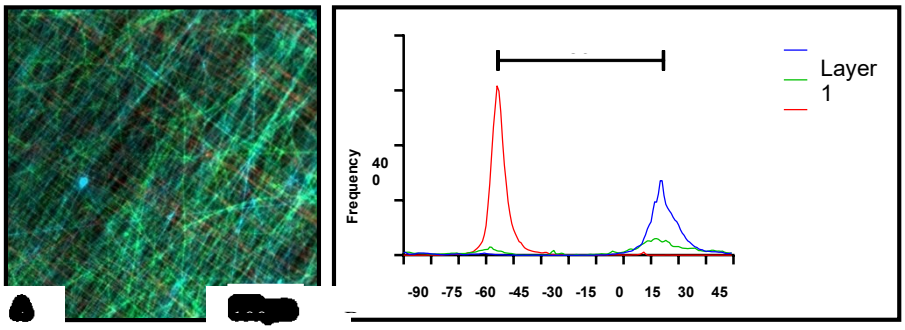

Figure 1: Fluorescence microscopy of triple-layer and orientation analysis[2]

After two weeks of cell culture a confluent layer of fibroblasts covered the scaffolds with collagen deposition on the surface and reaching into the scaffold. Thus the current study aims at producing a TAVI for TE which mimicsthe natural layers of human heart valves by using three-dimensional, triple-layered, electro-spun scaffolds as leaflets.

3D printed leaflet inserts were successfully integrated into the rotating mandrel of the electrospinning rig to spin scaffolds of various orientations and thus offers a working method to fabricate multi-layered, three-dimensional scaffolds and to readily manipulate mechanical strength and shape of resulting specimen.

Prototype valves were successfully manufactured using both planar and three-dimensional leaflets (Figure 2). The performance (effective orifice area, regurgitation fraction, leaflet motion, pressure gradient) as well as the fatigue life of valves currently under production and analysis will be reported.

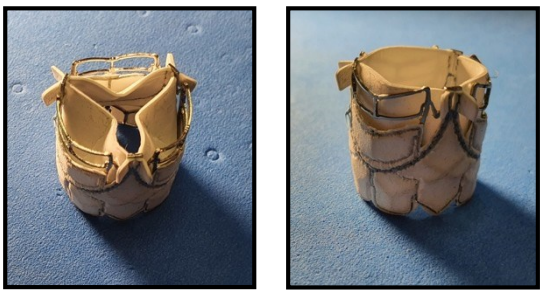

Figure 2: Prototype of single-layered heart valve

Conclusion and Outlook

The combination of 3D printing and electrospinning offers many benefits for the production of custom-made, three-dimensional, multi-layered TE scaffolds by combining this method with suitable biodegradable material, it is useful not only for the development of TE heart valves, but also for other TE applications where complex structures are required.

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One-year performance of an electrospun Coronary Artery Bypass Graft in an ovine model

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Keywords: Restorative, electrospun, coronary artery bypass graft, revascularization

Introduction
Saphenous veins are included in 80% of coronary artery bypass graft (CABG) procedures, despite suboptimal chronic patency, progressive vascular pathology and painful complications associated with vein harvesting. No synthetic coronary artery bypass grafts are currently approved for clinical use. The aim of this study was to assess the safety and performance of a restorative coronary artery bypass graft in a challenging ovine CABG model. Xeltis Restorative Coronary Artery Bypass Graft (XABG), 15cm long, 4mm inner diameter, n = 13) were electrospun from a supramolecular polyurethane with an embedded nitinol microskeleton. One graft per animal was implanted as a bypass graft from the descending aorta to the left anterior descending coronary artery, in an adult sheep model, with a maximal follow-up of 12 months. Autologous saphenous vein grafts (SVG) were used as controls (n = 3).

Results and Discussion
Implantations were successful in 11 of 13 XABG animals. Chronic aggregated patency was 91% (10/11) at 6-months and 73% (8/11) at 12-months. All SVGs (3/3) remained patent until 12-months. After an initial decrease in lumen diameter, the XABG showed a stable lumen size at 3-months and onward, while the SVG progressively dilated with time. At 12-months follow-up the XABG were characterized by a uniform graft diameter and wide-open anastomoses (Fig.1). Mid graft SEM and histology demonstrated progressive graft polymer replacement by recipient tissue and a smooth lumen surface, covered by an endothelial cell-like (vWf positive by immunohistochemistry) lining at mid-graft.

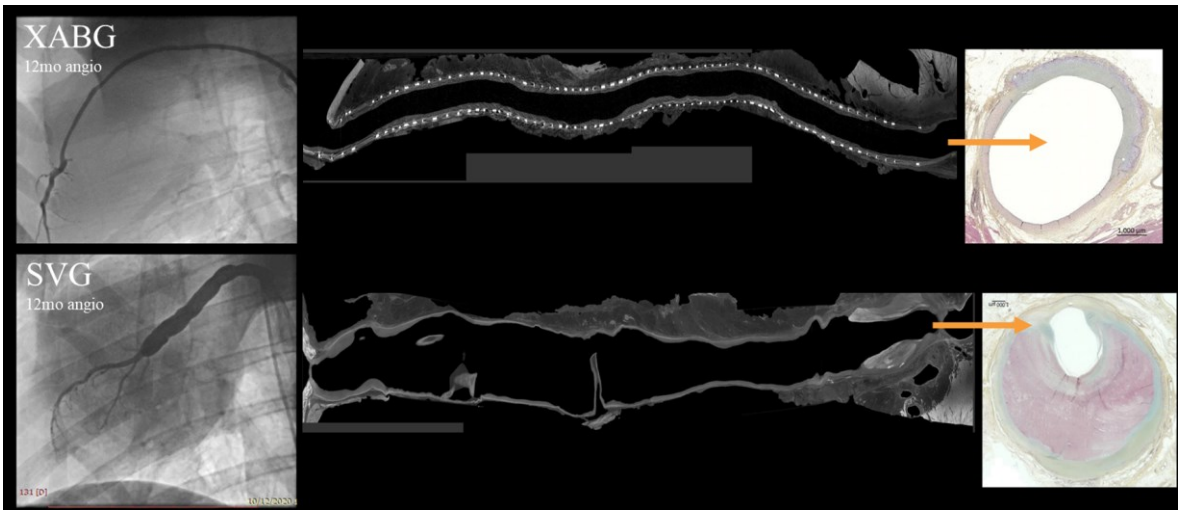

Figure 1: Xeltis Restorative Coronary Artery Bypass Graft (XABG) compared to Saphenous vein graft (SVG) at 12 months. Left: angiogram, Middle: longitudinal section per µCT and Right: histogram of the distal anastomosis.

Conclusions and/or Outlook
XABG patency was demonstrated out to 1 year in a challenging ovine model with observations of advanced healing and remodelling, suggesting adequate preclinical safety and performance of the XABG for further evaluation in clinical coronary revascularization.

Disclosure: Van den Bergh, Cox and El-Kurdi are employed by Xeltis. Prof. Serruys and Dr. Virmani are paid advisors to Xeltis.

Session 8 – Biofabrication

Advances in Natural-based Biomaterials and Bioinks for Biofabrication of Complex Tissues

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Keywords: Bioinks, Bioprinting, Complex tissues, Natural-based hydrogels, Tissue engineering

Introduction

We are now assisting to a paradigm-shift in medicine which involves the end of universal therapy and its substitution by customized therapy. The 3D bioprinting enables the recapitulation of important tissue features with precision, namely, biological components and mechanical cues, reproducibility and conformation. Despite the promise of 3D bioprinting, to create proper 3D bioprinted complex tissues, it is crucial to select the most suitable biomaterial. Hydrogels have been already extensively studied in the tissue engineering (TE) since they promote a 3D flexible environment with a high-water content that emulates the natural cells' surrounded environment. Hydrogels also hold the particularity of being stress, T, pH, and light responsive, which enlarge their application for engineering different tissues. Typically, the composition of hydrogels varies from natural and synthetic or formulation of different types of polymeric matrices. Natural-based hydrogels such as silk fibroin (SF) and gellan gum (GG) have been gaining a great deal of attention since they present key properties as shear thinning ability and adequate viscosity that allow its injectability, which is very attractive for 3D bioprinting techniques. Importantly, the intrinsic biochemical and mechanical characteristics of the hydrogels can also influence the cells functions. The recent reports dealing with the application of biomaterials/bioinks and processing methods for biofabrication of complex tissues are discussed herein. An overview of the several types of natural-based bioinks developed at the 3B's Research Group (University of Minho) and applied to TE of meniscus and intervertebral disc is presented.

Results and discussion

Among the gelation processes for obtaining natural-based bioinks, enzyme-mediated *in situ* hydrogelation reactions are appealing due of their specificity, efficiency, and non-cytotoxic nature. Different studies, including our work, have shown that polymers or proteins containing tyrosine groups can be used to prepare these *in situ* forming hydrogels mediated by the horseradish peroxidase (HRP) and hydrogen peroxide (H_2O_2) complex. Interestingly, we have demonstrated for the first time the possibility of applying HRP-crosslinked SF as fast-setting bioinks for 3D printing [1]. The SF bioinks have shown unique biocompatible, elastic and adhesion properties capable of being printed in an amorphous state. Although the formed structures have shown excellent mechanical properties and memory-shape features after processing, some stability issues can be found during the printing process. Hence, the possibility of controlling the conformational (random coil to beta-sheet, *i.e.* from amorphous to crystalline) transitions in the HRP-crosslinked SF hydrogels, and improve their structural stability as bioinks and injectable matrices, will make these system fundamental for the translational research and creation of mimetic complex tissues. The gelation of SF by means of combining different methods as heating, photo-crosslinking, or enzymatically crosslinking has been also reported by Costa *et al.* [2]. In that study, an hybrid approach has been innovatively explored and opened up new possibilities for the simultaneous printing of two different bioinks, *i.e.* one SF-based and a second one based on gellan gum, when complex tissue biofabrication is envisioned.

Conclusions and/or Outlook

The use of bioprinting techniques in tissue engineering and medicine has been providing diverse breakthroughs, with incomparable advantages over biofabrication methods. The natural-based bioinks and hybrid strategies developed at 3B's Research Group showed the promise of the new generation of bioinks for bioprinting of complex tissues, in particular for engineering fibrocartilaginous tissues.

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Mechanically-instructive Scaffolds to Steer Tissue Regeneration: Merging Mechaniobiology with Biofabrication

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Keywords: Additive manufacturing; biofabrication; 3D scaffolds; stem cells

Organs are complex systems, comprised of different tissues, proteins, and cells, which communicate to orchestrate a myriad of functions in our bodies. Technologies are needed to replicate these structures towards the development of new therapies for tissue and organ repair, as well as for 3D in vitro models to better understand the morphogenetic biological processes that drive organogenesis. To construct tissues and organs, biofabrication strategies are being developed to impart spatiotemporal control over cell-cell and cell-extracellular matrix communication, often through control over cell and material deposition and placement. To achieve these targets, the spatiotemporal control over biological signals at the interface between cells and materials is often aimed for. Alternatively, biological activity can be triggered through the control of mechanical cues, harnessing more fundamental know-how in mechanobiology that could be combined with biofabrication strategies. Here, I present some of our most recent advancements in merging mechanobiology with biofabrication that enabled the control of cell activity, moving towards enhanced tissue regeneration as well as the possibility to create more complex 3D in vitro models to study biological processes.

Biomolecular organization and cell behaviour at nanostructured surfaces

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Keywords: proteins, peptides, immobilization, molecular orientation, collective behaviour

In recent years, the role of nano-features at surfaces has been thoroughly investigated with respect to their effect on the amount and structural state of immobilized biomacromolecules as well as the related cell "lodging". In fact, well-established experimental evidence has unequivocally shown that the specific features induced by the interaction of the adsorbed layers with the substrate determines the event cascade leading to cell seeding, spreading and differentiation on suitably functionalized scaffolds, with obvious implications for tissue engineering strategies.
More in particular, the nanoscale bio-patterning of biomaterial surfaces has been shown to imply not only the success of early cell adhesion and spreading, but also of the late events as cytoskeletal organization and cell differentiation, overall suggesting a close relationship between the surface nanostructures and the nanosized "biomolecular" machinery that allows cells to respond to surficial and extracellular stimuli.

In this framework, the present lecture will discuss recent experimental results, based on techniquesas quartz crystal microbalance with dissipation monitoring, nanoplasmonic sensing, time-of-flight secondary ion mass spectrometry, molecular dynamics simulations, etc…, stressing the importance of driving both the adsorption selectivity as well as a proper exposure of the bioactive sites of adsorbed biomolecules (peptides and proteins). Examples will be shown of targeted chelation-basedmethodologies to anchoring well-defined amounts of oriented biomolecules [1,2], of counter-intuitive size-related protein trapping selectivity of nanowells [3], of a curvature-related "geometrical" resonance of nanostructure and biomolecules size determining their adsorption [4], in view of the related cell behavior [5-8].

It is to note that, overall, the reported experiments rely on a kind of "single-molecule-limited" view of the functionalization of the surfaces, which, owing to the influence of the pervasive use of self-assembled monolayer-based strategies of functionalization, are often considered as mere assemblies of single-responding functional molecules. However, an emerging view may be conceived to consider the structure and functions of the adsorbed layers as governed by the Inter-molecular systems interactions. In this perspective, the collective character of the response of peptide systems will be reported, suggesting that the biological response may be strongly affected by them [9, 10].

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Skeletal muscle tissue restoration using functionalized biomaterials

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Keywords: Skeletal Muscle, Myogenesis, Biomaterials, Tissue engineering, Regenerative Medicine

Introduction

At present, skeletal muscle (SKM) loss is considered to be largely irreversible, and therapeutic options to restore muscle mass have been limited. A muscle defect results in functional impairment caused by the limited regenerative ability and fall short of surgical operations. Patients with SKM tissue wasting and loss of strength experience a condition that severely influences their quality of life and survival with significant long-term disfigurement and disability. SKM has good regenerative capacity due to the presence of resident stem cells, the satellite cells, located between the cell membrane and the basal lamina; however, *in vitro* studies demonstrated their poor ability to migrate and to self-renewal, which means: their intrinsic proliferative capacity is lost in normal 2D cell culture. New strategies are required to overcome these limitations; recently, various *in vitro* studies have focused on recreating the niche-like environment by combining biomaterials (tissue engineering) and cells (cell-based therapy). In particular, tissue engineering (TE) has been recognized as a promising tool and a new therapeutic approach for the renewal of SKM tissue *in vitro*. In this work, we examined the role of a poly-(L-lactic acid)-co-poly-(ε-caprolactone) electrospun nanofibrous scaffold coated with type I collagen (PLCL es-scaffold) as support for SKM reconstruction. Our present study gained a greater understanding of the myogenic process and clarified the external environment's repercussions on myoblast behaviour.

Results and Discussion

The designed nanostructure of the PLCL es-scaffold, which mimics the physical functions of the native SKM microenvironment, provided linear attachment points for cell adhesion, orientation, and elongation, influencing myoblasts morphology and activities. In general, we recognized that the timeline of myogenesis differs significantly between the C2C12 cultivated on the PLCL es-scaffold and 2D control; we observed undifferentiated C2C12 only 6 hours after seeding, while at T0, in 24 hours, cells have already elongated, developed cell-cell adhesions, and began to be fused to form multinucleated myotubes. This observation performed with SEM analysis showed that PLCL es-scaffold guided C2C12 morphogenesis and strengthened the idea of how this new approach of *in vitro* cell culture positively alters C2C12 behavior and fate. With the notion that our designed scaffold positively impacts on myoblast lineage, we monitored the expression of fundamental myogenic regulatory markers (MRFs) which showed how PLCL es-scaffold contributes to an early myoblast differentiation. In addition, we also detected a steady staining for Myosin Heavy Chain (MyHC-1), muscle-specific protein, in aligned myotubes on PLCL es-scaffold throughout the differentiation, proving that myoblasts benefit once again from the presence of the tissue-like support (**Figure 1**). Collectively, the designed PLCL es-scaffold acted as a carrier for cell vitality, proliferation, and myogenic differentiation during the early (T1), intermediate (T5) and late (T7;T10) stages of myogenesis accelerating SKM tissue formation.

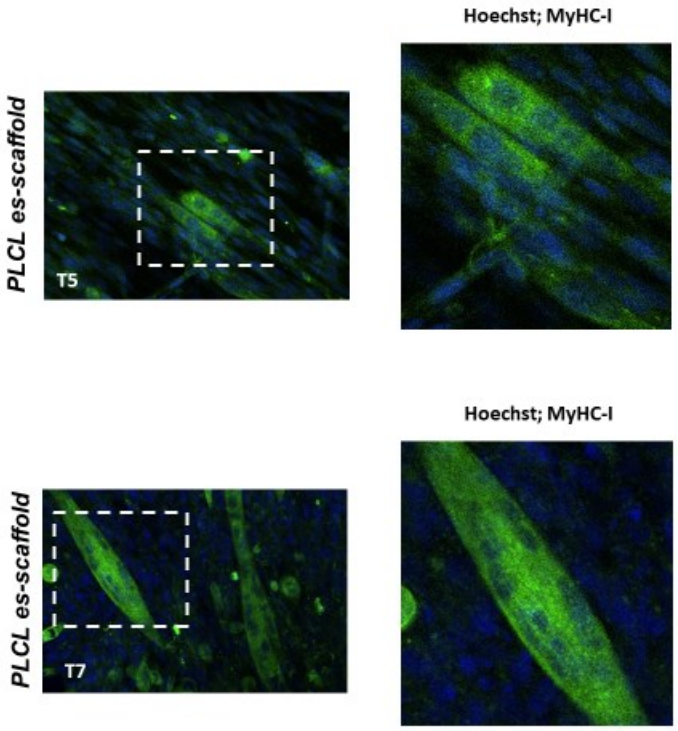

Figure 1: Myosin Heavy Chain expression towards C2C12 myogenesis in PLCL es-scaffold at T5 and T7

Conclusions and Outlook

Our results have shown the advantages obtained by culturing C2C12 on the PLCL es-scaffold, which provided a more suitable microenvironment for constructing functional SKM *in vitro*. Overall, we have clarified how the PLCL es-scaffold plays a key role during C2C12 myogenesis by promoting the development of cellular interactions and cellular adhesions with the activation of multiple signaling pathways fundamental at this stage. Myogenesis was affected by the presence of the PLCL es-scaffold, revealing significantly improved myogenic differentiation of C2C12 compared to the 2D control. In conclusion, we assumed that aligned and elastic nanofibers facilitate C2C12 organization, resulting in early myoblast differentiation by enhancing myocyte differentiation into maintenance myotubes. Hence, tissue engineering aimed at SKM tissue restoration acts as a booster for myoblast differentiation and tissue formation overcoming the conventional 2D tissue culture technique, the versatility of the system will be usued to create *in vitro* SKM disease models.

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A sound assembly platform to control tissue organization

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Keywords: sound assembly, morphogenesis, biofabrication, organoids, multicellular living systems.

Morphogenesis, a complex process, ubiquitous in developmental biology, tissue engineering, and many pathologies, is based on self-patterning of ensembles of cells. Such ensembles are gently orchestrated and perfectly arranged through chemical gradients, structural anisotropies, and hierarchical compositions.

Conventional tissue engineering approaches involve the development of physiologically relevant living microenvironments, by combining materials, cells, and biochemical factors to direct the generation of functional tissues. However, over the last few years, the application of remote fields, of extrinsic forces, is opening to exciting new perspectives to better control and reproduce the structural complexity of tissue organization toward the *in vitro* engineering of clinically relevant constructs.

Spatial patterns of cells, organoids, or inorganic particles can be forced on demand using acoustic standing waves, such as the Faraday waves. This technology allows tuning of parameters (sound frequency, amplitude, chamber shape) under contactless, fast, and mild culture conditions, for morphologically relevant tissue generation. We call this method Sound Induced Morphogenesis (SIM)[1,2]. Here we use SIM to achieve tight control over patterning of cells and spheroids densities within a hydrogel, enhancing tight cell-cell communication, with the endpoint formation of functional tissues.

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Tendon Biomimetic PLGA 3D Scaffold Enhances Amniotic Epithelial Stem Cells Biological Capability for Tissue Engineering Applications

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Keywords: tendon; stem cells; tissue engineering; scaffold; mechanotransduction.

Introduction

Tendon tissue engineering (TE) represents a promising treatment option for tendinopathies, which to date do not have a resolutive conventional solution. TE aims at creating successful implantable 3D biomimetic scaffolds with the physical, mechanical, biological, and functional properties of the natural tissue. These constructs can be engineered with stem cells to enhance their teno-differentiative properties [1–3]. In this context, amniotic epithelial stem cells (AECs), collected from the placenta, represent an attractive stem cell source due to their low immunogenicity, high immunomodulatory properties and tenogenic differentiative ability, as demonstrated through *in vitro* [4] and *in vivo* [5] studies, in which they were able to accelerate tendon regeneration. However, in the case of tendon ruptures, in order to rebuild the damaged tissue, it is necessary to engineer these cells within a scaffold.

Thus, the focus of this research was to develop bundle tendon-like PLGA 3D scaffolds resembling tendon macro and microarchitecture and biomechanics, and to evaluate their direct impact on AECs' biological potential in terms of teno-differentiation. Moreover, to acquire more insights into the biological results, the present research aimed to investigate the possible involvement of Yes-associated protein (YAP) activation, which represents a mechanotransducer implicated in the regulation of stem cell fate [6], and in particular in their tenogenic differentiation [7]. Finally, to simulate the *in vivo* microenvironment, in this study the AECs engineered scaffolds were tested, by using the *in vitro* co-culture system, for their teno-inductive potential on co-cultured cells.

Methods

PLGA fleeces with highly aligned fibres were produced via an electrospinning process through a rotatory collector. The obtained fleeces were then hand bundled to produce 3D tendon-like scaffolds, which were characterized *in vitro* in terms of ultrastructure (SEM analysis). Then, the constructs were tested for their teno-inductive influence on AECs. In detail, cells engineered on PLGA fleeces and 3D scaffolds were compared with respect to cells cultured on Petri dishes (CTR) through a confocal microscope after 48 h of culture for their morphological changes and for the cytoplasmatic expression of TNMD, a mature tendon protein, by using immunofluorescence assay (IF). In addition, AECs teno-differentiation on scaffolds has been also assessed by analysing the gene expression of tendon related markers (early: *SCX* and late: *COL1* and *TNMD*) by means of real time-PCR.

Moreover, the present research evaluated YAP protein activation in the seeded cells through an IF by assessing its localization within the cells' nuclei.

Finally, the AECs engineered fleeces and 3D scaffolds were co-cultured with AECs for 14 d to evaluate, through a morphological investigation and TNMD protein western blot quantification, if the differentiated cells engineered on scaffolds exerted a paracrine action on the co-cultured AECs.

Results and Discussion

The produced PLGA 3D scaffolds closely resemble tendon architecture, both macroscopically and microscopically, in fact, analysed through scanning electron microscopy, these showed high fibre alignment.

AECs seeded on the constructs exhibited an elongated tenocyte-like morphology already after 48 h of culture, with a lower aspect ratio with respect to CTR, which retained their characteristic polygonal morphology. The engineered AECs' phenotypic change was also confirmed by visualizing the cytoplasmic expression of TNMD protein and supported by tendon-related genes (*SCX*, *COL1*, and *TNMD*) significant upregulation already after 48 h of culture respect to CTR cells ($p<0.05$), which showed no TNMD protein expression or significant increase in tendon-related genes. Interestingly, AECs cultured on 3D scaffolds showed a more elongated phenotype and a significant upregulation of all the analysed genes with respect to cells seeded on fleeces ($p<0.05$), thus standing 3D scaffolds as greater teno-inductive stimuli for AECs.

Moreover, the highly aligned fibres of both fleeces and 3D scaffolds stimulated AECs in terms of cell cytoskeleton stress through the mechanosensitive YAP pathway. Indeed, the constructs significantly induced YAP nuclear

translocation, even if 3D scaffolds significantly exhibited a higher impact on YAP activation respect to fleeces (p<0.05), and CTR (p<0.05), in which YAP was exclusively localized in the cytoplasm.

The *in vitro* co-culture system allowed to verify that the microenvironment established by the engineered constructs with AECs were able to teno-differentiate the co-cultured cells through a paracrine stimulus. Indeed, by co-culturing the AECs engineered fleeces and 3D scaffolds with AECs, these last differentiated into tenocyte-like cells and also produced tendon-like structures that were not found in CTR. The co-cultured AECs' phenotypic change was also confirmed by quantifying the expression of TNMD through western blot analysis, showing a significantly higher protein expression in the teno-differentiated cells co-cultured with fleeces and even significantly greater with 3D scaffolds with respect to the CTR (p<0.05). This result is probably the effect of stimulating factors released by the teno-differentiated cells contained within the scaffolds' fibres.

Conclusion

Overall, these findings corroborate the biomimicry of 3D scaffolds in terms of macro and microstructure, as well as their significant teno-inductive potential on AECs. This boosted effect could be probably associated to YAP activation which recently raised to be related to the alignment of the scaffolds' fibres, which act through a mechanosensing stimulus on the AECs aligned along the construct [8]. Indeed, these latter not only differentiated into a tenogenic lineage, but also stimulated indirectly the surrounding cells to teno-differentiate even if not in contact with the scaffolds, potentially mimicking the *in vivo* microenvironment in which progenitor/stem cells should be stimulated to differentiate.

Thus, biomimetic PLGA 3D scaffolds stand as potential candidates for tendon regeneration.

Acknowledgments

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Effects of Snail Mucus on Porous 3D Agarose Scaffold for Tissue Engineering Applications

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Keywords: Snail mucus, chondrocytes, Biocompatibility, 3D Scaffold, Tissue engineering

Introduction

Natural and synthetic polymers have been widely reported for their use in tissue engineering and regenerative medicine.[1] From this group of polymers, the natural carbohydrate polymers have been proven to be essential for tissue engineering applications due to their nontoxic and biocompatible nature.[2] Perhaps, this explains why carbohydrate polymers are recently being explored for applications in the biomedical industry.[3] Some carbohydrate polymers that have been explored for biomedical applications include but are not limited to chitosan and alginate.[3–5] Agarose is another carbohydrate polymer reported for excellent mechanical properties and explored for tissue engineering applications.[6,7] It can form a hybrid or composite with other materials such as fibrin, collagen, and silk,[8,9] the results of which have been used for applications in tissue engineering and regenerative medicine involving cartilage regeneration, bone regeneration, angiogenesis, spermatogenesis, artificial pancreas, and wound healing. The mechanical property of agarose and its high water absorption capacity gives this natural polymer the ability to behave similarly to the natural extracellular matrix (ECM), and this helps in cell differentiation and proliferation. Despite the excellent properties of agarose, it lacks the ability to support cell adhesion without chemical modification of the polymer.[10] The cell adhesion property of agarose may also be improved by modifying the stiffness of the polymer through the formation of a hybrid or blend with other polymeric materials.[11] On the other hand, snail mucus or snail mucin is a complex substance that has been identified to contain glycosaminoglycans (GaGs), allantoin, hyaluronic acid, elastin, and glycolic acid.[12–14] These constituents, especially GaGs, reduce cartilage deterioration and inflammation. It has been reported for its antibacterial property due to the presence of achacin and has recently gained researchers' attention for biomedical applications. Specifically, snail mucus has been reported for wound healing, anti-inflammatory, and anti-aging properties.[15] Some of the composition of the snail mucus, especially, GaGs, mimics the ECM of cartilage; however, this biomaterial has not been fully explored for tissue engineering applications. This study aims to improve the bioactive properties of agarose by preparing agarose-snail mucus blends or composites to study the effects of snail mucus on the properties of agarose polymer. The agarose-snail mucus composites were prepared as a neat polymer 0% v/v, agarose blend with snail mucus 25% v/v of agarose, agarose blend with snail mucus 50% v/v of agarose, and agarose blend with snail mucus 75% v/v of agarose. The scaffolds were prepared using the freeze-drying method, followed by characterization and *in-vitro* studies.

Results and Discussion

Cylindrically shaped hydrogels were formed, transitioning from transparent agarose hydrogels to varying degrees of translucent hydrogels depending on the concentrations of snail mucus. This suggests a form of interaction between agarose polymer and snail mucus, which did not hamper agarose self-gelation or crosslinking properties. The FTIR data indicates the functional groups in the resulting composites. Specifically, peaks corresponding to -OH, N-H, C-H, C=O, and C-O were observed. The SEM of the scaffolds showed microporous structures for all the scaffolds but with varying degrees of porosity. It was, however, observed that the composite with 50% v/v had a more interconnected porous morphology. A compressive modulus test was performed to determine the mechanical strength of the scaffolds, and the results obtained indicate that the compressive strength of the scaffold is highest in 50% v/v with respect to 0% v/v and the other respective composite scaffolds. An MTT assay performed on the scaffolds showed that all the scaffolds are biocompatible and nontoxic to the human chondrocyte cell line used for the study as cell viability was observed to be higher than 100% throughout the 48 hrs study, suggesting that the scaffolds promote cell growth. This property is crucial for any material to be considered for use in tissue engineering applications.[16] Furthermore, an SEM of cells seeded on the scaffold showed that the cells were able to attach to the scaffolds much more than the cells on neat polymer. This suggests an improved capacity of agarose for cell attachment. This could be due to the bioactive nature of snail mucus which contains proteins and other constituents that may have contributed to the observed property.

Conclusion

In conclusion, our findings showed that snail mucus significantly increased the mechanical property of agarose and, at the same time, improved the bioactive property of the polymer, favouring cell attachment. Hence, snail mucus is a promising material with prospects for soft tissue engineering applications.

Acknowledgment
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Bioactive PEEK for Bone Tissue Engineering

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Keywords: Polyetheretherketon, Bone tissue engineering, Bioactive peptides, Osteoblasts, Covalent grafting.

Introduction

The clinical need for biomaterial therapies for bone is unrelenting. Polyetheretherketone (PEEK) is an ideal substitute material for bone tissue engineering. PEEK is a semi-crystalline polyaromatic linear polymer with $(–C_6H_4–O–C_6H_4–O–C_6H_4–CO–)_n$ chemical formula. It is finding popularity in bone replacement as it has been shown to have bio-mechanical properties close to human bones. It represents a promising polymeric alternative to metal implant components thanks to its excellent property combinations, of strength, stiffness, toughness, and environmental resistance. The Young's modulus of PEEK is much lower than any metallic material employed in common bone-implant and is tuneable through additive inclusion. It may increase up to 18 GPa, which is similar to the values of the cortical bone (18.4–20.7 GPa)[1], avoiding the effect of the stress shielding. PEEK is a thermoplastic polymer and in the range of its melting temperature, it can be processed using 3D-printing. The possibility to use PEEK polymer in the 3D printing process has increased scientific interest and impact future development [2].Furthermore, PEEK has interesting biological properties: it is highly resistant to thermal degradation, to the attack by organic and aqueous environments, and to biodegradation. Therefore, PEEK implants are biocompatible and biologically inert[3]. However, its inertness has constrained its potential applications. PEEK has a hydrophobic surface, which neither allows protein absorption nor promotes cell adhesion[4]. The aim of this research project is improving the biological properties. PEEK disk surface was functionalized with bioactive peptides a Vitronectin-derived sequence as adhesive peptide (HVP) and a self-assembling peptide (SAP) known to influence human osteoblast metabolic activity. Covalent and selective bonds were realized by reaction between amino-oxy groups inserted in the peptide N-terminus and the ketone groups of the PEEK surface. Preliminary biological tests have been carried out in order to evaluate: the best functionalization condition that maximizes human osteoblast adhesion, and improvements in adhesion, proliferation, and gene expression of Runt-related transcription factor 2 (RUNX2), Vitronectin (VTN), and Osteonectin (SPARC) of human osteoblasts on PEEK disks in the presence of either HVP or SAP.

Results and Discussion

Viability and metabolic activity of human osteoblasts in the presence of the two peptides was assessed with alamarBlue in order to optimize the best condition to maximize the yield of functionalization. Human osteoblast adhesion was evaluated on PEEK disks functionalized with HVP peptide at three different concentrations: 10^{-5}M, 10^{-4}M, and 10^{-3}M. As seen in **Figure 1**, all treated and untreated scaffolds significantly promoted cell proliferation. From day 3 to day 12 all the samples showed an increased cell activity. In particular, 10^{-4}M and 10^{-5}M test samples showed an increase in cell proliferation activity compared to 10^{-3}M and untreated. Overall, 10^{-4}M obtained the highest cell proliferation compared to the other groups, indicating that 10^{-4}M is having a greater stimulatory effect on osteoblast cells.

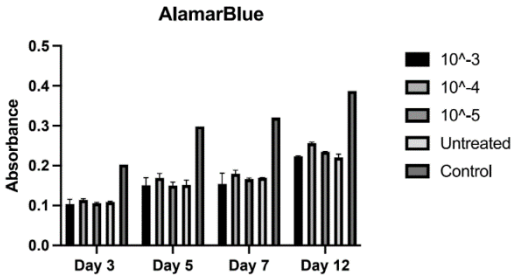

Figure 1: AlamarBlue assay at four time points on PEEK disks functionalized at three different peptide concentration (10^{-3}M, 10^{-4}M, 10^{-5}M), untreated PEEK disks and positive control

Further biological tests were performed at one time to further validate if the treatment of the PEEK surface with bioactive peptide would enhance the human osteoblasts activity. PEEK disks were functionalized with either 10^{-3}M

HVP solution or 10^{-3}M EAK solution. Both conditions showed significant improvements in adhesion after 2 hours (**Figure 2a**) and proliferation after 48 hours (**Figure 2b**), assessed through MTT assay and CFSE probe respectively.

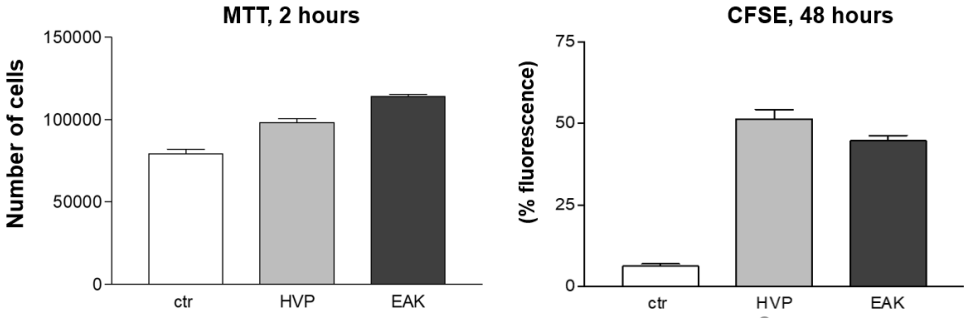

Figure 2: MTT assay on PEEK disks untreated and functionalized with HVP and EAK peptide at 2 hours (a); proliferation assay on PEEK disks untreated and functionalized with HVP and EAK peptide after 48 hours (b)

Regarding the gene expression (**Figure 3**), disks functionalized with EAK induced a significant increase in RUNX2 and SPARC; while the addition of HVP on the PEEK surface induced a significant increase in the VTN.

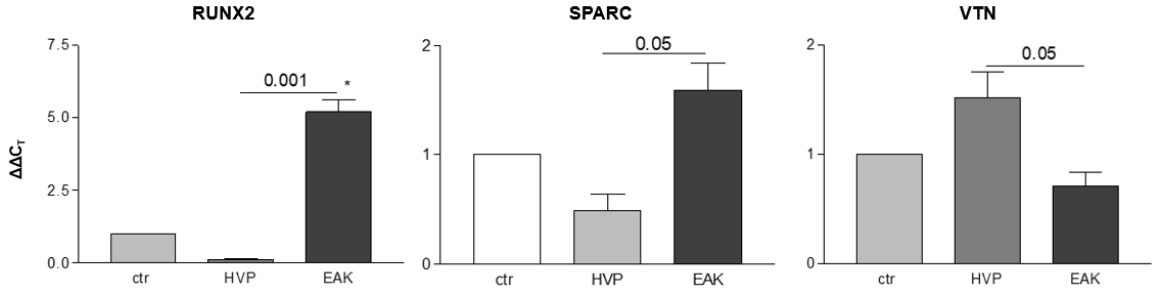

Figure 3: Assay on PEEK disks untreated and functionalized with HVP and EAK peptide evaluating gene expression of RUNX2 (a), SPARC (b), and VTN (c)

Conclusions

Despite their mechanical properties, PEEK implants are unable to promote cell adhesion, inducing a slow bone recovery from surgery. Our studies have shown that the biological properties of PEEK can be enhanced and this combined with its other properties, makes PEEK a favourable material for applications in bone tissue eningeering. PEEK disks functionalized with either HVP or EAK have both demonstrated an enhancement in biological activity and cell response as confirmed by adhesion, proliferation, and gene expression. Further tests are ongoing to exploit these properties e.g. peptides, such as a dimeric retro-inverted HVP analog as adhesive motif able to resist to protease-induced degradation, a peptide mapped on BMP-2 as differentiation molecule, are currently under more in depth, further biological analysis are ongoing to determine long term activity. In addition, thermoplastic properties of PEEK will be exploited through 3D-printing to modify the superficial pattern of PEEK scaffolds in order to improve human osteoblast interaction.

