National Research Council

2ND 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

2nd biennial International Conference BIOMAH

CONFERENCE PROCEEDINGS

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

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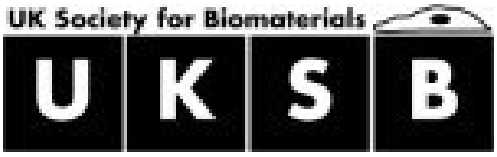

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INVITED SPEAKERS

Prof. M. Alini

(Switzerland)

Vice-Director AO Research Institute, Davos

Mauro Alini graduated in Chemistry from the University of Lausanne (Switzerland) in 1983. Since then he has been involved in connective tissue research, starting form his Ph.D. research work, done at the Laboratory of Cellular Pathology in Locarno (Switzerland), which focused on the isolation and characterization of proteoglycans extracted from both normal human mammary gland and carcinomas thereof. In September 1988, he joined the Joint Diseases Laboratory (under Dr. A. R. Poole's direction) at the Shriners Hospital in Montreal to work on quantitative and qualitative changes in extracellular matrix proteins (particularly proteoglycans and collagens) of the growth plate tissue before and at the time of cartilage matrix calcification during endochondral bone formation. In January 1995, he was appointed as an Assistant Professor at the Division of Orthopaedic Surgery of the McGill University (Chair Prof. M. Aebi) and head of the Biochemistry Unit of the Orthopaedic Research Laboratory, working to develop new biological approaches to treating intervertebral disc damage. Since July 2000, he is in charge of the Musculoskeletal Regeneration Program at the AO Research Institute (Davos, Switzerland), focusing on cartilage, bone and intervertebral disc tissue engineering. Since September 2009 is also the Vice-Director of the same Research Institute. He received the Marshall R. Urist Award in 2015 from the Orthopaedic Research Society (USA).

Prof. Luigi Ambrosio

(Italy)

Research Director, Institute of Polymers, Composites and Biomaterials,
National Research Council of Italy

Prof. Luigi Ambrosio received the doctoral degree in Chemical Engineering (1982) from University of Naples "Federico II'. He was Research Associate at University of Naples (1983-1985), Research Associate at University of Connecticut, USA (1985-1986), and Visiting Scientist at Kontron Medical Inc., USA (1986- 1988). Adjunct Professor of University of Connecticut, USA (1997-2003) and of University of Naples "Federico II" (1997-2010). Director of Institute of Composites and Biomedical Materials, National Research Council of Italy. (2008-2012). Director of Chemical Sciences and Materials Technology, National Research Council of Italy (2012-2017). Co-Director of "Multifunctional Polymers & Biomaterials Research Centre", Sichuan University-CNR, Chengdu, China (since 2013). He is member of Advisory Board and Guest Editor of International and National Scientific Journals, Vice-President of the Italian Society of Biomaterials (2006-2013), and President of the European Society for Biomaterials (2006-2013), Past President (2013-2017). He has been nominated Fellow of the American Institute for Medical and Biological Engineering (March 2001), and Fellow of Biomaterials Science and Engineering (May 2004). He received the APA Distinguished Award (2014) and the European Society for Biomaterials G. Winter Award (2015). Member of the European Commission Advisory Group of the FP7-NMP (2006-2008) and Member of High Level Group - Key Enabling Technologies- European Commission (2009-2015). Research interests include design and characterisation of polymers and composites for medical applications and tissue engineering, rheology of biological fluids, structural properties of natural tissue, properties and processing of polymers and composites and nanostructures, hydrogels and biodegradable polymers, additive technologies. Publications include over 300 papers on international scientific journals and book, 18 patents, more than 400 presentations at international and national conferences and over 120 invited lectures.

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. Avijit Banerjee

(United Kingdom)

Professor of Cariology & Operative Dentistry / Hon. Consultant and Clinical Lead, Restorative Dentistry at King's College London Dental Institute at Guy's Hospital (Guy's & St. Thomas' Hospitals Foundation Trust)
BDS MSc PhD (Lond) LDS FDS (Rest Dent) FDSRCS (Eng) FHEA -

Avijit is Professor of Cariology & Operative Dentistry / Hon. Consultant and Clinical Lead, Restorative Dentistry at King's College London Dental Institute at Guy's Hospital (Guy's & St. Thomas' Hospitals Foundation Trust). He holds the positions of Head of Conservative & MI Dentistry, Director of Education (UG) and Programme Director of the innovative KCL distance-learning Masters in Advanced Minimum Intervention Dentistry (open to dentists and therapists: google "KCL AMID" for more info). He also leads the Cariology & Operative Dentistry research programme at the DI (part of the Tissue Engineering & Biophotonics research division), researching, publishing and lecturing internationally about MI operative caries management and adhesive dental materials (>120 publications, >£2 million research income, supervision of 5 post-doctorate, 12 doctorate and 17 masters students to date). He acts as an international R&D KOL for many international Industry partners, including 3M Oral Healthcare, GCE, Septodont, Dentsply Sirona and P&G Oral B. Avijit is primary author of Pickard's Guide to Minimally Invasive Operative Dentistry (10th edition; OUP, 2015), a definitive and globally respected text in its field, amongst other book editorships (Minimally Invasive Esthetics, Elsevier (2015)) and chapter contributions (including, amongst others, a caries chapter in The Principles of Endodontics 2nd ed, OUP, 2013). He is editor-in-chief of Oral Health & Preventive Dentistry (Quintessence Ltd) and an editorial board member of Journal of Dental Research, Dental Update, BDJ, International Journal of Adhesion & Adhesives, and the Primary Dental Journal. He is a member of the BDA Health & Science Committee also, all whilst maintaining wet-fingered specialist practice in Restorative Dentistry, Prosthodontics & Periodontics.

Prof. Aldo Boccaccini

(Germany)

Head of Institute of Biomaterials, Friedrich-Alexander University of Erlangen-Nuernberg, Editor Materials Letters

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. Pedro L. Fernandez

(Spain)

Dept. of Pathology Hospital Germans Trias i Pujol, Badalona Universidad Autónoma de Barcelona, Barcelona
Senior Consultant Pathologist and Section Chief of Immunohistochemistry and Molecular Pathology of the Dept. of Anatomical Pathology, Hospital Clínico , Barcelona.
Full Professor, University of Barcelona Medical School.

Graduate in Medicine in 1985. . PhD in Medicine by the University of Granada in 1990.
Specialist in Anatomic Pathology, University Hospital of Granada, Spain, 1987-90.

Dr. Fernández has led 13 research projects funded by public and private agencies investigating breast and prostate cancer carcinogenesis and is author of 150 peer-reviewed papers, mostly in international journals. He has directed 6 doctoral theses, the last one dealing with the role of miRNA in breast cancer progression and holds a patent related to a method for molecular detection of prostate cancer in biopsy samples. He has also published 22 chapters in books and was in charge of the traslation of the 7th edition of Robbins and Cotran Pathologic Basis of disease. Dr. Fernandez has been coordinator of the Catalonian Tumour Bank network

Prof. Dr. Gultekin Goller

(Turkey)

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. 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 professional and scientific activity comprises: papers, which are cited over 1050 times, published in science citation index journals (95); papers published in international peer-review periodicals (7); the proceedings of international or national conferences (105); participating in different international or national research projects (48); author of several 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. Elizaveta Kon

(Italy)

Associated Professor Humanitas University, Milan

Orthopedic surgeon, Center of Functional and Biological Reconstruction of the knee, Humanitas Research Hospital, Milan
2nd Vice President of International Cartilage Repair Society (ICRS)
2009-2017 Director of Nano-Biotecnology Laboratory and orthopedic surgeon, Rizzoli Orthopedic Institute, Bologna.
2013-2017 Assistant Professor, University of Bologna
Coordinator of numerous research projects and clinical trials regarding biotechnology applications in orthopaedics, into the framework of Italian and European research.
Author of over 180 scientific articles in peer-reviewed journals and over 30 chapters in textbooks in orthopedic surgery (H-index 64).
Faculty of more than 400 society meetings all over Europe, Asia and America.
Associated Editor of BMC Musculoskeletal Disorders Journal, International Orthopedics, Journal of Experimental Orthopedics and Joints. Reviewer for more than 20 Orthopaedics Journals
Winner of several awards:

- Most cited publication 2011-2016 in Arthroscopy Journal
- Most downloaded publication 2011-2016 Arthroscopy Journal
- One of the most highly cited papers 2014-2015 in Arthroscopy Journal
- Leading article Knee Surgery Sports Traumatology Arthroscopy Journal, 2013 and 2016
- Poster Award Cum Laude, ICRS World Congress, 2015 and 2013
- Socio corrispondiente Sociedad Chilena de Ortopedia y Traumatología, 2014
- One of the ten top-cited articles published in the Arthroscopy Journal 2011-2012
- Most cited pubblication 2009-2010 in American Journal of Sports Medicine
- Best shientific paper, ORTOMED Meeting 2012
- Scientific exhibit award of excellence AAOS Annual Meeting 2011
- ESSKA/AOSSM European/North American Sports Medicine Traveling Fellowship, 2009
- ICRS Travelling Fellowship, North America, 2004

Prof. Alvaro Mata

(United Kingdom)

Professor in Biomedical Engineering and Director of the Institute of Bioengineering at Queen Mary University of London

Alvaro Mata's research is focused on developing supramolecular engineering strategies to create new biomaterials for tissue engineering, regenerative medicine, and *in vitro* models. He holds a Bachelor's Degree from the University of Kansas, a Master's Degree from the University of Strathclyde, and a Doctor of Engineering Degree from Cleveland State University. During his doctorate he worked at The Cleveland Clinic with Prof. Shuvo Roy and as a Postdoctoral Fellow with Prof. Samuel Stupp at Northwestern University. From 2008-2013 he was Head of the Nanotechnology Platform at Parc Científic Barcelona in Spain and is currently Professor in Biomedical Engineering and Director of the Institute of Bioengineering at Queen Mary University of London. He holds six patents or patent applications; publications in journals including Science, Nature Chemistry, and Nature Materials; and awards such as the Baxter Early Career Award in 2006, Ramon y Cajal Award in 2010, ERC Staring Grant in 2013, and Frontiers Innovator Award from the Wellcome Trust in 2015. More information can be found at: http://www.matabioengineering.com/, https://twitter.com/mata_lab.