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Three-Dimensional Tendon Biomimetic Scaffold Exerts a Boosted Immune Inductive Effect on Amniotic Epithelial Stem Cells

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Keywords: Immunoengineering, Tendon Tissue Engineering, 3D biomimetic scaffold, Immunomodulation, Amniotic Epithelial Stem Cells

Introduction

Tendinopathies still represent a public health concern both in human and veterinary medicine due to the unsatisfactory outcomes of the available conventional treatments [1]. In the last decades, relatively to the limited regenerative potential of tendons, much attention has been given to tendon tissue engineering (TE) that emerged as an advanced approach to deal with tendinopathies. TE aims to stimulate tendon regeneration through the production of functional implantable scaffolds [2]. Taking into consideration the crucial impact that the immune system has in tendon tissue regeneration, this latter can be achieved by designing scaffolds able to control the immune signalling through the creation of pro-regenerative environment [2]. Nowadays, immunoengineering has been introduced as a new discipline aiming at applying different immunoregenerative approaches to enhance the interaction between the transplanted scaffolds and the host tissues, which in turn could modulate the immune response and hinder the formation of fibrotic scar tissue within the regenerated site [2]. In fact, an ideal scaffold for tendon TE must be designed not only to mimic tendon architecture but to also switch the tendon pro-inflammatory response towards an anti-inflammatory one enhancing hence its regeneration [2]. This less explored feature in TE, especially for tendinopathies, might allow the modulation of the host inflammatory response and the stimulation of tissue regeneration or the potentiation of the immunomodulatory functions of stem cells. In this context, given the notable immunomodulatory properties of stem cells as the amniotic epithelial (AECs) ones demonstrated *in vivo* [3] together with their high teno-differentiative ability both *in vitro* and *in vivo* [4], the combination of this stem cell source together with tendon biomimetic scaffolds might represent an ideal microenvironment to modulate the immune cells and the regenerative process within the tendon injury site.

Starting from these premises, this study aimed at verifying the immune inductive potential of the fabricated tendon biomimetic 3D scaffold with highly aligned fibres on AECs by assessing the gene expression profile of anti- and pro-inflammatory cytokines as well as the released immunomodulatory molecules within the conditioned media (CM).

Materials and Methods

Bundled 3D PLGA tendon-like scaffolds were obtained by rolling up manually fleeces with highly aligned fibres fabricated through a rotatory collector using a commercial E-Spintronic electrospinning apparatus. The resulted constructs were characterized for their ultrastructure and biomechanical properties using scanning electron microscope (SEM) and uniaxial tensile test, respectively. The immune inductive potential of the fabricated constructs on the engineered AECs was assessed after 48h of culture by analysing the gene expression profiles of IL-10 and IL-12, anti- and pro-inflammatory cytokines, respectively, through real time qPCR. Moreover, the CMs were collected and analysed for the simultaneous expression profile of 40 immunomodulatory molecules, primary cytokines and chemokines, using Inflammation Antibody Array Membrane.

Results and Discussion

The fabricated 3D scaffold resembles macroscopically and microscopically the architecture of tendon tissues. In fact, the 3D scaffold was characterized by the high fibre alignment (SEM), as the native tissue. Moreover, the produced constructs had had significantly higher biomechanical properties in terms of Ultimate Tensile Strength and Young's modulus with respect to fleeces ($p<0.05$). The high fibre alignment of the fabricated constructs exhibited an immune inductive potential on AECs as demonstrated by the significant upregulation of IL-10, the anti-inflammatory cytokine, with boosted upregulation for 3D scaffolds at 48h ($p<0.05$). Interestingly, the expression of the pro-inflammatory IL-12, instead, was maintained at its basal levels within the 3D scaffolds. These results were confirmed by the analysis of IL-10/IL-12 ratio which showed a favourable pro-regenerative potential especially on 3D constructs ($p<0.05$) indicating the possibility of a better stimulation of macrophage skewing towards M2 phenotype when transplanted *in vivo*. Moreover, the immune-inductive role of the developed scaffolds on AECs was also confirmed by analysing the CM, which revealed a modulation of AECs' secretion profile of immunomodulatory molecules. In detail, CM

collected from AECs engineered 3D scaffold showed an overall enhancement in all analysed immunomodulatory factors, whereas a general down expression was revealed within the CM derived from engineered fleeces. In particular, in the CM collected from AECs engineered 3D scaffolds, all the analysed factors were mostly higher (1,5-fold) compared to the corresponding target expression levels in CM derived only from AECs (used as a control), with the only exception of the anti-inflammatory I-309 and TGF-1β and of the pro-inflammatory IL-8, MCP-1, IFN-γ, IL-7 and TIMP-2. On the contrary, CM derived from cells seeded on fleeces showed a down regulation of all the immunomodulatory factors with the only exception of the anti-inflammatory ones (IL-10 and IL-12 p40) and the pro-inflammatory ones (s TNF RI, MIP-1b, GCSF, ICAM-1, IL-17 and M-CSF). Interestingly, some of the upregulated immunomodulatory molecules within CM derived from AECs engineered 3D scaffolds, classified as pro-inflammatory ones including PDGF-BB, RANTES, IL-1α, IL-1b, IL-2, IL-6aR, IL-8, MCP-1, TNF-α, and IFN-γ are also known for their angiogenic role opening new insights on better understanding their involvement in the tendon resolution and regeneration.

Conclusions and/or Outlook

The presented data demonstrated the biomimicry properties of the fabricated 3D tendon-like scaffolds in terms of architecture and biomechanical properties. Moreover, the developed 3D scaffolds exhibited an immune inductive potential on AECs verified by the upregulation of anti-inflammatory cytokines as well as the release of immunomodulatory molecules within the CM. In future experiments, it will be explored the biological role of the secreted molecules within the CM on immune cells, as macrophages, to confirm their favourable immunomodulatory role.

These results might represent an innovative approach to design immune-informed scaffolds able to blunt pro-inflammatory response and improve tendon regeneration.

Acknowledgments

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Evaluation of 3D printed bone-like scaffolds in static and dynamic culture conditions

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Keywords: FDM, Bone Tissue engineering, Osteoporotic, Scaffold.

Introduction

Scaffolding is a key-element for bone tissue engineering (BTE), especially when large defects need to be clinically treated by prosthetic implants. The expected outcome should be driven by a functional substitute of the natural extracellular matrix (ECM), resembling the microarchitecture of the tissue to be healed to promote a proper integration between the scaffold and the surrounding biological environment [1,2]. In this work, 3D printed biomimetic polylactic acid (PLA) scaffolds for potential bone-related applications were fabricated by means of fused deposition modelling (FDM) technique, mimicking the physiological and pathological structures of cancellous bone. The biological evaluation of the proposed scaffolds was performed by means of osteoblast-like cells, the human osteosarcoma cell line SAOS-2, both in static and dynamic conditions to assess the role of external inputs on cell response.

Results and Discussion

By tuning the geometric characteristics of a computer aided design (CAD) model, it is possible to realize a 3D printed structures, in principle, of any desired shape. Two sets of biomimetic scaffolds are here proposed, characterized by different structural parameters and porosity degrees. In details, the porous pattern of the scaffolds was designed by Meshmixer 3.5 software (v.2018, Autodesk, San Rafael, CA, USA), firstly subtracting a three-dimensional random cluster of spheres from a box-shaped solid. Subsequently, the trabecular structure was obtained using the "Mesh + Delaunay Dual Edges" function, Voronoi's dual tessellation [3]. The resulting model files (.stl) were imported in IdeaMaker software (v 3.6.1, Raise 3D Inc., Irvine, CA, USA) to perform the slicing procedure and obtain the "GCode" file for the 3D printer. Models were sliced at 0.1 mm along the Z direction in order to reproduce the original CAD design as accurately as possible. The design parameters were varied to obtain two types of samples, increasing the trabecular spacing and reducing the trabecular thickness and thus dealing with bone physio-pathological conditions which imply an increase in total porosity [4]. The resulting models were labelled LP (low porosity) and HP (high porosity), respectively.

Figure 1 shows a picture of the 3D printed scaffolds while Table 1 summarizes the designed and measured porosities.

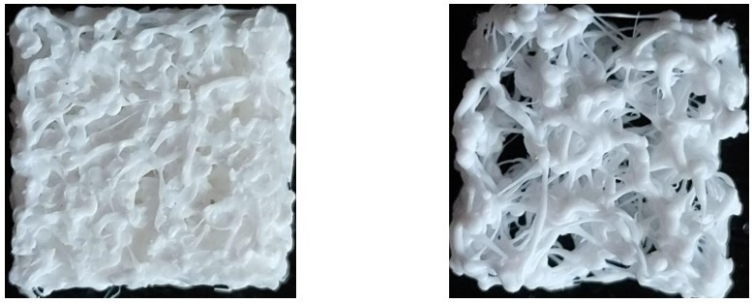

Figure 1: LP (left panel) and HP (right panel) scaffolds

Scaffold models	CAD Model Porosity [%]	Measured Porosity [%]
LP	53.8	45.7 ± 0.7
HP	83.0	81.0 ± 0.5

Table 1: Software estimated porosity and porosity measured by gravimetric.

Scaffolds were then used to perform static and dynamic cultures of SAOS-2 cells by means of a rotating bioreactor (Synthecon, Inc., Houston, TX, USA). The biocompatibility was evaluated by the analysis of TNF-α (Tumor Necrosis factor-α) secreted by the cells grown on the scaffolds. TNF-α release was measured in culture medium using human ELISA development kits (PeproTech® EC Ltd., UK), according to the manufacturer's instructions. The analysis of the secretion levels of TNF-α did not show detectable amounts of this pro-inflammatory factor in the cell medium, demonstrating an excellent biocompatibility of all substrates examined, without any inflammatory responses after 96h of culture. The cells growth was investigated by WST-1 colorimetric assay. As reported in Figure 2C, the SAOS-2 cells, seeded on high porosity scaffolds and cultured in dynamic conditions showed a higher growth rate compared to static conditions, but also compared to the other substrate.
These preliminary results indicate that the high porosity, by increasing the available surface area, improves the attachment of the cells to the scaffolds in dynamic cultures thanks to the continuous perfusion ensured the experimental setup. Otherwise, these specific culture conditions are not available in the static mode which may prevent an optimal cell-scaffold interaction, especially for the HP model.

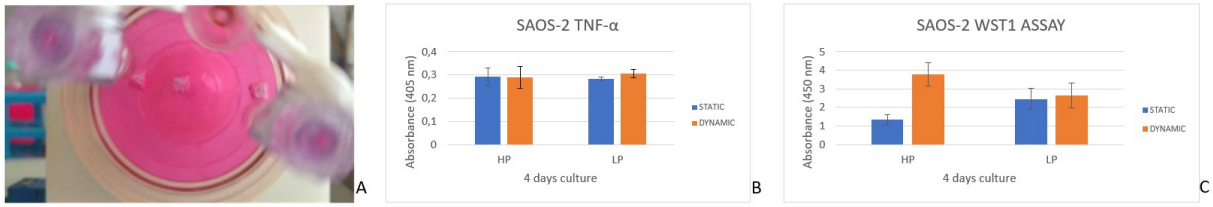

Figure 2. A) Bioreactor for dynamic cultures. B) TNF-α release by ELISA assay. C) Cell growth analysis by WST assay.

Conclusions and/or Outlook

Scaffolds for BTE with different porosity have been designed and fabricated. The effective porosity of the printed samples was in good agreement with the CAD model. All the tested scaffolds, under dynamic conditions, have shown a promising outcome in terms of metabolic activity without inducing inflammatory response. The HP model supported a valuable cell growth, probably due to the higher porosity. These preliminary results deal with an interesting approach to prepare 3D printed biomimetic bone scaffolds to be investigated in different experimental conditions. The osteogenic behaviour of SAOS-2, cultured on neat scaffolds and composites, combined with ad hoc surface treatments, is currently under investigation.

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Session 9 – Tissue Regeneration and Infection Treatment

Physico-chemical and structural features of calcium phosphates, relevant for inherent osteogenic and antibacterial properties

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Tissue regeneration, particularly when dealing with critical size bone tissue defects, is strongly related to the use of bioactive materials and 3D devices acting as a guide for cells and driving appropriate differentiation as well as new tissue formation and maturation. However, the surgical practice related to biomaterials implantation is frequently accompanied by the insurgence of infections, that often result in therapy failure, need of revision surgery and substantial raise of the patient suffering and healthcare costs. A major approach in this respect is the loading of devices with antibiotic drugs, however concerns related to inappropriate delivery profiles at the target tissue, and also the increasing bacterial resistance to antibiotic drugs often make ineffective therapeutic protocols (1). Therefore, the use of bioactive, regenerative devices capable of exerting resistance to bacterial proliferation and permitting sustained delivery of therapeutics is strongly desired.

The present work shows the effectiveness of nanostructured, ion doped apatites, as biomaterials capable of triggering osteogenic cell phenotype and, at the same time, in abating bacterial activity, as observed by testing with various pathogens, among the most diffuse in nosocomial infections (2,3). Apatite nanoparticles and self-hardening porous bone scaffolds obtained by the transformation of metastable, ion-doped calcium phosphate precursors (4), are here described in terms of physico-chemical and biological properties, this latter related to the bio-activation of human stem cells and also to specific antibacterial properties, related to both drug delivery ability and also to intrinsic compositional and microstructural features. We can hypothesize that structural and crystal modifications induced by multiple ions doping increase the surface charge and enhance the ability of ion exchange, giving enhanced bioactive effects, reported by higher stem cell viability and gene expression as well as by complete inactivation of all the tested pathogens. Ion doping plays also a relevant role in modulating the cross talk between various cell lines relevant for tissue regeneration (5), thus resulting in the ability to promote physiological bone metabolism, therefore being a promising solution to treat patients with impaired bone turnover such as for instance osteoporosis.

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Glycosaminoglycan-based functional biomaterials - promising options for therapeutic intervention in compromised tissue regeneration

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Keywords: Collagen, inflammatory macrophages, (sulphated) hyaluronan, wound healing

Introduction

The demographic development in western civilizations with an increase in multimorbid patients displaying compromised tissue regeneration, raises the need for novel innovative material concepts e. g. to tackle the growing number of chronic wounds. Non-healing wounds are stuck in a chronic inflammatory phase driven by uncontrolled infiltration and activation of macrophages leading to excessive tissue breakdown. Resolution of this unrestrained inflammatory loop is an unmet challenge in the treatment of non-healing wounds. Still, appropriate immunomodulating wound dressings for controlling macrophage functions in chronic skin wounds are lacking [1]. Using biomaterials based on extracellular matrix (ECM) components is a promising approach, since the ECM conveys fundamental biological functions regarding tissue homeostasis, cellular differentiation and angiogenesis [2]. The most abundant ECM molecules are collagen type I (coll) and proteoglycans. The latter have a protein core with glycosaminoglycans (GAG) bound to it. GAG itself can bind and protect growth factors and affect proliferation, cell adhesion and differentiation [2]. The GAG hyaluronan (HA) is an essential component of the ECM and known to regulate inflammatory processes. It is abundantly available and can be chemical derivatized in a controlled and reproducible manner (e. g. (meth)acrylation, sulphation) [2]. Hence, HA is suitable for use as functional biomaterial with tunable properties. Previously, HA/coll-based hydrogels (HG) successfully enhanced endothelial cell proliferation showing their capability to support the regeneration of vascularized tissue [3]. Coll-based materials are used as wound dressings to support healing and to avoid autologous skin transplantation. Still, their native structure is often affected during processing and purification leading to a foreign body reaction. Various options have been proposed for promoting the regenerative potential, such as surface modification and crosslinking. Chemical crosslinking, however, often results in an inflammatory reaction involving macrophages, while chemically sulphated hyaluronan (sHA) has potent anti-inflammatory activities towards inflammatory activated macrophages *in vitro* [4, 5]. In a translational approach, we implemented sHA in ECM-based biomaterials in two different ways for modulation of inflammatory macrophage activities: first, we included sHA in HA/coll-based hydrogels intended for continuous release of this immunmodulatory component into chronic wound sites. Second, we functionalized a clinically applied collagen-based scaffold (Mucograft®) with sHA via electrostatic or covalent binding. To evaluate the hypothesis that the inflammatory response is thereby modified, the cellular response was either studied in skin wounds of diabetic db/db mice, an established model for delayed diabetic wound healing, or in a subcutaneous rat model.

Results and Discussion

HA/coll-based hydrogels containing sHA demonstrated a continuous release of the immunomodulatory component over a period of one week [6]. Applying hydrogels at day post-wounding when inflammation in the wounds had already developed and wound analyses at day 10, when the impaired wound healing phenotype had fully developed, showed an improved wound closure in the diabetic mice. The healing response was characterized by i) reduced inflammation and augmented alternative macrophage activation (down-regulation of IL-1b and up-regulation of IL-10, Relma), ii) increased vascularization (up-regulation of VEGF, CD31) and iii) accelerated new tissue formation (up-regulation of EGF, Ki67, granulation tissue) [6].

Investigating the liberation of sHA from Mucograft® after electrostatic (sHA3/PBS) or covalent binding (sHA3+EDC/NHS) in comparison to unmodified and cross-linked controls (Ctrl and EDC/NHS Ctrl), revealed a low release of covalently linked sHA in contrast to the electrostatically bound form [7]. The former was structurally stable *in vivo* and showed integration into the implantation site after 30 days. SHA3+EDC/NHS reduced the proinflammatory macrophage response and showed no MNGC formation over 30 days, in contrast to sHA3/PBS and EDC/NHS Ctrl [7].

Conclusions and Outlook

In sum, our findings show that sHA modulates pro-inflammatory macrophage activity in favour of pro-resolution functions and has the capability to improve the outcome of disturbed skin wound healing either as wound dressings or for full-thickness skin regeneration in the future.

Acknowledgement:

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Polymeric nanosystems for targeted therapy of colorectal cancer

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Keywords: nanotechnology, colorectal cancer, targeted therapy, polymeric nanocarriers, drug delivery systems

Introduction

Despite the rapid development of innovative therapeutic methods and improvement in the quality of life achieved with better specialist healthcare, cancer will remain a major health problem of the modern medicine. According to the World Cancer Report [1-2] colorectal cancer is already the third leading cause of cancer death in the world, and its incidence is steadily rising in developing nations. Distant metastatic disease is present in approximately 35% of patients at initial diagnosis. Despite advances in cytotoxic therapy, resistance to chemotherapy remains one of the greatest challenges in long-term management of incurable metastatic disease [3]. The main drawbacks of chemotherapy are its poor bioavailability, high-dose requirements, adverse side effects, low therapeutic indices, development of multiple drug resistance, and non-specific targeting.

Therefore, the effectiveness of anticancer therapy significantly depends upon tumour-specific delivery of the drug The aim of the studies was the synthesis and determination of the structure as well as both chemical and biological properties of new polymeric nanocarriers for targeted therapy for metastatic colorectal cancer. Targeted delivery combined with controlled release of drugs plays a crucial role in future of personalized medicine. The main purpose of this study was to synthesized folic acid or biotin-functionalized polyesters as matrices for polymeric nanopartices (NPs) loaded with irinotecan as antitumor drug. Folic acid and its analogs can bind specifically to folate receptors overexpressed in colorectal tumor, enabling folic acid-modified nanoparticles to enter tumor cells through receptor-mediated endocytosis and enhancing the cancer targeting capacity of nano-drug delivery systems (DDS). Polymeric NPs are able to improve bioavailability, as well as provide precise distribution and suitable pharmacokinetics with the improved toxicological safety of the drugs. Moreover, polyesters degrade into acidic, non-toxic products and provide an appropriate microenvironment for biologically active lactone form of irinotecan. Irinotecan-loaded functionalized NPs were successfully obtained using the double emulsion solvent evaporation method.

The morphology (TEM) and physicochemical and biological properties (particle size, ζ potential, encapsulation efficiency, cytotoxicity) wf the NPs were assessed. The influence of topology of polyesters matrices on kinetics of the irinotecan release were alsoo investigated.

Results and Discussion

The results of the TEM investigations revealed that spherical PCL NPs were successfully obtained

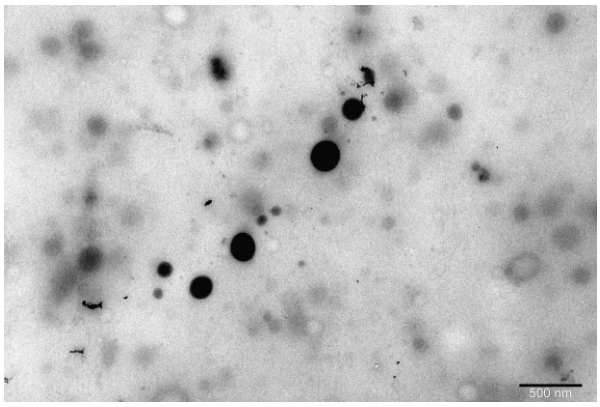

Figure 1: TEM image of irinotecan-loaded PCL NPs

The amount of the drug and polymer microstructures (the content of negative charged, linear PCLs chains and non-charged cyclic PCLs) affect on irinotecan encapsulation efficiency. Moreover, the crystallinity of polymer, presence

of the targeted molecules and hydrophilic-hydrophobic properties of the drug affect on kinetics of irinotecan release from new systems.

Conclusions

The results of the study show that PCL NPs could be a promising delivery system for targeted therapy for metastatic colorectal cancer.

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Endophytic fungi solvent extracts as a novel source of bioactive compounds in electrospun polycaprolactone wound dressing

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Keywords: endophyte, novel antibacterial additives, polymer wound dressings, electrospun fibers

Introduction

Although there are more than 3000 dressing types in the wound management market, still wound care is a global health issue with a financial burden equating up to US$96.8 billion annually just in the USA. Especially Chronic non-healing wounds, which show delayed and incomplete healing process. Developing efficient wound dressings requires embedding antibacterial additives against microbial infections. The lack of novel antibacterial agents and misusing the previous antibiotics have caused an increase in antimicrobial resistance (AMR) affecting ten million lives by 2050 worldwide. These ongoing challenges clearly indicate an urgent need for developing new antibacterial additives in wound dressings targeting reported microbial infections. Natural products and their derivatives have long been a significant source of pharmaceuticals against AMR. In this study, endophyte as a novel natural source of bioactive compounds is considered as a sustainable source for therapeutic applications resulting in an anti-AMR efficient antibacterial additive for chronic wound dressing.

Results and Discussion

In this study, 27 types of endophytic fungi originated from 12 Australian native plants studied for their antibacterial activity against targeted wound bacteria Staphylococcus aureus using two extraction methods of freeze-drying and solvent extraction. The most active sample was identified and used as an additive in processing Polycaprolactone electrospun fibers. X-ray Photoelectron Spectrometry (XPS), SEM, and disc diffusion data were used to investigate the surface characterization, presence of additives in the fiber, and its antibacterial activity consecutively.

Among all the 27 purified cultures, *Eremophila Longifolia* 19 had the best antibacterial activity with an inhibition zone of 17mm and a minimum inhibitory concentration of 0.156 mg/ml. Utilization of this extract as an additive was performed successfully and the XPS analysis approved the existence of antibacterial additive in electrospun fibers. In the final step, disk diffusion tests against the selected bacteria showed the antibacterial property of the fibers. **Figure 1** indicates the antibacterial activity of the fibers against selected bacteria and the SEM result of the fibers.

a) b)

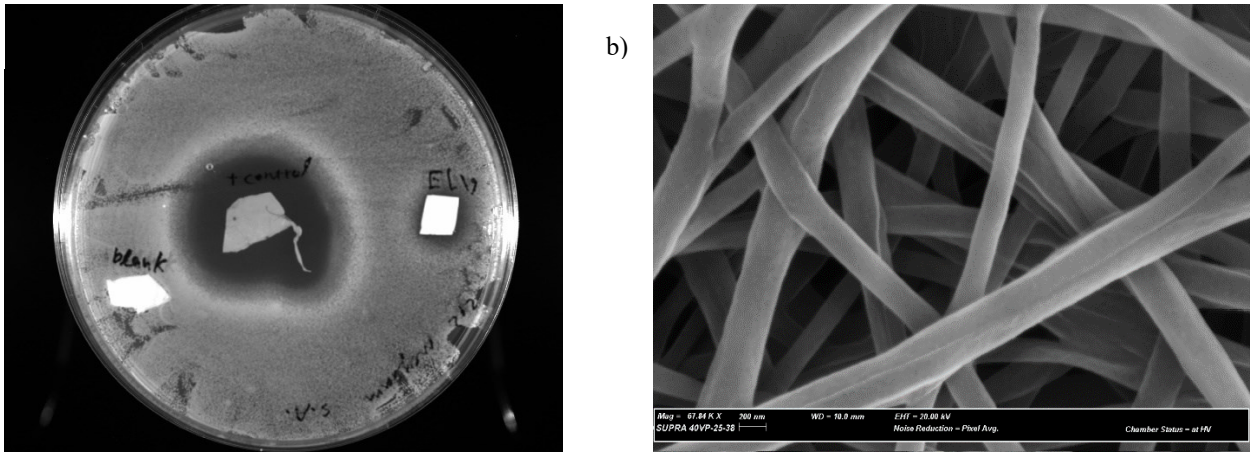

Figure 3 a)Disk diffusion result of the selected fibers against Staphylococcus Aureus b)SEM of the fibers

Conclusion and Outlook

After admitting the antibacterial activity of the selected endophytes, their utilization for the first time in electrospun fibers targeted for wound dressing was proved successfully. This sustainable source has a great potential to be used as a novel antibacterial additive for medical purposes.

Although the effectiveness of these bioadditives has been demonstrated, some steps need to be taken for future studies like the mode of action of the extracts as well as exploring and optimizing the functional properties of the fibers such as cytotoxicity and release profile.

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Study of the mechanical properties of new types of antimicrobial acrylic bone cement

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Keywords: bone cement, antimicrobial agents, mechanical properties, biocompatibility

Introduction

Poly(methyl methacrylate) (PMMA) bone cement is usually used to fix artificial joints, such as hip or knee prostheses. The main function of bone cement is to transfer body weight under different conditions (walking, standing, climbing stairs, etc.) from the prosthesis to the bone. Therefore, the mechanical properties of bone cement will determine its success as a structural material. The main problem when using a prosthesis is the risk of postoperative infection [1], a problem that could be solved through the development of some new antibacterial bone cement [2–4]. Usually, bone cement is loaded with antibiotics to prevent the infections associated with the clinical procedures. This way, the PMMA cement delivers a high quantity of drugs at the infection site without adverse effects on other human organs. However, there is still debate about the right concentration and amount of antibiotics to avoid harmful effects on the body [5]. Unfortunately, bacteria have developed a genetic and biochemical mechanism to fight against antibiotics, so therefore, other innovative solutions must be searched.

Considering the need to discover and develop new ways to combat postoperative infections and starting from the fact that nature is the best generator of solutions, we develop innovative modified PMMA-based bone cement consisting of silver nanoparticles incorporated in ceramic glass and peppermint oil included in hydroxyapatite - use. The impact of the addition of antimicrobial agents on the structure, mechanical properties and biocompatibility of new bone types of cement were quantified.

The experimental samples were obtained using a PMMA cement available on the market. As antimicrobial agents, we used silver nanoparticles incorporated in a ceramic glass matrix (as one commercial product from SANITIZED AG, Burgdorf, Switzerland), peppermint essential oil (from Hofigal Export Import SA, Bucharest, Romania) incorporated in hydroxyapatite (HAp, >95% purity, Plasma Biotal Limited, Tideswell, UK), and gentamicin (KRKA D.D.–- Slovenia). The silver nanoparticles were incorporated into the bone cement composition in a proportion of 2% (AM1 sample) and 4% (AM2 sample), respectively. Sample HUM contains 5% peppermint essential oil incorporated in hydroxyapatite, and sample GH contains 5% gentamicin. The commercial polymethylmethacrylate cement (R sample) was used as a reference.

The impact of the addition of antimicrobial agents on the structure, mechanical properties, and biocompatibility of new PMMA bone cements was quantified.

Results and Discussion

Morphological and structural characterization: SEM images show a good dispersion of the barium sulphate and antimicrobial additives into the PMMA-PBMA matrix (poly(methyl methacrylate)-poly(butyl methacrylate) ma-trix). A slight tendency of antimicrobial agents to form agglomerates can be observed, mostly in the bone cements samples containing 4% silver nanoparticles (sample AM2). The EDS analysis supports this observation. The Raman measurements showed only minor detectable differences within spectra, compared with the literature data [6,7]. The presence of stronger barite Raman signals for samples AM1 and AM2 could be interpreted as a better distribution of this component throughout the sample bulk/surface. The thermogravimetric analysis revealed that there is an improvement in the thermal stability of the cement by adding antimicrobial agents, except in the case of the HUM sample. The TGA curves for all the investigated samples were leveled at temperatures higher than 430°C, which indicates a low weight loss beyond this temperature. Adding peppermint essential oil incorporated in hydroxyapatite, the T10 was shifted to lower values, which is caused by the loss of volatile compounds in the oil. We can conclude that the addition of antimicrobial agents had no significant influence on the thermal stability of the experimental bone cement.

Evaluation of the properties of new bone cements: The *contact angle* measurements showed that the most hydrophobic character can be observed in the case of the GM sample. Samples HUM, AM1 and AM2 show a more hydrophilic character, which is caused by the ceramic components (hydroxyapatite and ceramic glass) that were added with the respective antimicrobial agents. The results show an increase in the *hydration degree* (Ha) of bone cements with the addition of antimicrobial agent and the amount of antimicrobial agent in AM1 and AM2 samples. Compared to the standard sample R, the values of the hydration degree (Ha) increased less in the case of the sample with gentamicin

(GM sample). The largest amount of water is subsequently absorbed during the first 7 days after immersion: the values remain almost constant in the case of the reference R, GM, and HUM samples.

Mechanical properties: At the *flexure tests*, all samples, failed almost immediately when the stress exceeded the elastic limit, without any significant signs of plastic deformation (Figure 1). The c*ompression test* proved that the addition of gentamicin (GH samples) does not influence the elastic modulus of the bone cement, while the addition of silver nanoparticles incorporated in ceramic glass (AM1 and AM2 samples) leads to a slight decrease, roughly 4% and 5% of its values comparing to the reference sample R. The addition of peppermint oil in hydroxyapatite shows a more substantial decrease, by roughly 9% reported to the sample R. Despite that in pure compression the addition of antimicrobial agents leads to a decrease in mechanical characteristics (Figure 2), this decrease is of an acceptable amplitude (maximum 12%, comparing to the reference sample R) and the strength values for the investigated bone cement still meet the minimum requirements stipulated by ASTM F451 standard (i.e., a strength of at least 70 MPa).

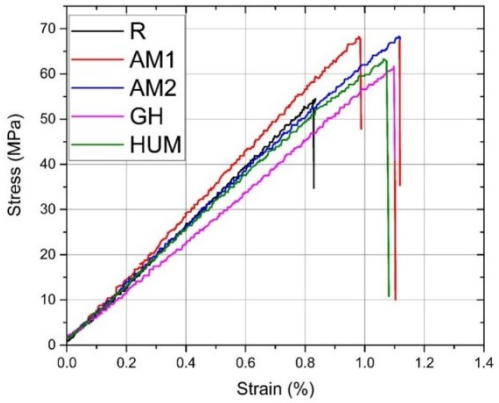

Figure 1. Selection of representative stress-strain curves in flexure

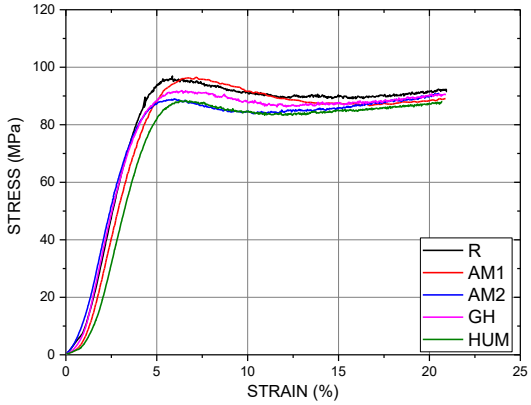

Figure 2. Selection of stress-strain curves in compression

Biocompatibility properties: Among the tested bone cement, AM1, HUM, and R exhibited the highest degree of biocompatibility, as revealed by the MTT and LDH tests. Samples GH showed the lowest cytotoxicity (as demonstrated by the low LDH values), yet it did not stimulate cell proliferation as much as the other materials tested. None of the materials tested induced significant changes in the cell morphology.

Conclusions

It has been shown that the addition of antimicrobial agents improves the flexural strength of the traditional PMMA bone cement, while the yield strength values show a decrease most likely because this agent acts as a discontinuity inside the material rather than as a reinforcing agent. In the case of all samples, the addition of antimicrobial agents had no significant influence on the thermal stability. The new PMMA bone cement showed a good biocompatibility and the possibility of osteoblast proliferation (MTT test) along with a low level of cytotoxicity (LDH test). Our research indicates that bone cements with peppermint essential oil incorporated in hydroxyapatite and silver nanoparticles incorporated in ceramic glass used as antimicrobial agents can be a viable solution that can be introduced in the near future in common clinical practice.

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Session 10 – Innovative Ceramic and Composite Materials

Calcium phosphate nanoparticles as nanocarriers of therapeutic peptides

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Keywords: calcium phosphates, antimicrobial peptides, cardiovascular diseases, nanoparticles, drug delivery

Introduction
Calcium phosphate is a natural biomineral and the major inorganic constituent of bones and teeth. Therefore, it possesses excellent biocompatibility as well as biodegradability. Due to their unique physiochemical properties, calcium phosphate nanoparticles (CaP NPs) are extensively exploited in the biomedical field as carrier of several biomolecules, including peptides, proteins, and nucleic acids. In this regard, peptides are exceptionally selective and efficacious for the treatment of a broad range of diseases. Peptide-loaded CaP NPs were interestingly prepared via biomineralization. The greatest advantage of biomineralization routes relies on the encapsulation of the selected biomolecules. The peptide can generally provide nucleation sites, so that inorganic mineral phase can form around the peptide itself creating a protective shell. Being trapped inside the nanoparticles, the bioactive agent is both stabilized and protected from degradation in biological fluids.

Results and Discussion
Among various peptides for biomedical applications, antimicrobial and cardio-specific peptides are particularly interesting since they represent a valuable alternative to conventional treatments. Moreover, they can contribute to overcome important clinical limitations including antibiotic resistance and non-specific biodistribution of traditional drug products. Within this context, we have investigated a new peptide-based therapeutic approach for the treatment of cystic fibrosis bacterial infections as well as cardiovascular diseases, using colistin, as a model peptide, which is also one of the most active antimicrobials against Gram-negative bacteria [1], and a therapeutic mimetic peptide that improves myocardial contraction and results in restoration of cardiac function [2], respectively. Peptide-loaded CaP NPs were prepared exploiting the biomineralization approach, using a mineralizing solution containing Ca^{2+}, Mg^{2+} and PO_4^{3-} ions. Several experimental conditions were tested, by varying the reaction time, temperature, and pH as well as the drug concentration. Colloidal stability, morphology, size as well as drug loading and release were evaluated to identify the best candidate to be tested *in vitro* and *in vivo*.

Conclusions
These results open the way for an innovative CaP-NPs based delivery system potentially able to counteract cystic fibrosis infections as well as cardiovascular diseases.

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Development and characterization of a composite material based on reduced graphene oxide (rGO) and nanocellulose (CNF)

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Keywords: graphene oxide, nanocelulose, domapine, polydopamine, film.

Introduction

Due to environmental and technological contingencies, materials science has recently focused on the development of environmentally friendly materials with abundant precursors in nature, at the nanometer scale.

Carbon is one of the most abundant elements in the biosphere, possessing hybridized orbitals sp, $sp2$, and $sp3$, which give it the ability to create endless interactions with itself and other elements. Carbon is capable of creating homoelemental networks called allotropic forms, among which are diamond, graphite and graphene [1]. After the oxidation of graphite, a compound rich in carbon-oxygen bonds is obtained, namely graphene oxide (GO), which after its reduction is transformed into reduced graphene oxide (rGO) [2].

In this study a composite material based on reduced graphene oxide (rGO) and nanocellulose (CNF) was synthetized by an in situ reduction by Dopamine (DA), and then characterized in terms of its morphological, physical and chemical aspects. The samples were named rGO@CNF1, rGO@CNF2, rGO@CNF3 and rGO@CNF4, in order to find an optimal amount of CNF, and in the controls there were CNF, GO and rGO.

Results and Discussion

As a result, a thin, resistant, black material, easy to adhere, was obtained. SEM characterization showed compact layers and nanofibers on its surface, the product of CNF anchorage in the matrix. The elemental presence of C, O and N was confirmed by EDS analysis. The reduction of GO was confirmed by spectroscopic characterization. In Raman spectroscopy a higher intensity of the D band was observed in comparison to the G band and a higher I_D/I_G, due to the recovery sp^2 hybridization[3]. In the FT- IR spectroscopy a decrease in the peaks belonging to the hydroxyl and epoxy groups was observed, due to the reaction with catechol, as well as a slight peak of the -C-N bond, confirming the crosslinking between rGO and PDA [4]. In the XRD analysis, peaks were observed evidencing the increase in the sp^2character of the structure [5]. Also, the increase in the width of the peaks indicated the interlayer incorporation of CNF and PDA [6].

As for the physical characterization, the contact angle was measured (Table 1) evidencing different behavior among samples, the CNF augment being the most hydrophilic and increasing the angle to the greatest degree. For the samples rGO@CNF2 and rGO@CNF4 ~~a~~ hydrophilic behavior was observed on one side of the plane -the opaque side- and the other side -the shiny side- was hydrophobic. This fact can be explained by the hydrophobic interactions occurring between CNF and rGO; and the heterogeneous ordering that distributes hydrophobic sections of rGO and CNF to one side of the film [7]. The swelling test showed that the material absorbs water, reaching a maximum capacity of 46.12%. The tensile test reported a high tensile strength, which increases with the CNF content, evidencing the anchorage to the surface, with a maximum of 28.42 mPa for rGO@CNF4. The maximum deformation showed no statistically significant changes when different amounts of CNF were added, reaching a maximum of 3.8% in rGO@CNF2.

Sample	Opaque side (n°±σ)	Shiny side (n°±σ)
CNF	43.5°±0.9	49.8°±4.4
rGO	59.9°±0.6	63.7°±2.6
rGO@CNF1	70.8°±2.9	77.0°±1.0
rGO@CNF2	73.4°±2.6	**94.3°±4.1**
rGO@CNF3	65.9°±2.9	75.1°±3.6
rGO@CNF4	86.0°±4.9	**92.9°±4.6**

Table 1. Contact angle for both side of materials

Conclusions

An rGO/CNF material has been developed by a simultaneous reduction and crosslinking method. It has the advantage of being environmentally friendly, with inexpensive reagents, and easy to prepare. The results obtained support the possibility of applying it as a biomaterial, specifically and in conjunction with other studies as a wound dressing, or in the field of electricity as an electrode or sensor, among others.

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The dependence of antimicrobial activity on the Cu^{2+} concentration in $Ca_{10.5-x}Cu_x(PO_4)_7$

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Keywords: calcium phosphate, copper substitution, copper doping, crystal structure, antimicrobial activity

Introduction

β-$Ca_3(PO_4)_2$ (β-TCP) crystalizes in the Whitlockite structural type. Compounds based on β-TCP are known as excellent biocompatible materials since the bone tissue is built from combination of whitlockite and apatite (Ap) ($Ca_{10}(PO_4)_6(OH)_2$) phases. Due to a wide range of isomorphous substitutions within the initial host-matrix it is possible to modify the crystal structure by varying the cationic and anionic sites to design the novel materials with the directly improved physico-chemical and biological properties. The β-TCP-type structure includes six cationic positions (M1-M6), where the M1-M3 (18-*fold*) and the M5 (6-*fold*) sites are always occupied, while the M4 site can vary its occupation from 0 to 1, and the M6 site is always vacant. The presence of a crystal site with variable occupation favors for substitutions by different ions in the structure, including Cu^{2+}.

Many studies were devoted to single ionic substitutions, such as Cu^{2+} and Sr^{2+} [2], Mg^{2+} [3], in tricalcium phosphate. It has been established that the incorporation of Cu^{2+} ions into the β-TCP-type structure can curb the growth of bacteria, which helps in protecting the sites with bone implant from the attack of microbes. Also, Cu^{2+} is not cytotoxic at low concentrations. The studies on single-doped Cu^{2+} phosphates [4,5] report on β-TCP-type with admixture of apatite and calcium pyrophosphate phases. Therefore, it is difficult to track the contribution of copper ions from β-TCP phase on the antimicrobial activity since apatite phase also can be doped by Cu^{2+} [6]. Also, the systematic investigation of Cu^{2+} concentration on the biological responses is absence.

In order to interpret the biological behavior of Cu^{2+} substituted β-TCP, it is of great importance to correctly characterize the structural location of the Cu^{2+} ions. The biological activity of the Cu^{2+} ions depends on occupied position and concentration.

Results and Discussion

Copper-substituted tricalcium phosphates $Ca_{10.5-x}Cu_x(PO_4)_7$ with x = 0, 0.001, 0.333, 0.667, 1, 1.333, 1.667 were synthesized by the high temperature solid-state synthesis. The synthesis procedures were carried out applying stoichiometric amounts of raw materials.

The possibility of the $Ca^{2+} \rightarrow Cu^{2+}$ substitution is in the agreement with calculation of the ratio percentage discrepancy (D_r). The D_r can be calculated using formula: $D_r = (R_h - R_d)/R_h * 100\%$. The continuous solid solution requires a similarity of ionic radius between doping ions (R_d) and substituted host ions (R_h). The value of D_r should be less than 30%. In the $Ca_{10.5-x}Cu_x(PO_4)_7$ the D_r value is 27% for the octahedral M6 site, so the Cu^{2+} ions can be successfully incorporated in the β-TCP structure in the octahedral position.

Powder X-ray diffraction (PXRD) patterns were collected on Rigaku SmartLab SE (3 kW sealed X-ray tube, D/teX Ultra 250 silicon strip detector, vertical type θ-θ geometry, HyPix-400 (2D HPAD) detector). The LeBail decomposition, quantitative phase analysis and structure refinement by the Rietveld method were applied using the JANA2006 software [7].

Fig. 1a shows the powder X-Ray Diffraction (PXRD) patterns of $Ca_{10.5-x}Cu_x(PO_4)_7$ obtained by the high temperature solid state reaction. According to the obtained data, the Cu-doped β-TCP are single phase. No impurities of hydroxyapatite, $Ca_3Cu_3(PO_4)_4$ or $Ca_2P_2O_7$ phases were detected up to x =1.33. However, the impurity of CuO was detected in the sample with x =1.33 (**Fig. 1**). The quantity of the CuO phase was 3%. The sample with x = 1.583 showed the presence of the impurity $Ca_3Cu_3(PO_4)_4$ phase (**Fig. 1**). These PXRD data approved the limit composition of $Ca_{10.5-x}Cu_x(PO_4)_7$ at x = 1.5, as it was established earlier [8].

The PXRD patterns were used to refine the crystal structure of samples with $0 \le x \le 1$. The free refinement of calcium positions showed the partial occupancy of M4 position by Ca^{2+} ions (**Fig. 2**). The final occupancies (a_i) of the M4 site were slightly varied for half-occupied (a_i(M4) ~ 0.5) (**Fig. 2**). No Cu atoms were found in the M4 site, since they are

totally located in M5 sites (**Fig. 2**). The composition $Ca_{9.5}Cu(PO_4)_7$ is characterized by complete occupation of the M5 site by the Cu atoms (**Fig. 2**).

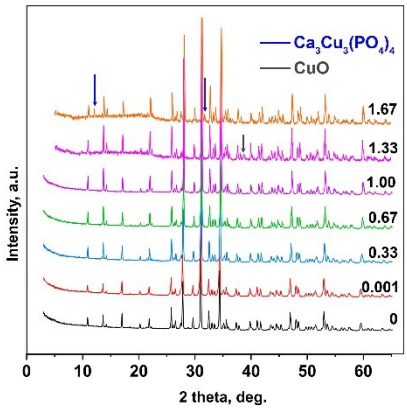

Figure 1: PXRD patterns of $Ca_{10.5-x}Cu_x(PO_4)_7$ with $0 \leq x \leq 1.583$ obtained by solid-state method. The impurity phases of CuO (PDF#2 80-1917) and $Ca_3Cu_3(PO_4)_4$ (PDF#2 №72-203) are highlighted.

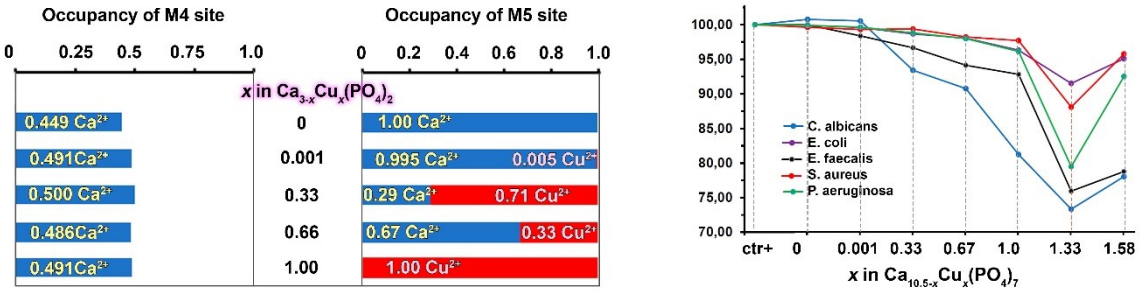

Figure 2: Occupancy of the M4 and M5 crystal sites by Ca^{2+} and Cu^{2+} ions in $Ca_{10.5-x}Cu_x(PO_4)_7$ (left). Results of the experiment on the inhibition of the growth of microorganisms grown for 24 hours in the presence of $Ca_{10.5-x}Cu_x(PO_4)_7$ (x = 0, 0.001, 0.33, 0.67, 1.0, 1.33, 1.58). The positive control (ctr +) is represented by the growth of each microorganism in the absence of compounds. The growth rate is calculated on the optical density measured at 600 nm, resulting from three independent experiments.

The study of antimicrobial activity was performed by growing of one fungus (*C. albicans*) and four bacteria (*E. coli, E. faecalis S. aureus* and *P. aeruginosa*) in the presence of $Ca_{10.5-x}Cu_x(PO_4)_7$ at different concentrations of Cu^{2+}: $0 \leq x \leq 1.583$. The obtained results regarding the inhibition can be observed from **Fig. 2**.

Conclusions

The influence of the Cu^{2+} concentration in $Ca_{10.5-x}Cu_x(PO_4)_7$ on the antimicrobial activity was studied. The strong dependence of the inhibition of the microorganisms's growth on the Cu^{2+} concentration was observed. The growth inhibition for *C. albicans* (6.59%) and *E. faecalis* (3.34%) is already highlighted in compound with x = 0.333. At x = 0.667 growth inhibition remains stronger for *C. albicans* (9.22%) and for *E. faecalis* (5.86%). At x = 1.00 all the microorganisms studied are clearly inhibited growth, in particular at this concentration of Cu^{2+} *C. albicans* has the strongest growth inhibition (18.77%), *E. faecalis* (7.18%), *E. coli, S. aureus and P. aeruginosa* had growth inhibition ranging from 2.29% to 3.68%. The greatest growth inhibition occurs at x = 1.33 for all microorganisms, especially *C. albicans* is 26.67%, *E. faecalis* is 24.06%, *P. aeruginosa* is 20.51 %, *S. aureus* is 11.91 % and *E. coli* is 8.49% after 24 hours of growth. This activity may be related to the presence of 3% CuO phase in the sample. The growth of the 5 microorganisms in the presence of $Ca_{8.917}Cu_{1.153}(PO_4)_7$ was inhibited to a greater extent for *C. albicans* (21.95%) and for *E. faecalis* (21.23%), while for *P. aeruginosa*, *E. coli* and *S. aureus* inhibition is between 4.91% and 7.48%, respectively.

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Tailor-made synthesis of bionic amorphous calcium phosphate

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Keywords: Amorphous calcium phosphate

Introduction

Amorphous calcium phosphate (ACP) is one of the most common forms of calcium phosphate (CaP) found in organism [1]. Previous studies have reported that calcium and phosphates undergo localised concentration in mitochondria of eukaryotes to form ACP which are then converted to hydroxyapatite. There are increasing evidence that ACP acts as a precursor phase of apatite growth for example in embryonic chicken long bone, developing mouse calvaria, growing bones of zebrafish, calcified soft tissue etc. It is theorised that sphericalACP acts as a transient phase which is easily transformed to platelet like morphology aligning along long axis ofcollagen fibres. The apatite in bone possesses unique chemical composition and geometry; it is calcium deficient commonly substituted by carbonate, possess imperfect platelet geometry and poorly crystalline with the crystal does not exceed size of 30–50 nm while maintaining thickness of ~2 nm. From these studies it can be stated that the spherical size ACP should fall in the range of 30–50 nm [2]. Synthesis of ACP is reported by two major approaches: by dry and wet route. Due to the metastable nature of the ACP dry state synthesis leads to formationof other phases of calcium phosphate therefore the wet route is more preferred. The synthesis by wet route is governed by the pH, time, as well as type and concentration of calcium and phosphate salts. Though there are numerous reports on wet route synthesis, there are variation in the synthesis pH, temperature, particle size, calcium to phosphate ratio. Herein, we report a standard protocol for synthesis of ACP in the range of 30–50 nm.

Results and Discussion

Synthesis of amorphous calcium phosphate was performed by double decomposition reaction between aqueous solutions of calcium and phosphate salts at alkaline pH (close to 11) under ambient temperature, with calcium to phosphate ratio of 1.5. The pH of the calcium salts was adjusted close to 11 by NaOH and rapidly added into phosphate solution. This results in precipitation of ACP which was filtered and washed thrice with distilled water. Further the precipitate was frozen in presence of liquid nitrogen and then lyophilised. The obtained powder was used for further characterisation. There are many factors that influence the synthesis of ACP. Initial concentrations of calcium and phosphate source are very crucial. At higher concentration (more than 750mM), crystalline phase is obtained whereas lower concentration (less than 250mM) gives rise to the amorphous phase as shown in Fig 1A. Secondly, pH also plays a decisive role. At alkaline pH (close to 11) there is abundance of PO_4^{3-} ions whereas acidic pH give rise to HPO_4^{2-} ions. Due to this, calcium to phosphate ratio in the final product is compromised. Temperature also plays a key role in synthesis of ACP. At elevated temperature (more than 40°C) crystalline phase was observed were as at ambient temperature (less than 30°C) amorphous phase was obtained. The ACP synthesised at optimum calcium and phosphate salt concentration, pH and temperature have revealed particle size of ~40 nm (Fig 1B).

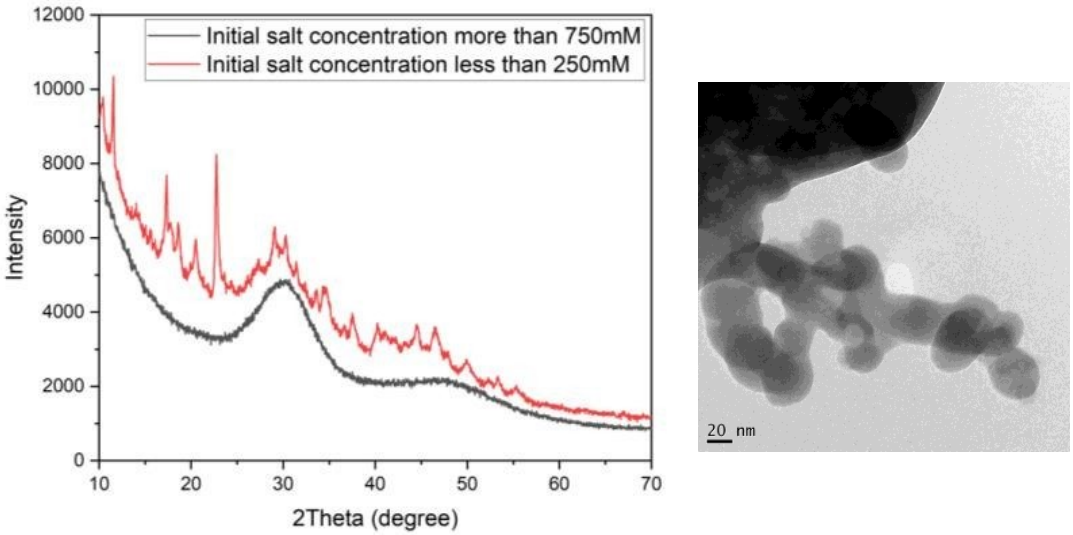

Figure 1: a) X-ray diffraction b) TEM of synthesised amorphous calcium phosphate.

Conclusions

Recent *in vivo* and *in vitro* studies have shown evidence that ACP acts as a precursor phase of apatite growth. This had made ACP a pivotal candidate in bone tissue regeneration application. The geometry of the apatite is 30–50 nm in size while maintaining thickness of ~2nm. Therefore, there is a need to synthesise ACP with particle size in this range. Factors such as temperature, pH, initial concentration of calcium and phosphate salts, affects synthesis of ACP which results in different particle sizes. Here we successfully demonstrate a standard procedure for synthesise ACP with suitable particle size required to form biological apatite.

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Cytocompatibility of calcium phosphate cements functionalized with biosurfactants

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Keywords: calcium phosphates, bone cements, biosurfactants, biocompatibility, antibacterial activity

Introduction

Over the years, to improve the properties of calcium phosphate cements (CPCs), various modifications have been applied. For example, different polymers [1], drugs [2], etc. were used to alter CPCs' properties. An interesting but still poorly studied group of CPCs modifiers are surfactants, i.e. surface active agents which can be used to obtain highly porous, foamed calcium phosphate bone cements (fCPCs) [3]. Many surfactants additionally possess interesting properties that can be transferred to the final materials. Biosurfactants, due to their biological activity such as antibacterial properties [4] are interesting compounds. In this study, two nonionic biosurfactants, namely a sucrose ester S0112 and saponin from *Quillaja* bark, were selected for foaming of CPCs. The research aimed to design and investigate the cytocompatibility of highly porous, foamed calcium phosphate-based cements (fCPCs) functionalized with biosurfactants.

Materials and Methods

The α-TCP powder, used as a solid phase of CPCs, was obtained by the wet chemical synthesis. The sucrose ester S0112 (SuE, sucrose palmitate min. 75%, Tokyo Chemical Industry Europe N.V., Belgium) and saponin from Quillaja bark S7900 (SAP, sapogenin min. 10%, Sigma Aldrich, Germany) were applied as biosurfactants. Cement without biosurfactant (fCTRL) served as a control material. The 2 $g \cdot L^{-1}$ aqueous solution of Na_2HPO_4 (POCH, Poland) was applied as a cement liquid phase. The foaming step was performed with the use of a food mixer (BOMANN, Germany). The cement foaming process depended on surfactants' solubility in water. Soluble saponin was introduced in the liquid phase (fSAP cement), whereas insoluble sucrose ester was introduced as a solid phase component (fSuE cement). To produce fSAP material, the mechanically foamed liquid phase was mixed with the solid phase (α-TCP). To obtain fSuE material, the solid phase of the cement (α-TCP, SuE) was mixed with the liquid phase and then the resulting cement paste was mechanically foamed. For all materials, the ratio of the liquid to the solid phase was 0.7 $g \cdot g^{-1}$. Indirect cytocompatibility studies of obtained cements were performed using MC3T3-E1 cells (mouse osteoblast-like cell line). Cell proliferation was studied by Alamar Blue assay, whereas their adhesion and morphology were evaluated by fluorescence stereomicroscope (Zeiss Axio Zoom.V16) using Hoescht 33342 staining, and scanning electron microscope (NovaNano SEM 2000).

Results and Discussion

The results of the Alamar Blue assay (**Figure 1**) show that up to 96 hours of incubation number of cells on the cement with sucrose ester was comparable to their number on the control cement without any surfactant (fCTRL). Interestingly, after 7 days of incubation, the absorbance for fSuE was 29% higher than for the control material. It was probably due to the ingrowth of cells into the three-dimensional porous fSuE cement after the initial lag phase. In the case of cement with saponin (fSAP) until 96 hours of incubation absorbance was reduced if compared with the fCTRL. After 7 days of incubation, it started to increase, but not as significantly as for fSuE cement. It was 6% higher than for the control material. Also, in this case, it was most likely caused by the cell ingrowth into the pores of the fCPCs.

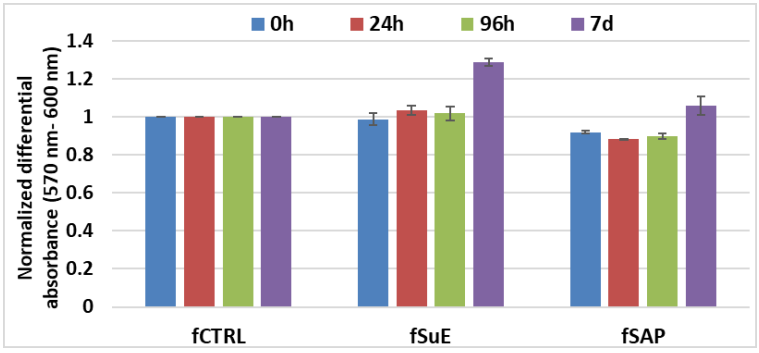

Figure 1: Cell proliferation on cement samples presented by normalized differential absorbance (Alamar Blue assay).

MC3T3 cells on the cement samples were visualized also by fluorescence and scanning electron microscopy examinations (**Figure 2A-B**).

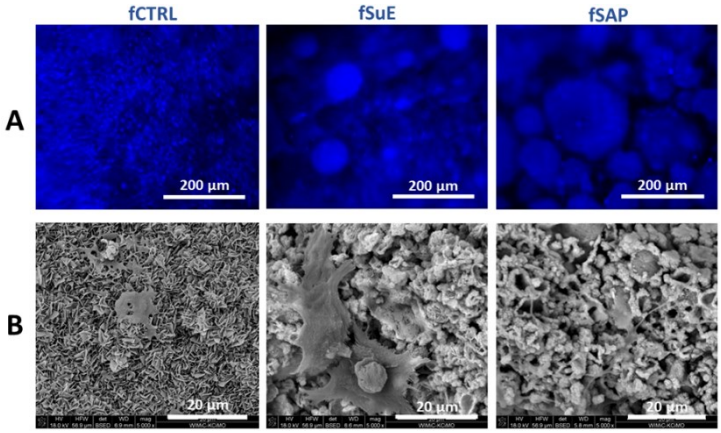

Figure 2: Cement samples with fixed cells visualised by (A) fluorescence microscopy – cells dyed with Hoechst 33342, (B) scanning electron microscopy.

They were the most numerous on the surface of fCTRL – probably because in the case of highly porous biosurfactant-foamed cements the cells were mainly located inside the pores.
The SEM images show strong anchorage of osteoblasts cultured on the studies cements, especially in the case of fSuE. Cells exhibited numerous and sometimes very long filopodia, particularly on the cement with saponin. The results suggest that obtained biosurfactant-functionalized fCPCs are fully cytocompatible concerning MC3T3 cells.

Conclusions and/or Outlook

In our study, biosurfactants such as saponin and sucrose ester were used to obtain functionalized, highly porous, foamed calcium phosphate bone cements (fCPCs). Biosurfactants, due to their biological activity, are an interesting alternative to foaming agents previously used in the manufacturing of these materials. fCPCs with biosurfactants (especially with sucrose ester) were cytocompatible and positively influenced MC3T3-E1 cells. Nevertheless, to confirm the materials' biocompatibility further *in vitro* and *in vivo* studies should be performed.

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Influence of tetraethoxysilane (TEOS) on physicochemical properties of α-tricalcium phosphate-based injectable bone substitutes

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Keywords: calcium phosphates, silane coupling agents, bone substitutes, natural polymers

Introduction

Although a variety of different commercial bone substitutes are available on the market, the problem of effective reconstructive treatment remains challenging, and new bone replacement materials are needed. An important group of them are calcium phosphate cements (CPCs), characterized by excellent bioactivity and similarity of their chemical composition to the inorganic part of human bone [1]. CPCs consist of a solid and a liquid phase which, when mixed, form a plastic paste with self-setting properties. Despite their unique biological properties, CPCs based solely on α-tricalcium phosphate (α-TCP) are usually brittle and non-injectable materials. To improve mechanical properties and obtain injectable pastes different natural polymers can be added to cement formulation (i.e. sodium alginate, citrus pectin, methylcellulose). Unfortunately, polymeric additives, besides providing beneficial properties to the material, often disrupt CPCs hardening by elongating their setting process [2, 3]. An innovative approach to overcome these issues is the use of silane coupling agents (SCAs) as modifiers of α-TCP. SCAs are chemical substances that can increase the adhesion between the individual material components by creating chemical and/or physical bonds between them. It is hypothesized that due to the SCAs' use obtained materials will be characterized by acceptable setting times while maintaining high mechanical properties and injectability. SCAs possessed strong cytotoxicity, according to the literature. However, their hydrolysis completely changes their nature and they become not toxic. In the case of developed CPCs, hydrolysis of the SCAs will occur during the mixing of the powder and liquid phases, thus before their application and potential contact with the cell environment.
In this study, the influence of tetraethoxysilane (TEOS) on the physicochemical properties of α-TCP-based bone substitutes was investigated.

Materials and Methods

The highly reactive α-TCP powder modified with 1 wt%, 2 wt%, or 5 wt% of TEOS and citrus pectin powder (4 wt%) were used as a powder phase (P) of the developed biomaterials. The 2 wt% aqueous solution of disodium hydrogen phosphate (Na_2HPO_4) was used as a liquid (L) phase (L/P = 0.5 g/g). The phase composition, setting times, compressive strength, and SEM microstructure were studied. Furthermore, the cell viability assay of the obtained materials was also investigated.

Results and Discussion

Studies have shown that due to the modification of α-TCP with SCA the obtained bone cements were characterized by altered properties if compared to the control material without coupling agent. The XRD analysis of obtained materials has revealed two crystalline phases – α-TCP and hydroxyapatite (HAp). The quantitative analysis showed that the amount of α-TCP was in the range of 88.3 – 92.7 wt% and the hydroxyapatite phase in the range of 7.3 – 11.7 wt.%. It was observed that silane coupling agent accelerates α-TCP hydrolysis to non-stoichiometric hydroxyapatite. The setting times of investigated CPCs were in the range of 4 - 11 ± 1 min and 8 - 19 ± 1 min. It was confirmed that an increasing amount of TEOS shortens both, initial and final setting time of α-TCP-based materials. The microstructure of the TEOS-modified material looks different in comparison to the microstructures of control material cement with unmodified α-TCP (Figure 1).

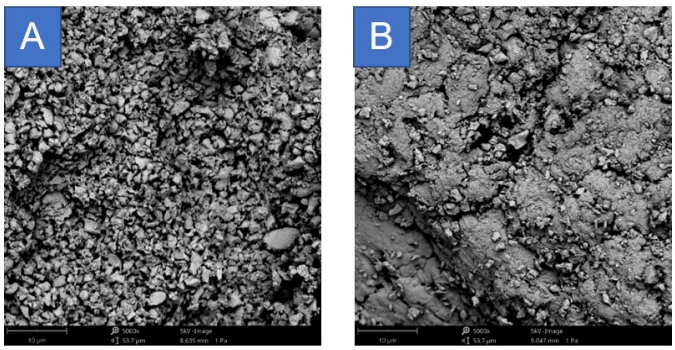

Figure 4. Microstructure of CPCs based on α-TCP (A) and TEOS-modified α-TCP (B)

It was observed that the individual α-TCP grains were more integrated with each other. What is more, the modified materials revealed better adhesion between the polymer and α-TCP grains, probably due to the formation of interactions between these components.

As predicted, the SCA modified materials showed no negative effect on the cells (Figure 2).

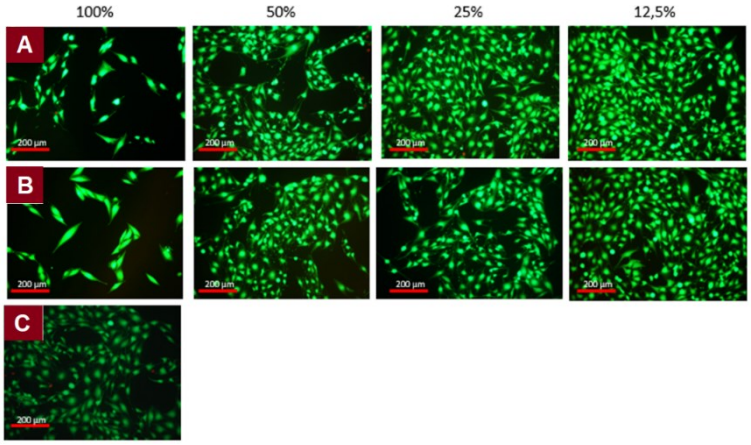

Figure 5. Live/dead cell viability assay of MG63 cells cultured in extract from: control unmodified α-TCP cement (A), TEOS-modified α-TCP cement (B), minimum essential medium (C)

The obtained results revealed that the use of TEOS resulted in the presence of different interactions between the coupling agent and other components of the developed CPCs. It has been showed thar the increasing amount of SCAs caused more significant changes in the materials properties. The unique properties of the developed materials result from the hydrolysis of α-TCP to non-stoichiometric hydroxyapatite, and hydrolysis of TEOS.

Conclusions and/or Outlook

The innovative usage of TEOS as modifier of α-TCP allows to obtain novel cementitious-type bone substitutes with beneficial properties, such as high compressive strength and injectability. Obtained materials combine beneficial properties of bioceramics and biopolymers and can be prospective self-setting biomaterials. Preliminary *in vitro* cell tests demonstrated the bioactive potential of obtained CPCs and paves the way for further *in vitro* and *in vivo* studies.

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Ultrasonic-assisted Micro-Arc Oxidation Deposition of Calcium Phosphate Coatings For Local Drug Delivery Applications

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Keywords: ultrasound-assisted micro-arc oxidation (UMAO); calcium phosphate coating; fluorouracil; interferon α-2b; vancomycin.

Introduction

Conventional treatment of bone disease, is usually connected with systematic supplemental treatment with drugs which could lead to some unwanted side effects such as systemic toxicity, short-term benefits, and patient inconvenience [1]. Implants that could provide localized therapy by carrying the needed drug to the implantation site and delivering it promptly devoid of these problems. However, there is a constant need for improvement in terms of more controlled drug release and precise admission of high local drug concentrations. The problem of pulse or burst release of drugs is a major obstacle for drug delivery systems and drug-loaded implants. From the point of perspective of new materials for drug delivery systems, calcium phosphate (CaP) based materials are amongst the most interesting. There is a still major challenge to manufacturing an efficient drug carrier system, especially for bone treatment applications. Here surface engineering of existing metallic implants plays a vital role. An electrochemical family of methods for metallic implant surface engineering is considered one of the most promising approaches to manufacturing drug delivery systems. The micro-arc oxidation (MAO) method, also known as plasma electrolytic oxidation (PEO), is a complex plasma-chemical and electrochemical method for the surface modification of medical implants. The MAO makes it possible to create strong, thick, and uniform CaP coatings with high adhesion to the metallic substrates having complex shapes. Recently, a modified MAO method where during the micro-arc discharges ultrasonic (US) vibrations in an electrolyte are introduced has been extensively researched [2, 3]. US modified MAO (UMAO) process that is designed for the drug delivery application have not been yet reported. In the present work we show the results of UMAO coating deposition with subsequent drugs immobilization and discuss the results of their performance *in vitro*.

Results and Discussion

In our work three types of coatings were deposited using: conventional MAO process, UMAO [3], and new hybrid method of UMAO deposition (UMAOH) where UMAO with US for 8 min and subsequent CMAO without US for 2 min was performed. The influence of the US field resulted in a slight decrease in thickness from 50 to 60 μm when comparing CMAO with UMAO or UMAOH types of coating. An increase in internal porosity from 28% to 42% and a decrease in surface porosity from 29 to 12% were attributed to the effect of US influence that resulted in the formation of macropores and areas of distorted spheres and their residues (**Figure 1**). The surface morphology of the MAO coating includes the structural spheroidal elements (spheres or hemispheres) with the pores located between spheres and across their morphology. The applied US during the course of UMAO at different powers did not influence on the size of the structural elements. Destruction of structural elements and a filling of pore spaces with fragments under the action of US vibrations led to an increasing the heterogeneity of the surface morphology, and, as a consequence, to an increase in the surface roughness (Ra) from 3.0 to 4.5 μm. Structural elements on the surface of coatings can be destroyed as a result of the impact of shock waves appearing during the collapse of cavitation bubbles in the electrolyte irradiating with ultrasound. The US application during the MAO led to formation of local macro-pores with the sizes of 15-30 μm in the coatings near the interface with the substrate. From **Figure 1** it could be concluded that the UMAOH mode of deposition is a perspective mode of deposition for drug-carrying purposes as the inner porosity and hence the volume where the drug could be stored is significantly increased.

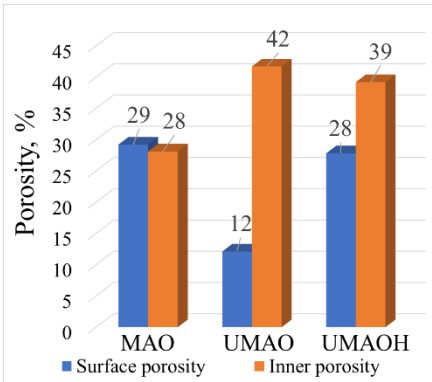

Figure 1. The surface and inner porosities of the MAO, UMAO, and UMAOH coatings

The XRD patterns of the US-assisted MAO coatings included both the diffuse scattering region and weak reflection peaks of the CaHPO4 (monetite, ICDD #00-009-0080) and β-$Ca_2P_2O_7$ (ICDD #00-009-0346). In this case, the intensity of the Ti reflection peaks decreased in the XRD patterns of the US-assisted MAO coatings compared to the control MAO coating. This may be result of the increasing the MAO/US thickness. It has been established that UMAOH coatings are characterized by a sufficient set of morphological and structural characteristics among the three studied groups such as Ra roughness equal to 3.0-4.0 μm, thickness in the range of 50-55 μm, and a combination of high values of internal and surface porosity which are equal to 39 and 28 %, respectively.

After that the UMAOH coatings were used substrates for drug delivery. Three groups of drug solution series were prepared by subsequent dilution: Vancomycin (VMN) (Elfa Laboratories, Russia) of 10, 25 and 50 mg/ml; 5-Fluorouracil (5-FU) (Lens-Pharm, Russia) of 10, 25 and 50 mg/ml; Interferon α-2b (IFNα) (Reaferon EC 5 x 10^6 IU, Vektor-Medika, Russia) of 0.1 x 10^6, 0.5 x 10^6 and 1 x 10^6 IU/ml. The UMAOH coated samples were soaked in 1 mL of drug solutions of each of the abovementioned concentrations for 1 hour at 25 ºC. It resulted in drug adsorption to the surface of the coating and inside the pore volume.

FT-IR spectra of as-deposited UMAOH CaP coating and the ones loaded with VMN, 5-FU, and IFNα drugs show bonds ascribed to PO_4^{-3} ions which are proved by an absorption band region in the range of 1031-1140 cm^{-1}. It has become evident that the signal from VMN and 5-FU drugs are superimposed with the signal from as-deposited UMAOH coatings. However, a a noticeable absorption in bonds related to C=O stretching at 1658 cm^{-1} for VMN and 5-FU loaded UMAOH CaP has been detected. This is a well-reported indication for drug adsorption that has been reported multiple times in the literature.

Kinetic curves of drug release are characterized by an initial burst, after which a sustained release begins. After 2 hours, the concentration of VMN for samples impregnated by immersion with drug concentrations of 10, 25, and 50 mg/ml is 0.006, 0.014, and 0.031 mg/ml, respectively; after 48 hours - 0.012, 0.022 and 0.36 mg / ml, respectively. The concentration of 5-FU in solution after 2 hours of exposure resulted in the following. The samples impregnated by immersion with drug concentrations of 10, 25 and 50 mg/ml, was 0.018, 0.036 and 0.036 mg/ml, respectively; after 48 hours - 0.046, 0.051 and 0.065 mg / ml, respectively. Hence, the therapeutic dose could be delivered to an implantation site and local tissues.