Prof. Horatiu Moldovan

(Romania)

Faculty of Medicine, Titu Maiorescu University, Bucharest,
Faculty of Materials Science and Engineering, University Politehnica of Bucharest
Sanador Clinical Hospital, Bucharest

Horatiu Moldovan received the M.D., Ph.D. and Postdoc degrees in Medicine and Cardiovascular surgery at University of Medicine and Pharmacy Carol Davila, Bucharest. Since 1990, he has been appointed as university assistant and later, Associated Professor of Cardiovascular surgery at the Faculty of Medicine of the University of Medicine and Pharmacy Carol Davila, Bucharest. In the meantime, he worked as cardiovascular surgeon at the C.C.Iliescu Institute of Cardiovascular diseases and from 2006 untill 2015 as Head of the Cardiovascular surgery department. Since 2016, he is appointed as Assoc. Professor of Cardiovascular surgery at Faculty of Medicine, Titu Maiorescu University; and Assoc. Professor of Biomaterials at the Faculty Materials Science and Engineering of University Politehnica, Bucharest. He is also Head of the department of Cardiovascular surgery in Sanador Hospital, Bucharest. Dr. Moldovan has published over 30 papers in peer-reviewed journals and conference proceedings, 1 patent, several books (like Surgery of Thoracic Aortic Aneurism) and over 20 invited lectures at conferences focused on cardiovascular surgery. He is currently full member of the Romanian Academy of Medical Sciences, Vice-President, and Council Member of the Romanian Society for Cardiovascular Surgery (SRCCV), member of European Association of Cardiothoracic Surgery (EACTS) and member of International Society of Cardiovascular Surgery (ISCVS)..

He's also member of the International Society of Endovascular Surgery (ISES). Research interests include major vascular surgery, hybrid procedures in cardiovascular surgery and cardiovascular biomaterials

Prof. Giovanna Orsini

(Italy)

DDS, MSc, PhD, Associate Professor of Dental Materials and Restorative Dentistry at the Faculty of Medicine of the Polytechnic University of Marche, Department of Clinical Sciences and Stomatology, Polytechnic University of Marche, Via Tronto 10, 60126 Ancona

1994: Degree in Dentistry, University of L'Aquila, Italy;
1996: Residency in Esthetic Dentistry, UCLA, Los Angeles, USA;
2000: MSc in Biomedical Sciences, University of Montreal, Canada;
2005: PhD in Stomatology and Oral Sciences, University of Chieti-Pescara, Italy.
She is now Associate Professor of Dental Materials and Restorative Dentistry at the Faculty of Medicine of the Polytechnic University of Marche, Italy.
Dr. Orsini's research interests focus on dental biomaterials, oral calcified tissues, nanotechnology, tissue engineering, clinical trials and aesthetic dentistry. She has received several research awards and has authored or co-authored over 115 papers published in international peer-reviewed journals. Invited speaker in numerous International Congresses, member of numerous Editorial Boards of International Journals, Active member of several Dental Societies, she is presently the Italian representative of the Management Committee for the European Action COST CA16234.

Dr. Gaetano Paolone

(Italy)

Restorative and Conservative dentistry

Adjunct Professor, Restorative Dentistry, Università Vita Salute San Raffaele, Milano.
Active Member: AIC (Italian Academy of Restorative Dentistry) http://www.accademiaitalianadiconservativa.it.
Active Member: IAED (Italian Academy of Esthetic Dentistry http://www.iaed.it).
Active Member: AIOM (Italian Academy of Microscopic Dentistry http://www.aiom.net).
Private Practice, Rome, Lecturer, Master in Esthetics and Restorative Dentistry, University of Bologna, Italy.
Author and co-author of several publications in scientific international journals.
Lecturer in national and international courses and congresses.
Author of chapter 6 of "Endoprotetyka", a book from Maciej Zarov and “Moderna odontoiatria estetica Workflow dalla A alla Z”, books from Quintessence pub.

Prof. Francesco Pavone

(Italy)

Director of the European Laboratory for Non Linear Spectroscopy, University of Florence

Francesco Saverio Pavone was born in Bari the 23th March 1962. In 1989 he obtained his Laurea degree in Physics at the University of Florence. In 1990 he became Research Officer at the European Laboratory for Non-Linear Spectroscopy (University of Florence). In 1993 he obtained a Ph.D. in Optics at the National Institute for Optics (Florence, Italy). In 1997 he spent one year and half as "Maitre de Conférences Associe au College de France", Paris, with experimental work at the "Ecole Normale Superieure" (ENS) of Paris with Prof. Claude Cohen-Tannoudji (1997 Nobel price in Physics). In 1998 he became associate Professor of physics at the department of physics of the University of Perugia, Italy and Scientific director of the section of Atomic and Molecular Physics at the European Laboratory for Non-Linear Spectroscopy (LENS), Florence, Italy. In 2001 he moved as associate professor to the university of Florence (dept. of physics) and became scientific responsible of the "biophysics laboratory" of the European Laboratory for Non-Linear Spectroscopy (Florence, Italy). In 2005 he became full professor .

As scientific experience, from 1990 to 1995 he worked in the field of "Atomic and molecular spectroscopy". From 1995 to 1999 he worked in the field of Atomic physics, and since 1999 in Biophysics. Currently he is directing a research group of more than 40 people working in the field of biophotonics on single molecule biophysics, microscopy imaging-spectroscopy techniques, biomedical imaging, laser manipulation of bio-samples.
In particular, he is developing new microscopy techniques for high resolution and high sensitivity imaging, and for laser manipulation purposes. These techniques have been applied both for single molecule biophysics, single cell imaging and optical manipulation. Together with this, Pavone is working also in the field of tissue imaging, where non linear optical techniques have been applied for tissue pathology detection or for neural or cardiac imaging. In this area, many contributions have been realized with new techniques based on imaging and spectroscopic content to detect neural and cardiac activity together with whole organ imaging to connect structure and functionality.

Pavone is authors of many internationals papers and editor of international books. He has more than 100 invited talk and he is editors of international journals. He coordinates several European projects, he is member of the Scientific Infrastructure Board of the European Flagship "Human Brain Project" and he has organized several international congresses: He is also director of the European Laboratory for Non Linear Spectroscopy in Florence and winner of a European Research Council Advanced Grant. He has an *h-index* equal to 45 (Google scholar). He is in the evaluation panel of the European Research Council (PE3) and the DFG (Germany), fellow of SPIE and fellow of the AIMBE. Finally, Pavone is founder of the academic spin off company "Light4tech" (ww.l4t.it) and author of few patents.

Prof. Federico Quaini

(Italy)

Associate Professor of Oncology, University of Parma; Director of the Cardiac Stem Cell Center CISTAC, University of Parma; Expert Manager on Regenerative Medicine, University Hospital Parma; Coordinator School of Specialization in Oncology

1979-1985: Assistant Professor of Medicine, University of Parma,
December 1981 and January - July 1984: Research Assistant Professor, Department of Anatomy, New York Medical College
1984: Adjunct Assistant Professor of Histology, City College University of the NYU
1985-1996: Associate Professor of Medicine, University of Parma
1987: Research Assistant Professor, Department of Anatomy, NY Medical College,
1994, 1997, 2001-2007: Visiting Professor, Cardiovascular Research Institute, Department of Medicine, NY Medical College
Associate Professor of Oncology, University of Parma 1996-
Teaching positions: Head of the Course in Hematology-Oncology, Faculty of Medicine-University of Parma; Course in Regenerative Medicine, Faculty of Biotechnology-University of Parma. Coordinator of the Board in Oncology-University of Parma
Editorial Board PloSOne
Reviewer for: PloSOne, Stem Cell, Biochemical Pharmacology, Stem Cell Translational Medicine
Supported Research:
-Co-Principal investigator of the European Project N° 214539 FP7-NMP-2007: BIOSCENT BIOactive highly porous and injectable Scaffolds controlling stem cell recruitment, proliferation and differentiation and enabling angiogenesis for Cardiovascular ENgineered Tissues, 2008-2013
-Co-Principal Investigator of Regional Project on Regenerative Medicine, 2008-2012
- Co-Principal Investigator of AIRC 2017 project: Study of PD-L1 and other immunotherapy efficacy predictors on cytology and circulating tumor cells in advanced NSCLC
Membership - International Association for the Study of Lung Cancer, European Society of Medical Oncology, Council Basic Cardiovascular Science, Italian Society of Cardiovascular Research

Statement of Interest: Translational Research in Normal and Cancer Stem Cells, Tumor Microenvironment, Regenerative Medicine, Identification of the role of stem cells in several pathologic states including cardiovascular and lung diseases.

Prof. Franco Rustichelli

(Italy)

Polytechnic University of Marche, Ancona (Itay)

Prof. Franco Rustichelli studied Physics at SCUOLA NORMALE SUPERIORE di Pisa and is Professor of Physics at the Polytechnic University of Marche (Italy) and is Guest Professor at the University of Krakow (Poland) and at the University Steel and Alloys of Moscow (Russia). He worked for the EUROPEAN ATOMIC ENERGY COMMISSION for about 20 years. He has a vast experience in fields like material science, biophysics, biomaterials and stem cells research. He is using for these investigations in addition to conventional laboratory techniques, like X-ray diffraction and electron mi croscopy and AFM, especially X-ray and neutron scattering techniques available at European large Scale Facilities like Euro - pean Synchrotron Radiation Facility (ES - RF) and High Flux Reactor of ILL, build in Grenoble (France). He published more than 300 papers on international journals in different fields. He is currently present ing invited talks at International Conference especially related to Nanoscience and Nanotechnology and to Stem Cell Research. He was/is member of several international scientific committee and member of editorial board of scientific journals. He was chosen to be an evaluator for European Projects, Soros Foundation Projects and Projects of foreign countries. He was the scientific director during several years of an International Summer School on Advanced Material Science and Technology in Jesi (Ancona). He has taken part to more than 40 EU Projects, in some of them as coordinator or task coordinator, exploiting his experience in experimental investigations us ing small angle scattering (SANS/SAXS), microtomography and other techniques, available at European Large Scale Facilities, for microstructural studies of materials for different applications in several industrial and biomedical fields.