The results of the MTT-test indicate a weak cytotoxic effect of UMAOH CaP coatings in relation to represented cell lines (3T3, HeLa, MCF-7). Samples loaded with VMN show a weak cytotoxic influence on the 3T3 and Hela cell lines but to a greater extent affect the viability of the MCF-7 cell line, the number of living cells was 70, 71, and 54%, respectively. 5-FU have a significant cytotoxic effect in all studied cell lines. Viability diminished to 30 % in the case of the MCF-7 cell line. Furthermore, IFNα drug alone caused in vitro cell death of 3T3, HeLa, and especially MCF-7 tumour lines in the range of 10-20 % as compared with 100% viability in control cell culture. The antibacterial activity of VMN-loaded UMAOH coatings was proved for methicillin-resistant strain of S. aureus (MRSA ATCC4300) during the 7 days in disk-diffusion assay.

Conclusions

It has been established that UMAOH coatings deposition is a good candidate for drug delivery system when compared to other conventional MAO deposition route due to increase pore volume and therefore increased volume for drug storage. Drug loading into the UMAOH CaP coatings was confirmed by FT-IR, UV-vis desorption kinetics, and enzyme-linked immunosorbent assay in the case of IFNα drug. Kinetic curves are characterized by an initial burst, after which a sustained release of VMN or 5-FU begins. The cytotoxic effect of as-deposited UMAOH CaP coating was not observed for 3T3, HeLa, and MCF-7 cell lines. However, it was established that the UMAOH CaP coatings loaded with VMN of 5-FU enhanced caused cell damage in all cell lines which could be potentially used for enhancement of the cytostatic activity of drugs. Antibacterial activity of VMN-loaded UMAOH CaP coatings reached its peak at 24 hours of incubation, after which a monotonous decrease in activity was observed.

Acknowledgments

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Poster Session 1

The use of Bottlenose Dolphin umbilical cord mesenchymal stem cells in regenerative medicine and diagnostic tests

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Keywords: Bottlenose Dolphin stem cells, Regenerative Medicine, establishment cell line

Introduction
Mesenchymal stem cells (MSCs) have become a promising tool due to their peculiarity of being undifferentiated and pluripotent cells that can differentiate into many specialized cells. In marine mammals, studies on stem cells are sporadic, and there are little references available. To increase the knowledge of the stem cells in the cetaceans it is important to have the availability of established cell lines to use for toxicology and diagnostic assays. Here we describe the isolation, differentiation and immortalization of a cell line derived from the umbilical cord (UC) of a captive female bottlenose dolphin (Tursiops truncatus) collected during labour.

Results and discussion
In the laboratory, UC was submitted to enzymatic collagenase digestion (IA·Sigma-Aldrich) and was resuspended in growth medium (DMEM + 10% FBS; 100 U/ml penicillin and 100 µg/ml streptomycin, Sigma-Aldrich) at 37 °C with 5% CO_2 atmosphere. Cells isolated from the dolphin's umbilical cord (DOC) can proliferate and generate homogeneous colonies as well as prove to meet all the criteria for stem cells: cells are plastic adherent and they have a spindle-shaped morphology. Evaluation of osteogenic, chondrogenic and adipogenic differentiation, after addition of specific differentiation media, was performed by cytochemical staining to confirm presence of calcium production, intra- and extracellular glycosaminoglycan residues and intracellular accumulation of lipid-rich vacuoles. DOC-derived cells were transfected with a plasmid encoding the hTERT, adding the hygromicin-resistance gene (Addgene #1773). All hygromicin-resistant clones were isolated and expanded, and all of them proliferated at a faster rate than non-transfected DOC cells, which exhibited signs of senescence after 32 passages.

Conclusions
The DOC-MSC provides a novel *in vitro* model for cell and gene therapy, cloning and biotechnological applications. DOC-MSC can constitute the basis for biological treatments in wound healing and tissue regeneration in dolphins. In addition, due to the growing interest in viral diseases of aquatic mammals, these cells could represent the ideal substrate for in vitro virus isolation. Indeed, DOC-MSC could also be used as a fundamental tool for toxicology assays, as an alternative to animal models, to identify the cytotoxicity levels caused by chronic exposure to environmental pollutants (organochlorines and heavy metals), providing valuable information regarding conservation and protection of marine mammals.

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Incorporation of borate-based bioactive glass particles as inorganic filler in alginate-based hydrogels for bone tissue regeneration

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Keywords: bone tissue engineering, hydrogels, borate based bioactive glass particles, inorganic fillers

Introduction

Current research in tissue engineering (TE) is focusing on cell containing biomaterials, which have the ability to mimic human organ functions [1]. Bioactive glasses (BGs) can be used as inorganic fillers incorporated into alginate-based hydrogels to create composite hydrogels for bone TE with the ability to induce mineralization. To date mostly silicate based BGs have been considered as fillers in hydrogels ([2,3]. Sodium alginate is a well known biomaterial frequently used in drug delivery systems and/or in 3D bioprinting approaches [2,4]. However, due to its chemistry alginate shows a slow degradation rate and decreased cell interaction properties. In this work, an oxidized form of alginate, namely alginate dialdehyde (ADA), is used. The oxidation leads to the development of free aldehyde groups, which provide the ability to bind to proteins like gelatine (GEL) via Schiff's Base formation, forming ADA-GEL, a cell attractive hydrogel [2]. Inorganic fillers, such as borate based bioactive glass particles (size ~50 µm) (70-BBG, 70-B_2O_3-30-CaO mol%) synthesised by sol-gel represent an alternative to silicate BGs to enrich ADA-GEL hydrogels. The main goal of this study was to investigate the impact of these novel glass particles on ADA-GEL properties in terms of degradation/swelling behaviour and bioactive properties. Moreover, a plant-based biomolecule was incorporated into ADA-GEL-70-BBG hydrogels to investigate how the presence of BG particles would affect the release properties of ADA-GEL hydrogels. Furthermore, a crosslinking degree investigation was performed in the presence of 70-BBG to assess the influence of the particles on the covalent bonding between ADA and GEL. Moreover, a direct cell viability testing of cell containing films and 3D bioprinted scaffolds was performed by incorporation pre-osteoblast MC- 3T3 cells directly within ADA-GEL-70-BBG bioinks.

Results and Discussion

To investigate the impact on degradation and swelling behaviour of borate glasses within ADA-GEL films a degradation/swelling study was performed. Degradation/Swelling results revealed that none of the hydrogel-bioactive glass films started degrading after 28 days. It can be concluded that the incorporation of borate-based bioactive glass particles have no or little influence on the degradation behaviour of the ADA-GEL hydrogel films compared to pure ADA-GEL films.

In order to assess the bioactive properties of these novel glass particles a bioactivity study was performed in simulated body fluid (SBF). Two different concentrations of 70-BBGs were incorporated into ADA-GELhydrogel films, which were immersed in SBF solution for over 28 days. After 7 days of incubation FTIR spectra and XRD patterns revealed the formation of a hydroxyapatite (HAp) layer on the surface of the films. Moreover, SEM images and EDS analysis were used to confirm the mineralization effect provided by 70-BBG particles.

The release of phytotherapeutic agent from pure ADA-GEL films and 70-BBG-containing ADA-GEL films in HBSS was determined to estimate the impact of borate-based bioactive glasses on the release properties of ADA-GEL hydrogels. The lowest release was seen in samples without any BBG-particles, meaning that the presence of bioactive glass can promote a more rapid release. This effect may be caused by the particles disrupting the network created by ADA and GEL resulting in an accelerated FA release.

In this regard, a TNBS assay was performed in order to investigate the crosslinking between ADA and GEL in dependence of 70-BBG borate glass content. The results indicated a decreasing crosslinking degree with higher concentration of 70-BBGs. This effect might be explained due to higher presence of particles in the network which leads to weaker stability of ADA and GEL binding. Regarding this result, it may also be concluded that a limited concentration of 70-BBG particles within ADA-GEL hydrogels is required, in this case it was 0.5% (w/v).

In order to determine the cell viability of pre-osteoblastic MC-3T3 cells a calcein-DAPI staining of 3D printed scaffolds and films was performed. In both cases a high cell viability of the BBG-containing scaffolds could be observed compared to pure ADA-GEL considered as reference.

Conclusions and outlook

The study confirmed the advantages of incorporating inorganic fillers, such as borate-based bioactive glass particles, into ADA-GEL hydrogels. It can be concluded that these particles have no negative influence on the degradation and swelling properties of ADA-GEL hydrogels. The bioactive properties of these particles were

confirmed by a successful formation of a HAp layer on the surface of ADA-GEL films after immersion in SBF solution for 28 days. Moreover, borate-based particles seem to be a suitable incorporation to accelerate the release of phytotherapeutics from ADA-GEL films. Furthermore, a crosslinking investigation indicated a decreased crosslinking degree with increased borate-based particles content, therefore confirming that a limited concentration of 70-BBG within ADA-GEL hydrogels is required. All used bioinks showed a high cell viability when MC-3T3 cells were embedded in ADA-GEL films as well as in 3D bioprinted scaffolds. Therefore, the development of BG containing composite hydrogels should receive more attention especially in the fields ofbone tissue engineering, drug delivery approaches and wound healing applications.

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Ozoile-alginate microspheres for the therapeutic management of Crohn'sdisease

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Keywords: Ozonides; Ozoile; Hydrogel microspheres; Stimuli-responsive microspheres; Drug delivery systems.

Introduction

Ozone is a highly unstable gas with strong oxidizing capacity that can be successfully applied for medical treatments. The ozone effects on wounds healing and tissues repairing have been widely studied, whereas its oxidizing capacity has been tested in order to verify its disinfectant and sanitizing properties. Ozone reacts with the olephinic bonds of olive oil forming the ozonides, a class of chemical compounds that represent an alternativeto drug therapy due to their features. In this work, we used stable ozonides (Ozoile®) produced in a patented process. Ozoile® releases molecular oxygen driving the production of radical species with the generation of moderate oxidative stress in the environments characterized by a protonic increase, such as ischemic, hypoxic, ordamaged tissues [1].

Moderate oxidative stress stimulates the expression of numerous antioxidant enzymes by the activation of the nuclear factor-erythroid 2-related factor 2 (Nrf2). The generation of a moderate pro-oxidant environment leads tothe stimulation of molecular mechanisms that are able to suppress the inflammatory response. Moreover, the oxidative stress may induce the expression of the hypoxia-inducible factor-1α (HIF-1α), which is a transcription factor mainly associated to the adaptive responses of cells to hypoxia. HIF-1α induces the expression of hundredsof genes, among these there are several growth factors such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), transforming growth factor-α (TGF-α), and TGF-β, which are involved in the complex multi-step process of tissue regeneration [2].
In this work, Ozoile® was embedded into stimuli-responsive sodium alginate microspheres (SRAMs) with the aimto reach targeted inflammatory sites (**Figure 1**). In this perspective, Ozoile-SRAMs represent a new and smart technology for drug delivery in the therapeutic management of Crohn's disease (CD), which is a chronic inflammatory bowel pathology mainly affecting the ileum and colon. This disease is characterised by intestinal ulcers that can cause stenosis or fistulas. Here, the Ozoile-SRAMs could be effective releasing the active ozonideprinciple thus favouring the healing.

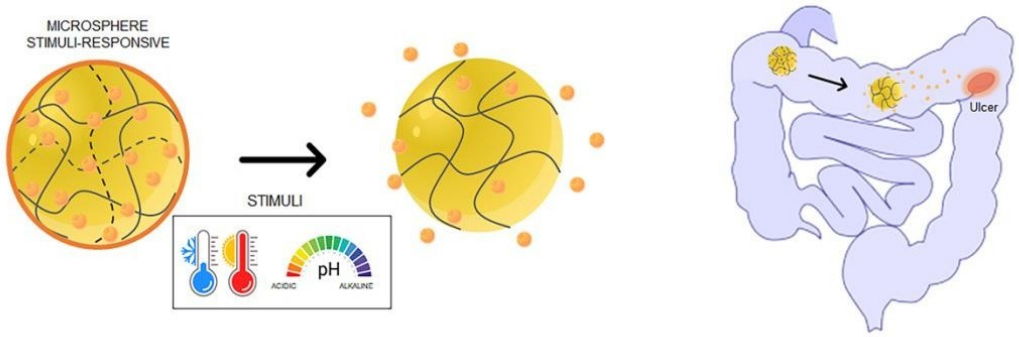

Figure 1. General mechanism of drug release in bowel by means pH and temperature-responsive microspheres.

Results and Discussion

In this work, we fabricated polymeric hydrogel microspheres based on sodium alginate and Ozoile® for potential use as an advanced drug delivery system in the bowel ulcers caused by Crohn's disease. The use of alginate provides several advantages including biocompatibility, ease of preparation, non-toxicity and biodegradability [3]. For this purpose, Ozoile® was dispersed in the alginate solution forming an emulsion using the high-intensity ultrasound (HIU) method. The physical effects of ultrasound induced by acoustic cavitation increase the breakdown of oil droplets, facilitating the formation of stable O/W emulsions with small droplet sizes [4]. The microspheres were prepared from an alginate-based emulsion with different concentrations of Ozoile® (from 10

to 50%). The emulsification process was optimised by varying the types and concentrations of surfactants and stabilisers. For the fabrication of Ozoile-SRAMs, the extrusion-dripping technique was used [5]. In addition, the Ozoile-SRAMs were coated by a chitosan layer, with the aim to improve and control the drug release capacity.

Moreover, poly(N-isopropylacrylamide) (PNIPAm) was also integrated in the microspheres, in order to make them responsive to temperature variations as well. **Figure 2** shows a schematic representation of the fabrication processof the Ozoile-SRAMs. Due to the response to specific internal or external triggers, these microspheres can increasethe drug targeting efficacy, and at the same time, they can reduce the side effects/toxicities of payloads, which arethe key factors for improving the patient compliance.

The chemical properties, thermal stability, microstructure and morphological changes of the Ozoile-SRAMs were evaluated by Fourier transform infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC), and scanning electron microscopy (SEM). Overall, the results from this experimental characterization were useful to assess the potential use of the Ozoile-SRAMs in the therapeutic management of Crohn's disease.

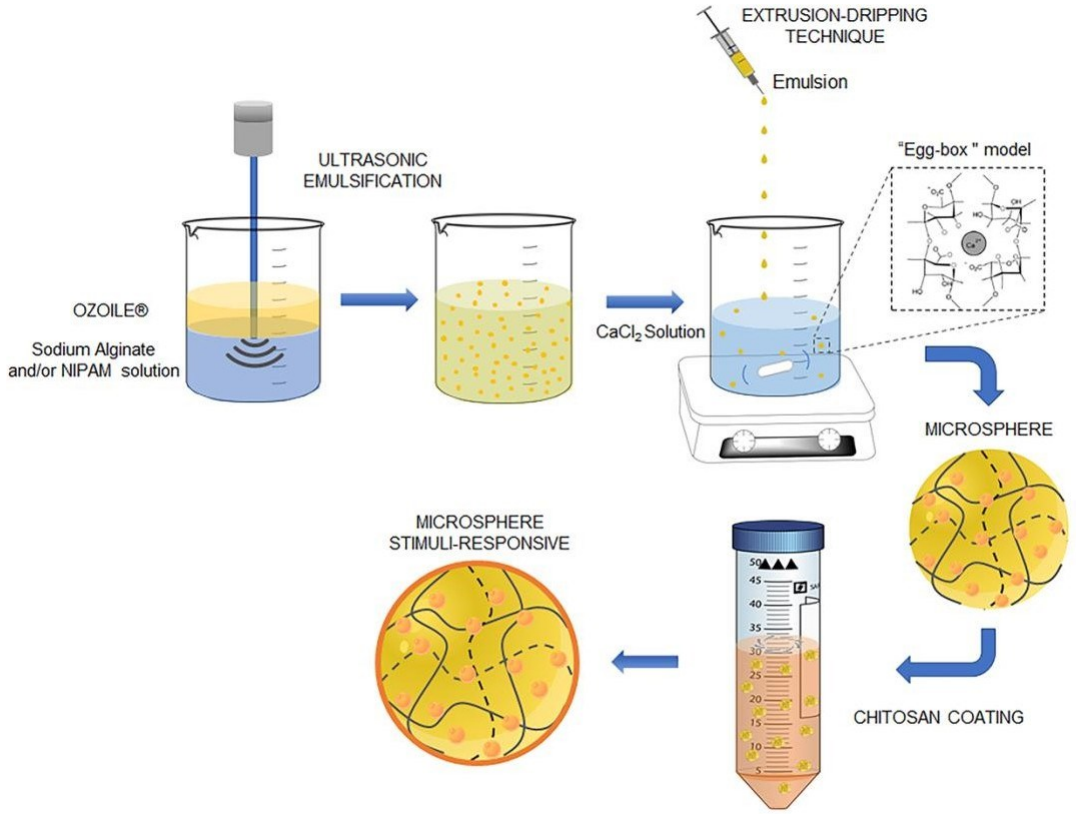

Figure 2. Schematic representation of the fabrication process of Ozoile-SRAMs.

Acknowledgments

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Synthesis and chracterization of rGO/PDA/alginate hydrogel as possible wound dressing

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Keywords: hydrogel, alginate, reduced grapehene oxide, conductive, self-healing

Introduction

Wounds in general are a set of complex pathologies affecting humans, linked to expensive and extensive treatments around the world and producing an economic, medical, and personal burden. The lack of therapeutical approaches to treat both chronic and acute wounds make it essential to search for new materials to generate adequate tissue recovery, to create a favorable environment for healing, and to improve the patient's experience [1]. Hydrogels are promising candidates for these purposes, mainly because of their absorption capability and their functionalization with other compounds[2]. In this study, biomaterial was developed from reduced graphene oxide, (rGO), polydopamine (PDA) and alginate to create a hydrogel dressing that promotes wound healing and this biomaterial was physicochemically characterized.

rGO gives conductivity and mechanical resistance characteristics to the material, necessary to promote cell power in the healing process[3]. PDA, for its part, provides adhesive characteristics on practically any surface and behaves as a reducing agent for graphene oxide[4]. Finally, the alginate gives the hydrogel the biocompatibility and bioactivity necessary to avoid generating any type of alteration in the living tissue, and it also acts as a thickening and gelling agent in the mixture[5].

A method for the synthesis of rGO/PDA/alginate hydrogel is therefore proposed through a functionalization process of rGO with PDA, a mechanism of reduction and the coating of GO through oxidative self-polymerization of DA and a subsequent secondary functionalization with alginate, through physical crosslinking and electrostatic interactions. Important factors to control in this process are the pH, temperature, proportion of GO-DA oxide and the type of crosslinking. The hydrogels were characterized by FT-IR spectroscopy, Raman spectroscopy, UV-Vis spectroscopy, scanning electronic microscopy (SEM), swelling to different pH, self-healing, conductivity, among others.

Results and Discussion

The chemical analysis of materials shows in the FTIR spectrum, comparing the rGO with GO spectrum, a decrease in the 3370 $cm{-}1$ peak, demonstrating that the hydroxyl group was significantly removed by reduction. Furthermore, in comparison with the GO, the intensity of the 1716 $cm{-}1$ peak almost completely disappeared, which represents the C=O stretching of the carboxyl. In addition, this spectrum exhibits a small peak at 1462 $cm{-}1$, which represents C=C aromatic stretching and N-H bending caused by DA, and the peaks around 1000 and 1300 $cm{-}1$ were weakened due to the removal of oxygen-containing groups, checking the reduction of GO.

Then, UV-vis spectroscopy shows the characteristic peaks of GO at 300 nm and 253 nm. The rGO presented only one peak at 252 nm, this shift being explained by a change in the electronic configuration of graphene, which is restored after reduction with DA.

In the rGO Raman spectrum, the 2D band decreased, caused by a lower presence of oxygenated functional groups in the structure through the GO reduction. The ratio ID/IG was 1.013 for GO, which increased after reduction to 1.026.

The rGO morphology after SEM analysis shows a large number of sheets, which after the PDA modification were observed to be finely wrinkled in irregular and disordered layers, which confirms the PDA coating on GO.

The swelling capacity of the hydrogel (Figure 1) was different depending on pH, being 577% for water (pH 5.7), 619% for basic solution (pH 10) and 415% for acid solution (pH 3). This difference is caused by the acidic or basic functional groups in their structure, which accept or donate protons in response to a change in solution pH.

The self-healing test demonstrated that the hydrogels are capable of compose without the need for an external agent, thanks to the multiple and reversible interactions of the components.

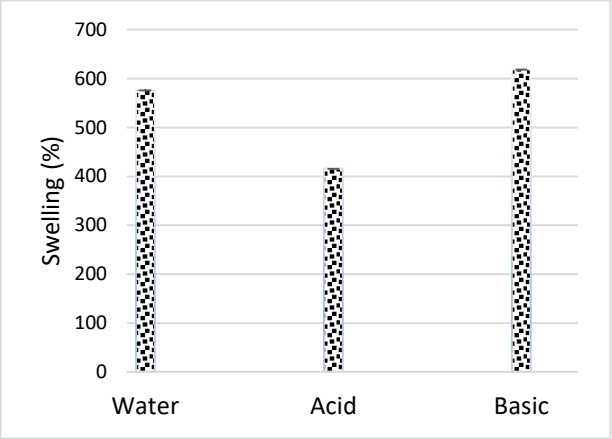

Figure 1: Swelling capacity of the hydrogel at the equilibrium, in water, acid and basic media.

Conclusions and/or Outlook

The designed hydrogel presented properties of mechanical resistance, adhesiveness, swelling capacity, conductivity and self-healing, all of which are desirable in dressings for chronic and acute wounds.

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Regenerative Medicine by stem cells and bioinformatics study:repair of articular hyaline

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Keywords: Stem cells, adipose tissue, bone regeneration

Introduction

This study aimed at isolating cells from equine adipose tissue and evaluating their ability to differentiate and establish an alternative approach to the treatment of osteochondral lesions. Briefly, it highlights how the potential of chondrocytes in vitrogrowth can be inserted in a possible site of inoculation of traumatic injury.
Furthermore, through bioinformatics studies, an algorithm was developed to identify genes, named "leader genes", which play a key role in osteogenesis given their high number of interactions with the other genes involved in the same process.

Results and Discussion

A horse with a fracture of the carpal joint cartilage was selected for the study to evaluate the reparative ability of in vivo tracheal chondrocytes. The differentiation potential of horse stem cells towards lineages, osteogenic, adipogenic and chondrogenic was evaluated during stimulation.
The protocol allowed to isolate a homogeneous population of cells with fibroblastoid-like morphology from collected tissue samples.
At same time genes involved or potentially involved in osteogenesis were identified with systematic interrelated queries of several databases until identification of a final set. Interactions among genes were mapped and given a significance score using STRING database. The genes in the highest cluster were termed as leader genes.
The chondrocytes stimulated by osteogenic differentiation showed a change of morphology and an extracellular matrix mineralization. In chondrogenic differentiation, deposits of intra- and extra-cellular glycosaminoglycans were observed.
Adipogenic differentiation was observed in rounded cells with lipid vesicles in the cytoplasm.
Leader genes were identified through a bioinformatic study. In particular, our interest focused the genes more closely relatedto production and differentiation of stem cells such as COL2A1.

Conclusions

The histological investigations prove the successful achievement of a significantly high degree of hyaline over fibrous cartilage.

A validated bioinformatic algorithm was applied to increase our knowledge of molecular mechanisms of osteogenesis and toperform targeted experimentation focused on leader genes. Immunocytochemistry characterization was suggested by the results of the investigation on the peculiar role of the leader genes identified by our bioinformatics approach.

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Melt-electrowritten PCL scaffolds loaded with methacrylated fibroin and human bone powder for bone tissue engineering applications

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Keywords: Melt-electrowriting, bone, osteoblast, fibroin, tissue engineering

Introduction

Melt electrospinning and melt electrowriting (MEW) are two fabrication techniques that are acquiring an increasing relevance in the field of tissue engineering. MEW not only enables the production of physiological 3D scaffolds with cell-invasive ability, but also to obtain structural features in the macro and in the micro scale, such as scaffold porosity, fiber diameter and fiber alignment to match tissue-specific requirements (1).

MEW can be successfully employed in bone tissue engineering for guided bone regeneration (GBR) a procedure of a ridge augmentation technique that allows to regenerate enough bone on the damaged site for a successful implant placement. However, MEW involves a complex interaction between different variables such as material properties, processing parameters and ambient conditions. Therefore, the possibility to establish a stable charged jet and a successive control over fiber diameter necessitate an understanding of these variables and their effects for an optimization of the process. Moreover, to improve the osteoconductivity of synthetic scaffolds the capacity of combining materials and bioactive components is critical to achieve increased bone regeneration.

The final objective of this work is to evaluate the possible combination of melt electrowritten Polycaprolactone (PCL) synthetic scaffolds and extruded naturally derived materials such as methacylated silk fibroin (SilMA) along with decellularized bone matrix starting from singular components.

Results and Discussion

Scaffolds were fabricated using the previously described Melt Electrowriting technology applied to a medical grade PCL formulation, through the RegenHU 3D discovery bioprinter. In an iterative way, different trials were performed in order to obtain the right processing parameters allowing a stable charged jet and an adequate fiber diameter. After some optimization procedures, an adequate set of parameters was found, enabling the fabrication of 100 layers - constructs and reproducibility of the process. To find an optimal balance among biological and mechanical behaviours of scaffolds, two different pore geometries were considered. A square pore geometry was produced alternating vertical filling paths with line space of 500 μm and a triangular porosity was also investigated. In this last case, scaffolds were fabricated alternating 30 horizontal filling paths with line space of 500 μm and 60 diagonal filling paths, 30 out of 60 oriented at 60° and the rest at 120° with respect to the horizontal axis with the same line space. Representative pictures taken with SEM are reported in Figure 1.

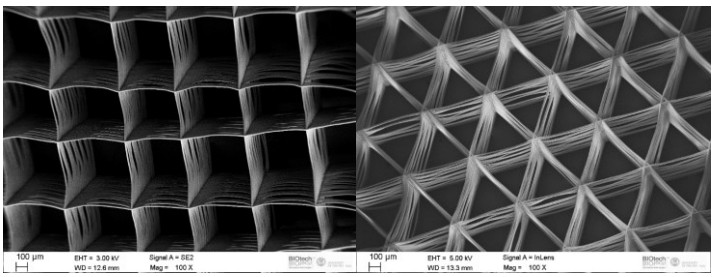

Figure 1: representative pictures taken with SEM of square and triangle-shaped constructs

To prove the feasibility of the manufacture of hybrid scaffold endowed with osteogenic ability, constructs were filled with bone powder previously micro-fragmentated and mixed with the hydrogel SilMA. Tensil stress tests were performed on scaffolds to understand the effect of the number of layers and the addition of Sil-MA/bone powder suspension. As expected, thicker samples show better mechanical properties than samples with a lower number of layers. Furthermore, the addition of Sil-MA/bone powder suspension on MEW PCL scaffolds, at equal number of layers, lead to an increase in all the studied parameters confirming the improved mechanical properties.

Finally, biological tests were performed using adipose-derived stem cells seeded on the hybrid scaffold; viability and cell proliferation assays were performed and revealed that the presence of Sil-MA with or without bone particles increased significantly the cellular proliferation as compared to PCL-scaffold alone.

Conclusions and/or Outlook

In conclusion, we have exploited a MEW approach to obtain PCL- scaffolds that in a second stage, were combined with Sil-MA and fragmentated bone powder to produce a hybrid scaffold suitable for applications of tissue engineering. Geometrical and structural parameters were optimized to achieve best mechanical properties and to allow cell seeding. This work represent a valuable proof of concept for further development of hybrid scaffolds based on MEW-technology

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Improving the printability of hyaluronic acid/gelatin bioinks by pre-crosslinking

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Keywords: hydrogels, printability, oxidized hyaluronic acid, gelatine

Introduction

Biomaterials frequently used in 3D printing and biofabrication are hydrogels. Hydrogels are defined as water-swollen hydrophilic polymers that are crosslinked to form a 3D matrix. Through their microstructure they mimic the native extracellular matrix (ECM) in the body, thus providing an artificial matrix for the encapsulation of cells, enabling the opportunity to fabricate engineered tissue structures. Hydrogels are highly variable in chemistry, mechanical properties and degradation/sweeling rate and can hence be modified towards different applications. For biofabrication, hydrogels are required to have an ideal viscosity with shear-thinning properties while providing the possibility to be post-crosslinked to achieve a stable hydrogel after printing [1]. For the incorporation of cells all printing and crosslinking processes should be cell-friendly and thus exclude high temperatures (> 37°C), toxic chemicals and exposure to strong UV light [2]. Hyaluronic acid (HA) is a biocompatible and biodegradable polysaccharide present in the human body ECM with a high water affinity [3]. Through an oxidation process aldehyde groups are formed in the HA chain which can be used to covalently crosslink the oxidized hyaluronic acid (OHA) to amino groups in proteins, such as gelatine by Schiff base formation [3]. To improve the viscosity of the OHA hydrogel precursor for bioprinting, pre-crosslinker can be added prior to printing, partially crosslinking the polymer solution. Adipic acid dihydrazide (ADH) has been used previously in conjunction with OHA in the development of an injectable hydrogel system [4]. Upon the addition of ADH, which contains amino groups, free aldehyde groups are crosslinked with each other through Schiff's base formation [5]. Microbial transglutaminase (mTG) is an enzyme native to the human body catalyzing the formation of an amide crosslink and can hence be used for the connection of free amino groups in proteins, such as gelatine. These agents can be used to pre-crosslink the OHA and gelatine hydrogel in an *in-situ* approach. In this study, different concentrations of ADH and mTG were incorporated into the hydrogel material and the effects on printability were examined. Frequency sweep experiments were performed to compare the shear thinning properties and viscosity of the hydrogel precursors containing different ratios of pre-crosslinkers. Standardized tests for the determination of printability were used to find the ideal composition of pre-crosslinker, including manual dispension [6], filament fusion test (FFT), filament collapse test (FCT), and grid structure test (GST) [7]. The pre-crosslinked inks and pre-crosslinker free control were extruded into well plates and post crosslinked. The samples were incubated at cell culture conditions for several weeks to investigate stability by determining degradation and swelling by change in weight.

Results and Discussion

Rheological measurement revealed a significant increase in viscosity and shear-thinning behaviour at 37°C upon the addition of an increasing amount of pre-crosslinker. After 40 min of crosslinking time the viscosity increased from 1.7 Pas for pre-crosslinker free hydrogel to 5.2 Pas, 13.2 Pas, and 186 Pas for 5%, 10%, and 15% of mTG added respectively, for an angular frequency of 1 rad/s. This result indicates an increasing formation of imide bonds between free amino groups of the gelatine chains. Further, printability tests at 37°C after 10, 20, 30, 40 and 50 min showed improved printability with increasing crosslinking time. Compared to the pre-crosslinked samples, hydrogels without pre-crosslinker were not printable at 37°C due to a low viscosity.

By the degradation study, the pre-crosslinked constructs were found to be more stable over a course of 28 days at cell culture conditions compared to the pre-crosslinker-free control. The results indicates overall a more homogenous and higher density of crosslinking points in the hydrogel constructs

Conclusions and Outlook

In the course of this study different agents were investigated towards their use as pre-crosslinker for OHA and gelatine hydrogel inks. Frequency sweeps and printability tests showed an increasing viscosity with increasing amount of crosslinker, as well as crosslinking time and hence an improved printability at 37°C. These results indicate the possibility to modify the crosslinking time and viscosity towards different requirements of applications. Further, the possibility to print at 37°C, which is usually not possible for gelatine containing inks, offers a wide range of advantages towards the encapsulation and viability of cells. The decrease of degradation by pre-crosslinking further improves the applicability of this OHA and gelatine hydrogel for the application in bioprinting and tissue engineering.

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Effect of gallium on physicochemical characteristics of hydroxyapatite

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Keywords: hydroxyapatite, gallium, substitution, wet chemical precipitation

Introduction

Hydroxyapatite (HAp) is one of the most widely used biomaterials for bone regeneration. Substituted HAp nanoparticles with biologically active cations, such as Mg^{2+}, Zn^{2+} and Sr^{2+} etc., are considered as valuable sources for a continuous and sustainable release/delivery of these ions [1]. Ga^{3+} ions have been reported to be clinically effective against bone resorption and for the treatment of osteoporosis and cancer-related hypercalcemia [2]. In addition, antibacterial Ga-based materials have a great potential in bone tissue engineering [3]. However, the delivery of Ga^{3+} needs to be finely tuned. Incorporated in HAp, Ga gives extended possibility for a controlled and local ion release. Thus, the Ga-containing HAp can be considered as an alternative way to deliver Ga^{3+} ions at a site of defect, where they might assist in regeneration of bone and inhibiting the occurrence of biomaterials-related infections. In this study, we focus on the evaluation of the effect of Ga on the physicochemical properties of HAp such as phase purity, crystallinity, morphology, Ga^{3+} ion release, *etc.*

Results and Discussion

Low crystallinity Ga-containing HAp powders were synthesized using wet chemical precipitation by adding various amounts of Ga (0, 2, 4, 6.3 and 8 wt%). In the XRD patterns only characteristic HAp peaks were detected. Broadening of the peaks with increasing Ga content was observed indicating decrease in crystallinity.

FTIR analysis confirmed the phase purity of the products as only absorbance bands for HAp chemical groups were detected: $[PO_4]$ at 472, 560, 600, 963, 1020, 1090 cm^{-1}, $[HPO_4]$ at 875 cm^{-1}, and [OH] at 630, 3570 cm^{-1}.

The actual Ga content in the synthesis products was measured by ICP-MS and the specific surface area was determined by BET method (SSA_{BET}), the particle sizes (d_{BET}) calculated by taking into account the true density (ρ) determined by He pycnometry. Measured Ga concentrations were slightly lower in comparison with the nominal ones. This could indicate that part of the Ga^{3+} ions remained in the synthesis medium (liquid phase) and was removed by filtration. All synthesized powders were nano-sized (d_{BET} in a range from 18 to 31 nm). A significant effect of Ga content on the morphology was observed, namely, the SSA_{BET} decreased and, accordingly, d_{BET} increasd. However, it cannot be unequivocally stated from the results described above that Ga is incorporated into the structure of HAp. The observed effect of Ga can have two potential explanations that need to be confirmed by additional analyzes: (a) Ga replaces Ca as a result of heterovalent substitution and consequently produces distortions in the framework of HAp matrix due to different ionic radius (0.62 Å for Ga^{3+} and 0.99 Å for Ca^{2+}) and charge; (b) Ga remains fixed in the form of solid solution of intercalation.

Ga^{3+} ions' release from the HAp nanoparticles in Dulbecco′s Modified Eagle′s Medium (DMEM) was measured up to 21 days and the results are summarized in Table 1. The amount of Ga^{3+} ions released is directly proportional to the Ga concentration in the HAp nanoparticles.

Ga concentration in the HAp nanoparticles [wt%]	Total released Ga^{3+} ions in 21 days	
	[%]	[mg]
1.6 ± 0.1	42.9 ± 0.9	0.222 ± 0.004
3.3 ± 0.4	36.4 ± 2.7	0.377 ± 0.028
5.5 ± 0.1	36.4 ± 1.1	1.056 ± 0.033
6.9 ± 0.5	42.2 ± 0.8	1.309 ± 0.023

Table 1: Ga^{3+} ions' release from the HAp nanoparticles in DMEM

Conclusions

Nano-sized hydroxyapatite with various gallium concentration was successfully synthesized. The most significant effect of gallium on the hydroxyapatite was reflected in the decrease in crystallinity with the increase in gallium content, as well as in the kinetics of ion release. The obtained nanosized hydroxyapatite particles assure a prolonged supply of Ga^{3+} ions.

Acknowledgement

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A gradient mineralisation technique for fascicle-inspired electrospun bundles to be used for enthesis regeneration

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Keywords: Electrospun scaffolds; gradient mineralization; enthesis; ligament and tendon regeneration;

Introduction

The regeneration of an injured tendon or ligament (T/L) using artificial grafts is a complex process pertaining to the graft's biomimetic morphology and mechanical properties[1]. The part of T/L that attaches to bone (i.e., enthesis) is critical since it has been observed to have a progressive gradient of mineral content. This gradient of composition also corresponds to a gradient of mechanical properties as mineralised fibroblasts/tenocytes from T/L merges with osteocytes in the bone tissue[2].

Electrospinning is a versatile technique to obtain polymer nanofiber-based scaffolds mimicking the collagen fibril's hierarchical organisation of T/L and their enthesis[3]. Resorbable electrospun nanofibrous scaffolds mimicking the morphology of tendon fascicles were developed by some of the authors in a previous study, where a physiological 3D arrangement of the fibres with adequate cell adhesion was demonstrated[4].