Prof. Salvatore Sauro

(Spain)

Dental Biomaterials, Preventive and Minimally Invasive Dentistry (Línea Bilingue) Departamento de Odontología, Facultad de Ciencias de la Salud, Universidad CEU-Cardenal Herrera

Salvatore Sauro (Orcid number: 0000-0002-2527-8776) is currently professor in dental biomaterials and minimally invasive dentistry at the faculty of health sciences, coordinator of the "Dental Research" and Director (Principal Investigator) of the research group "In Situ Dental Tissues Engineering and Minimally Invasive Therapeutic Adhesive Rehabilitation" at the University CEU Cardinal Herrera of Valencia. Dr Sauro is also honorary senior lecturer, Biomaterials, Biophotonics and Tissue Engineering , King's College London Dental Institute (KCLDI) at Guy's Hospital, London (UK) and Visiting Professor, Federal University of Ceará, School of Dentistry, Fortaleza, Brazil. He obtained his Ph.D (2009) in "Dental Biomaterials Research Pre-clinical Dentistry", and post-doctorate in "Dental Biomaterials/Pre-clinical Dentristy" at King's College London Dental Institute, London. Prof. Sauro has been working in dental biomaterials, preventive and minimally invasive dentistry research for 15 years (JCR - H-Index: 25), He collaborates with internationally renowned researchers, and he has published more than 100 articles in international peer-review journals with high impact in the dental field, more than 150 abstracts of research in international conferences, two international patents and chapters in scientific books.

Prof. Sauro is also part of the "editorial board" of the International Journal of Adhesion and Adhesive, Oral Clinical Investigation, International Journal of Endodontics, Journal of Endodontics and the Journal of Dentistry and a member of the "IADR - International Association of Dental Research" and "AMD - Academy of Dental Materials".

Dr. Anna Tampieri

(Italy)

Institute of Science and Technology for Ceramics of the National Research Council (ISTEC-CNR)
of ISTEC-CNR and Head of the Department of Bio-ceramics and Bio-hybrid composites.

Research Topic: Nanomaterials for regenerative medicine and Theranostics

Anna Tampieri, Chemist, 30 years of experience in Material Science, particularly addressed to biomimetic materials and devices for regeneration of hard and soft tissues and organs.
She authored more than 200 scientific papers published on peer-reviewed Journals and about 20 book chapters (H index = 45 based on Scopus).
She is inventor of 16 National and International patents, several of which are licensed to companies acting in the biomedical fields and translated to 7 commercial products.
She is Editor of a monography dealing with bio-inspired approaches in regenerative medicine, and Guest Editor of several international scientific journals.
Tutor of 11 Ph.D, 14 M.Sc students, and more than 20 National and International fellowships.
Coordinator of 8 EC-funded Projects belonging to the 6th and the 7th European framework programmes, and WP Leader in 6 EC-funded Projects. Coordinator of several national projects. Since 2009 she is member of the "European Technology Platform for Nanomedicine".
She is Scientific Advisor of European Commission for funding scheme ERC-projects.
Organizer and Chair of several National and International Symposia, Schools and Conferences on Biomaterials, among which the "International Conference of Materials in Medicine (MiMe)", Faenza (Italy), 2013; the Symposium "Regenerative Medicine", Global Biotechnology Congress, Boston (USA), 2013; the Symposium "Biomimetic Materials for Biomedical Applications", EUROMAT2009, Glasgow (UK) 2009.
Since 2011 is Senior Affiliate Member at the Methodist Hospital Research Institute, Houston, U.S.A.
Associated Professor in Medical Science and Applied Biotecnology, since 2014.

Founder of the company FINCERAMICA Biomedical Solution S.p.A, she was the Idea-woman, then President and today is the Head of the Scientific Advisory Board.
Consultant for several chemical, biochemical and pharma companies (e.g. Johnson&Johnson, FIN-CERAMICA Biomedical Devices, Menarini Pharma).
Former scientific advisor of the Italian Ministry of Economic Development and Industry, and of the Ministry of the French Industrial Research in 2011.
Awarded by the TIME Magazine for "from Wood to Bone" as the 30° research among the most important 50 researches in 2009. Awarded from Massachusetts Institute of Technology Review for the project GreenBone (biomimetic bone implants).
Founder of the Start UP GreenBone Ortho Srl in 2014.

Prof. Nicola Tirelli

(Italy)

Laboratory of Polymers and Biomaterials, Italian Institute of Technology, Genova

EDUCATION

1992-1995 PhD in Industrial Chemistry, Dept. of Chemistry & Industrial Chemistry, University of Pisa (Italy)
1992 Chartered chemist (Italy)
1986-1992 Master in Chemistry, Dept. of Chemistry & Industrial Chemistry, University of Pisa (Italy), (110/110 summa cum laude)

APPOINTMENTS

2017-present Senior Researcher, Director of the Laboratory of Polymers and Biomaterials
2014-2017 Academic Director of the NorthWest Centre for Advanced Drug Delivery (NoWCADD), UoM
2014-2017 Deputy Director, then Director of the Centre of Doctoral Training in Regenerative Medicine, UoM
2005-present Chair of Polymers and Biomaterials, various Schools, UoM
2003-2004 Senior Lecturer, School of Pharmacy, University of Manchester (UoM)
1998-2002 Oberassistent, Department of Materials, ETH Zurich

AWARDS/PRIZES

2005-10 EPSRC Advanced Research Fellow.
2005 Fellow of the Royal Society of Chemistry (RSC)
2004 Friedrich Wilhelm Bessel Research Award, from the Alexander von Humboldt Foundation

ACADEMIC OUTPUT To May 2018:

- 126 research papers, 12 peer-reviewed proceedings, 10 reviews. 6 patents/patent applications
- h-index: 40 (Google Scholar) / 36 (Scopus/Web of Science)

Prof. María Vallet-Regí

(Spain)

Full Professor of Inorganic Chemistry at Universidad Complutense de Madrid (UCM), Spain, leader of the Smart Biomaterials Research Group, Group leader of the Biomedical Research Networking centre in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), and of the Research Institute of the Hospital 12 de Octubre. Recipient of an ERC Advanced Grant: Polyvalent mesoporous nanosystem for bone diseases.

María Vallet-Regí has published more than 675 peer-review research papers. Author of 7 books and 32 book chapters and editor of 8 books. **h-index: 82** (Google Scholar) and 70 (ISI Web of Science). **Cites: >30800** (Google Scholar) >23500 (ISI WoS). She is also among the top 50 European scientists in the field of Materials Science, and one of the top 5 European scientists working in Biomedical Materials. Her multifaceted research work is reflected in more than 30,000 citations in the fields of materials Science, Chemistry, Pharmacy and Medicine.
Full Member of the Spanish Royal Academies of Pharmacy (**RANF**) and Engineering (**RAI**). Fellow of Biomaterials Science and Engineering, appointed by the International Union of Societies, Biomaterials Science & Engineering (**FBSE**). Member, College of Fellows of the American Institute for Medical and Biological Engineering (**AIMBE**)
Among the received awards, there should be highlighted the French Spanish Award of Societé Française de Chimie in 2000, RSEQ 2008 Award in Inorganic Chemistry, National Research Award 2008, FEIQUE Research Award 2011, RSEQ Gold Medal awarded in 2011. Doctor *Honoris Causa* by the Basque Country and Jaume I Universities. IUPAC 2013 Distinguished Women in Chemistry/Chemical Engineering and Miguel Catalán Research Award 2013. Lilly Distinguished Career Award in Chemistry 2016 and Julio Peláez Award to Pioneer Women in Sciences, Physics, Chemistry and Mathematics, 2017.

Acknowledged as pioneer in the field of mesoporous ceramic materials with biomedical applications. Her research revealed, for the first time, the potential biomedical applications of these materials, particularly in the fields of bone regeneration and controlled drug release systems (*Chem. Mater. 13,308-311, 2001; 1574 citations*). Her novel line of work lead to the development of a new field of research (more than 5,500 published papers so far). She has supervised 30 works of undergraduate students, 10 Master of Advanced Studies of graduate students and 20 Ph.D. works.

She has put in place different cooperations with various research institutions, where pre and post-doctoral members of her research group have carried out stays in order to complete their scientific curricula. Several Ph.D. students have successfully entered the professional arena, holding positions in academia or research, as well as in private institutions.

Prof. Thomas J. Webster

(USA)

The Art W. Zafiropoulo Professor and Chair, Department of Chemical Engineering, Northeastern University, Boston
President, U.S. Society For Biomaterials
Founding Editor, International Journal of Nanomedicine

Thomas J. Webster's (H index: 84) degrees are in chemical engineering from the University of Pittsburgh (B.S., 1995) and in biomedical engineering from Rensselaer Polytechnic Institute (M.S., 1997; Ph.D., 2000). He was appointed Department Chair of Chemical Engineering at Northeastern University in 2012 in which the Department broke the record for the fastest increase in *U.S. News and World Report* ranking over a five year period. Prof. Webster has graduated/supervised over 149 visiting faculty, clinical fellows, post-doctoral students, and thesis completing B.S., M.S., and Ph.D. students. To date, his lab group has generated over 13 textbooks, 68 book chapters, 376 invited presentations, at least 503 peer-reviewed literature articles and/or conference proceedings, at least 767 conference presentations, and 42 provisional or full patents. He is the founding editor-in-chief of the *International Journal of Nanomedicine* (pioneering the open-access format). Prof. Webster currently directs or co-directs several centers in the area of biomaterials: The Center for Natural and Tropical Biomaterials (Medellin, Colombia), The Center for Pico and Nanomedicine (Wenzhou China), and The International Materials Research Center (Soochow, China). Prof. Webster has received numerous honors including but not limited to: 2012, Fellow, American Institute for Medical and Biological Engineering (AIMBE, representing the top 2% of all medical and biological engineers); 2013, Fellow, Biomedical Engineering Society; 2015, Wenzhou 580 Award; 2015, Zheijang 1000 Talent Program; 2016, International Materials Research Chinese Academy of Science Lee-Hsun Lecture Award; 2016,

International College of Fellows, Biomaterials Science and Engineering; 2016, Acta Biomaterialia Silver Award; and 2018, Fellow, National Academy of Inventors.

He also served as the President of the U.S. Society For Biomaterials. He has appeared on BBC, NBC, ABC, Fox News, the Weather Channel, the Discovery Channel, and the recent special 'Year Million' TV series on National Geographic talking about the future of medicine and science

INVITED TALKS

Advanced Biomaterials for Minimally Invasive Surgery

*M.G. Raucci, A.Gloria, I. Fasolino, T. Russo, L. Ambrosio**

Institute of Polymers, Composites and Biomaterials, National Research Council
Viale J.F. Kennedy, 54, Mostra d'Oltremare, Pad.20,
80125 Naples, Italy
**e-mail: luigi.ambrosio@cnr.it*

Keywords: hydrogels, calcium phosphate, sol-gel, bone, intervertebral disc, injectability.

Introduction

The implementation of a personalised therapy approach together a less invasive surgery for the restoration of human tissues is becoming an *appropriate* strategy to mitigate costs of the modern health care system together the maintenance of health and quality of life.

The current challenge is to inject the material at the site of surgery by a minimally invasive technique in an easy way and able to fill complex 3D regions of tissues.

Selection of a suitable injectable is often based on material characteristics (including mechanical properties, drug release kinetics and degradation) that serve for the specific treatment function. Micro or nano-structured materials in the form of gels, nanoparticles, nano-fibers and nano-composites have gained increasing interest in regenerative medicine because they are able to mimic the physical features of natural extracellular matrix (ECM) at the sub-micro and nano-scale level.

By a careful selection of materials and processing conditions it is possible to finely control characteristic shapes and sizes from micro to sub-micrometric scale and to incorporate bioactive molecules such as proteins or growth factor to develop active platforms to support the repair/regeneration of different tissues such bone, and intervertebral disc (IVD).

It has been shown that the use of minimally invasive bone cement injection to treat vertebral fracture has significant clinical potential. To achieve the ideal properties of bone filler, i.e. osteoconduction, osteoinduction and ease of handling, investigators have been developing products based on natural and synthetic polymers, ceramics, and composites. In the last four decades, ceramics mainly based on calcium phosphates have been explored due to their chemical composition that is similar to mineralized bone. Recently, efforts have been paid to optimize injectable calcium phosphate cements (CPC) which have been recognized as excellent alloplastic material for osseous augmentation because of their unique combination of osteoconductivity, biocompatibility and mouldability. The sol-gel synthesis approach appears to be the most suitable route towards performing injectable CPC.

Intervertebral disc (IVD) degeneration is one of the main causes of low back pain. Current surgical treatments are complex and generally do not fully restore spine mobility. Development of injectable extracellular matrix analogue-based hydrogels offers an opportunity for minimally invasive treatment of IVD degeneration.

Results and Discussion

Calcium phosphate obtained by sol-gel synthesis combines hydroxyapatite (HA) with other calcium phosphate phases such as dicalcium phosphate (DCP), a precursor of natural HA in bone. A calcium phosphate (CaP) gel with different level of DCP can be produced by varying the pH of the system thus providing an opportunity to modulate the material resorption *in vivo*. Here, an overview of different strategies used to prepare bioactive and osteoinductive injectable calcium phosphates was reported. CaP gels complexed with phosphoserine-tethered poly(ε-lysine) dendrons (G3-K PS), hyperbranched molecules designed to interact with the ceramic phase and able to induce osteogenic differentiation of human mesenchymal stem cells (hMSCs) was reported [1]. Furthermore, injectable hybrid material based on graphene oxide nanosheets and hydroxyapatite prepared by sol-gel approach was described. It was found that the spindle like Hydroxyapatite nanoparticles with a diameter of about 5±0.37 nm and a length around 70±2.5 nm were intercalated between GO nanosheets. The oxygen-containing functional groups, such as hydroxyl and carbonyl, present on the basal plane and edges of the GO sheets play an important role in anchoring calcium ions as demonstrated by FTIR and TEM investigations. The presence of GO increase the bioactive and osteogenic properties of materials [2,3]. Recently, attention has been given to the modification of hydroxyapatite with Strontium (Sr) due to its dual mode of action, simultaneously increasing bone formation (stimulating osteoblast differentiation) while decreasing bone resorption (inhibiting osteoclast differentiation). Moreover, an injectable Sr-containing calcium phosphate bone cement (CPC) could meet the requirements of vertebroplasty because its radio-opacity was three times that of cortical bone. Here, the effect of systems based on strontium modified hydroxyapatite (Sr-HA) at different composition (0-5-10-15-20 mol%) on proliferation and osteogenic differentiation of hMSC was described [4]. One more approach is based on the use of antimicrobial injectable materials. It is well known that, bacteria and fungi can often adhere to biomaterials and have the capability of forming biofilms on foreign bodies. The subsequent detachment of cells from these biofilms can result in the development of highly resistant local or systemic infections in patients. It has been demonstrated that some imidazolium, pyridinium and quaternary ammonium ionic liquids (IL) have antimicrobial activity against a different clinically significant bacterial and fungal pathogens (*i.e. E. Coli, S. typhimurium, S. Aureus and Candida Albicans*). Here, we report several systems based on IL at different alkyl-chain length incorporated in Hydroxyapatite (HA) through the sol-gel process to obtain an injectable material with simultaneous opposite responses toward osteoblasts and microbial proliferation.

Due to the complex structure and function of the IVD, the repair/regenaration of a disc substitute represents a challenge from mechanical and biological (nutrition and transport) points of view. Hyaluronan gels are very promising for the specific application as nucleous substitute [5].
Here we analyze a specific formulation of hyaluronan (HA) polymeric substitute materials HYAFF 120 (an ester of HA), HYADD3 (an amide of HA), and collagen-low molecular weight hyaluronic acid (LMW HA) semi-interpenetrating network (semi-IPN) loaded with gelatin microspheres as a potential materials for tissue engineering of the nucleus pulposus (NP).
The material displayed a gel-like behavior [6], it was easily injectable as demonstrated by suitable tests and did not induce cytotoxicity or inflammation. Importantly, it supported the growth and chondrogenic differentiation potential of mesenchymal stem cells (MSC) in vitro and in vivo.
The properties of the semi-IPN hydrogel were successfully combined with TGF-b3 delivery by gelatin microspheres, which promoted the chondrogenic phenotype. Collagen-LMW HA loaded with gelatin microspheres represents a good candidate material for NP tissue engineering as it combines important rheological, functional and biological features.
For the in vivo study, the two hyaluronan derived polymeric substitute materials, HYAFF120 and cell-loaded HYADD3, were injected into the NP of the lumbar spine of female mini-pigs in which a nucleotomy had also been performed. Homologous bone marrow stem cells, obtained from the bone marrow three weeks before spinal surgery, were included in the HYADD3 material (1 × 106 cells/ml).
After 6 weeks of implantation, nucleotomy resulted in loss of normal IVD structure with narrowing, fibrous tissue replacement and disruption of the bony end-plates. By contrast, both HYAFF 120 and HYADD 3 treatment prevented this change. The injected discs had a central NP-like region which had a close similarity to the normal biconvex structure and contained viable chondrocytes forming matrix like that of normal disc.

Conclusions and/or Outlook
The proposed systems demonstrated the feasibility of minimally invasive surgery by designing multifunctional injectable biomaterials with therapeutic reparative and regenerative behaviors system thus providing an unique therapeutic strategy.

Acknowledgements
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[2] Ambrosio Luigi, Raucci Maria Grazia, Longo Angela, Carotenuto Gianfranco, Giugliano Daniela A biocomposites of biomineralized graphene oxide and its use for bone tissue engineering. International Publication number WO 2017/017610 A1.
[3] Raucci, M. G., Giugliano, D., Longo, A., Zeppetelli, S., Carotenuto, G., & Ambrosio, L. Comparative facile methods for preparing graphene oxide-hydroxyapatite for bone tissue engineering. *Journal of Tissue Engineering and Regenerative Medicine*, 2017;*11*(8), 2204-2216.
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Bio-inspired 3-D nano-ceramics directing hard tissue regeneration

Anna Tampieri[1], Simone Sprio[1], Monica Sandri[1], Alessio Adamiano[1], Monica Montesi, Silvia Panseri[1]

[1] ISTEC-CNR, Via Granarolo 64, 48018, Faenza, Italy
anna.tampieri@istec.cnr.it

Introduction

New approaches for tissue regeneration aim to recover the original tissue functionality and reduce the societal impact, particularly related to degenerative pathologies affecting bone and osteochondral tissues. Advances in this field are strictly related to progress in materials science aiming to develop new smart, stimuli-responsive bio-devices. In fact, to trigger the correct cascade of biological events that lead to tissue regeneration, cells need to be exposed to chemical-physical, structural and morphological signals whose presentation follows precise spatial and temporal patterns. Nanotechnologies are today strongly addressed to this target, particularly to develop innovative and safer nanoparticles with new smart functionalities. On the other hand the achievement of nanostructured and nanocrystalline materials in the 3-D state is a great challenge to obtain porous scaffolds retaining physico-chemical-mechanical cues relevant for correct activation of the regenerative cascade. In fact, thermal consolidation of bioceramics destroys the bioactivity and bio-resorbability of biomimetic ceramics such as nanocrystalline, multi-doped apatite (HA). Biomineralization [1] and biomorphic transformation processes [2] are relevant examples of this concept, also enabling implementation of the scaffolds with smart functionality for remote activation.

Materials and methods

Biomineralization process induces and directs the self-assembling of collagen matrices by pH control, and the simultaneous heterogeneous nucleation of quasi-amorphous apatite crystals, occurring under several control mechanisms exerted by the collagen macromolecule. Biomorphic transformation process bases on a sequence of heterogeneous reactions at the interface between a natural porous template and a reactive gas, generating a highly reactive precursor which can then be transformed into 3-D biomimetic apatite with excellent bioactivity and damage-tolerant mechanical performance. Controlled substitution of Ca^{2+} ions with $Fe^{2+/3+}$ ions in the HA structure, with specific Fe/Ca and Fe^{2+}/Fe^{3+} ratios, generates a superparamagnetic, bioactive and bioresorbable apatite phase (Fe-HA) [3] that can be obtained as nanoparticle, or heterogeneously nucleated onto self-assembling collagen matrix as a bioactive scaffold capable of remote magnetic activation and controlled delivery of bioactive factors.