Results and Discussion

In this study, a polymer solution of Poly-L-Lactic acid (PLLA) blended with collagen was electrospun on a drum collector to obtain aligned nanofibers which were then rolled manually to produce fascicle-inspired bundles. These bundles were then crosslinked with Ethyl (dimethylamino propyl) carbodiimide (EDC) and N- hydroxysuccinimide (NHS) to retain the collagen inside the fibres.

Mineralization of these bundles involves depositing Calcium and Phosphate compounds on the surface of the nanofibers to increase adhesion and differentiation of osteoblasts upon implantation. This was achieved by suspending the bundles in a supersaturated calcifying solution5 of Calcium and Phosphate ions at 37 C to facilitate a gradient coating of minerals on the surface of the nanofibers.

Optimised immersion of the nanofibrous bundles in supersaturated calcifying solution provided a homogenous gradient coating of minerals on the surface of the nanofibers (Figure 1). The deposits consisted of star-like formation of calcification spread across the aligned nanofibers as viewed at 8000x magnification using scanning electron microscope. Energy dispersive X-ray spectroscopy analysis was performed to confirm the presence of calcium and phosphate on the nanofibers.

Cytotoxicity analyses were performed after sterilization of the bundles with gamma rays. The cytotoxicity assay was conducted according to the ISO 10993 protocol to measure the cell viability based on absorbance values in three different settings of cell contact with the bundles (direct, indirect, and extract). It was observed that the difference in cell viability between control groups and experimental groups were negligible, i.e., the bundles had no significant toxicity towards adherent cells in all the three tests. Preliminary mechanical tensile tests were carried out confirming an increment in stiffness of the scaffolds.

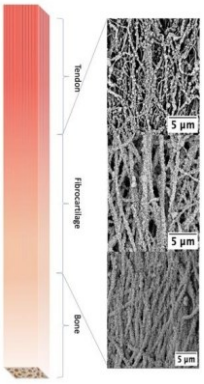

Figure 1: SEM images (8000x) of mineralised bundles of PLLA-Collagen displaying gradient deposits of calcification

Conclusion and Outlook

These preliminary results suggest that using a supersaturated solution is effective to form gradient mineralisation on a biomimetic scaffold to be used for enthesis regeneration.

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Injectable biomimetic microparticles for *in situ* myocardial regeneration: a comparison between traditional and microfluidic fabrication techniques

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Keywords: alginate, gelatin, emulsion, microfluidic, H9C2

Introduction

Heart disease is a leading cause of morbidity and mortality worldwide. As cardiac cells are completely differentiated shortly after birth, after a massive cell loss due to ischemia, the myocardial tissue lacks intrinsic regeneration capability. Currently available clinical treatments do not directly repair damaged myocardium, therefore patients may experience other debilitating cardiac conditions as time progresses. Therefore, scientists and surgeons are continuously looking for new strategies to regenerate the injured heart. In the last decade, cardiac tissue engineering has made considerable progress [1]. Among the different cardiac tissue engineering approaches, the implantation on the damaged cardiac tissue of injectable scaffolds, which create a friendly environment for the proliferation and differentiation of implanted or migrated cells, is receiving a great interest. This approach is in fact clinically appealing, as it is minimally invasive, differently from the traditional approach based on the epicardial implantation of an *in vitro* engineered cardiac patch. Traditionally, injectable scaffolds are obtained using strategies such as thermoreversibility, *in situ* cross-linking and self-assembling peptides [2]. The use of injectable microparticles is an innovative strategy. Microparticles offer several advantages to favour cell adhesion and tissue growth, such as a high surface to volume ratio and the possibility to be easily loaded with bioactive agents. Few studies in literature report the application of microparticles as injectable scaffolds in cardiac tissue engineering [3-5]. Ideally, polymeric microparticles intended for use as support for tissue growth should have a regular shape and homogeneous dimensions. The mostly investigated technique in literature for obtaining polymeric microparticles is emulsion; however, one of the main limitations of this procedure is the difficulty to produce particles with a narrow dimensional range. This drawback could be overcome using an innovative and more advanced technique for the production of polymer microparticles, based on the use of microfluidic platforms. These latter are devices containing micrometric channels, that allow the manipulation of small volumes of fluids [6]. Microfluidic devices can offer several advantages with respect to the emulsion techniques for the manufacture of microparticles, including: more stable reaction conditions; less reagent consumption; production of microparticles with controlled shape, size and composition; fabrication of monodisperse microparticles; precise regulation of particle properties. These conditions can be simply achieved by adjusting parameters like channel geometry, flow rates, solution viscosity, wettability and surface tension. In addition, the mild reaction conditions permit the entrapment of easily denaturable bioactive agents and particles with highly reproducible release profiles can be obtained.

In this work, biomimetic microparticles to be used as injectable scaffold for myocardial tissue engineering, were produced both by traditional and microfluidic techniques in order to obtain a comparison of their properties. A blend of alginate and gelatin, mimicking the composition and the interactions occurring among components in the extracellular matrix of the native cardiac tissue, was used as injectable scaffold material. A similar blend was already successfully investigated by our research group as material for the fabrication of preformed three dimensional spongy scaffolds [7]. Obtained microparticles were characterized by morphological, physicochemical and functional analyses. Preliminary cell culture tests with H9C2 cardiomyocytes were also carried out.

Results and Discussion

Alginate/gelatin microparticles were firstly produced by a water-in-oil emulsion. The water phase was made by a 2% (w/v) solution of the two biopolymers (weight ratio 20/80 between alginate and gelatin, respectively) in bi-distilled water; the oil phase was a solution of isooctane, containing Span 80 as emulsifier. Obtained microparticles underwent a double cross-linking treatment, to ionically cross-link alginate and chemically cross-link gelatin, and finally freeze-dried.

Morphological analysis was carried out by scanning electron microscopy. SEM images revealed that the prepared microparticles had a spherical shape and a quite large size distribution.

Particles were also observed under an optical microscope, to evaluate their average dry diameter and size distribution. After the determination of the average dry diameter, a swelling test was carried out. Particles were immersed in phosphate buffer solution (pH = 7.4) for 1 h. Then they were observed again under the optical microscope and the average diameter of swollen particles was determined. Obtained particles had an average dried diameter of 147 ± 56 μm and an average swollen diameter of 352 ± 68 μm. The swelling degree was therefore of 139 %.

Fourier transformed infrared analysis was carried out. The microparticles spectrum confirmed the presence of the typical adsorption peaks of gelatin and alginate. Sample homogeneity was also investigated by Chemical Imaging.

Spectra derived from the chemical map confirmed the presence of the two biopolymers in all the analyzed volume. Moreover, the chemical map showed the morphology of solid particles.
The *in vitro* hydrolytic degradation properties of alginate/gelatin microparticles were evaluated in PBS. Microparticle weight loss during degradation was determined by measuring changes in dry weight after incubation for fixed time periods. The degradation kinetic was characterized by a first quick phase (during the first 3 days), with a percentage of weight loss of 12 ± 3%. Then, the degradation occurred more slowly, reaching 37 ± 5% of weight loss after 30 days.
A preliminary *in vitro* cell proliferation test was performed in static conditions, using rat embryo ventricular cardiomyocytes of the H9C2 cell line. Microparticles were sterilized by washing with 70% (v/v) ethanol solution in sterile water, followed by UV-exposure for 15 min. Then they were placed in a 24-well plate and seeded with H9C2 cardiomyocytes. Cells were also cultured directly on the wells of the tissue culture plates, as control. At appointed times, cell viability was evaluated by MTS test. The cell proliferation level observed on the particles was comparable to that observed on the control, suggesting the potential use of alginate/gelatin microparticles as support for cardiac tissue engineering.
The following step of our work was the production of injectable alginate/gelatin microspheres by microfluidics. Microparticles were produced using an alginate/gelatin (with 20:80 weight ratio, respectively) aqueous solution, as dispersed phase, and a vegetal oil with Span 80 as emulsifier, as continuous phase, in a microfluidic flow focusing device. The device was connected with syringe pumps through plastic tubings and fixed to a microscope to allow for real-time observation of microparticles formation. Several tests were performed varying different parameters, such as the concentration of the dispersed phase, the surfactant concentration, the flow rate of the continuous and dispersed phase. The experimental setup was defined first in a device with 1 mm square channels and then moving to a microfluidic device with channels of 100 μm. Alginate/gelatin droplets, exiting from the outlet and passing through the connected plastic tubing, were then collected in a cross-linking bath, to stabilize both the protein and the polysaccharide components.
Preliminary morphological tests showed the obtainment of highly monodispersed microparticles, with a spherical shape and an average diameter of 92 ± 3 μm.
All the other characterization tests, previously performed on alginate/gelatin microparticles produced by emulsion, are currently ongoing on microparticles produced by microfluidics.

Conclusions and Outlook
In this work, biomimetic injectable microparticles for cardiac tissue engineering applications were fabricated by traditional and microfluidic techniques. Microparticles produced by traditional emulsion showed adequate physicochemical, functional and biological properties for the aimed application. However, they were characterized by a quite large size distribution. The employment of a microfluidic flow-focusing device permitted to overcome this limitation, producing highly monodispersed microparticles. To the best of our knowledge, this is the first report on microfluidic application to the fabrication of injectable scaffolds for cardiac tissue engineering. We expect that further characterization, currently ongoing, could demonstrate that microfluidic fabricated microparticles are a promising alternative to conventional injectable scaffolds.

Acknowledgments
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Novel 3D approach for osteosarcoma research comprising bioactive composite scaffolds in conjunction with perfusion bioreactors

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Keywords: 3D microenvironment, macroporous scaffolds, biomimetic bioreactor, bone tumour engineering

Introduction

Osteosarcoma (OS) is the most common bone tumour in patients of all ages with 5-year survival rate of ~ 60% [1]. First-line care for OS is neoadjuvant chemotherapy followed by surgical removal and adjuvant chemotherapy after surgery [2]. In order to improve and simplify the current therapy, great efforts are directed towards understanding the etiology, development and growth of OS. However, OS is still incompletely understood due to traditional research based on 2 dimensional (2D) cell culture systems that are unable to adequately mimic the tumour environment, leading to questionable and misleading results. This problem is tackled by applying tissue engineering principles to create artificial *in vivo*-like tumour environment based on biomaterials as cell carriers that imitate native extracellular matrices (ECMs) and biomimetic bioreactors providing physiologically-relevant conditions for bone tumour. Such environment is expected to recapitulate all key features of tumours *in vivo* and provide a platform for reliable antitumour drug testing as well as development of personalized medical therapies. In this work, we have addressed osteosarcoma tumour engineering by a novel 3D approach comprising macroporous composite alginate scaffolds with embedded inorganic particles (hydroxyapatite (HAP) and β-tricalcium-phosphate (β-TCP) doped with Mg^{2+}) as an artificial ECM for immobilized cancer cells and biomimetic perfusion bioreactor ("3D Perfuse", Innovation Center of the Faculty of Technology and Metallurgy, Belgrade, Serbia) that imitates environment of highly vascularized tissues such as bone tissue. The scaffolds were previously shown to be bioactive under medium flow in bioreactors by transformation of β-TCP to HAP, the native bone tissue component [3]. The present work, describes optimization of cell seeding and short-term cultivation studies of seeded scaffolds under continuous flow in biomimetic perfusion bioreactors.

Results and Discussion

Macroporous scaffolds with open and connected pores based on alginate hydrogels with uniformly incorporated bioactive powders HAP or HAP/β-TCP (2 wt.% alginate and 2 wt% powder) were prepared as previously described [3] by a slightly improved procedure including simple controlled gelation and freeze-drying method followed by rehydration in the culture medium. HAP and β-TCP particles were preserved during the scaffold production, as confirmed by the Fourier transform infrared-attenuated total reflectance spectroscopy (FTIR-ATR) and field-emission scanning electron microscopy (FE-SEM). The porosity of scaffolds after partial rehydration of 4 h was ~60 % with the majority (~60 %) of pores being larger than 1 mm in length. The murine K7M2-wt osteosarcoma cells (ATCC® CRL-2836™) were suspended in the culture medium (high glucose (4.5 g dm^{-3}) DMEM with 10 % FBS, 1 % L-glutamic acid (200 mM), 1×penicillin-streptomycin-amphotericin B). The cell suspension was seeded manually onto the scaffolds (~9.5 mm in diameter, ~4.5 mm thick disks, $15x10^6$ cells cm^{-3} scaffold volume) and left for 24 h in the culture medium under static conditions. The cells adhered to the scaffolds, on the surfaces and in the pores as individual cells and as aggregates as determined by histological (H&E stain) and FE-SEM analyses of scaffold cross-sections (**Figure 1a**). Also the cells exhibited high metabolic activity as confirmed by the MTT assay (**Figure 1b**), while cell seeding efficiency was above 80%. The obtained scaffolds with osteosarcoma cells were further cultivated in a biomimetic perfusion bioreactor at continuous medium flow of 0.27 cm^3 min^{-1} corresponding to the superficial velocity of 40 μm s^{-1} in short term studies up to 7 days, while cultures under static conditions served as a control. The scaffolds were examined regarding the cell metabolic activity by MTT assay as well as viability, morphology and distribution within the scaffolds by histological and FE-SEM analyses. The cells stayed on the surfaces, as well as in the pores with the negligible cell loss (~1 %) after 7 days of cultivation under continuous medium flow. In addition, the cells retained their viability and exhibited higher metabolic activity as compared to the cells in static cultures. The obtained results could be explained by positive effects of medium flow on the cultivated cells due to providing efficient mass transport of nutrients and oxygen by convection, as well as controlled levels of hydrodynamic shear stresses exhibited on cells [4]. In addition, sampled scaffolds from each culture were dissolved and the cells were retrieved and cultured in Petri dishes under static conditions in order to examine ability of cells to adhere to the culture plastic and retain proliferative capacity after 3D cultures. The retrieved cells retained their morphology and viability, attached

to the culture plastic and proliferated, confirming biocompatibility and supporting environment of the investigated macroporous composite scaffolds. In the next steps, the novel 3D approach will be evaluated in anticancer drug screening by assessment of cell viability and resistance-related gene expressions after doxorubicin treatment.

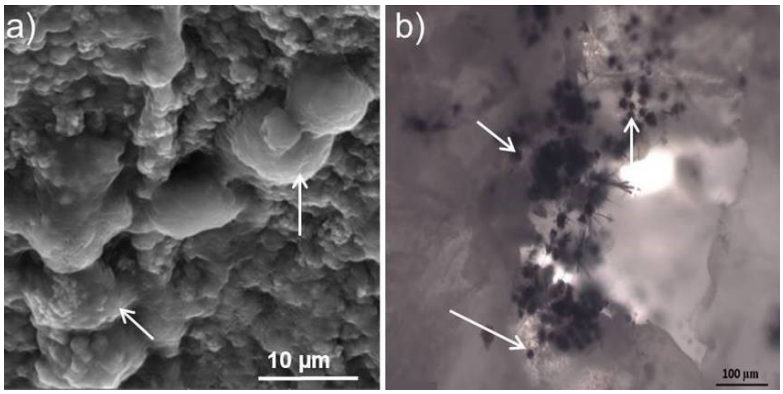

Figure 1: Macroporous composite scaffold after 24h of K7M2-wt osteosarcoma cell seeding: a) FE-SEM micrograph of a representative cross-section of the scaffold (scale bar = 10 µm); b) optical micrograph of MTT-stained viable cells within the scaffold (scale bar = 100 µm); white arrows designate individual cells

Conclusions

The present work presents cultures of OS cells in macroporous alginate scaffolds containing mineral particulate phase in conjunction with medium flow in perfusion bioreactors, thus imitating the native tissue structure and composition as well as interstitial fluid flow in bone tissue. The cell seeding method onto the scaffolds was shown to be fairly efficient while the bioreactor cultures supported cell viability and metabolic activity. Thus, the overall results have shown potentials of the novel 3D approach as a platform for OS tumour engineering.

Acknowledgments

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The ExcellMater project for advancement of novel bioceramic and composite biomaterials for medical applications

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Keywords: bone implants, dental inserts, osteosarcoma culture model, twinning project

Introduction

Biomaterials engineering is one of the strongest research areas at the Faculty of Technology and Metallurgy (FTM), University of Belgrade including development of novel biomaterials for orthopaedic and dental applications. Especially, synthesis and physico-chemical characterization methods are well established resulting in variety of novel inorganic, polymer and composite biomaterials produced in different forms with controlled compositions and structures. Still, expertise in further advancement of the research results towards utilization in medical devices is lacking. The ExcellMater Horizon 2020 project aims to further advance the scientific and technological capacity of the Faculty in this area by twinning the knowledge from the three European partners, internationally leading institutions, with recognized expertise in biological characterization methods, intellectual property protection and utilization, evaluation of health technologies and regulatory aspects of medical devices. The aim of the project is also to increase visibility of scientific research and enhance collaboration of engineers at FTM with life scientists, medical doctors and pharmacists as well as with industry. Here, we will present some of the novel bioceramic and composite biomaterials developed at FTM aimed for potential applications in orthopaedics, dentistry, bone tissue engineering, and cancer research.

Results and Discussion

Biological apatite contains different trace elements such as: Mg, F, Si, Sr, Zn, Mn, etc., which enhance bone formation. By introduction of these elements into the hydroxyapatite (HAP) structure during synthesis, *i.e.* HAP doping, it is aimed to mimic the biological apatite, so to stimulate a specific biological response in the body upon implementation. Usually, HAP is doped in order to improve its biocompatibility and/or to achieve antimicrobial activity.

HAP powders doped with Ag^{+}, Zn^{2+} or Cu^{2+} ions (all at 0.4 mol. % regarding to Ca) were hydrothermally synthesized and further annealed at 1200 °C for 2 h in order to obtain HAP/α-tricalcium phosphate (α-TCP). The powders were then used to produce ceramics by conventional and microwave sintering [1]. Doping with Ag^{+} and Zn^{2+} led to pore formation and ceramics with worsened mechanical properties, while Cu-doped ceramics, on the contrary, have shown the highest values of hardness and fracture toughness. Next, simultaneous doping of hydrothermally synthesized HAP with Cu^{2+} (0.4 mol. % regarding to Ca) and Mg^{2+} (1-20 mol. % regarding to Ca) was performed in order to achieve good mechanical properties at optimal antimicrobial activity and bioactivity of the biomaterial as magnesium is known to promote bone mineralization and osteoblast-like cell proliferation. The ceramics obtained by conventional sintering for 2 h at temperatures ranging 750–1200 °C have shown satisfactory antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis.* Also, the ceramics have exhibited excellent mechanical properties based on the measured hardness by Vickers of 4.96 GPa and fracture toughness of 1.75 MPa $m^{1/2}$ [2]. Presence of Mg^{2+} ions was found to favour HAP to β−TCP transformation during the synthesis and stabilize β−TCP during sintering by suppressing β− to α−TCP transformation [2]. Co-doping of HAP by Sr^{2+} and Mg^{2+} led to formation of β-TCP phase resulting in biphasic calcium phosphate powders. In the next step, sintered compacts were obtained exhibiting high potentials for use as dental inserts and dentin substitutes based on measurements of the material hardness and bonding strength to a commercial dental composite [3].

HAP/β-TCP powders obtained by HAP doping with Mg^{2+} were also used for creation of macroporous composite alginate hydrogels [4] attractive for application as scaffolds in bone tissue engineering or for 3D osteosarcoma cell culture models. The scaffold composition imitates that of bone tissue, Ca-alginate corresponding to the organic phase with embedded mineral particles that transform to HAP over time. The HAP/β-TCP powder was shown to be biocompatible in monolayer cultures of murine K7M2-wt osteosarcoma cells (ATCC® CRL-2836™) as the cells migrated underneath the powder and stayed viable and metabolically active over 24 h (**Figure 1a**). The scaffolds (1 wt.% alginate and 1 wt% powder) were produced by controlled gelation and freeze-drying followed by rehydration. HAP/β-TCP particles were preserved during the scaffold production and transformation to HAP was investigated in perfusion bioreactors ("3D Perfuse", Innovation Center of the Faculty of Technology and Metallurgy, Belgrade, Serbia) in the simulated body fluid over 28 days [4]. It was shown that enhanced mass transport by the fluid flow facilitated HAP formation within the scaffolds as compared to static conditions yielding improved mechanical properties [4]. In addition, seeding of scaffolds with murine K7M2-wt and human Saos-5 (ATCC® HTB-85™)

osteosarcoma cells has shown that cells successfully attached to the pore walls and stayed viable in static cultures (**Figure 1b,c**). Thus, the scaffolds were shown to be suitable to support osteosarcoma cell cultivation, which may further lead to establishment of 3D *in vitro* models for reliable anti-cancer drug screening.

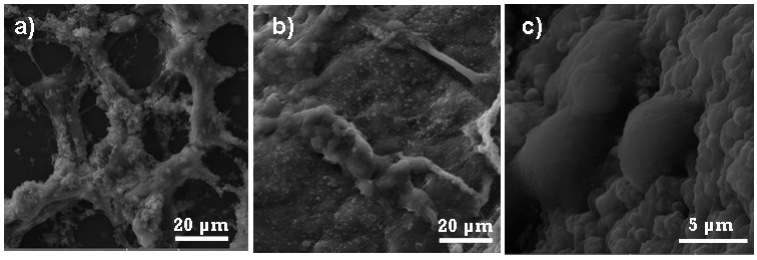

Figure 1: FE-SEM micrographs of osteosarcoma cells: a) murine K7M2-wt osteosarcoma cells attached to the tissue culture plastic covered with HAP/β-TCP powder after 24 h culture (scale bar = 20 µm); b) murine K7M2-wt osteosarcoma cells attached to the pore wall within a macroporous composite alginate scaffold after 7-day culture (scale bar = 20 µm); c) human Saos-5 osteosarcoma cells attached to the pore wall within a macroporous composite alginate scaffold after 5-day culture (scale bar = 5 µm)

Outlook

Various promising biomaterials have been synthesized at FTM including different HAP powders and bioceramics for orthopaedic and dental applications as well as composites aimed for bone tissue engineering and osteosarcoma *in vitro* models. Collaboration within the ExcellMater project is expected to further advance this research and bring at least some of the most promising candidates to practical utilization in medical devices.

Acknowledgments

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Fabrication and Characterisation of Hydroxyapatite Reinforced Collagen Porous Composite Beams for Bone Tissue Engineering

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Keywords: Porous biomaterials, Composite beam, Fracture mechanism, Bending deformation

Introduction

Over the past few years, the fabrication of porous hydroxyapatite (HA) based scaffolds have been rapidly increasing as they have become one of the promising biomaterials for the reconstruction of bone damage [1],[2]. Their unique properties such as biocompatibility, bioactivity and osteoconductive have become the main reason for scaffolds fabrication [3],[4]. Therefore, many researchers have been trying to develop highly porous HA scaffolds using some effective methods to mimic the microenvironment of the bone, such as the template method [5]. Nonetheless, insufficient mechanical properties and low fracture energy of pure HA scaffolds have become the major problem in tissue engineering. Hence, it is necessary to improve the mechanical and fracture properties of the pure HA scaffolds to be practically functional in actual clinical situation. In this paper, porous HA/Col composite beam were fabricated by using a combine technique of template and freeze-drying process. Three-point bending tests were used to assess its mechanical and fracture properties. The scanning electron microscopy (SEM) was also used to examine the microstructures and fracture mechanisms.

Materials and specimen fabrication

A combination of template and freeze-drying techniques were employed in the fabrication of the HA/Col composite beams [6]-[8]. Briefly, 7g micro-HA powder (Sangi Co., Ltd.) was mixed with 7g, 5wt% PVA (Wako Chemical Industries Ltd.) to prepare 1:1 HA slurry. PU sponges of 10mm x 10mm x 70mm dimensions were immersed in the HA slurry and dried for 48 hours. The coated sponges were then undergoing sintering process at 400°C and 1100°C for 6 hours and 4 hours, respectively, to prepare pure HA beam specimens. Next, the HA beam specimens were dipped in the Type I Collagen (Cellmatrix Type 1-A, Nitta Gelatine Co., Ltd.) and vacuumed for 1 hour to remove trapped air, then were frozen overnight at -80°C in the freezer. Finally, the specimens were freeze-dried at -50°C for 24h to create the secondary collagen porous structures.

Results and Discussion

The SEM micrographs of HA/Col composite beam can be seen in **Figure 1 (a)**. It was observed that the composite beam produced a two-phase continuous porous structure, namely (1) HA porous structures created through sintering process and (2) honeycomb-like porous collagen phase formed via freeze-drying process. The average pore sizes and porosity of HA structures (larger pores) are found to be 420µm and 84%., respectively, which are sufficient for the vascularization and formation of new tissue. However, the average pore sizes of the secondary collagen phase were found to be smaller than 100µm, mainly due to the solid-liquid phase separation during the freezing process. The fracture surfaces of HA/Col composite beam shown in **Figure 1 (b)** can be characterised as ductile bending deformation with a rough surface structure. The collagen cell wall ruptured; however, many pores are still visible and intact, as a result from high ductility.

Figure 2 depicts the load-displacement curves of HA/Col composite beam in comparison with pure HA beam. Both beams exhibit a linear elastic deformation with modulus of elasticity, E_{HA} = 3.16 MPa and $E_{HA/Col}$ =1.64 MPa for pure HA beam and HA/Col composite beam, respectively. Although the value of E obtained from the HA/Col composite beam is lower than the pure HA beam, it was found that the maximum stress, σ_{max} of HA/Col is 6 times greater than the pure HA beam. The fracture absorbed energy, G (calculated from the area under the curve) given by HA/Col is also higher when compared to pure HA beam (G_{HA} = 1.73 J/m^2 and $G_{HA/Col}$ = 305.8 J/m^2), suggesting that HA/Col composite beam has a better resistant to fracture. It was confirmed that the introduction of collagen as a secondary porous phase resulted in high flexibility, thus increasing the mechanical properties of pure HA beams. It was also observed that HA/Col beam did not show any crack initiation and propagation until the final fracture. On another note, it is interesting to note that the microdamage formation was first initiated by the cracking of HA struts, followed by the deformation and fracture of the collagen phase.

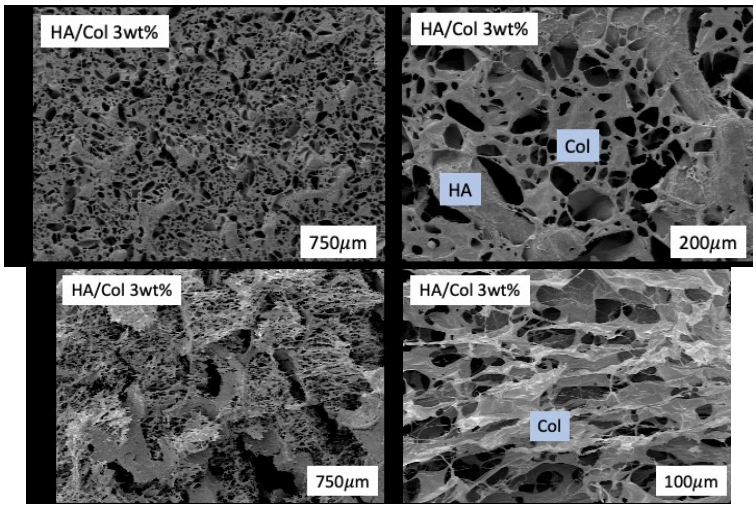

Figure 1: SEM micrographs of HA/Col: (a) microstructures and (b) fracture surface

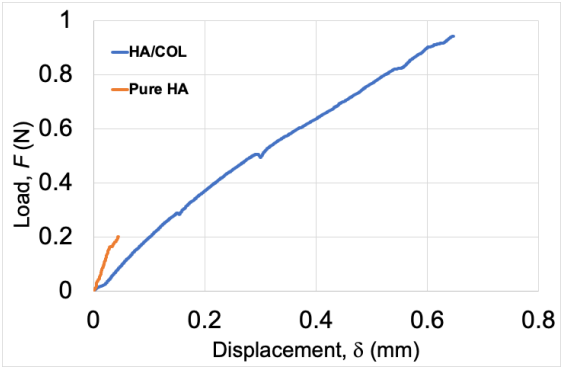

Figure 2: Load-displacement curves

Conclusions and/or Outlook

A highly interconnecting porous composite beam, namely HA/Col was successfully fabricated with improved mechanical properties and fracture energy. Three-point bending tests were performed to assess the mechanical properties and SEM imaging was used to evaluate the microstructures and fracture mechanism. It was observed that both pure HA and HA/Col beams exhibited linear elastic deformation and no initial cracking took place until the final fracture. It was found that HA/Col composite beam possessed a higher modulus and fracture energy compared to the pure HA beam, as a result of the introduction of collagen as secondary porous structures within the HA frame. It can be confirmed that the collagen phase has greatly support the whole beam structure from an abrupt bending fracture of the composite beam.

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Fabrication and Evaluation of HA/pearl Composite Particles and Chitosan Based Scaffolds

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Keywords: Pearl powder, bone repair and regeneration, bioactive scaffolds.

Introduction

Chitosan and its derivative have gained considerable attention as the popular tissue-engineering strategy for bone repair and regeneration, drug delivery and wound healing. It is composed of *N*-acetylglucosamine (GlcNAc) and glucosamine (GlcN) residues and demonstrates several ideal properties, for instance, low toxicity, biocompatibility, biodegradability, and osteogenesis [1]. At the same time, the imperfections of its chemical properties resulting in an unfavourable mechanical strength, which is detrimental when designing for bone regeneration scaffolds. In the recent decade, complex formulations of scaffold based on chitosan incorporating certain particles that improves osteogenesis and angiogenesis have been studied and documented. Previous works demonstrated that bioactive particles which derived from stiff particles, e.g., ceramic, enhanced the mechanical properties when incorporated with the soft polymer [2-4]. Such particles, including bioactive particles, such as pearl powders and, have gained wide acceptance and rising interest to enhance the biological and mechanical performance of chitosan based bioactive scaffolds.

Pearl is a natural bioactive mixture of inorganic and organic components, similarities of biological properties and physicochemical properties resembling human bone tissue making it a suitable candidate for bone regeneration compared with other inorganic materials. Like bone crystals, pearl or nacre is a natural mineral with different crystal structures and compositions. Hydroxyapatite (HA) also exhibits biocompatibility, biodegradability with significant enhancement of mechanical strength. In comparison, pearl contains growth factors which support the proliferation of osteoblasts even more directly. However, there are only few studies discussed on chitosan and its derivatives-based scaffolds with pearl powders.

In this study, the incorporation of pearl in chitosan scaffolds with and without HA has been developed by using freeze-drying technique and chemical, physical and mechanical properties of these bioactive composites. Fourier transform infrared (FTIR), Raman spectroscopy, x-ray diffraction (XRD) scanning electron microscopy (SEM) and mechanical testing, respectively.

Results and Discussion

The acquired HA/pearl composite particles were synthesized via three different methods including precipitation method, hydrothermal technique and sol-gel strategies as described below in Figure 1.

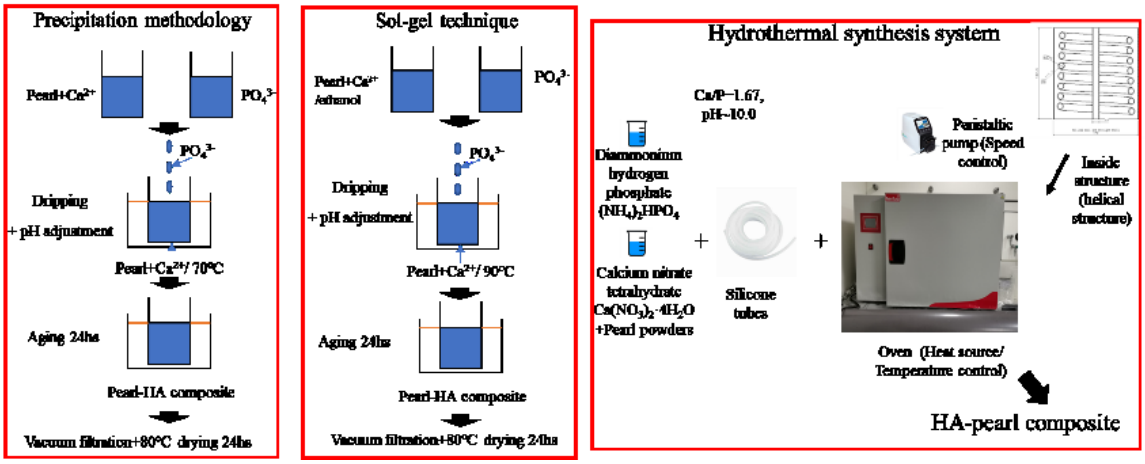

Figure 1: Schematic diagram of three different methods employed for synthesizing HA/pearl composite particles

Each composite particles acquired from different methods were characterized by XRD and FTIR photoacoustic (FTIR-PAS) spectroscopy, respectively, which are shown in Figure 2.

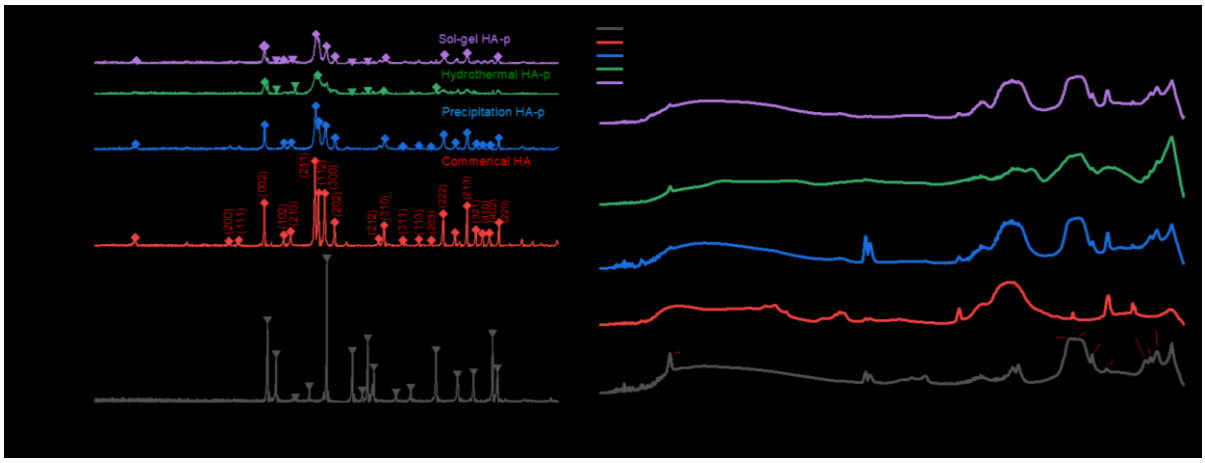

Figure 2: XRD results and FTIR-PAS results of synthesized HA/pearl particles

Physical and chemical properties of 3 different HA/pearl composite particles with commercial pearl and HA particles were analysed by XRD and FTIR-PAS. XRD patterns and the infrared spectra confirmed the presence of HA and pearl powder in the resultant scaffolds, FTIR-PAS spectra provided more detailed chemical information of the composites, presence of PO_4 and CO_3 peaks, as well as amide peaks were clearly evident. Spectral peaks at 630 cm^{-1} and 873 cm^{-1} observed in precipitation and hydrothermal samples, indicated the potential chemical groups replacement between CO_3 and PO_4 in accordance with the previous study [5]. Further SEM studies in Figure 3 provide evidence of attachment between HA and composite particles involved in chitosan scaffolds.