Results and Discussion

Biomineralization process generates hybrid bone scaffolds with high mimicry of mineralized and non-mineralized tissues of osteochondral regions and showing excellent regeneration of critical bone and hyaline cartilage defects, demonstrated in a large number of patients in orthopaedic, spinal and dental surgery. Biomorphic transformation generates multi-doped apatite phase directly nucleated in the 3-D state, as scaffold exhibiting bone-mimicking compositional and nanocrystalline features inducing outstanding bioactivity and regenerative ability reported by in vivo tests in sheep. Biomechanical performance are assured by nanotwinning phenomena and multi-scale hierarchical structure giving damage-tolerant behaviour. Implementation of biomimetic scaffolds with magnetic properties opens to a variety of new application fields in regenerative medicine. Besides, Fe-HA in form of nanoparticles can replace magnetite for breakthrough applications in nanomedicine, with enhanced safety. New guiding systems for cells can be established by enabling selective internalization of Fe-HA by mesenchymal cells and magnetic guiding.

Conclusions

The use of scaffolds exhibiting real mimesis of natural tissues can fuel new improved and personalized therapies for bone regeneration. To this, nature-inspired synthesis approaches are an elective way to bypass the limitation of ceramic processing and develop bioceramics showing innate cell-instruction ability.

References.
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Supramolecular tools to engineer advanced biomaterials to tissue engineering

A. Mata

Professor in Biomedical Engineering and Director of the Institute of Bioengineering at Queen Mary University of London

Nature engineers functional materials with a high level of molecular organization across scales, which results in properties such as outstanding stiffness and elasticity, the capacity to grow and self-heal, or the ability to acquire complex hierarchical structures. The talk will present supramolecular engineering strategies that incorporate dynamic self-assembly, disordered proteins, or bioprinting to develop materials with advanced properties in a simple and tunable manner. These approaches are being used to create complex scaffolds that can guide mineralization, stimulate bone formation, grow vascular-like structures, and recreate complex biological environments.

Two Decades of Commercializing Nanomedicine for Improving Human Health

A-Andrew D. Jones, III.[a,b], *Gujie Mi*[a], *Thomas J. Webster* [a,*]

[a] Chemical Engineering
[b] Mechanical & Industrial Engineering
Northeastern University
**e-mail: th.webser@northeastern.edu*

Keywords: Nanomaterials, Implanted Medical Device, Commercialization

Introduction

Nanomedicine, or nanotechnology applied to medicine, has been anticipated to revolutionize treatments and diagnostics due to the distinctive physicochemical and biological properties that arise at the nanoscale. Nanomedicine has captured the attention of researchers, legislatures, manufacturers, doctors, and the public for the past 40 years. In that time, 200,000 research articles were published (**Figure 1**). Nanomaterials improve a body's acceptance of transplants, artificial bone materials, and other implanted medical devices [1]. From 1986 - 2016, over 1000 peer reviewed articles on nanostructured materials in medical devices were published (**Figure 1**), 88 manufacturers created, and 992 news articles published (Lexis-Nexis search "nano" and "medical device"). In 2016, $500 million was invested by the US Department of Health and Human Services [2], $316 million by venture capital and the Freedonia Group estimated that the market for medical devices containing nanomaterials is between $2.4 - $24 billion annually [3]. Despite these investments, estimates and anticipation, there is a lack of product realization and regulatory oversight is seen as a primary bottleneck. However, we have found that nanomaterials in medical devices, from a manufacturing and product safety standpoint, require reduced geographical and knowledge separation between researchers, manufacturers, and clinical servicers to achieve similar levels of nanomedicine commercialization to that of other medical devices. Nanomedicine in other arenas, such as drug delivery, infectious disease and cancer therapeutics, good lab practices and good manufacturing practices (GLP and GMP) and better models - both biological and mathematical - for conducting *ex vivo* experiments - are needed to achieve the level of success of other medical technologies.

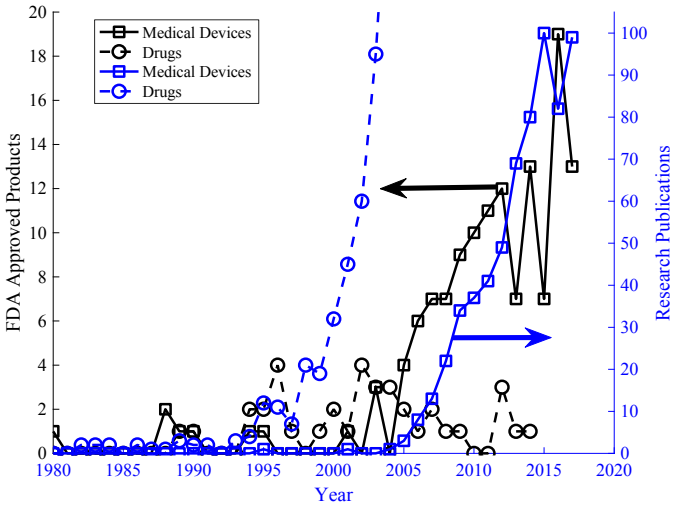

Figure 1: Academic publishing and manufacturing trends in devices containing nanostructured material. FDA 510 (k) cleared products by 72 unique manufacturers, including those later acquired, where either the manufacturers or the product name contains "nano." Research publications in the PubMed database from 1980 -2017 containing "nanoparticle" and "implant" but not containing "drug delivery." Compared to nano based drugs, from PubMed "nanoparticle" and "drug delivery" extends to 3171 by 2017 and product data from Weissig and others, limiting the data set to 2014.[4] Figure reprinted from[5]

Results and Discussion

Results from our lab have led to the formation of 11 different companies and 4 FDA approved products based on nanomaterials. Some of these have benefited from the geographical proximity, for example partnerships between Webster Nanomedicine Lab at Brown University and clinical trials initiated by the Rhode Island Hospital system. Others have benefited from the foundries and innovation economy developed by Northeastern and the biotechnology space in the Boston area. Further still, knowledge exchange of lab alumni and trainees between the Webster Nanomedicine Lab and the Webster lab at Wenzhou Medical University in China have accelerated this exchange. While our lab's examples provide anecdotal evidence, the advantages of these exchanges and geographical proximity have been well documented in the literature for other research intensive industries.[5] We and others are leading efforts to increase the exchange of experts between countries between academia and industry.

Nanomaterials will still incur similar clinical testing costs to those of pharmaceuticals even if geographical proximity and exchange of experts is increased between academia, industry, and venture capital firms. To reduce this cost, there are efforts to produce organoids[6] and organs-on-a-chip to improve *in vitro* testing. In addition to more accurate biological models, such as organoids and organ-on-chip devices, mathematical models of nanomaterials and their interaction with a pathogen, tumor, host and host environment can increase the success rate of clinical trials[7]. These models can be based on statistical tools similar to existing bioinformatics approaches to cancer gene therapies and hereditary models. Other models can be based on molecular dynamic simulations of how particles interact with cells. Further models still can use hybridized approaches of improved deterministic models for *in vitro* models merged with statistical tools, similar to the models we are developing for bacteria interacting with nanoparticles, **Figure 2**.

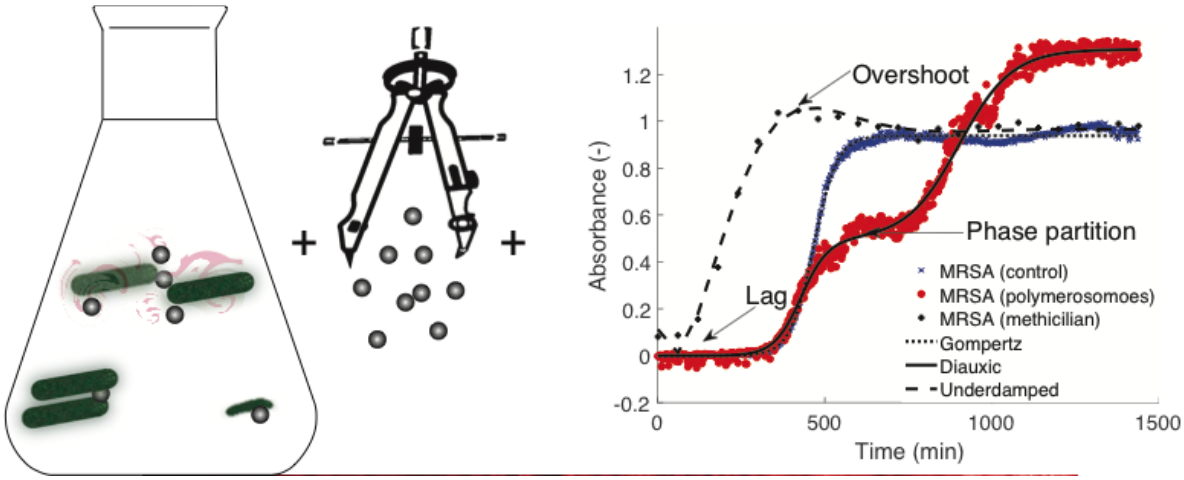

Figure 2: The illustration represents the three discovered modes of bacteria-nanoparticle interaction: cell death, no-effect, or the secretion of exopolymer substances that lead to biofilm formation and/or neutralizing of bactericidal effects. When combined with measurements of nanoparticle properties and diverse models of bacteria growth can lead to predictions for nanoparticle design.

Outlook

The pace of commercializing nanomedicine has increased dramatically in the last 20 years. Part of our lab's success and a roadmap we recently published is the creation of geographically close nanoparticle foundries, industries, clinical sites, and academic institutions. The exchange of experts across these sectors has greatly benefited our success. Furthermore, the use of novel in vitro models and mathematical models will reduce the rate of products that fail in clinical trials leading to a healthier marketplace.

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Doping bioactive glasses with biologically active ions for emerging tissue engineering applications

Aldo R. Boccaccini

[a]Institute of Biomaterials, University of Erlangen-Nuremberg, 91058 Erlangen, Germany
**e-mail: aldo.boccaccini@ww.uni-erlangen.de*

Keywords: bioactive glasses, ion release, angiogenesis, tissue engineering, bioactivity

Introduction

"Bioactive" materials, defined as those biomaterials with highly bioreactive surfaces that interact with cells and tissues to induce tissue growth and regeneration, are at the centre of current research efforts. Prominent representatives of bioactive materials are bioactive glasses (BGs) which are also considered a class of "third generation biomaterials" for regenerative approaches [1]. Biochemical interactions at the interface between BGs and the biological environment, specially related to the release of ionic dissolution products at the glass–tissue interface, mark the special characteristics of BGs for both hard and soft tissue regeneration [2, 3].