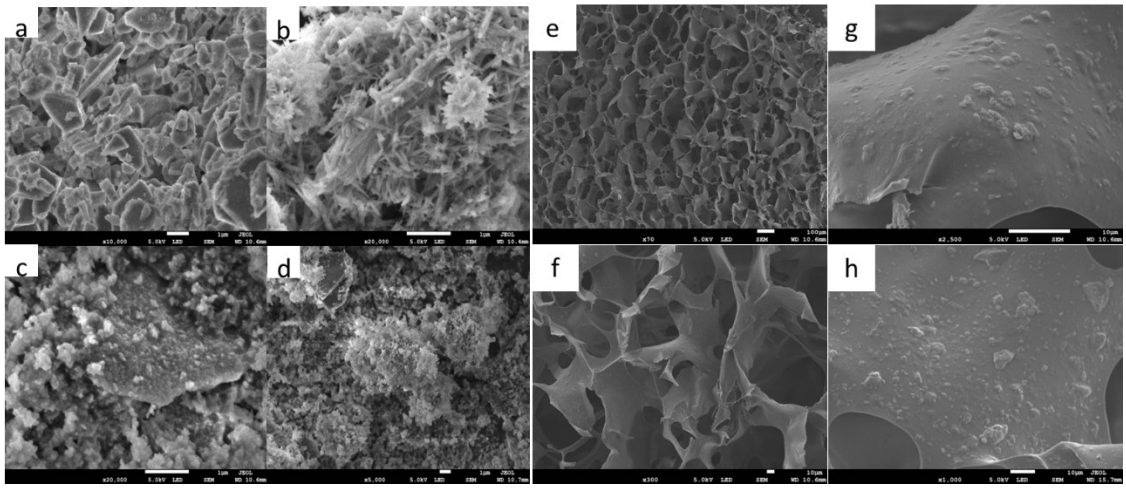

Figure 3: SEM results of pearl, HA and HA/p composite samples (a-d) and internal morphological studies of chitosan HA/p composite scaffolds (e-h). (a) the pearl particles at x10,000 magnification; (b) commercial unsintered HA particles at x20,000 magnification; (c,d) HA/pearl composite particles at x20,000 and x5,000 magnifications, respectively; (e-h) cross-section of HA/p chitosan scaffold

Conclusions and Outlook

A novel method to obtain HA/pearl composite particles and the incorporation of composite particles in chitosan scaffolds via the freeze-drying strategy has been developed. SEM studies indicate that optimal interconnectivity and porosity is sufficient to support osteogenesis and angiogenesis for further *in-vitro* and *in-vivo* biological studies.

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Cytocompatibility of Sol-Gel Coatings Containing Carbon Nanoparticles

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Keywords: antimicrobial coatings, sol-gel, nanodiamond, carbon nanoparticles, cytotoxicity, cell proliferation

Introduction

Investigations of various research groups are focused, particularly in recent years, on preventing of bacteria spreading on various surfaces at sensitive areas, e.g. hospitals, clinics, public transport, shops, schools, etc., in order to reduce a potential epidemiological threat. This is an important problem, especially in large human agglomerations and due to people migrations (in EU millions of people travel from place to place every day). What is more, bacteria transferred through continents, brought with tourists and immigrants might be difficult to identify and treat. A potential solution of this problem is the application of antimicrobial coatings to objects and items that are in contact with human.
In the undertaken project, coatings obtained by sol-gel method that contain carbon nanoparticles (CNPs) are developed. The aim of this study was surface visualization and potential cytotoxicity investigation of nanolayers obtained by sol-gel method containing CNPs of three allotropic types. The rationale is that the antibacterial coatings should be durable and safe, that is non-toxic for the user.

Results and Discussion

Three types of carbon nanoparticles: nano-diamond (DND), reduced graphene oxide (rGO) and carbon nanotubes (CNT) were used. Nanolayers on the stainless steel AISI 304 plates of 15 x 40 mm and thickness approx. 2 mm were prepared as follows: proper amount of nanoparticles were mixed with the sol of 3-(trimethoxysilyl)propyl methacrylate and tetraethoxysilane in isopropyl alcohol. Then the layers were obtained by: 1/ dip-coating (DC) and 2/ spin-coating (SC). Subsequently, after evaporation of isopropyl alcohol (RT, 15-30 min.), the samples were placed in a dryer at 150°C/30 min to complete the polymerization process. Characterization of hybrid layers was done by the Zeiss ultra plus scanning electron microscope to examine the quality of the layers.

Normal Human Primary Dermal Fibroblast (cell line ATCC-PCS-201-012) were used for cytotoxicity/ vibility testing using assays of XTT and Live/Dead™ Viability/ Cytotoxicity two-color (green-fluorescent calcein-AM and red-fluorescent ethidium homodimer-1). Study was done with fluorescent microplate reader (BioTek, Synergy HT) and fluorescent microscope (Zeiss, Axio Observer). Image analysis was performed using a custom-built workflow in Matlab 2019a (The MathWorks, Inc.), the human fibroblasts cells properties (live/dead) on the surfaces in the fluorescence images were analyzed. The ratio of green-stained cell areas (live) to red stained cell areas (dead) was assessed. Two parameters were used because of the aggregation of cells into colonies (area and total cell count). Other parameters as perimeter, roundness, length and width were determined as well. The steps were conducted within program script.

There are some challenges in preparing sol-gel nanolayers containing nanoparticles mainly due to incomplete particles dispersion and agglomeration. Two coating methods (dip-coating and spin-coating) have been used in parallel, whereas these methods provide usually different layer quality. In the case of DND, the initial dispersion was already ineffective. The layers contain predominantly clumps (both deposition methods). We assume, that DND are be firmly fixed in the layer. rGO initial failed to disperse; the layers contain only clumps (both deposition methods). The clusters of graphene were evidently very poorly wetted in the sol. The particles were spread onto the surface of the layers, where they appear as separate particles in the final stage of layer preparation. The best results were obtained with carbon nanotubes CNT. Most of the nanotube aggregates were dispersed well during the initial dispersion. Both deposition methods gave similar results and are well applicable.

The results of the XTT test are presented in the Figure 1. According to ISO 10993-5, a reduction in cell viability below 70% means a cytotoxic effect, thus the tested materials did not show cytotoxic activity. Life/Dead assay results showed that the layers containing DND and rGO somewhat restrict proliferation of cells, and CNT containing layers might be even harmful to cells.

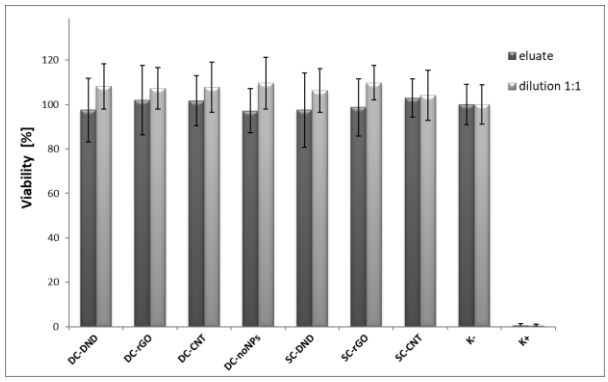

Figure 1: Viability of human fibroblasts after 48 h incubation in the presence of eluates of investigated samples, K^- culture medium, K^+ 0.01% Triton X100 solution

Outlook

The production method: dip-coating and spin-coating does influence on the quality of the coating on steel plates. All obtained sol-gel nanolayers containing nano-diamonds (DND), graphene (rGO) and carbon nanotubes (CNT) are not toxic on human fibroblast cells. Nevertheless, the sol-gel layers containing carbon nanoparticles do not support proliferation of cells. This does not restrict potential application of sol gel coatings with carbon nanoparticles on surfaces in sensitive public areas.

Acknowledgements

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Poster Session 2

Development of a functional resistance zirconia graded bioactive composite

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Keywords: mechanical strength, antibacterial, composites, therapeutic, remineralization, zirconia

Introduction

Many current dental materials are developed to be neutral and other "bioactive" dental materials have an "active" behavior, not "passive", participants in the restorative process. The requirements imposed on them are: non-toxicity, biocompatibility, bioactivity and resistance to mechanical wear. If the first two criteria are achieved, the last ones are precarious, with either bioactivity or mechanical strength.

The aim of the proposed study is to test with the help of an *in vitro* model the mechanical, and antibacterial properties of a bioactive composite resin mixed with zirconium powder.

Material and method

In this regard, the in vitro study aimed to create a bioactive restorative dental material with improved mechanical and physical properties, being "fortified" with zirconium oxide powder. Changes in the ionic, antibacterial and mechanical properties of the material was analyzed. The incorporation of a monolithic crystalline ceramic into the bioactive composite resin aims to significantly increase the strength of the new material while inducing remineralizing and antibacterial properties.

Bioactive composite resin mixed with zirconium dioxide powder (ZnO_2) discs (n = 100) were milled for this investigation and polished for three-point flexural strength testing. The specimens were divided into 4 groups, depending on the concentration of zirconium powder included, as follows: Group 1 (G1; n = 25; 0% ZnO_2), Group 2 (G2; n = 25; 5% ZnO_2), Group 3 (G3; n = 25; 10% ZnO_2), Group 4 (G4; n = 25; 15% ZnO_2). Microhardness (Vickers indentation), as well as quantitative (energy dispersive spectroscopy) and qualitative (scanning electron microscopy) structural analysis were conducted on fracture surfaces. Data were analyzed by one-way ANOVA and Tukey HSD post-hoc test (p = 0.05).

Results

One-way ANOVA showed that ZnO_2 percentage had a significant effect on the fracture strength ($p < 0.0001$). Tukey HSD post-hoc test between paired groups showed that G4 and G3 had significantly higher fracture strength values compared to the first two groups. However, no significant difference was found in fracture strength between G1 and G2 or between G3 and G4.

The flexural strength values of measured disc specimens were significantly different among all groups of materials ($p < 0.0001$), with G4 showing the highest flexural strength value (199 MPa ± 20) followed by G3 (169 MPa ± 20), G2 (111 MPa ± 20), and G1 (103 MPa ± 18). For the flexural modulus, which defines the tendency of a material to bend under an applied force, one-way ANOVA showed significant difference among material groups. Tukey HSD post-hoc test between paired groups showed that G3 had a significantly lower flexural modulus (5.1 GPa ± 0.3), indicating the higher flexibility of the bioactive composite material improved with ZnO_2 compared to each of the other groups ($p < 0.0001$). From lowest to highest value, G3 was followed by G4 (7.9 GPa ±1.3), G2 (8.7 GPa ± 2.4), and G1 (9.4 GPa ± 2).

The results of the EDS for quantitative chemical composition analysis of the study materials are: all the groups contained fluoride, silicon, and zirconium. Aluminum and potassium were also detected in all groups except in G1.

Conclusions and/or Outlook

With the evolution of caries management shifting to "minimally invasive" techniques, restorative materials are endowed with increasing expectations for therapeutic effects and for mechanical properties. Most of the studies on developing a new generation of antimicrobial, therapeutic, and bioactive resins are *in vitro*and *in vivo* studies are still needed.

Nonetheless, through multidisciplinary research, the new generation of antimicrobial and bioactive/therapeutic resins is expected to offer tremendous benefits to oral health.

Acknowledgment. The study was supported by the Internal Research Grant no. 10127/18/17.12.2020 of the *George Emil Palade University of Medicine, Pharmacy, Science and Technology of Targu Mures, Romania.*

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Preparation of Uncalcined Hydroxyapatite Microspheres and In Vitro Dissolution Assement

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Keywords: Uncalcined hydroxyapatite, Microspheres, In vitro dissolution

Introduction

Since hydroxyapatite is the same material as human bones and teeth, it has excellent biocompatibility and is not toxic. Hydroxyapatite can be controlled its properties such as shape, crystallinity, and size through various synthetic methods. Hydroxyapatite is usually produced through a calcination process of 800 to 1,400 degrees. When calcium phosphate-based ceramics are calcined at 800 degrees or higher, the particles are tightly attached to each other in the process and solidified to remove carbonic acid groups. However, as human bones are known to contain some carbonate, the biological behaviour of uncalcined hydroxyapatite produced without calcination may be closer than that of calcined hydroxyapatite. Moreover, bone for human replacement is classified into permanent bone that replaces a part of the body according to the period of use and temporary bone that is temporarily replaced for the purpose of treatment, wherein the temporary bone is made of a biodegradable material. Since biodegradable materials are gradually decomposed and absorbed into the body after treatment, there is no need for additional surgery, reducing the risk and side effects. However, there are few studies on the degradability of hydroxyapatite, and further research is needed to be used as a main material for biodegradable materials.

Results and Discussion

The uncalcined hydroxyapatite (un-HAP) microspheres were prepared by precipitation process using a diammonium hydrogen phosphate and calcium nitrate in aqueous ammonia. The particle size, Ca/P ratio, and crystallinity of un-HAP were obtained using a SEM, ICP, and XRD analysis. Figure 1 shows the comparison of physical properties of un-HAP comparing the calcined hydroxyapatite (cal-HAP). The morphologies of both particles are not needle shape as shown the inserted image in Fig. 1(a). The crystallinity of un-HAP and cal-HAP particles was calculated by XRD (Fig. 1(b)) and are 85.8 %, and 92.8 %, respectively. Moreover, Ca/P ratios was 1.48 and 1.68 for un-HAP and cal-HAP. The average particles size was 2 μm and 16 μm for un-HAP and cal-HAP as shown in Fig. 1(c). Compressive strength was measured using a single axis hydraulic press and showed 43.1 MPa, and 50.2 MPa for un-HAP and cal-HAP as shown in Fig. 1(d).

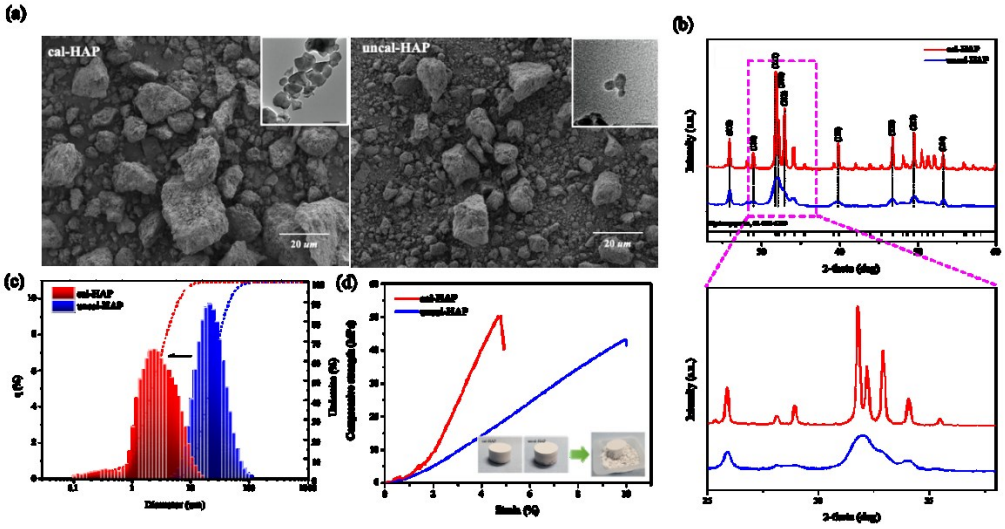

Figure 1: (a) SEM images, (b) XRD analysis, (c) particle size distribution, and (d) compressive strength of calcined and uncalcined HAp

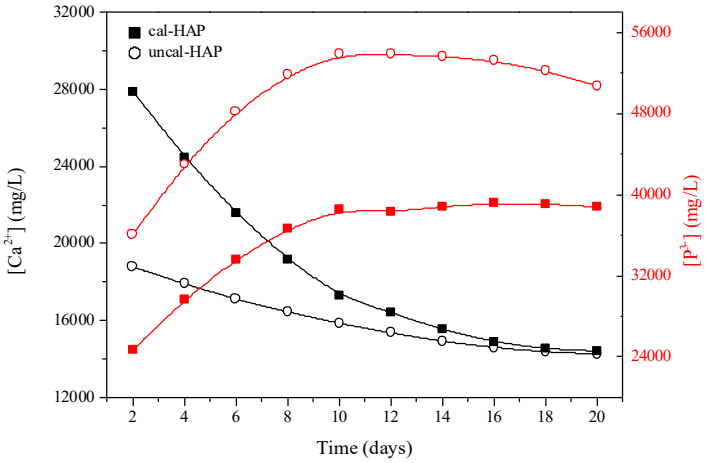

Figure 2: Dissolution profile of calcium and phosphorous ion in calcined and uncalcined HAp as a function of time.

Figure 2 shows the changes in the concentration of calcium and phosphorus ions due to the dissolution of calcined and uncalcined HAp for 20 hours using a citrate buffer in accordance with ISO 10993-14. As the elution time increases, the amount of dissolved calcium ions decreases and the amount of phosphorus ions increases. The decrease in the concentration of calcium ions is due to the formation of apatite such as hydroxycarbonated apatite through reactions with carbonate ions present in the body as they are dissolved. In particular, the reason that the amount of calcium ions dissolved from the uncalcined HAp is smaller is because the calcium ions dissolved are used to form a hydroxycarbonated apatite. Therefore, the uncalcined HAp means that the crystal strength is lower than that of the calcined HAp, which means that it is easily dissolved. For this reason, the dissolution profile of phosphorus ions appears more in uncalcined HAp than in calcined HAp.

Conclusions

Various morphological hydroxyapatite (HAp) and uncalcined hydroxyapatite (un-HAp) were synthesized in different preparation procedures due to the increasing demands for higher biocompatibility, mechanical property, and bio-decomposition. In addition, these osteo-conducive materials were characterized using several instruments such as FE-SEM, TEM, XRD, and UTM for morphological characterization, crystallinity, and mechanical property, respectively. Especially, we demonstrated the decomposition of HAp and un-HAp with citric acid for 20 days as standard method. The concentration of released Ca ions and phosphate ions of the materials are determined using ICP analysis, and wight change of the materials are also calculated during the dissolution periods. The release of Ca and phosphate ions are preferred in un-HAp rather than in HAp, and the resorption ability of Ca ions is also preferred in un-HAp.

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The dependence of antimicrobial activity on the Cu^{2+} concentration in $Ca_{10.5-x}Cu_x(PO_4)_7$

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Keywords: calcium phosphate, copper substitution, copper doping, crystal structure, antimicrobial activity

Introduction

β-$Ca_3(PO_4)_2$ (β-TCP) crystalizes in the Whitlockite structural type. Compounds based on β-TCP are known as excellent biocompatible materials since the bone tissue is built from combination of whitlockite and apatite (Ap) ($Ca_{10}(PO_4)_6(OH)_2$) phases. Due to a wide range of isomorphous substitutions within the initial host-matrix it is possible to modify the crystal structure by varying the cationic and anionic sites to design the novel materials with the directly improved physico-chemical and biological properties. The β-TCP-type structure includes six cationic positions (M1-M6), where the M1-M3 (18-*fold*) and the M5 (6-*fold*) sites are always occupied, while the M4 site can vary its occupation from 0 to 1, and the M6 site is always vacant. The presence of a crystal site with variable occupation favors for substitutions by different ions in the structure, including Cu^{2+}.

Many studies were devoted to single ionic substitutions, such as Cu^{2+} and Sr^{2+} [2], Mg^{2+} [3], in tricalcium phosphate. It has been established that the incorporation of Cu^{2+} ions into the β-TCP-type structure can curb the growth of bacteria, which helps in protecting the sites with bone implant from the attack of microbes. Also, Cu^{2+} is not cytotoxic at low concentrations. The studies on single-doped Cu^{2+} phosphates [4,5] report on β-TCP-type with admixture of apatite and calcium pyrophosphate phases. Therefore, it is difficult to track the contribution of copper ions from β-TCP phase on the antimicrobial activity since apatite phase also can be doped by Cu^{2+} [6]. Also, the systematic investigation of Cu^{2+} concentration on the biological responses is absence.

In order to interpret the biological behavior of Cu^{2+} substituted β-TCP, it is of great importance to correctly characterize the structural location of the Cu^{2+} ions. The biological activity of the Cu^{2+} ions depends on occupied position and concentration.

Results and Discussion

Copper-substituted tricalcium phosphates $Ca_{10.5-x}Cu_x(PO_4)_7$ with x = 0, 0.001, 0.333, 0.667, 1, 1.333, 1.667 were synthesized by the high temperature solid-state synthesis. The synthesis procedures were carried out applying stoichiometric amounts of raw materials.

The possibility of the $Ca^{2+} \rightarrow Cu^{2+}$ substitution is in the agreement with calculation of the ratio percentage discrepancy (D_r). The D_r can be calculated using formula: $D_r = (R_h - R_d)/R_h * 100\%$. The continuous solid solution requires a similarity of ionic radius between doping ions (R_d) and substituted host ions (R_h). The value of D_r should be less than 30%. In the $Ca_{10.5-x}Cu_x(PO_4)_7$ the D_r value is 27% for the octahedral M6 site, so the Cu^{2+} ions can be successfully incorporated in the β-TCP structure in the octahedral position.

Powder X-ray diffraction (PXRD) patterns were collected on Rigaku SmartLab SE (3 kW sealed X-ray tube, D/teX Ultra 250 silicon strip detector, vertical type θ-θ geometry, HyPix-400 (2D HPAD) detector). The LeBail decomposition, quantitative phase analysis and structure refinement by the Rietveld method were applied using the JANA2006 software [7].

Fig. 1a shows the powder X-Ray Diffraction (PXRD) patterns of $Ca_{10.5-x}Cu_x(PO_4)_7$ obtained by the high temperature solid state reaction. According to the obtained data, the Cu-doped β-TCP are single phase. No impurities of hydroxyapatite, $Ca_3Cu_3(PO_4)_4$ or $Ca_2P_2O_7$ phases were detected up to x =1.33. However, the impurity of CuO was detected in the sample with x =1.33 (**Fig. 1**). The quantity of the CuO phase was 3%. The sample with x = 1.583 showed the presence of the impurity $Ca_3Cu_3(PO_4)_4$ phase (**Fig. 1**). These PXRD data approved the limit composition of $Ca_{10.5-x}Cu_x(PO_4)_7$ at x = 1.5, as it was established earlier [8].

The PXRD patterns were used to refine the crystal structure of samples with $0 \le x \le 1$. The free refinement of calcium positions showed the partial occupancy of M4 position by Ca^{2+} ions (**Fig. 2**). The final occupancies (a_i) of the M4 site were slightly varied for half-occupied (a_i(M4) ~ 0.5) (**Fig. 2**). No Cu atoms were found in the M4 site, since they are totally located in M5 sites (**Fig. 2**). The composition $Ca_{9.5}Cu(PO_4)_7$ is characterized by complete occupation of the M5 site by the Cu atoms (**Fig. 2**).

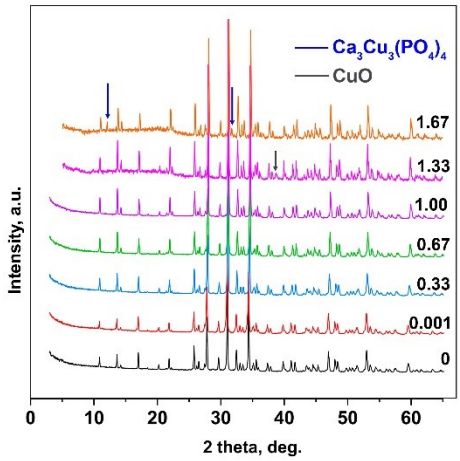

Figure 1: PXRD patterns of $Ca_{10.5-x}Cu_x(PO_4)_7$ with $0 \le x \le 1.583$ obtained by solid-state method. The impurity phases of CuO (PDF#2 80-1917) and $Ca_3Cu_3(PO_4)_4$ (PDF#2 №72-203) are highlighted.

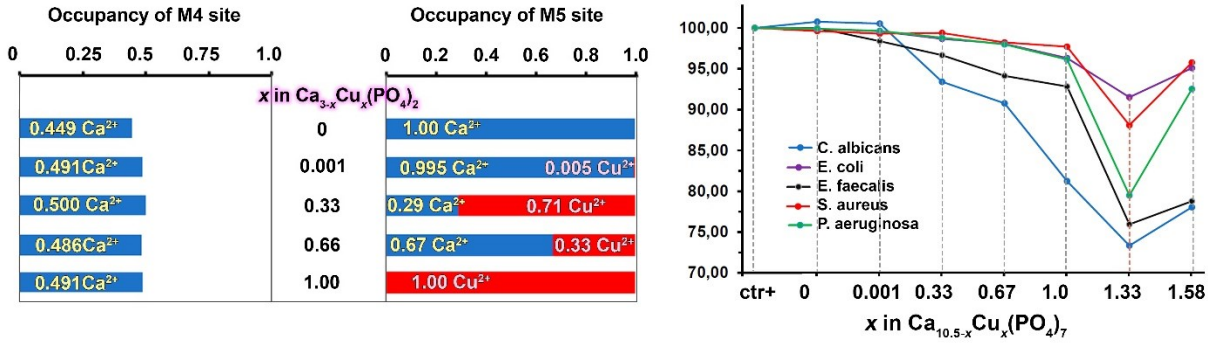

Figure 2: Occupancy of the M4 and M5 crystal sites by Ca^{2+} and Cu^{2+} ions in $Ca_{10.5-x}Cu_x(PO_4)_7$ (left). Results of the experiment on the inhibition of the growth of microorganisms grown for 24 hours in the presence of $Ca_{10.5-x}Cu_x(PO_4)_7$ (x = 0, 0.001, 0.33, 0.67, 1.0, 1.33, 1.58). The positive control (ctr +) is represented by the growth of each microorganism in the absence of compounds. The growth rate is calculated on the optical density measured at 600 nm, resulting from three independent experiments.

The study of antimicrobial activity was performed by growing of one fungus (*C. albicans*) and four bacteria (*E. coli, E. faecalis S. aureus* and *P. aeruginosa*) in the presence of $Ca_{10.5-x}Cu_x(PO_4)_7$ at different concentrations of Cu^{2+}: $0 \le x \le 1.583$. The obtained results regarding the inhibition can be observed from **Fig. 2**.

Conclusions

The influence of the Cu^{2+} concentration in $Ca_{10.5-x}Cu_x(PO_4)_7$ on the antimicrobial activity was studied. The strong dependence of the inhibition of the microorganisms's growth on the Cu^{2+} concentration was observed. The growth inhibition for *C. albicans* (6.59%) and *E. faecalis* (3.34%) is already highlighted in compound with $x = 0.333$. At $x = 0.667$ growth inhibition remains stronger for *C. albicans* (9.22%) and for *E. faecalis* (5.86%). At $x = 1.00$ all the microorganisms studied are clearly inhibited growth, in particular at this concentration of Cu^{2+} *C. albicans* has the strongest growth inhibition (18.77%), *E. faecalis* (7.18%), *E. coli, S. aureus and P. aeruginosa* had growth inhibition ranging from 2.29% to 3.68%. The greatest growth inhibition occurs at $x = 1.33$ for all microorganisms, especially *C. albicans* is 26.67%, *E. faecalis* is 24.06%, *P. aeruginosa* is 20.51 %, *S. aureus* is 11.91 % and *E. coli* is 8.49% after 24 hours of growth. This activity may be related to the presence of 3% CuO phase in the sample. The growth of the 5 microorganisms in the presence of $Ca_{8.917}Cu_{1.153}(PO_4)_7$ was inhibited to a greater extent for *C. albicans* (21.95%) and for *E. faecalis* (21.23%), while for *P. aeruginosa*, *E. coli* and *S. aureus* inhibition is between 4.91% and 7.48%, respectively.

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Modelling the behaviour of two titanium-based alloys with an equivalent circuit

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Keywords: circuit titanium alloy EIS spectroscopy

Introduction

The objective of the present study is to obtain information on how two alloys composed mainly of titanium react to corrosion in a biological environment and to model an equivalent circuit that describes the observed behavior. In addition to titanium, the second most present element in each of the two alloys is manganese (4 % and 3 %); the one with 3 % manganese also has 0.2 % vanadium and the one with 3 % manganese also has 1 % iron. Both alloys have 0.6 % aluminum in their composition. To carry out the tests, the materials were first coated with epoxy resin, then they were roughed with a series of emery papers of increasing sizes and finally on a polishing cloth with a suspension of alpha-alumina. Finally, the samples of the materials were immersed in a solution of Ringer Lactate, connected with a series of electrodes to perform the analysis. A saturated calomel electrode was used as reference electrode, a Ti-10Al-0.5V electrode as working electrode and the counter electrode used is made of platinum.

The method used to obtain the information was electrochemical impedance spectroscopy (EIS)[1]. This method is based on making use of different potential values, supplying them to the samples to obtain how the materials would react to different electrical voltages. With the information obtained from the spectroscopy it was possible to obtain equivalent circuits for each of the data series obtained, modelling by means of the ZSimpWin software, as has been done previously in other studies [2]. The most positive value of applied potential and the most negative value of applied potential were used to analyse if the assumed equivalent circuit correctly defines the behaviour of the materials. These values of potential were 1.2 V and -1 V.

Results and Discussion

Different possible equivalent circuits were considered, the one that was found to best describe the behaviour of the two alloys is an R(CR(CR(QR))(CR) circuit, as shown in **Figure 1**. In this model, the blue region corresponds to the Ringer's solution were the samples are placed, the red region corresponds to the porous outer layer on the surface of the alloys, the yellow one to the compact layer of the titanium alloy and the green one to the internal composition of the alloys. As can be seen, four resistors, two capacitors and one current element are used.

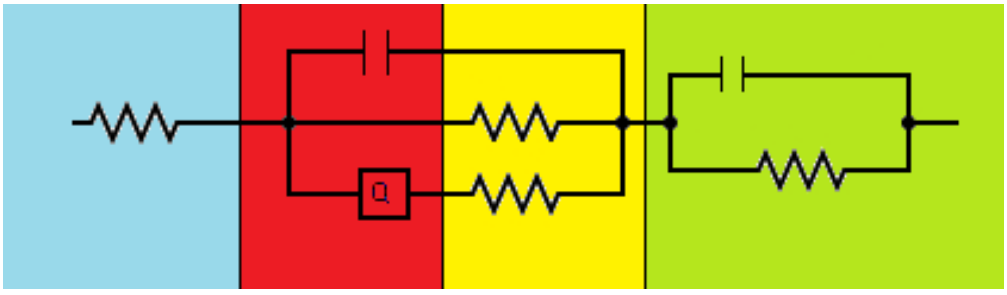

Figure 1: Equivalent circuit that describes the behaviour of the two alloys

This equivalent circuit was chosen because it is the one that recreates the behaviour of the two alloys with more precision. Proof of it can be observed in **Figure 2** and **Figure 3**, where the experimental data of the alloys when the EIS was applied is compared with the prediction calculated by ZSimpWin software.

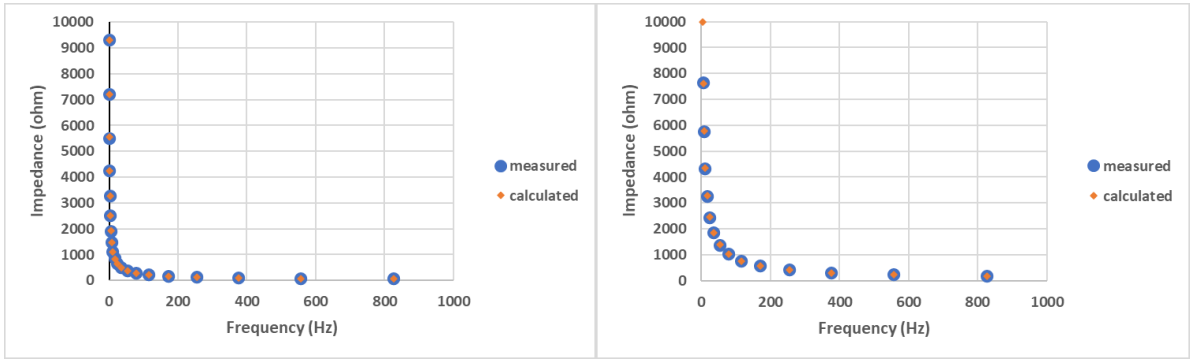

Figure 2: Comparison of experimental and fitted data by ZSimpWin software for the alloy Ti4Mn at: a) -1 V; b) + 1.2 V

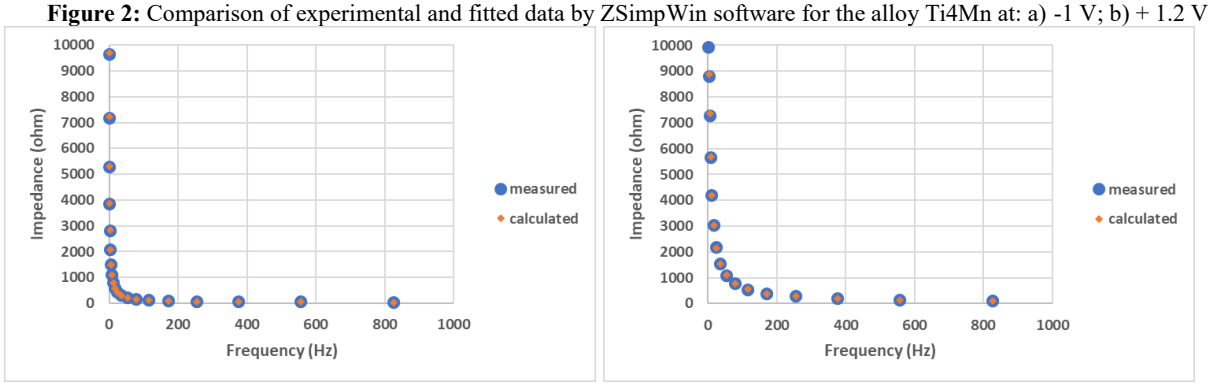

Figure 3: Comparison of experimental and fitted data by ZSimpWin software for the alloy Ti3Mn at: a) -1 V; b) + 1.2 V

It can be seen from the figures that the values calculated by the software are very similar to those obtained experimentally, suggesting that the behaviour of the alloys describes the same behaviour as a circuit with the arrangement presented in **Figure 1**. The chi-square value in all four cases is low, being 0.0009847 compared to the values obtained when applying the 1.2 V potential in the Ti4Mn sample and 0.0008263 compared to the values obtained when applying the -1 V potential. In the case of the Ti3Mn sample, chi-square values of 0.0007115 were obtained for the 1.2 V potential and 0.0005876 for the -1 V potential.
Other circuits such as an R(CR) circuit and an R(CR)(CR) circuit were tested. However, the values obtained deviated significantly from the values obtained in the experiments, ruling out these circuits as possibilities. A circuit with an R(CR)(QR)(CR) arrangement was considered, which initially gave promising results for the 3 % manganese alloy, but the results provided by the software deviated significantly when compared to those obtained experimentally for the 4 % manganese alloy, ruling out that other possible configuration.

Conclusions

The main conclusion that can be drawn for the two titanium-based materials studied is that they behave very similar to a circuit with an R(CR(QR))(CR) arrangement. This is advantageous for knowing how the alloys will react when a potential is applied to them. These results could be used as a reference for similar materials, by testing with a configuration like the one used here in the ZSimpWin software to see if this behaviour is common among other materials with similar characteristics, such as similar amounts of titanium or manganese.
Further research is needed to see to what extent this equivalent circuit would be useful in defining the behaviour of the materials. In this case, a maximum potential of 1.2 V for the materials and a minimum of -1 V was used. Whether the material would react the same at higher potentials, such as 50 V, or lower potentials, such as -40 V, needs to be tested experimentally.

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Influence of Zn on properties evaluation of Mg-0.5Ca-xZn biodegradable alloys

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Keywords: Mg-Ca-Zn biodegradable alloys; microstructure; mechanical analysis; electrochemical evaluation

Abstract
Magnesium based biodegradable alloys have an important approach in the biomedical domaine. The main advantages of Mg alloys are: good biocompatibility, similar Young modulus with the human bone and second surgery removal. However, one major disadvantage is shown by the lower corrosion rate in specific medium. Many researchers have studied these biodegradable materials, as medical applications, in past decades. In order to improve the mechanical properties and corrosion rate, Mg based materials must be alloyed with biocompatible and non-toxic elements. These novel types of Mg-alloys improve the biocompatibility and can be used in the field of orthopaedic applications [1-3]. The aim of this research is to investigate the aspects concerning the microstructure, tribological aspects and electrochemical response (corrosion resistance) of a new experimental Mg-based biodegradable alloy—Mg–0.5%Ca with controlled addition of Zn as follows: 0.5, 1.0, 1.5 and 2.0 wt.%—in order to obtain the corrosion rate and some mechanical properties. For this purpose, scanning electron microscopy (SEM), energy-dispersive spectroscopy (EDS), light microscopy (LM) and X-ray diffraction (XRD) were used for determining the microstructure and chemical composition of the studied alloy and the linear polarization resistance (LPR) method was used to calculate the corrosion rate for the biodegradability rate assessment. The research led to the discovery of a dendritic α-Mg solid solution, as well as a lamellar Mg2Ca and a $Ca_2Mg_6Zn_3$ intermetallic compound. The Mg-Ca-Zn alloy shows generally better biocorrosion behaviour than the Mg-Ca due to its lower content in the Ca2Mg6Zn3 phase and thus reduced galvanic corrosion. The mechanical properties are improved with the addition of Zn content. The Mg-Ca-Zn system presents high potential for usage as implants in medical domain.

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Bioactive antibiotic-eluting hydroxyapatite-based coatings obtained by electrophoretic deposition on titanium

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Keywords: biomaterial, electrophoretic deposition, antibacterial activity, bioactivity.

Introduction

The osteoarticular implants are meant to enable complete recovery of the lost function and secure successful implant fixation. Inevitably, problems might occur that lead to implant failure due to infection at the implantation site and aseptic loosening. The aging population worldwide is in dire need of these medical devices, but especially vulnerable and prone to infections due to compromised immune systems by the implant presence and subsequently even a few bacteria could easily latch to the solid substrates, and rapidly multiply to form a highly resistant biofilm. These problems could be so severe and potentially life-threatening wih the occurrence of multidrug-resistant bacterial strains. The damage that methicillin-resistant *Staphylococcus aureus* can cause to the human body is sadly evidenced by many medical reports. Therefore, a successful design of an implant should incorporate a potent drug e.g., wide spectrum antibiotic and the composition of the biomaterials that would secure functionality while promoting lasting osteointegration. Since osseointegration implies a direct, firm, and lasting bonding of the periimplant bone to the implant, the process depends on surgical positioning of the device, followed by the cellular response at the bone-implant interface, crucial for the bone healing. Therefore, surface of an implant has to be modified so it would provide osteoinductive and antibacterial properties at the same time.

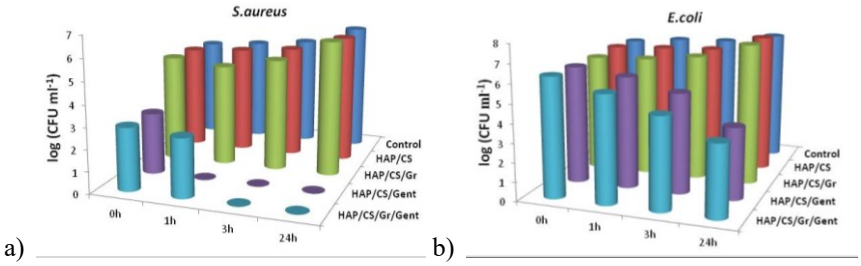
Due to similarity in chemical composition, synthetic hydroxyapatite (HAP) is commonly applied as a metallic implant surfaces' modifier or as a bone filler material since its porous structure could have favorable effect on tissue integration of the implants. Hence, a good combination of HAP biocompatibility and the excellent mechanical properties of metals are considered a promising approach to fabricate more suitable bone implants. However, HAP itself shows poor mechanical properties and is very brittle, so it is necessary to develop HAP-based composites with the addition of certain polymers. The spontaneous formation of passive film is crucial for the long-term stability of metals in human body. The conditions of simulated physiological solutions have been enriched by the additions of synovial fluid, which is the natural environment of joint. The most recent research delt with the problem of drug release and synergistic effect of chitosan and gentamicin showing the effective drug release from the polymeric component of the composite coating assembly on the surface of Ti [1-3]. Chitosan (CS) is a natural polymer, a cationic polysaccharide, obtained by partial deacetylation of chitin. CS shows biocompatibility, biodegradability and antimicrobial action while providing good adhesion and could act as a carrier of antibacterial agents. One of the antibiotics that are often used to treat in-hospital complications is gentamicin. Gentamicin (Gent), a water-soluble aminoglycoside antibiotic, is known to have very potent antibacterial activity for the treatment of wide range of infections, caused by both Gram-negative and Gram-positive bacteria. The reason for gentamicin high potency lies in its mechanism of action since, like most aminoglycosides, the drug irreversibly binds to 30S ribosomes thus inhibiting bacterial protein synthesis. Therefore, gentamicin is indicated for acute serious infections.

The original approach of the presented research is the single-step electrophoretic deposition (EPD) on Ti plates of thus prepared biocomposite that would allow for on-site release of the drug. EPD has numerous advantages comparing to the other techniques, e.g., uniform composition and thickness of the deposited coating, the possibility of deposition on substrates of complex geometries, even inner surfaces and deposition at room temperatures. We successfully obtained hydroxyapatite/chitosan biocomposite coatings with the addition of graphene, and loaded with gentamicin, using EPD and investigated their bioactivity, biocompatibility and antibacterial properties The overall construct on the substrate in such a form would be well-advanced arrangement for future medical device improvement of skeletal implants.

Results and Discussion

Figure 1 depicts the effect of HAP/CS, HAP/CS/Gent, HAP/CS/Gr, and HAP/CS/Gr/Gent coating against *Staphylococcus aureus* TL and *Escherichia coli* ATCC 25922 in phosphate buffer, respectively, using test in suspension, simulating static *in vitro* conditions. Bacteria incubated without any coated sample (control) and bacteria in the presence of HAP/CS and HAP/CS/Gr follow the same growth pattern. Immediately after inoculation HAP/CS/Gent and HAP/CS/Gr/Gent coatings expressed strong bactericidal effect against *S. aureus*, reducing the initial bacteria count by 2 logarithmic units supporting the "burst" release of antibiotic. The complete reduction of bacterial cells was achieved within 1h (HAP/CS/Gent) and 3h (HAP/CS/Gr/Gent) *i.e.,* the effect is highly bactericidal. Retained antibacterial effect of HAP/CS/Gent and HAP/CS/Gr/Gent (Figure 1b) coatings was clearly seen against *E.*

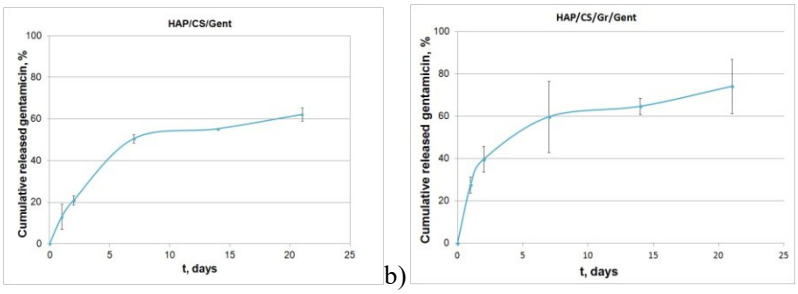
coli. In the initial period after inoculation the release of the drug hardly affected the bacteria, their sensitivity toward antibiotic was low. For the duration of the experiment (24 h post incubation) substantial decline of surviving *E. coli* cells was observed. Comparing the effects of these composite coatings and based on the kinetics testing against *E. coli*, since the reduction in bacterial cells was less than 3 logarithmic units, HAP/CS/Gent and HAP/CS/Gr/Gent composite coatings were classified as bacteriostatic.



a) b)

Figure 1: Reduction of viable cell number of: (a) *Staphylococcus aureus* and (b) *Escherichia coli* after contact with HAP/CS, HAP/CS/Gent, HAP/CS/Gr and HAP/CS/Gr/Gent coatings for 0, 1, 3, and 24 hr in PB compared to the control

The amount of loaded gentamicin in the composite coating, and the corresponding release profiles were obtained by high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS). Gentamicin release from composite HAP/CS/Gent and HAP/CS/Gr/Gent coatings were studied during 21-day immersion in deionized water and measurements were done at predetermined periods of time (24 h, 48 h, 7 days, 14 days, and 21 days). The cumulative release profiles are represented in **Figure 2** as an average of three samples.



a) b)

Figure 2: Cumulative gentamicin release during 21 days in deionized water at 37 °C. (a) HAP/CS/Gent coating (b) HAP/CS/Gr/Gent coating

Gentamicin-release profiles verified the initial burst-release effect of gentamicin from the composite coatings, i.e., more than 50% of the loaded antibiotic was released within 7 days in the case of HAP/CS/Gent coating and at the similar rate for HAP/CS/Gr/Gent coating ~60%. This initial 7-day period was followed by a slower release pattern, only 13% and 14% respectively, of the released drug until day 21. Considering the gentamicin-release profiles, the obtained composite coatings could have potential use as prolonged drug-delivery systems for treating orthopedic infections.

Conclusions and/or Outlook

Titanium surface was modified by applying new composite, biocompatible, and bioactive hydroxyapatite/chitosan coatings with and without gentamicin using the electrophoretic deposition technique (HAP/CS, HAP/CS/Gent, HAP/CS/Gr, and HAP/CS/Gr/Gent). HAP/CS/Gent and HAP/CS/Gr/Gent coatings presented strong activity against both bacterial strains tested and even noticeably more against *S. aureus*, while their counterparts without the antibiotic did not exhibit antibacterial properties. The rapid and strong antibacterial effect monitored during the first 24 h coincides well with the initial burst effect of gentamicin observed on the cumulative release profiles. Presented results are a solid confirmation that bioceramic HAP/CS/Gent and HAP/CS/Gr/Gent composite coatings electrodeposited on Ti are promising biomaterials for further investigation and potential biomedical application as orthopedic and dental implants.

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Chitosan-metal nanoparticles composite with enhanced antibacterial activity

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Keywords: Biopolymer, Chitosan, AgNPs, Antimicrobial, Coating

Introduction

Chitosan is a polysaccharide obtained from chitin through a process involving deproteinization, demineralization, discoloration and deacetylation. The interest for chitosan in biomedical applications such as tissue-cartilage engineering, wound healing and orthopedic applications [1] is due to its high biocompatibility, biodegradability, porous structure, suitability for internal cell growth, osteoconduction and intrinsic antibacterial nature [2]. Moreover, chitosan is used as coating material of bone implants with antibiotic drugs or other biopolymers, in fact it possesses a wider spectrum of efficacy, a higher microbial killing rate and lower levels of toxicity for mammalian cells [3] with respect to other natural antimicrobials. The mechanism of bactericidal action considers the interaction between the positively charged chitosan molecules and the negatively charged microbial cell membranes, resulting in protein and other intracellular components leaking [4].
Silver nanoparticles (AgNP) are increasingly used in various fields due to their unique physical and chemical properties such as: optical, electrical, biological and thermal properties, high electrical conductivity [5,6]. In particular, their remarkable antimicrobial properties have favoured their use as antibacterial agents. AgNP act by indirect damage, generating reactive oxygen species and by direct damage, interacting with the bacterial membrane causing cytoplasm to escape and cell death. [7]. Considering the properties of the two compounds, it was thought to produce a nanostructured composite material that combined the film-forming properties and the increased antimicrobial activity for gram positive bacteria of chitosan [4] with the marked microbicidal capacity for gram negative bacteria of silver nanoparticles [8]. The obtained composite will have highly adaptable antibiotic characteristics and could be used as a coating for metallic bone implants. The chitosan/AgNPs composite was prepared by direct synthesize the metallic particles in a chitosan solution by Laser Ablation in Liquid (LAL) technique.
The obtained composite was characterized by ultraviolet visible spectroscopy (UV-vis spectroscopy), X-ray diffractometry (XRD), Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), transmission electron microscopy (TEM).
Finally, the antimicrobial activity of the solutions obtained on Gram positive and Gram negative bacteria was tested by scalar dilutions method in liquid.

Results and Discussion

Uv-Vis spectrum of the silver nanoparticles obtained by laser ablation in the chitosan solution presents the characteristic band at 400 nm. The absorbance and shape of the band remains unchanged for several days (30) confirming the stability of the obtained solution. AgNPs, observed by TEM, are spherical and well dispersed in the polymeric matrix and have a mean diameter of 18 nm and 15 nm for 1 g/L and 0.1g/L solution, respectively. XRD analysis confirmed the synthesis of a composite material where chitosan and metallic silver characteristic features can be recognized. To evaluate the possible modification of chitosan during laser ablation, XPS and FTIR analysis were carried out. Both analysis confirm that the characteristic groups of chitosan are present also in the polymeric/metallic composite. Antimicrobial tests shown, Figure 1, a bactericidal activity of both components against both gram positive and gram negative with an MIC between 0.5 and 0.25 g/L and 0.5 and 0.125 g/L for gram positive and gram negative, respectively. Moreover, an enhanced antimicrobial effect can be observed for composite solutions with respect to both chitosan both silver used individually, in the same concentration range.

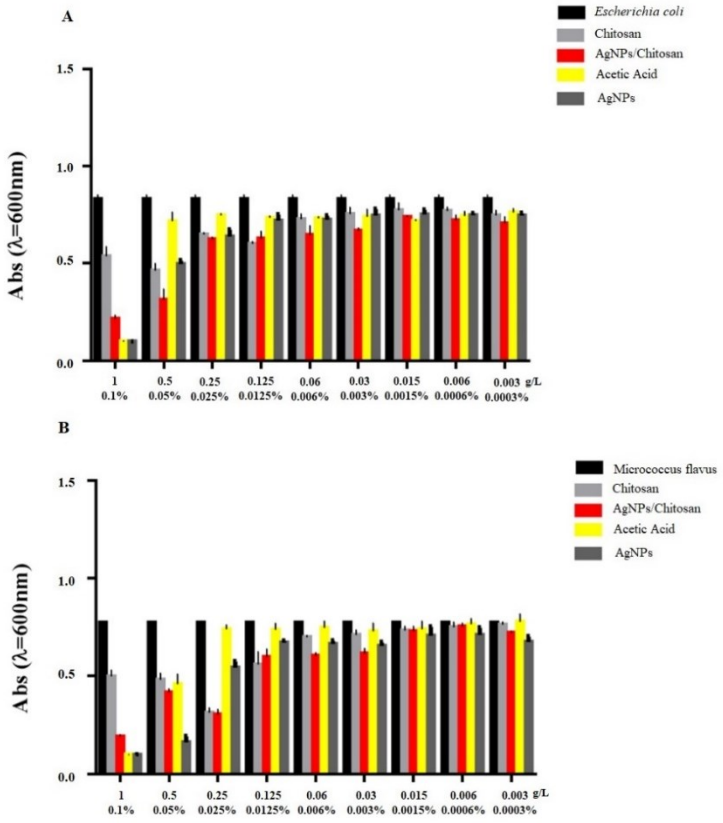

Figure 1: antibacterial activity of chitosan, AgNPs and chitosan/AgNPs composite in Gram negative and Gram positive bacterial cultures (panel A and panel B, respectively). Concentrations expressed as g/L refer to the concentration of chitosan, while those expressed as a percentage refer to the concentration of acetic acid.

Conclusions and/or Outlook

A composite polymeric/metallic material has been synthesized by nanosecond laser ablation of silver in acid chitosan solutions. The characteristic functional groups of the polymer remain unchanged during the AgNPs generation. Chitosan and AgNPs have a synergy of effect, demonstrated by the greater antibacterial activity of the composite with respect to both component individually.

The antimicrobial activity of silver nanoparticles could be improved, as reported in several studies [9], by the formation of nanoparticles with smaller average size.

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Microstructure and mechanical properties of new Ni-Cr and Co-Cr dental alloys

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Keywords: biomaterials, dental alloys, corrosion, metallography, three-point bending.

Introduction

Metals, as well as their alloys, have played an important role in reconstructive surgery as structural biomaterials [1]. Dental implants have recently emerged as a viable oral rehabilitation therapeutic option [2]. Biocompatibility, non-toxicity, safety, and stability, in addition to mechanical qualities, are all required for metals used in dental applications [1].

The most often utilised alloys for the manufacture of permanent and removable dental prostheses are nickel- based and cobalt-based alloys [3,4]. Because of their low cost, outstanding clinical performance, strong corrosion and wear resistance, and capacity to modify immunological response, nickel-chromium based alloys have been the metal framework material of choice for porcelain-fused-to-metal permanent dental prostheses and for deposition applications [5,6]. However, the emission of metal ions has some harmful consequences [5,7]. As a result, several nations have opted to replace these metals with cobalt-chromium alloys for prosthesis fixation [8,9].

Cobalt-based alloys, on the other hand, are known for their great creep, corrosion, wear, and temperature resistance, as well as their hardness, non-magnetic characteristics, and cytocompatibility [8,10,11]. Although the number of people experiencing allergy responses as a result of using cobalt-chromium alloys is relatively uncommon, the discharge of metal ions from these alloys might trigger specific reactions in the human body [12].Metallography, electrochemical, and three-point bending tests were used to assess and compare the corrosion effects and mechanical characteristics of two CoCr and NiCr-based samples.

The samples were inserted in an epoxy resin cylinder for further cutting and polishing using progressive grain silicon carbide emery sheets and 0.1 micrometres alumina suspension before using the study methodologies. Thesamples were then chemically etched after being bathed in an ultrasonic machine to eliminate any signs of dirt. The samples were subjected to metallography tests to examine their surface structure, corrosion tests to determine their corrosion resistance, and three-point bending tests to determine their modulus of elasticity.

Results and Discussion

The crystalline structure of the NiCr and CoCr samples after chemical etching under the microscope at different magnifications can be shown in **Figure 1** after the procedures were employed in the metallographic test.

In addition, the samples were submerged in Grifols Ringer's solution, which mimics the physiological fluid ofthe human body, during the corrosion testing. To acquire the data and graphs, the EC Lab software was used to process all electrochemical experiments. The passivation or corrosion propensity of each sample is determined by plotting E-potential versus time. To get the Bode Impedance plots, the EIS approach is applied to each specimen at various potentials. The higher the applied voltage, the higher the impedance and phase angle values were also verified.

In the three-point bending test, however, the NiCr specimen utilised a lower support spacing than the CoCr specimen due to the shorter length of the specimens. The modulus of elasticity values of the tested NiCr specimens were greater than those of the CoCr specimens for the same support spacing.

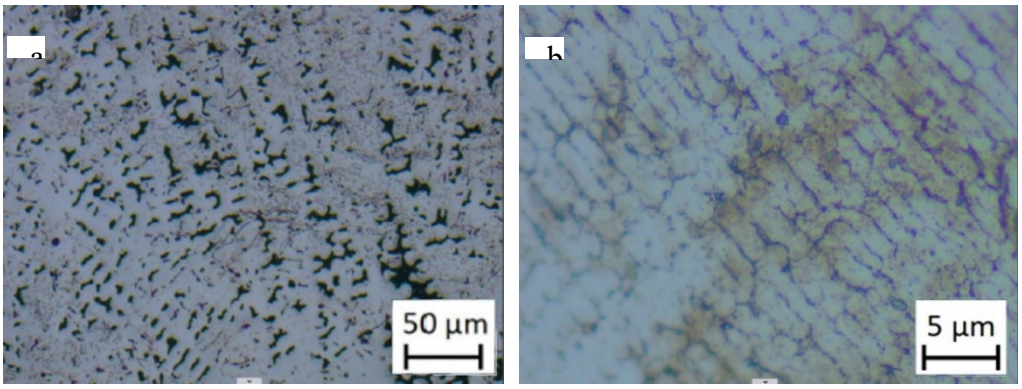

Figure 1: Optical microstructure after reagent etching at x5 magnification for NiCr alloy (a) and at x50 magnification forCoCr alloy (b).

Conclusions and/or Outlook

The corrosion behaviour and mechanical characteristics of dental alloys based on NiCr and CoCr were examinedand compared in this study.

The metallography test revealed that both samples have a dendritic structure, despite certain flaws. When it comes to determining the corrosion potential, the samples tend to passivate but not stabilise, thus this test should be repeated over time to determine if a stable potential is established. The greater the result of the impedance value, the stronger the corrosion resistance, according to the electrochemical impedance spectroscopy (EIS) technique and Bode impedance graphs. As a result, the CoCr alloy has stronger corrosion resistance than Ni-Cr alloy because it has a higher impedance value and a higher positive corrosion potential.

Furthermore, the CoCr alloy had a lower modulus of elasticity in the three-point bending test, although the results of both samples were widely spread.

Nonetheless, no apparent association between the degree of molybdenum and silicon concentrations in the alloysand the electrochemical corrosion behaviour has been established.

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The Effects of Ultrasonic Scaling and Air-Abrasive Powders on the Decontamination of 9 Implant-Abutment Surfaces: Scanning Electron Analysis and In Vitro Study

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Keywords: abutment surface, abutment connection; peri-implant response; implant decontamination.

Introduction

One of the most investigated aspects in contemporary dentistry is the long-term maintenance of dental implants and the prevention of peri-implantitis. This condition is characterized by inflammation of the peri-implant connective tissue and by a non-linear, accelerated bone loss supporting pattern. A study by Derks et al. reported that 45% of implanted patients are affected by peri-implantitis. Several factors can influence this susceptibility to peri-implantitis, increased by poor patient compliance, such as poor oral hygiene and smoking habits. Furthermore, uncontrolled diabetes, periodontitis history and biphosphonates are factors that can lead to an altered immune response. Shifting out attention from the host to the implant-supported restoration, it is possible to observe different aspects that are not present in natural teeth. Factors responsible for bone reconstruction failure, can be incorrect 3D implant positioning, incorrect soft tissue management, abutment unscrewing, or overload induced implant fracture, incorrect finishing line positioning with soft tissue margin line, infection of internal tolerance spaces at the connection and presence of non-resorbable cement. All the implants have a macro- and micro-geography of surface that does not make the biofilm decontamination easy [1]. In addition, the implant/abutment junction has a mismatch that favors the accumulation of bacteria within the implant connection. In less-stable prosthetic connections, this causes a pumping effect of the junction which leads to the accumulation and release of bacteria [2]. For this reason, bacterial colonization of the connection inner portion can be associated with peri-implant bone loss and peri-implantitis. From a clinical point of view, in case of peri-implantitis and mucositis, it would, therefore, be desirable not to simply debride the connection with ultrasound and airflow, but remove and decontaminate the prosthetic connection and abutment. For many clinicians, the treatment of mucositis and peri-implantitis is a challenge. In fact, the surface micro-topography can influence the biofilm in terms of bacteria adhesion and release of pro-inflammatory and necrotizing cytokines. It can often result in the formation of ecological niches that are difficult to decontaminate. Many authors have proposed the use of powders and ultrasounds for biofilm removal [3]. Often this practice can result in incomplete removal of the biofilm, which can be responsible for incomplete healing. In fact, in the deeper pockets the action of ultrasound loses its effectiveness [4], whereas the airflow can lead to the formation of emphysema. Some authors have investigated the ability of different air powder abrasive systems to decontaminate different surfaces.

In this study, attention was focused on the use of ultrasound and bicarbonate powders because, among general dentists, they are common materials in a dental office. The purpose of the in vitro study is to understand from a microscopic point of view whether the use of bicarbonate powders associated with ultrasonic instruments can decontaminate nine different kinds of surface used for the manufacturing of abutment/implant junction. Furthermore, attention will be paid to the regrowth of fibroblasts above the decontaminated surfaces in order to understand if there are significative differences in terms of biocompatibility of the different substrates.

Results and Discussion

After taking samples of patient plaque, nine different surfaces were contaminated and analyzed by SEM, then their wettability was evaluated. Fibroblasts were cultured on the decontaminated surfaces to understand their ability to establish a connective tissue seal after decontamination. The results were analyzed from a statistical point of view to hypothesize a mathematical model capable of explaining the properties of the surfaces. A negative cor- relation between roughness and contamination has been demonstrated, whereas a weak correlation was observed between wettability and decontamination capacity. All surfaces were topographi- cally damaged after the decontamination treatment. Grade 5 titanium surfaces appear tougher, whereas anodized surfaces tend to lose the anodizing layer.

Figure 1: Micrographs of the sterile, contaminated by plaque and decontaminated surfaces.

- *Titanium Grade 4: RS: machined surface; RS-GI: machined anodized surface; UTM: "micro-grooved" Ultrathin Threaded Microsurface; UTM-GI: "micro-grooved" Anodized Ultrathin Threaded Microsurface; XA: "micro-grooved" Thin Machined surface; XA-GI: Anodized "micro-grooved" Thin Machined surface; ZT: zirconium oxide-sandblasted and mineral acids-etched surface.*
- *Titanium machined Grade 5 Surface: RS-GR5.*
- *Yttria-stabilized zirconia ceramic surface: ZR.*

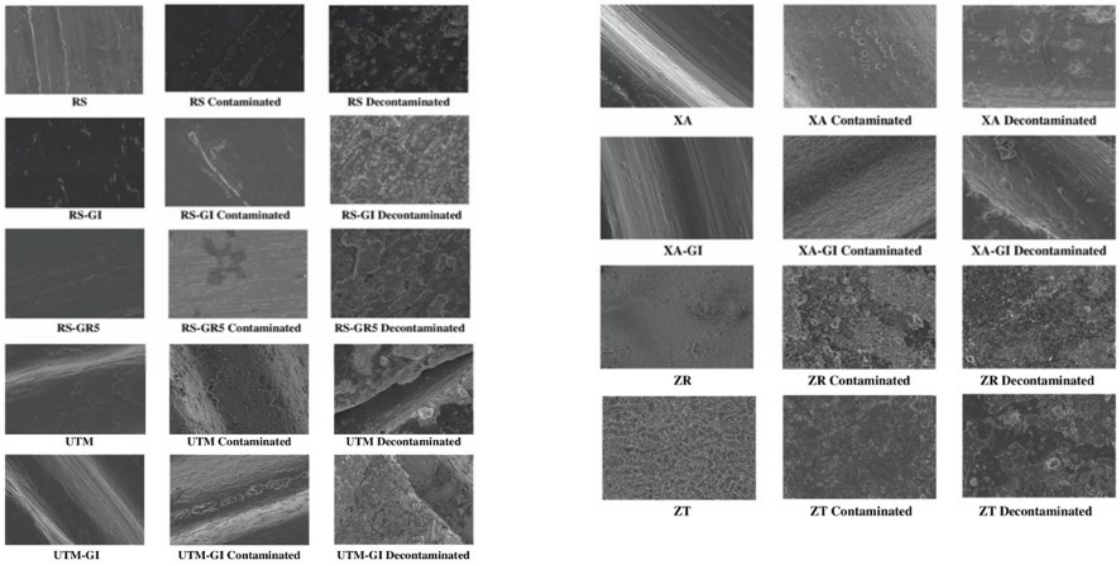
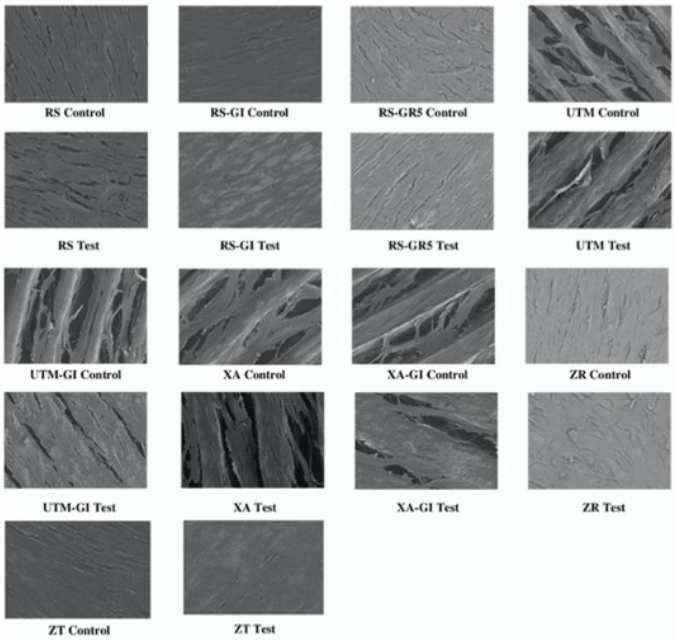


Figure 2: Micrographs of growth of fibroblast on the nine sterile (control) and decontaminated (test) surfaces.



Conclusion

The following study demonstrated how nine different types of surfaces used to pro- duce implants and abutments react differently to bacterial colonization and decontamination with powders and ultrasounds. A negative correlation between roughness and the possibility of contamination has been demonstrated. The growth of fibroblasts gave discor- dant but statistically significant results. Future studies will be needed to fully understand how these decontaminated surfaces affect the adhesion, proliferation and differentiation of fibroblasts and osteoblasts.

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3D printing of porous calcium phosphate ceramics

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Keywords: Extrusion-based 3D printing technique, Calcium phosphate ceramics, Bioactivity

Introduction

In recent years, significant attention has been paid to ceramic materials for bone tissue engineering. Among the many biomaterials, calcium phosphate ceramics has been widely used for this purpose due to its bone regenerative property [1, 2]. The using of 3D printing makes it possible to obtain individual implants for each patient, as well as significantly reduce manufacturing lead-times [2–4].

The aim of this work was development of compositions and technological parameters for the production of calcium phosphate ceramics, establishing the relationship between physicochemical properties of the synthesized materials, their structure and composition of initial mixture.

Results and Discussion

In this study hydroxyapatite was synthesized by aqueous precipitation according to the following equation:

$$Ca(NO_3)_2 + 6(NH_4)_2HPO_4 + 8NH_4OH \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 20NH_4NO_3 + 6H_2O.$$

Particle size of synthesized powder was between 0.05–50 μm (Analysette 22), X-ray phase analysis (diffractometer DRON-2) indicated only the hydroxyapatite phase.

The synthesized hydroxyapatite was used to prepare of slurry for 3D printing. Calcium dihydroorthophosphate, amorphous silicon dioxide nanoparticles, calcium pyrophosphate nanoparticles, aluminium oxide nanoparticles, sol-gel derived quartz glass or bioglass were used as additives. The amount of additives was 2.5–10.0 wt.% over 100 %. The optimal physico-technical properties of slurries allowed 3D printing were as follows: dynamic viscosity – 20–30 Pa·s; pH – 7; moisture content – 45–50 wt.%. In this study, an 20×20×20 mm^3 model was created. The printed samples were dried at 50–70 ºC for 24 h and fired at 500–1200 ºC for 8 h.

Physical and chemical properties of the samples:

	water absorption, %	apparent porosity, %	bulk density, kg/m^3	compressive strength, MPa
Without additives	10.0–49.6	23.8–59.7	1200–2380	4.1–41.8
Calcium dihydroorthophosphate	32.0–51.8	48.3–61.6	1510–1167	1.6–11.2
Amorphous silicon dioxide nanoparticles	18.9–54.8	35.7–61.4	1110–1885	3.3–27.1
Sol-gel derived quartz glass	25.0–51.6	43.4–60.7	1142–1745	1.5–19.3
Sol-gel derived bioglass	18.0–44.0	34.9–56.3	1276–1940	2.9–26.7
Aluminium oxide nanoparticles	29.7–53.3	47.5–62.1	1152–1602	2.7–14.3
Calcium pyrophosphate nanoparticles	9.8–57.2	23.3–62.2	1076–2388	2.3–31.1

It was established that ceramics without additives had the maximum values of compressive strength, however, its open porosity was low. For example, the open porosity of calcium phosphate ceramics, obtained without additives at 1150 ºC, was 38.19 %, and the compressive strength – 35.2 MPa. In comparison, additives of bioglass and silicon dioxide provided higher values of open porosity (47.8–50.7 %) and compressive strength was about 20.8–22.6 MPa (Fig.1).

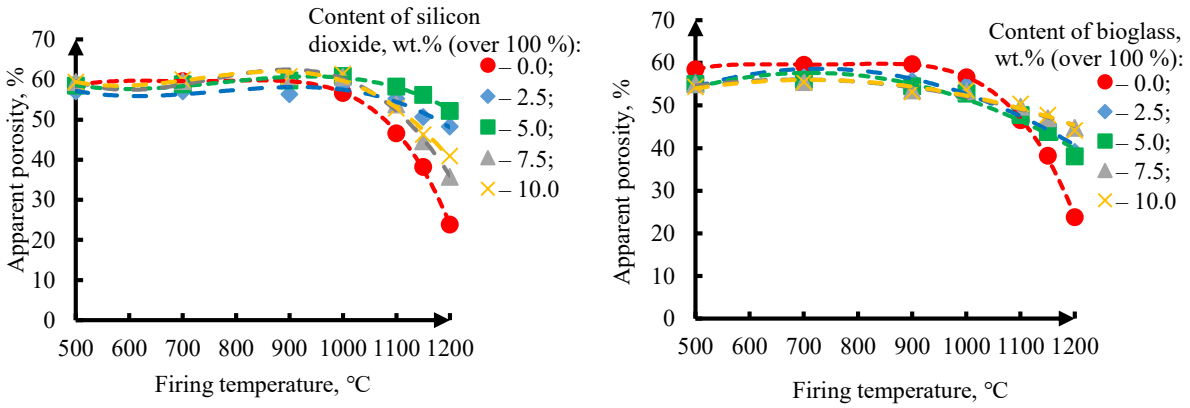

Figure 1: Apparent porosity of fired sample with respect to firing temperature

XRD study revealed that major crystalline phases were represented by hydroxyapatite and at firing temperatures above 1100 °C tricalcium phosphate β-$Ca_3(PO_4)_2$. The pore size (JSM-5610 LV scanning electron microscope) was approximately 0.5–20.0 μm. An SBF cultivation experiment was used to evaluate the biological activity. It was found that the deposition layer, after simulation body fluid.

Conclusions and/or Outlook

The obtained porous material seem to be a promising bone substitution material.

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Does a nano bioceramic surface enhance bone integration with titanium alloys?

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Keywords: titanium alloy, osseointegration, biocompatibility, bioceramic surface

Introduction

BioCera Medical have developed a process of electrochemical oxidation with elementary co-deposition, that builds nano-structured ceramic layers with desired functionality. Coatings were developed with objective of providing a robust wear resistant ceramic surface thoroughly integrated into the titanium alloy surface to stimulate apatite mineralization and osteogenic differentiation of stem cells, thus accelerating the formation of bone in intimal contact with the implant surface.

Ti_6Al_4V substrates: disks of 25 mm diameter and thickness of 5 mm were used for characterisation and *in vitro* experiments; Ti_6Al_4V wire of 0.6 mm diameter was used for in vivo investigation. Test substrates were coated using various parameters to vary, thickness, porosity and composition. Four types of coatings were made in different electrolytes:

Type 1: hydroxide and phosphate of alkaline metals, zirconium salt
Type 2: Type 1 + Zirconium oxide nano powder
Type 3: Type 1 + Titanuim oxide nano powder
Type 4: Type 1 + Hydroxyapatite nano powder

The test piece surfaces were evaluated to determine *in-vitro* bioactive response to the differentiation of soas-2 cells. Cell viability was determined by alamar blue assay after 1, 14 and 28 days of culture. Mineralization on the surfaces was analysed by SEM and energy-dispersive X-ray spectroscopy (EDX). The in vivo response of Ti_6Al_4V coated rods were evaluated in a rat tibia fracture model, evaluating bone contact at 48 days.

Results and Discussion

The ALP activity of all Soas-2 cells for all groups increased with time compared with control samples (plain tissue culture plastic and bare Ti alloy (Figure 1). Cell proliferation and ALP activity results indicated that these bio-ceramic coatings on Ti substrate advanced cell proliferation and cell differentiation with an increase over time (Figure 2). Explanted analysis by micro CT analysis after 48 days demonstrated high percentage of bone volume fraction (BV) and bone implant contact (BIC) that was 65% higher than for uncoated Ti sample.

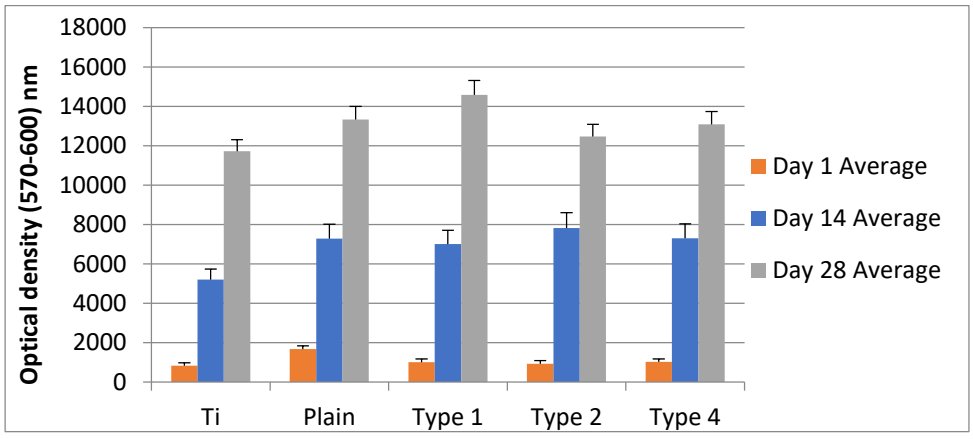

Figure 1: Alamar blue test of Soas-2 cells after being cultured with three types of coatings

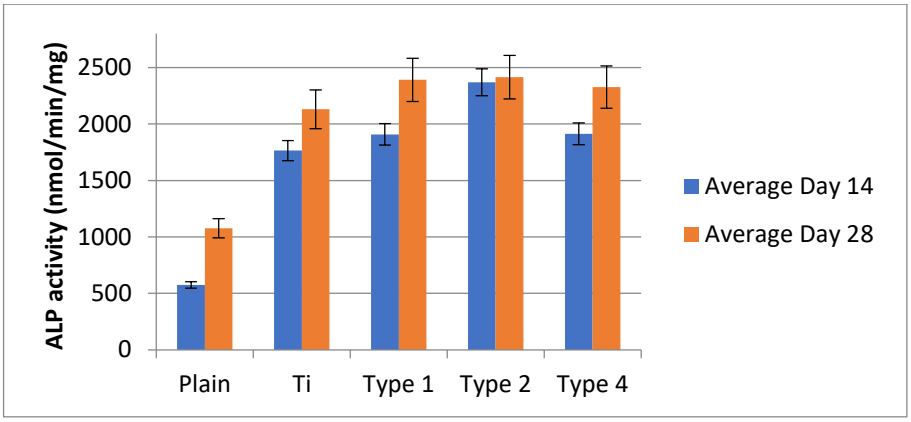

Figure 2: ALP activity of Soas-2 cells cultured for 14 and 28 days

The study was designed to test an implant with modified surface treatment. ZrO_2 and TiO_2 nanoparticles have been formed on the surface of titanium implant (Figure 3). High flexural strength, biocompatibility, bone generative and regenerative of these nanomaterials have been observed. Four types of coatings have been produced to test different concentration of Zr and Ti in the coatings for further investigation of their biocompatibility, osseointegration, hardness, and corrosion resistance.