Results and Discussion

Based on own results and literature data, the development of ion doped BGs (considering silicate, phosphate and borate BG compositions) are discussed, covering current research in the field. The focus will be on the effect of the release of biologically active ions on specific cellular pathways. Selected in-vitro and in-vivo results that demonstrate the suitability of the ion dissolution during BGs degradation in different tissue engineering strategies are discussed, including studies investigating vascularization, wound healing and nerve tissue repair [3]. A series of in-vitro studies has demonstrated the effects of BG dissolution products on cell behaviour in relation to angiogenesis, in particular considering novel BG compositions incorporating selected ions such as Co, Cu, Li, Nb, Mn and B. In-vivo investigations assessing vascularisation of tissue in contact with BG scaffolds are available, which highlight the importance of BGs of different compositions to affect different biological markers such as increase of vascular growth factor release and induction of hypoxia conditions that affect endothelial cell behaviour. The combination of BG particles and biopolymers to create rigid or flexible bioactive composite scaffolds for hard or soft tissue repair, respectively, will be shown.

Conclusions and/or Outlook

The vascularisation potential of bioactive glasses incorporating and releasing metallic ions was discussed. How BGs can be effectively used to tackle the current challenge in the field, e.g. the development of vascularised tissues, can be illustrated based on recent in-vitro and in-vivo results. Areas of future research in the field of biomaterials for regenerating (complex) tissues, wound healing and soft and hard tissue repair include the concept of biofabrication, e.g. processing cells in bioinks, which can be enhanced by incorporating BG (nano)particles.

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Stem cells use for cartilage pathology - clinical prospective

Kon E[1,2], Vitale ND[1,2], Di Matteo B[1,2], Marcacci M[1,2]

[1] Department of Biomedical Sciences, Humanitas University
[2] Humanitas Clinical 2and Research Center, Rozzano, Milan, Italy

The application of MSCs has emerged as a promising option for the treatment of articular chondral defects and early osteoarthritic processes. Several preclinical studies revealed the beneficial actions of these cells on all articular tissues, promoting their clinical use.
Different cell-sources have been investigated in a growing number of clinical trials, especially targeting knee or ankle cartilage disease. The most exploited cell types are those derived from bone marrow and adipose tissue. Adipose cells have been applied mainly through cell concentration in a one-step procedure, while cells derived from bone marrow are currently applied both after expansion or concentration.
The small number of patients treated and the presence of several confounding factors (PRP concomitant use, cell use in combination with scaffolds, etc.) are the main limitations of these studies. Furthermore, the tissue harvest procedure poses practical and ethical concerns which prevent from performing studies with a blinded design, therefore leaving an important bias related to the placebo effect, which is an important issue in this field of new fashionable regenerative treatments. All these factors impede the establishment of a clear support for the use of these cells in the therapy of cartilage diseases.
On the other hand, the available studies still allow to draw some indications on the clinical use of MSCs.
First, the use of MSCs in the clinical setting can be considered safe, since no major adverse events related to the treatment nor to the cell harvest have been reported. Second, a clinical benefit of using MSCs therapies has been reported in most of the studies, regardless of cell source, indication, or administration method. This effectiveness has been revealed in terms of clinical improvement but also through positive MRI and macroscopic findings. Third, different studies also gave a few indications regarding the patients who might benefit more from MSCs treatment: young age, lower BMI, smaller lesion size for focal lesions. However the available data strength does not allow to define clear cutoff values.

Stem Cells and Tissue Engineering for Cardiac Repair

[1]Gallia Graiani, [1]Caterina Frati, [2]Elisabetta Rosellini, [3]Aldo Boccaccini, [1]Denise Madeddu, [1]Angela Falco, [4]Diana Massai, [5]Alberto Saiani, [1]Federico Quaini

1.Department of Medicine and Surgery, University of Parma; 2. Department of Civil and Industrial Engineering, University of Pisa; 3. Department of Materials Science and Engineering University of Erlangen-Nuremberg; 4. Department of Mechanical and Aerospace Engineering, Politecnico di Torino; 5. School of Materials, The University of Manchester, Manchester, UK

The intensive search for regenerative approaches is mainly addressed to overcome the limited ability of adult human tissues to repair severe ischemic or non-ischemic damage. Successful regeneration of injured organs may be achieved by the combination of biomaterials, progenitor cells and bioactive elements able to modulate tissue reconstitution. Recent advances in stem cell biology and tissue engineering have increased the success in reversing or replacing the unfavorable microenvironment, which hampers cell growth and differentiation and functional tissue organization.
Different strategies have been investigated to regenerate the damaged myocardium, including cell, gene or biologic therapy and the application of passive or bioactive materials, such as patches or injectable materials (hydrogels, microspheres).
Here we describe the in vitro characterization of injectable biomaterials and cardiac patch prototypes to provide methodological tolls to test their efficacy to repair the damaged heart. Data on the ability of biomaterial to promote progenitor cells mobilization, homing and engraftment in vivo are also proposed. In addition, the cellular basis of the physiologic and pathologic myocardial growth will be critically revisited to provide important clues in understanding cardiac regenerative processes.

Application of bio-adhesives in cardiovascular surgery

H. Moldovan

Faculty of Medicine, Titu Maiorescu University, Bucharest, Romania
Faculty of Materials Science and Engineering, University Politehnica of Bucharest, Romania
Sanador Clinical Hospital, Bucharest

Advances in surgery inherently brought new surgical technologies. The introduction of surgical bio adhesives for reinforcement of various type of anastomoses was a major step towards achieving better results in major vascular surgery. As soon as cardiac surgery became widespread, when complex procedures became routine rather than exceptions, (repair of thoracic aortic aneurysms /dissections is just one example), new methods and approaches needed to be implemented to allow less experienced surgeons to achieve reproducible, acceptable outcomes.
We review the use of surgical adhesives like, GRF, albumin and fibrin glues and reflects our opinion about their utility in major cardiovascular procedures. We present the advantages and disadvantages of these types of glues and report on their different components such as gelatin, formalin, bovine albumin, fibrinogen, and bovine thrombin.
These adhesives are mainly used as adjunctive technological products to reinforce the surgical suture lines and anastomosis in the treatment of severe aortic conditions like aneurysms and aortic dissections.

The new COST project BIONECA connecting physical sciences with regenerative CARDIOLOGY and NEUROLOGY

Franco Rustichelli

Department Di.S.C.O., Università Politecnica delle Marche, Ancona, (Italy)
[b] Istituto Nazionale Biostrutture e Biosistemi, Rome, (Italy)

Cardiovascular diseases are the leading cause of death in the western world. A progressively ageing population is increasingly affected by neurological diseases, which brings a negative impact on European economies with more than 1 billion euros cost per year. Faced with too slow and expensive progress in development of new therapies, new approaches in development of new therapeutic protocols are urgently needed. One of the most promising strategy is based on stem cell applications for cardiovascular and neurological diseases and on the employement of biomaterials for supporting cultivation and integration of stem cells in disease-affected tissues. On the basis of some experiments we performed in cooperation with cardiologists and neurologists, we conceived a project having the title: **Biomaterials and advanced physical techniques for Regenerative Cardiology and Neurology** (BIONECA), which was written with several colleagues of different disciplines, being approved by COST Organization, and commencing on March, 15 2017, lasting 4 years. At present **36 countries** joined BIONECA.
The main goal of BIONECA is to establish an intensive interaction among top-level European Institutions of different scientific communities in order to induce significant progresses in Regenerative Cardiology and Regenerative Neurology with a consequent reduction of deaths and costs associated to brain and heart diseases.
A way to fulfil the main goal is to proceed to an exchange of information, a networking activity and a process of mutual knowledge among scientists of the following disciplines: physics, chemistry, mathematics, informatics, biomaterials science, material engineering, nanotechnology, surface science, rapid prototyping, advanced imaging technology, cell biology, molecular biology, tissue engineering, regenerative cardiology and regenerative neurology.
In order to achieve the COST ACTION objectives five different Working Groups were established, with a strong interconnection among them.
WG1: Processing of Biomaterials, aiming to improve the processing of Biomaterials,taking into account the specific needs for applications in Regenerative Cardiology and Neurology.
WG2: Characterization and visualization of Biomaterials and Stem Cells, with use of advanced techniques, like microtomography and holotomography, based on X-Ray synchrotron radiation.
WG3: Modelling, in order to obtain an integrated understanding of essential processes, which include nutrient transport and utilization, matrix formation, cell population dynamics, cell attachement and migration, and local cell-cell interaction.
WG4: Stem Cells and Neurology, aiming to essential progresses in these disciplines, as a result of mutual interaction and exploitation of results produced in the other WGs.
WG5: Stem Cells and Cardiology

Biomaterials discoveries to the clinic: are there alternatives to animal models?

Mauro Alini

AO Research Institute, Davos Platz, Switzerland
mauro.alini@aofoundation.org

Keywords: Organoid, Bioreactor, In Silico,

Introduction

With a growing acceptance that two-dimensional (2D) *in vitro* models are an oversimplification of the human body response and animal models do not, in most cases, represent human conditions, more complex systems are needed to recapitulate human physiology / pathophysiology. Although three-dimensional (3D) cell culture systems have shown promise, they fail to completely recapitulate the complexity of native tissues (i.e. including all cell types and acellular extracellular matrix components involved). Therefore, whole tissues and even whole organs have been used as advanced *ex-vivo* models. Over the last decades, bioreactors have been successfully used to further improve *in vitro* and *ex-vivo* culture conditions, to investigate fundamental questions on specific cell functions and tissue development, and to develop standardized, up-scalable, and safe tissue growth systems. A "bioreactor" can be broadly defined as a system that allows the culture of cells or tissues under defined biological conditions. By testing regenerative therapies under conditions that are closer to the ones encountered *in vivo*, bioreactors can provide a useful screening tool for the evaluation of various cell types / biomaterials / drugs / tissue engineered products prior to animal testing.

For the future, there is a high need for predictive tools based on experimentally-validated *in silico* models and bioreactors (especially in high-throughput platforms) can provide a useful tool to validate computational models.[84] A huge challenge is the development of models capable of linking *in vitro, ex-vivo, in vivo* outcomes. The organ on chip technology may provide a remarkable tool for this purpose.[85]

The goal of this is to open a discussion on the various systems used to evaluate biomaterials, drugs and cell therapies that could have a higher relevance for humans compared to conventional 2D and 3D cultures and in certain cases to animal experimentation.