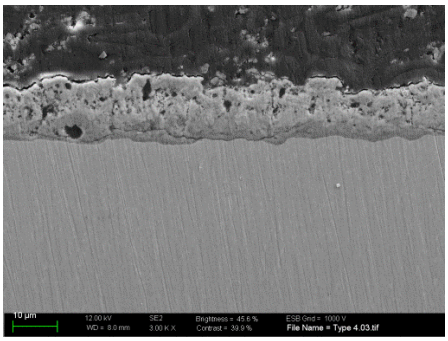

Figure 3. Example of surface structure by cross-sectional SEM of Type 4 sample

Conclusions and outlook

Four types of coatings have been produced in order to test different concentration of Zr and Ti in the coatings for further investigation of their biocompatibility, osseointegration, hardness, and corrosion resistance. Metallic leakage from implants is one of the main concerns over the long term as Al^+ release is caused by corrosion of implant. In this study, we have demonstrated a multifunctional implant surface with good osseointegration and corrosion resistance properties which could further improve the implant life and performance of medical grade titanium alloys.

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Surface Characteristics Evaluation of Translucent Multilayered Dental Zirconia Subjected to Different Methods of Aging

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Keywords: multilayered zirconia, surface roughness, autoclaving, thermocycling

Introduction

Considering that the microstructure and the surface characteristics are relevant factors that can affect the strength and the appearance of a material, the porpose of this study was to analyse the differences in terms of surface roughness and topography between layers of three types of multilayered dental zirconia with various Y_2O_3 and cubic phase amount, respectively translucencies, and the effect of finishing-performed by polishing or glazing-before and after aging process obtaining through autoclaving or thermocycling. The microstructural differences may influence the certain properties of the material: toughness, behaviour to aging and translucency [1]. Previous studies revealed that zirconia with higher cubic fraction is least affected by aging process, which is usually associated with degradation and surface roughening [2]. The monolithic multilayered zirconia are widely used in prosthodontics and the layers can have the same or various chemical composition and microstructure. Polishing and glazing procedures as surface treatments are applied to enhance aesthetics. The slight polishing may induce an increase in surface roughness and the gentle grinding no evident flows. Glazing seals the pores and smoothens the surface, on the contrary, defects may occur due to the lack of uniformity of the layer [3].

Materials and methods

Three multilayered dental zirconia were investigated: Ceramill Zolid fx ML (Amman Girrbach, AG, Koblach, Austria)-CE, IPS e.maxZirCAD CEREC/in Lab MT Multi (IvoclarVivadent AG, Schaan, Liechtenstein)-IP and STML (Katana, Kuraray Noritake Dental, Tokio, Japan)-ST. 64 plate-shaped samples of each material were cut, polished and sintered; the final dimension was 16×14×1 mm. For each material, 32 sample were polished and the 32 glazed and equally divided into two control groups and four aged groups. The aging procedures: autoclaving (1 h, 134 ◦C, 0.2 MPa) and thermocycling (30.0000 cycles in a 5˚C and a 55˚C bath with distilled water). The surfaces were assessed by microroughness and atomic force microscopy on three areas corresponding to the layers: incisal, cervical, medial. Statistical analyses were performed (α=0.05).

Results and Discussion

Mean roughness values (Ra=arithmetic average surface roughness) were recorded in cervical, medium, incisal areas on the glazed and polished samples, before and after aging (autoclaving, termocycling)-Figure 1:

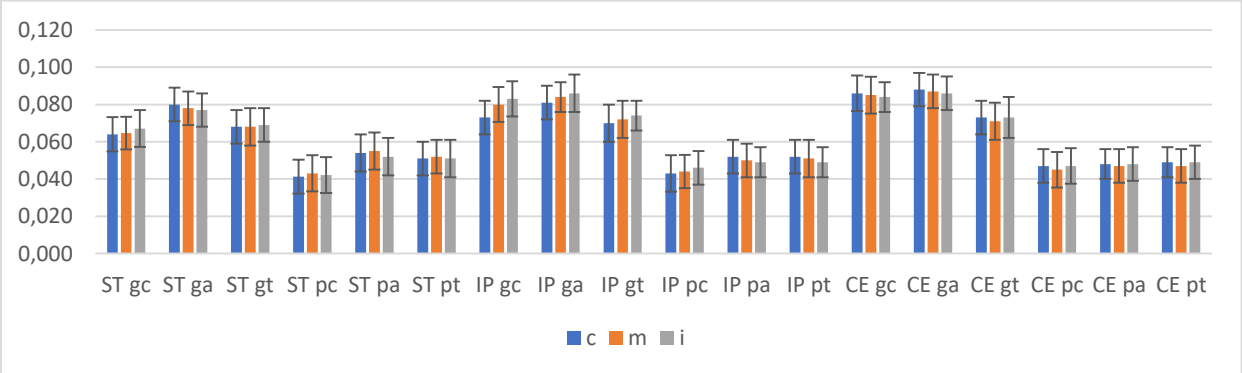

Figure 1. Mean Ra values with standard deviations, before and after aging for all groups: IP = IPS e.maxZirCAD CEREC/in Lab MT Multi, ST= STML, CE=Ceramill Zolid fx ML; gc=glazed-control, pc=polished-control, ga=glazed-autoclaving, pa=polished-autoclaving, gt=glazed-thermocycling, pt=polished-thermocycling, m=medium, c=cervical, i=incisal.

Before aging, between materials, on glazed and polished samples, the highest Ra values were found for CE and the lowest for ST. For both before and after aging stage, Ra on glazed specimens are higher than for those polished; the two-way ANOVA test

reported no significant differences between incisal-medium-cervical areas individual on glazed or polished sides; the unpaired t-Test revealed a significant difference ($p<0.05$) among the same areas of polished and glazed samples, for all materials. After aging, for autoclaved specimens, statistical paired t-Test showed on the glazed sides-no significant increase in values for CE and a significant increase for ST ($p<0.05$) and for polished sides-the Ra values for ST increased more than those for CE. After thermocycling, the surface roughness decreased for CE and IP (however, remain the roughest) and increased for ST on glazed samples and for all materials for polished samples. For a better surface topography assessment, an atomic force microscope (AFM) was used. The images revealed before aging -fine and deeper irregularities, scratches and sharp peaks, on both treated sides, for all three materials, however for CE the striations are more obvious. Both after autoclaving and thermocycling, on the glazed surfaces, the highest nanoroughness values (Sa=arithmetical mean height) were for CE and the lowest for ST and on the polished surfaces- the highest for ST and the lowest for CE; the statistical t-Test reported a significant increase on both sides. The 3D visualization of the glazed and polished ST and CE specimens, before (control) and after aging (autoclaving, thermocycling) are presented in Figure 2:

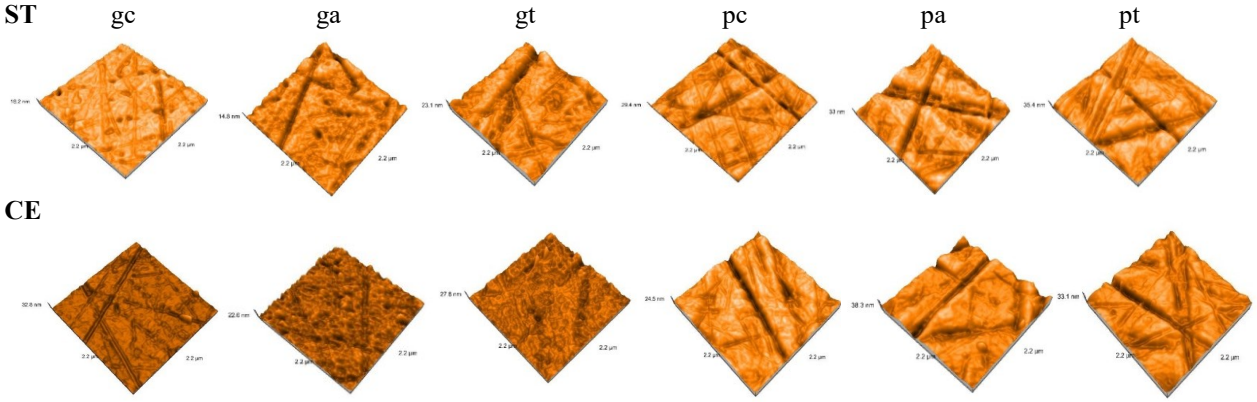

Figure 2. 3D AFM images of ST, CE glazed and polished samples; gc=glazed-control, pc=polished-control, ga=glazed-autoclaving, pa=polished-autoclaving, gt=glazed-thermocycling, pt=polished-thermocycling.

Conclusions

1. Concerning surface roughness, no significant differences among layers were recorded on polished or glazed surfaces.
2. Glazing or polishing had a significant impact on surface roughness, both before and after aging, glazing increased the surface roughness.
3. After aging an increase in roughness on polished and glazed samples was registered. ST (4 mol%Yttrium) was more affecting by aging (autoclaving, thermocycling) then CE (5 mol%Yttrium).

Author Contributions: Conceptualization, F.R.T. and L.P.; methodology, F.R.T. and L.P.; software, F.R.T. and L.C.M.; validation, F.R.T. and L.P.; investigation, F.R.T., M.I.B., I.-D.U. and R.D.V.; writing—F.R.T.; writing—review and editing, L.P.; supervision, L.P. All authors have read and agreed to the published version of the manuscript.
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Informed Consent Statement: Not applicable.
Data Availability Statement: Not applicable.
Conflicts of Interest: The authors declare no conflict of interest.

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Polyaniline colloidal dispersions with immunomodulatory effect prepared by enzyme-catalysed polymerization

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Keywords: Polyaniline, enzyme, colloidal dispersion, cytotoxicity, antibacterial effect

Introduction

The discovery of intrinsically conducting polymers (ICPs) opened the door to research of an attractive new material exhibiting interesting properties (e.g. intrinsic conductivity) and suggesting numerous possibilities with respect to its application. Polyaniline (PANI) as representant of ICP exhibits advantages, such as the ease and low cost of synthesis, the ability to electrically switch between its conductive and resistive states, and stability. PANI is commonly prepared by the reaction of ammonium persulfate with aniline in an acidic aqueous medium, where the PANI precipitates as a powder 4. Unfortunately, such PANI powder is insoluble and difficult to process. Although the solubility of PANI in aqueous environment or organic solvents can be improved, the utilization of toxic organic solvents or severe functionalization conditions make these processes inconvenient for commercial purposes, increase the environment burden and raise concerns about biocompatibility of product. One way to avoid these complications is to prepare water-dispersible PANI colloidal particles which can be routinely synthetized by the polymerization of aniline in presence of oxidizing agents and steric stabilizers, these including wide variety of water-soluble polymers and biopolymers, such as poly(vinyl alcohol) (PVA) [1], poly(vinyl pyrrolidone) 8, and poly(2-acrylamido-2-methyl-1-propanesulfonic acid) 9, among others. In addition, some of them have shown low cytotoxicity on the one hand [2].
Application of enzymes – peroxidases under synthesis of colloidal PANI appears to be an efficient and environmentally friendly procedure, which can be an alternative to chemical or electrochemical synthesis route of these systems. The advantages of this enzyme-catalysed method are the milder conditions under reaction where the oxidation rate mainly depends on the amount and activity of the enzyme on the other side, common chemical oxidation of aniline is a non-autocatalytic reaction [3]. In addition, during enzymatic oxidation supported by hydrogen peroxide only limited concentration of potentially harmful by-products (e.g. the contamination of the reaction media with salts) is formed [4], and limiting increase in acidity is observed in comparison with chemical route of synthesis [5].
In this work we prepared and characterized colloidal particles of PANI by an environmentally-friendly approach based on the enzyme-assisted polymerization of aniline in aqueous media with the aid of horseradish peroxidase, using either chitosan or PVA as a steric stabilizer. On the basis of our previous knowledge and published studies [6] we anticipated that the colloidal particles exhibit antioxidant properties, which can induce immunomodulatory effects on macrophages and neutrophils.

Results and Discussion

The colloidal particles exhibited different sizes depending on the type of polymer used for their stabilization. The PANI-PVA colloids were smaller in size (206 ± 2 nm) than samples prepared in the presence of chitosan, showing a z-average diameter of 585 ± 10 nm. The widths of distributions expressed as PDI were 0.55 and 0.45 for PANI-PVA and PANI-CHIT, respectively.
The antioxidation activity of PANI-CHIT and PANI-PVA samples, which were both capable of scavenging DPPH radicals were confirmed. Furthermore, the analyses revealed that the samples´ activity differed. The efficacy of PANI-CHIT was higher immediately after mixing with DPPH$^{\bullet}$; however, with increasing time the activity reduced and reached plateau. The activity of PANI-PVA, on the other hand, was not as high as for PANI-CHIT in short reaction times, but it gradually increased with prolonged time of contact with radicals. Earlier onset of antioxidation activity for PANI-CHIT samples can be correlated with their higher concentration of PANI (791 μg L^{-1}) in comparison with PANI-PVA (308 μg L^{-1}). Differences in the course of PANI-PVA and PANI-CHIT scavenging activities might also be correlated with differences in their particle size. In summary, PANI-CHIT and PANI-PVA scavenging activities were of 82 and 67 % after 60 min, respectively.
The non-cytotoxic threshold was achieved at dilutions of colloidal dispersion in cultivation medium lower than 10 %, except for PANI-CHIT on NIH/3T3 cell lines. Here, cell viability slightly decreased below the non-cytotoxic threshold at 10% sample dilution.
The immunomodulatory activity of PANI-PVA and PANI-CHIT was studied using two types of selected professional phagocytes, neutrophils (acting in blood circulation) and macrophages (acting in the tissues). Both types of colloidal particles significantly reduced the amount of detectable ROS produced by neutrophils spontaneously or after their activation. PANI-PVA significantly reduced ROS production in all tested concentrations. In contrast, 1% PANI-CHIT significantly up-regulated spontaneous ROS production at other tested concentrations of PANI-CHIT, the ROS production was reduced. At 5 % dilutions of all tested samples (a PANI concentration of 15.4 μg mL^{-1} in PANI-PVA and 39.6 μg mL^{-1} in PANI-CHIT) spontaneous ROS production was reduced. When OZP was used as the activator, tested colloidal particles were able to significantly reduce ROS

production in a dose-dependent manner. Considering the antioxidant activity of colloidal particles (Figure 5), the effect is related to the inactivation of ROS, not to the interaction between colloidal particles and neutrophils itself.

Conclusions

The original approach to synthesis used here, employing environmentally-friendly and biocompatible horseradish peroxidase, demonstrates a smart way of preparing conducting particles with unique properties, which can be further modified by the stabilizers used. Overall, the results of this study indicate that colloidal particles based on conducting polyaniline and chitosan (or PVA) produced by enzymatic synthesis are promising systems for biological applications.

Funding Sources

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Multidoped tricalcium phosphate/polyhydroxynonanoate-based bone substitutes

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Keywords: calcium phosphates, multidoped materials, scaffolds, polyhydroxyalkanoates

Introduction

In bone tissue engineering, considering the biomimetic approach, inorganic-organic materials containing bioceramics and biopolymers are frequently used. Among bioceramics, calcium phosphates (CaPs), due to their bioactivity and composition similar to bone apatite, are commonly applied. To achieve novel functionalities of CaPs, such as enhanced cell proliferation or antibacterial properties, modifications with various nanoparticles or ions can be considered [1], [2]. The introduction of Ag^{+} or Cu^{2+} can lead to additional antibacterial effect, whereas Si^{4+} or Ti^{4+} can improve the bioactivity of final material. Other advantage, the enhancement of mechanical properties of bioceramics can be reached by developing ceramic-polymer composites. Recently, in biomaterials applications, much attention has been paid to biopolymers of bacterial origin – polyhydroxyalkanoates (PHAs). PHAs are characterised by biocompatibility, bioresorbability and can serve as carriers of biologically active substances, i.e. hormones or drugs [3]. In biomedical applications, mostly short chain-length PHAs were applied. Nevertheless, nowadays also medium and long chain-length PHAs are considered as biomaterials.

The study aimed to design and investigate the inorganic-organic composite scaffolds, consisting of multidoped silver-silicon β tricalcium phosphate (2/3AgSi-βTCP), and an elastic biopolymer – medium chain-length polyhydroxynonanoate (PHN).

Materials and Methods

AgSi-βTCP powders were synthesized by the wet precipitation method. During synthesis, silver nitrate and silicon tetraacetate were used as Ag and Si sources, respectively. βTCP powders were doped with 2 or 3 %wt. of silver and 0.3 wt.% of silicon. To obtain ceramic scaffolds, the polyurethane foams were immersed in ceramic slurry containing multidoped βTCP powder. Green bodies were dried and sintered at 1150 °C (3h). To manufacture the inorganic-organic composites, the ceramic specimens were infiltrated with 5% PHN solution in chloroform, dried and subjected to further studies. The phase and chemical composition of developed scaffolds were investigated by X-ray diffraction (XRD), X-ray fluorescence method (XRF) and scanning electron microscopy (SEM). The compressive strength (universal testing machine Instron) as well as chemical stability and bioactivity *in vitro* of composites were studied.

Results and Discussion

Bioceramic scaffolds (AgSi-βTCP) consisted of one crystalline phase - βTCP. XRF and SEM-EDS studies revealed the presence of silver and silicon in doped CaP bone substitutes (Fig. 1 a,b). The structural studies confirmed the introduction of dopants into the βTCP structure. Elemental cell parameters in case of multidoped materials were different if compared with unmodified βTCP (higher parameter *a,* lower parameter *c*). According to the literature reports, calcium ions are replaced Ag^{+} [4] whereas SiO_4^{4-} groups take the place of PO_4^{3-} groups [5]. EDS mapping revealed that silver and silicon were evenly distributed. Microstructural observations demonstrated that the scaffolds were uniformly covered with PHN (Fig. 1 c, d). Polymer coating allow improving the materials' compressive strength from 4.6 ± 1.0 MPa to 5.8 ± 1.0 MPa. Furthermore, composites after mechanical tests maintained their integrity. Scaffolds were chemically stable during 28 days of incubation in simulated body fluid (SBF). After incubation in SBF, an apatite-like layer was formed on the surface of the multidoped tricalcium phosphate/polyhydroxynonanoate-based bone substitutes, confirming their bioactive potential.

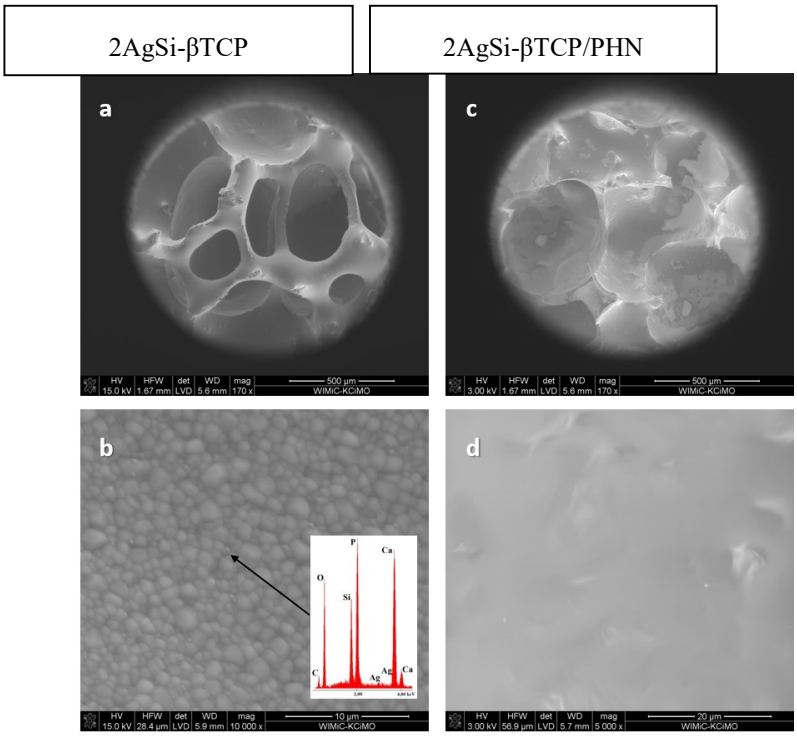

Figure 1: Microstructure of bioceramic - 2AgSi-βTCP (pictures a and b) and composite - 2AgSi-βTCP/PHN (pictures c and d) scaffolds

Conclusions and/or Outlook

The inorganic-organic composites based on multidoped with silver and silicon tricalcium phosphate (AgSiβTCP) and polyhydroxynonanoate (PHN) were successfully obtained. The introduction of dopants to bioceramic material was confirmed. The simultaneous doping of βTCP with Ag and Si and the use of bacteria-derived PHN as a coating of the ceramic scaffolds lead to the improvement of composites properties such as enhanced bioactivity and mechanical properties. We hypothesized, that by the introduction of silver into the βTCP structure, antibacterial activity of obtained materials can be achieved. Nevertheless, further studies should be performed.

Acknowledgments

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Characterization of the cellulose acetate coatings for magnesium alloys

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Keywords: Mg-alloys; composite coatings; Mg substituted hydroxyapatite; in vitro testing

Introduction.

Magnesium (Mg) plays a key role in bone growth and metabolism by modulating osteoblast and osteoclast activity in biological apatite. Its level increases as calcification progress and decreases as calcifications take place. Although research on the role of Mg on bone health is still ongoing, most studies report a positive effect on all stages of skeletal metabolism and bone health, particularly in the process of osteoporosis. Various types of polymers-based materials are extensively used in the biomedical field due to their biodegradability, nontoxicity, and compatibility with human tissues. A special place among the biomaterials of interest is taken by those used in osseointegration processes as coatings for metallic implants. This field has been in continuous development due to the practical necessity for a number of emerging biomedical applications. The most important known and used coatings for metallic implants used in orthopaedic surgery are bioceramics like calcium phosphates and bioactive glasses. The use of bioactive glass as a coating is justified by its ability to deliver, within biological fluids of the implantation surrounding site, ions leading to the hydroxyapatite formation and bonding the metal implant to bone. Besides the role that these ions have in the formation of hydroxyapatite, they also present an antibacterial effect, but it depends on the ion concentration and the pH value. The polymeric coatings for metallic implants are usually made of biodegradable and bioresorbable polymers like polylactic acid, collagen, or cellulose, designed to promote osteoblast proliferation at the implantation site through the porosity they possess and also welding the bone to the implant. The success of composite coatings has been emphasized by the development of micro and nano chemical species' synthesis: carbon nanotubes, fullerenes, graphene, hydroxyapatite, calcium phosphates, and bioglasses.

The aim of this study was to develop new composite coatings made by polymeric materials reinforced with Mg and Mg substituted hydroxyapatite, deposited on magnesium biodegradable implants with superior osseointegration potential. Used polymeric materials will be cellulose acetate, reinforced with Mg and Mg-substituted hydroxyapatite particles. Composite coatings for osseointegration were designed to be used at the interface between implants and bone in order to facilitate and improve the integration of implants in bone.

The obtained composite coatings were evaluated using electron scanning microscopy (SEM), energy dispersed X-ray spectroscopy (EDS), and FTIR spectroscopy. The degradation rate in PBS and cytotoxicity tests (MTT test, Calcein cell viability test) were also realized.

Results and Discussion

SEM images reveal pores on the surface of the composite coating, pores with irregular sizes, and distributed in regions of different densities. Compared to the cellulose acetate polymer coating, the coatings containing reinforcing elements show an increase in porosity. Reinforcing elements influence the degradation behaviour of coatings differently. Mg substituted hydroxyapatite improves the degradation behaviour, while Mg particles cause more pronounced degradation.

In terms of cytotoxicity tests, for two of the tested materials, cell viability values >80% were obtained, which indicates their non-cytotoxic character. Cell viability values < 80% could be explained by the following aspects observed during the tests: the existence of impurities left over from the process of obtaining the materials and for some materials, a slightly acidic character was observed, which led to the acidification of the culture medium.

Conclusions

The presence of Mg-substituted hydroxyapatite and Mg particles in the polymeric matrix of the synthesized coating has been shown to have an effect on the morphology and degradation rate of the polymeric coatings.

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Copper-based composite coating as an Antimicrobial Surface

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Keywords: Antimicrobial copper, copper coatings; coating methods, antimicrobial properties

Healthcare-associated infections in all hospitals generate important economic consequences for the health system of each country. No matter how much the surfaces are disinfected, they are permanently contaminated with pathogens, which often generate infections associated with healthcare, which in turn generate additional costs, increase the length of hospitalization, and sometimes increase morbidity. About 20–40% of healthcare-associated infections are due to direct contact with contaminated surfaces or affected patients via health workers in the hospital [1]. As a result, research has focused on the development of antimicrobial agents, especially antibacterial ones, and many studies have been conducted on the antibacterial effects of metals such as copper [2]. Copper particles have been scientifically proven to have strong antimicrobial activity [3]. In 2008 copper was recognized by the United States Environmental Protection Agency (EPA) as the first metallic antimicrobial agent, which generated much research into the various properties of copper as an antibacterial, antifungal, and antiviral agent [3]. In recent years, experimental research has shown that surfaces coated with at least 70% copper eliminate bacteria associated with nosocomial infections, microbes on medical devices and high-touch surfaces in the hospital environment, influenza virus, HIV, and fungi such as Candida albicans [4–6].

Due to its antimicrobial properties, copper is of great interest as a coating material. There are several methods of effectively depositing copper-based antimicrobial coatings on a variety of surfaces, including thermal spray techniques, electrodeposition, electroless plating, chemical vapor deposition, physical vapor deposition, and sputtering techniques.

This composite could be used as a self-sanitizing layer to complement infection control strategies in medical clinics, in crowded spaces such as public transportation, airports, and train stations, for clinics, for the food and pharmaceutical industry, and also in domestic applications.

The effectiveness of copper coatings on Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli Listeria onocytogenes and Candida albicans has been demonstrated.

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Comparison of Physicochemical Properties of Calcined and Uncalcined Hydroxyapatite Nanospheres

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Keywords: Hydroxyapatite, Uncalcined, Calcined, Spherical, Nanospheres

Introduction

Since hydroxyapatite has excellent biocompatibility, high mechanical strength, and no biotoxicity, it has been widely used as a biomaterial for orthopedics and dentistry for tissue regeneration or bone replacement. The general synthesis of hydroxyapatite is obtained by calcining at a temperature of 800° C or higher by various methods such as dry, wet, and high temperature. The shape of hydroxyapatite is a needle or rod-like before calcination, but it changes to a polygonal shape by volume reduction due to heat. In addition, hydroxyapatite produced through the calcination process can produce various calcium phosphate-based ceramics other than hydroxyapatite. As a result, mechanical strength, bioactivity, etc. are greatly affected, and it is difficult to control desired physical properties.

As human lifespan increases, bio-absorption researches on the implants or bone substitutes have been interested. Bioabsorbable materials are intensively focused on the biodegradable polymers until now. Recently, many researches are reported on biodegradable composite materials between polymer and hydroxyapatite with high bioactivity. However, the conventional hydroxyapatite has a degradable problem because smooth and hard crystals are formed through the interaction of single particles during the calcination process.

In this study, we prepared the calcined and uncalcined hydroxyapatite nanospheres, and then demonstrated the various physicochemical properties of these materials.

Results and Discussion

Figure 1 shows the results for hydroxyapatite, which are the particle shape results using transmission electron microscopy and diffraction pattern and crystallinity results using x-ray diffraction. Calcined HAP in (a) is a polygonal shape with a size of about 200 nm, un-calcined HAP in (b) is a spherical shape with a size of about 20 nm. Both calcined and non-calcined HAP show main peaks (121), (300), and (202) that exist between 32 and 34 degrees in (c). This is the same as the main peak in the hydroxyapatite crystal phase. According to the XRD database, it can be said that calcined was JCPDS#01-074-0565, and non-calcined had a single phase of JCPDS#01-080-6199. In addition, a relatively gentle peak appeared in non-calcining compared to calcining, which is due to the difference in crystallinity as shown in (d). The degree of crystallinity was 91.5% for calcined and 84.8% for non-calcined.

Figure 2 shows the content of calcium and phosphorus ions due to powder of hydroxyapatite using inductivity coupled plasma optical emission spectroscopy and compressive strength curves using universal testing machine. Ca, P content and Ca/P molar ratio in (a) were 42.51 wt%, 19.85 wt%, and 1.66 in calcination, respectively, and were 51.47 wt%, 27.23 wt%, and 1.46 in non-calcining, respectively. This was included in the Ca/P molar ratio range of 1.40 to 1.70 of calcium phosphate-based ceramics to which hydroxyapatite belongs. The sample used for the compression test in (b) was a green body of hydroxyapatite, and was manufactured in a ratio of 2:1 in width and length. Compressive strength was about 5% deformed when a maximum of 54.171 MPa was applied in calcination, and about 10% deformed when a maximum of 43.108 MPa was applied in non-calcining. When used as a substitute for human bone, adequate mechanical strength is required. Therefore, in the case of high strength, it causes stress shielding, so this compressive strength is a result that does not put any strain on the human body.

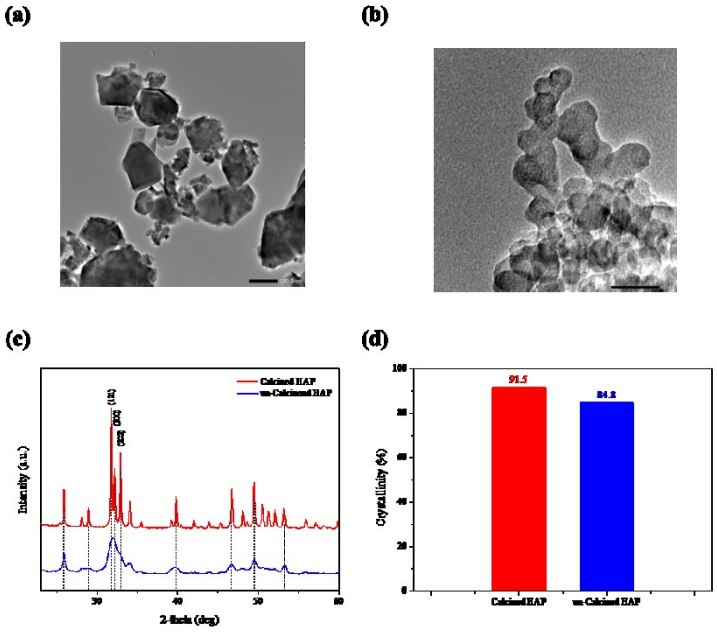

Figure 1: The result of hydroxyapatite: (a) Calcined HAP and (b) un-Calcined HAP of TEM image, (c) diffraction pattern, (d) crystallinity

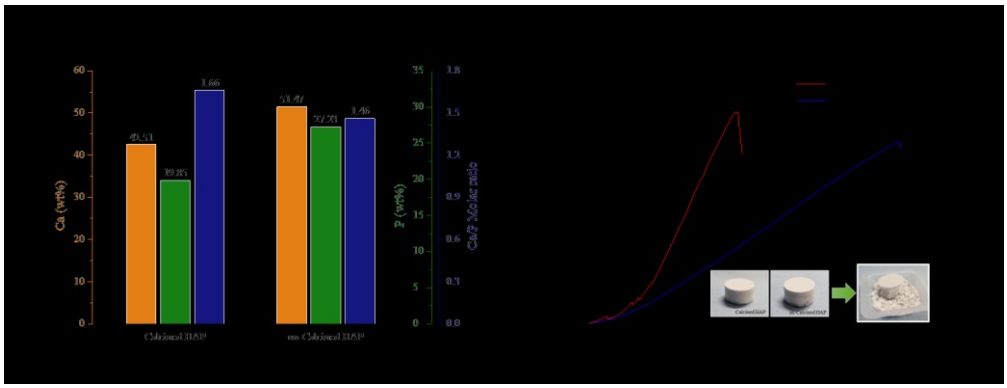

Figure 2: The result of hydroxyapatite: (a) Ca and P content and Ca/P molar ratio, (b) compressive strength-stain curves

Conclusions

The difference between calcined and un-calcined is an important variable that determines the morphology of hydroxyapatite. The hydroxyapatite single phase may be obtained without calcination, but the degree of crystallinity may be relatively low. Hydroxyapatite contained in human bone is calcium deficient including carbonate, which is lower than the Ca/P molar ratio of 1.67 of synthetic hydroxyapatite. And materials with high mechanical strength cause osteoporosis. Therefore, uncalcined hydroxyapatite may be more suitable for the human body than calcined hydroxyapatite.

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Novel copper and rare earth elements substituted whitlokite-type phosphates promissing for biomedical implant applications

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Keywords: tricalcium phosphate, whitlockite, rare earth elements

Introduction

Calcium phosphates with whitlokite-type structure (TCP) are promising material for various biomedical applications. Previously [1], the antibacterial activity of the coper-substituted was investigated against *S. enteritidis*, *E. coli* and *P. aeruginosa*. That coper-substituted phosphate exhibits a distinct antibacterial activity. In contrast, the pure TCP does not possess intrinsic bacterial properties against these *S. enteritidis*, *E. coli* and *P. aeruginosa.* It was shown that with substitution Ca^{2+} to Gd^{3+} in initial phosphate host might also provide the antimicrobial activity. The Gd-substituted powders demonstrate a good inhibition of growth for the microorganism species (*S. aureus*, *E. coli*, *S. typhimurium*, *E. faecalis*, and *C. albicans*) *The*[2].
In this case, it is necessary to obtain and investigate novel co-doped Cu^{2+} and REE^{3+} substituted whitlockite-type phosphates with general formula $Ca_8CuR(PO_4)_7$, where R – rare earth elements.

Results and Discussion

Novel phosphates $Ca_8CuR(PO_4)_7$ (with R – Eu, Tb, Dy, Ho, Er) were synthesized by high temperature solid-state method from stoichiometric mixtures of $NH_4H_2PO_4$ (99.9%), $CaCO_3$ (99.9%), CuO (99.99%), R_2O_3 (99.99%) at 1173 K for 30 h. The powder X-ray diffraction (PXRD) patterns of the precursors and the prepared compounds were checked using JCPDS PDF-2 Database. The precursors did not indicate any reflections of the impurity phases. All samples were compared with early synthesized whitlockite-type phosphate $Ca_8MgEu(PO_4)_7$ (PDF-2 Card No 45-551). All peaks in the synthesized $Ca_8CuR(PO_4)_7$ were indexed in whitlockite-type structure. The results of the Le Bail decomposition revealed *R*3c space group. So, all *as*-prepared phosphates are crystalize in the whitlockite-type structure.

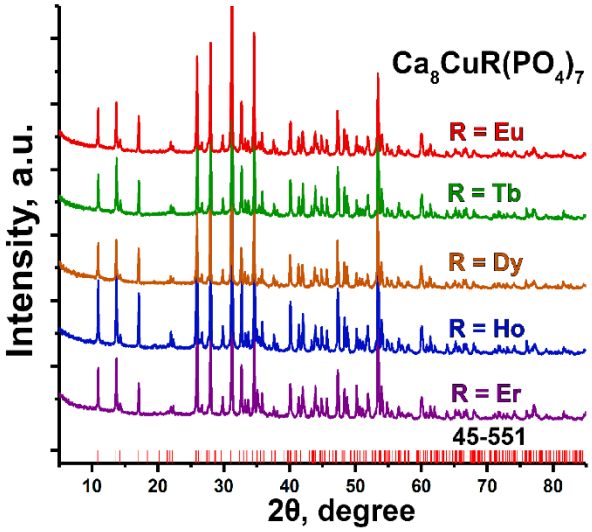

Figure 1: The XPRD patterns of $Ca_8CuR(PO_4)_7$ (R – Eu, Tb, Dy, Ho, Er) and Bragg reflections of pure $Ca_8MgEu(PO_4)_7$ (PDF-2 Card No 45-551).

All *as*-prepared phosphates may provide the antimicrobial activity, due to content of coper and REE in whitlockite-type matrix.

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