Research Studies on Biocomposites

Gultekin Goller

Istanbul Technical University, Department of Metallurgical and Materials Engineering, Maslak, Istanbul, Turkey

This entry will provide a detailed overview of the processing techniques and properties of bioceramics, with special emphasis on nearly inert ceramics like alumina based composites and bioactive ceramics like hydroxyapatite 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. Further physical, microstructural and mechanical characterization techniques are discussed. Biological behavior and bioactivity measurements of bioactive hydroxyapatite based composites and zirconia toughened alumina composites in in-vitro environments such as cell viability assays are also elucidated.

Bioceramic Coatings on Magnesium Alloys for Medical Application: Trends and Techniques

Iulian Antoniac

Materials Science and Engineering Faculty, University Politehnica of Bucharest, Romania
antoniac.iulian@gmail.com

Magnesium alloys have attracted interest as biomaterials for biodegradable metallic implants due to their biodegradability into the human body, and as their mechanical properties match closer to bones than other biodegradable materials or currently used metals for metallic implants. Metallic implants made by biodegradable magnesium alloys have several advantages over other implantable metals currently in use, such as eliminating both the effects of stress shielding, corrosion and the requirement of a second surgery for implant removal. Unfortunately, the fast degradation rates of magnesium alloys impose some limitations on their clinical applications. This necessitates development of implants with controlled degradation rates to match the kinetics of bone healing. Surface coatings to control biodegradation of magnesium-based alloys offer the flexibility to be easily modified for specific applications and have significantly less investment. Since different bioceramics like hydroxyapatite, calcium phosphate or bioactive glasses are well tolerated by living organisms, they appear to be the excellent candidates for coatings on magnesium alloys. There are several methods that could be used for bioceramics coatings on biodegradable magnesium alloys. 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 proper surface design and bioceramic coatings, magnesium alloys could be considered a very promising candidate for biodegradable implants used in orthopedics, dentistry, and neurosurgery.

Effects of therapeutic operative strategies combined with bioactive/biomimetic ion-releasing materials on dentine permeability and remineralization

Salvatore Sauro

Dental Biomaterials, Preventive and Minimally Invasive Dentistry. Departamento de Odontología, Facultad de Ciencias de la Salud, Universidad CEU-Cardenal Herrera. C/Del Pozo s/n, Alfara del Patriarca. 46115 - Valencia, Spain
Hon. Senior Lecturer. Tissue Engineering and Biophotonics Research Division. King's College London Dental Institute (KCLDI).Floor 17 Tower Wing, Guy's Hospital. Great Maze Pond. London SE1 9RT (UK)

Keywords: Remineralisation, Biomimetic, Bioactive, Dentine, Minimally Invasive and Preventive Dentistry

Introduction

Therapeutic operative strategies combined with resin-based ion-releasing materials may interact and remineralise mineral-depleted dental hard tissue such as dentine and enamel. Dental adhesive systems (DAs) have improved substantially over the past few years. However, such DAs may create hybrid layers characterised by enzymatic collagen degradation and polymer hydrolysis. It has been advocated that it is possible to improve the durability of resin-dentine bonds by using therapeutic bonding strategies and ion-releasing materials that can interact with mineral-depleted dentine. Moreover, fully remineralisation of hybrid layers may be achieved when using bioactive resin-based materials along with "smart" primers containing biomimetic analogues of phosphoproteins. Furthermore, air-abrasion/polishing performed with innovative bioactive glass powders can create a therapeutic "bioactive smear-layer-covered surface", which reacted with body fluids, evoking hydroxyapatite (HAP) precipitation, and hence remineralisation of mineral-depleted dentine and reduction of dentine hypersensitivity. These outcomes are especially evident when dentine air-abraded with bioactive glasses is protected with GIC-based materials. However, the use of experimental resin-based systems containing bioactive fillers in combination with biomimetic primers doped may evoke a "bottom-up" dentine remineralisation that restored the modulus of elasticity of demineralised dentine [1,2].

Results and Discussion

Several experimental therapeutic approaches were tested in our studies to remineralise mineral-depleted (i.e. acid-etched) resin-dentine interface. For instance, dentine specimens were first air-abraded with bioactive glass 45S5 (BAG) and then restored with fluoride-releasing glass ionomer cements (GIC) or resin-modified glass ionomer cements (RMGIC). Moreover, experimental etch-and-rinse and self-etch adhesive systems containing different bioactive fillers were first formulated and then applied onto demineralised a dentine pre-treated with or without different biomimetic primers doped with sodium trimetaphosphate, aspartic acid (PASA) and/or poly(acrylic acid) (PAA). All the bonded-dentine specimens were prepared and aged in simulated body fluids (SBF, 24h or 6 months). These specimens were finally processed and assessed for microtensile bond strength, AFM nano-indentation, XRD, FTIR-ATR, FEG-SEM (fractographic analysis), TEM, dye-assisted confocal microscopy and Raman microscopy as well as those of cell differentiation and biocompatibility with stem cells (Figure 1). All necessary statistical analysis was performed on quantitative data [2,3].

The air-abrasion technique performed with BAG was able to create a therapeutic "bioactive smear-layer-covered surface" for bonding procedures, which reacted with body fluids, evoking hydroxyapatite (HAP) precipitation, and hence remineralisation of mineral-depleted resin-dentine interfaces. These outcomes were especially evident when BAG air-abraded dentine specimens were bonded using GIC or RMGIC [1]. Moreover, the results of this study showed that the use of experimental adhesive systems containing bioactive fillers in combination with biomimetic primers doped with PAA/PASA and TMP evoked "bottom-up" dentine remineralisation that restored the modulus of elasticity of water-rich/resin-poor dentine-bonded interfaces. These latter specimens showed no bond strength after 6 months of storage in SBF [3,4].

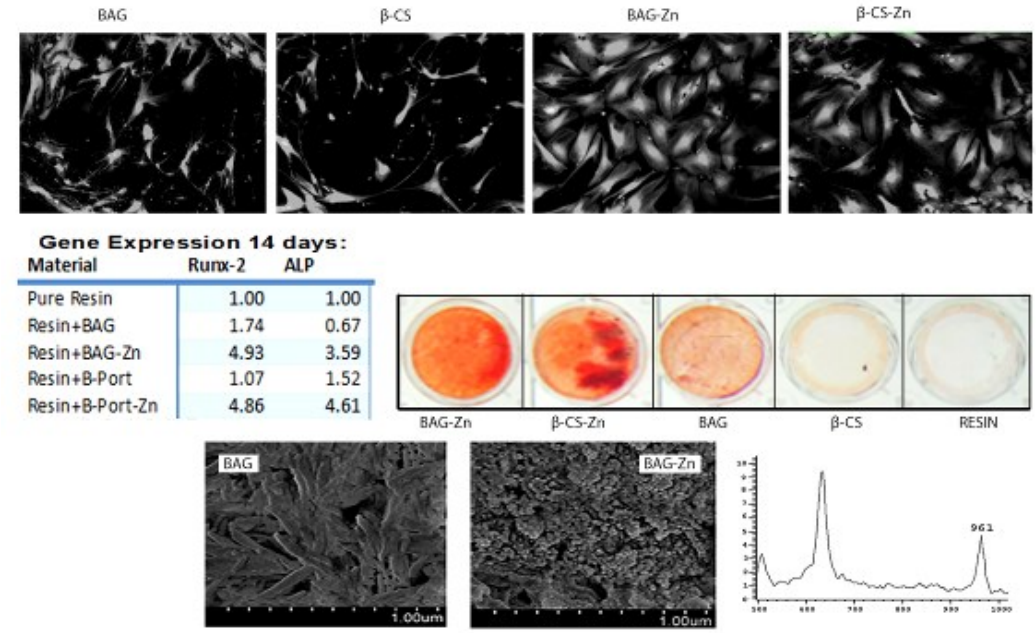

Figure 1: Results obtained in our studies

Conclusions

The application of preventive and minimally invasive therapeutic operative strategies along with the use of current or innovative ion-releasing containing biomimetic materials may represent a suitable strategy to fully remineralise demineralised dental hard tissues, reducing the dentine hypersensitivity. Moreover, innovative resin-based materials such as ion-releasing flowable composites and "smart" adhesive systems containing biomimetic reagents may represent a suitable strategy to fully remineralise and prevent degradation of resin-dentine bonds to enhance their clinical longevity.

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Minimally invasive management of deep carious lesions - the scientific and clinical rationale

A Banerjee [a]

[a] Conservative & MI Dentistry, King's College London Dental Institute at Guy's Hospital, King's Health Partners, London, UK
**e-mail: avijit.banerjee@kcl.ac.uk*

Keywords: carious lesions, selective caries removal, infected dentine, affected dentine, bacteria, collagen, caries

Introduction

The management of deep, cavitated carious lesions has evolved with the advent and gradual introduction of minimally invasive (MI) operative dentistry principles. The traditional approach to treating dental caries as if it were gangrene, with complete surgical excision of the carious dental tissues to sound margins, is now neither recommended nor should be practised.

A review of the literature has showed that the traditional surgical approach to dealing with carious tissues was influenced by three factors:

1. The level of knowledge of the caries process and its histopathological spread through the dental tissues
2. The non-selective surgical technologies available to excavate the tissues, and
3. The relative paucity of choice of restorative materials to repair the created cavities, which had the ability to seal the cavity-restoration interface and bio-interact with these tissues to aid tissue healing and regeneration.

On top of the factors listed above, the clinical skills of the operator (including their knowledge and understanding of cariology and dental biomaterials, operative skills and material handling skills) and the behaviour of the recipient patient in terms of taking responsibility / managing their oral health, oral hygiene and diet, both play a significant part in ensuring the caries management process is complete and continues long term, so increasing the longevity of the tooth-restoration complex in clinical function.

Contemporary cariology research now appreciates an interlinking of certain factors to manage the most prevalent non-communicable condition affecting humankind on the planet (**Figure 1**). In terms of the minimally invasive restoration of existing carious lesions, the factors to consider are:

1. The quantity and quality of the bacteria retained within the tissues
2. The seal and adhesion of the bio-interactive restorative material to tooth structure.

Clinical evidence now exists to show that retaining bacterially-contaminated (caries-infected) and demineralised (caries-affected) dentine after carious tissue excavation over the underlying pulp in close proximity, is not detrimental to the pulp sensibility as long as the cavity is restored carefully using a sealed, adhesive, bio-interactive material which ionically crosslinks with the tissues. These materials need to be reviewed *in vivo* with regular patient recall consultations to ensure their mechanical integrity with the tooth structure and to ensure the patient upkeep is optimal. The regular review of the tooth-restoration complex will allow an MI management regime to be used which encourages review, refurbishment, resealing and repair rather than opting for complete replacement.

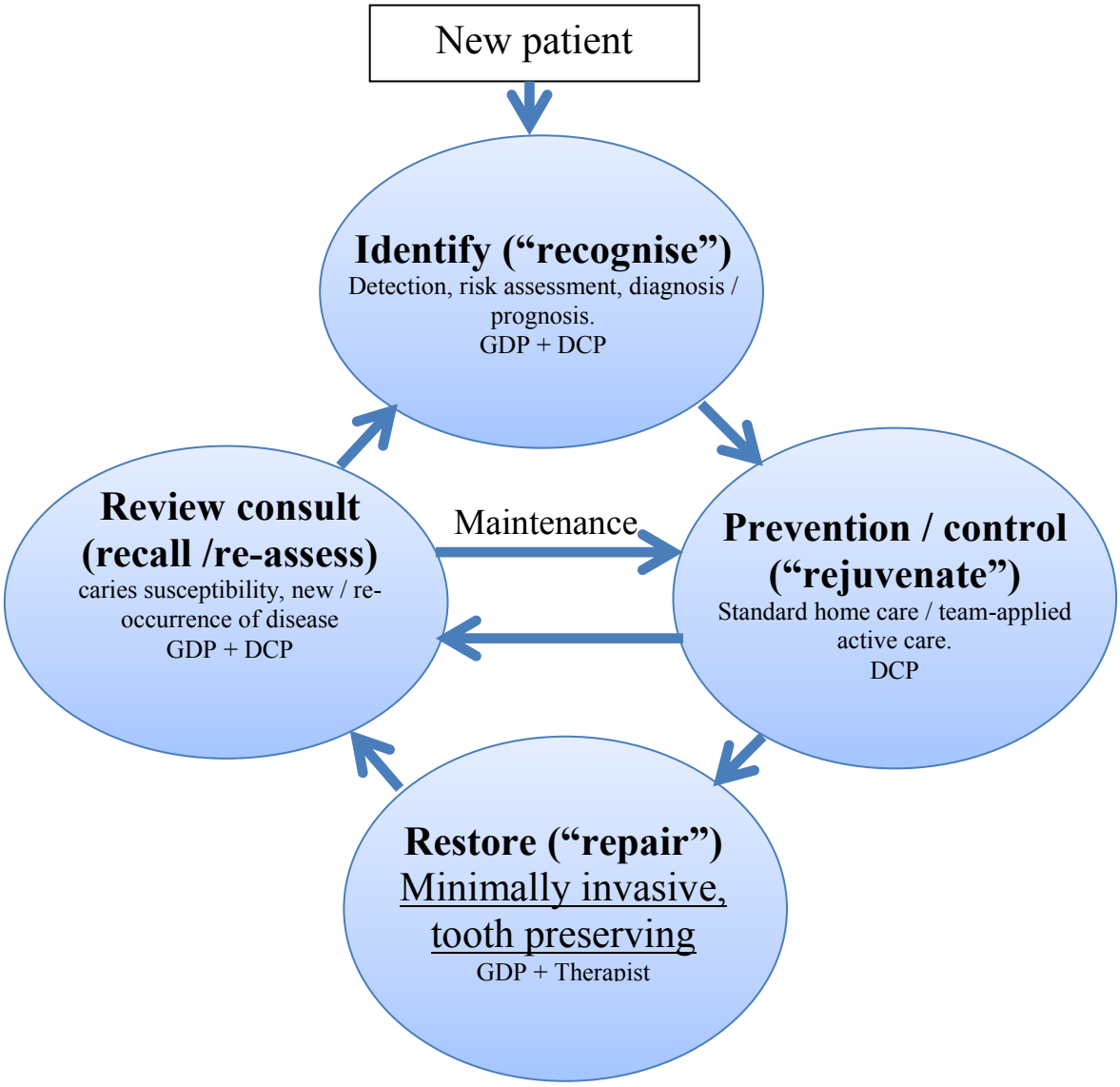

Figure 1: The minimum intervention care planning cycle showing the four interlinking stages of patient assessment, diagnosis, non-operative prevention, minimally invasive operative intervention and review (recall). The arrows indicate the direction of patient flow through this cycle and within each bubble an indication is given of the members of the dental team who might be included (GDP - general dental practitioner; DCP - dental care professional (includes oral health educator-trained nurses, hygienists, therapists, practice managers, reception s

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Design of drug nanocarriers

María Vallet-Regí

Department of Chemistry in Pharmaceutical Sciences, School of Pharmacy, Universidad Complutense de Madrid, Instituto de Investigación Sanitaria Hospital 12 de Octubre i+12, Plaza Ramón y Cajal s/n, Madrid, 28040 Spain. Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain

Mesoporous silica nanoparticles have already proven to be adequate nanocarriers for various chemical and biological species (1). For instance, they are valuable tools when carrying antitumor agents selectively to a tumor tissue, and releasing them there thanks to the application of an external stimulus. We use the term smart because those nanocarriers are able to release the drugs when and where they are needed. The surface of our nanosystems can be decorated with molecules able to recognize specifically tumor cells and to trigger the penetration of nanocarriers into them. The main advantage of developing selective nanocarriers able to accumulate only in tumor tissues are: increased selectivity of the therapy, which allows reducing the cytotoxic dosage; higher control over the administered doses; and the reduction of side effects, because the drugs will not be distributed throughout the whole body. Taking into account that most anticancer drugs are cytotoxic, their release must take place only inside tumor cells (2, 3,4).

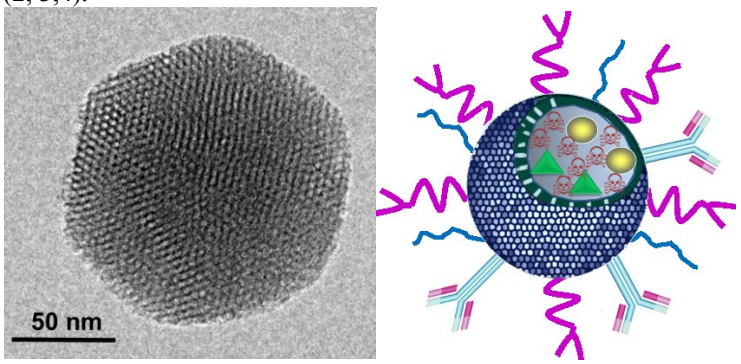

Figure 1. TEM-image of a mesoporous silica nanoparticle and schematic layout of potential modifications to its inner and outer surfaces.

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Fibrin engineering through knob-hole interactions: a versatile platform of in situ gelling matrices

Nicola Tirelli

Laboratory of Polymers and Biomaterials, Italian Institute of Technology, Genova (Italy)

Fibrin recapitulates all the features of an ideal artificial matrix: in situ-gelling (injectable) and biocompatible process of formation, adhesive to cells, angiogenic, easily degradable. Further, it has a history of clinical use, primarily as a sealant/haemostatic agent (fibrin glues). Here, we deal with fibrin (bio)functionalization and modulation of both its physical properties and its properties as a cell-hosting matrix. Specifically, we use an innovative technique to introduce additional components, which initially bind the fibrin precursor (fibrinogen) in solution, and then remain bound to fibrin fibres in the final gels. The binding is based on affinity interactions identical to those presiding to fibrin own self-assembly, which are typically referred to as knob-hole interactions. In this communication, we use poly(ethylene glycol) (PEG) as an example of secondary component introduced through knob-hole interactions. PEG chains are functionalized with two different peptides, which allow them to bind to two different sites in the D globular domain of fibrinogen. The introduction of bound PEG chains does not significantly alter the overall network organization or the size of fibrin fibres, but introduces defects within the fibres; qualitatively these defects appear to depend on the nature of the peptide used, but always have a profound effect on the mechanical properties: defective fibres lead to a decrease in both shear and compression moduli. This offers a versatile way to control the mobility of cells moving through these gels: fibroblasts migrate through fibrin in a non-protease-dependent fashion that is strictly dependent on matrix stiffness (more rapid at lower modulus) and can therefore be easily modulated via knob-hole fibrin engineering.

Frontiers in Clinical Advances of Dental Biomaterials

G. *Orsini*

Faculty of Medicine of the Polytechnic University of Marche, Department of Clinical Sciences and Stomatology, Polytechnic University of Marche, Via Tronto 10, 60126 Ancona, Italy

Biomaterials used for dental and craniofacial treatments are currently facing new developments due to nanotechnology and tissue engineering technology. Modern biomaterials can enhance clinical performance in all fields of dentistry from preventive and restorative applications to implant dentistry and oral/maxillo-facial reconstruction, up to tissue engineering approaches coupled with stem cell biology. Substantial improvements in materials for enhanced healing of dental and oral tissues have been performed through the incorporation of filler elements possessing at least one dimension in the nanometer range. Nanometric surface modifications of existing materials are increasing to ameliorate physical as well as biological properties in order to improve the quality and the duration of tissue repair. Cutting-edge properties of different biomaterials will be evaluated, including morphological, antibacterial, mechanical, remineralization and regeneration potential of polymeric, metallic and inorganic nano-based materials, as well as their employment as microcluster fillers, in nanocomposites, mouthwashes, dentifrices, biomimetic dental materials, and bio-engineered scaffolds for tissues repair and reconstruction. Perspectives of research in this arena, clinical outcomes and the most modern procedures to use the biomaterials in dental clinics will be discussed.

Esthetic adhesive restorations

Gaetano Paolone

University Vita Salute San Raffaele
e-mail: gaetano.paolone@gmail.com

Direct restorations are very often considered an artistic work.
Although some clinicians may be more skilled than others, a proper technnique can be useful to obtain great results. When planning a restoration, the color of the substrate should be taken into account in order to achieve a satisfactory esthetic outcome. In some clinical situations, clinicians are asked to hide the substrate with the restoration; in other situations, clinicians can take advantage of an unaltered color substrate and therefore select less opaque materials that exploit the color of the underlying tissue. Differences in direct anteriors and direct posteriors will be outlined during the lecture.

Optical early diagnosis of tissue disease

Francesco Pavone

Director of LENS - European Laboratory for Non-Linear Spectroscopy, University of Florence, Physics department

In the first part of this talk a brief review on the non linear laser imaging techniques will be displayed. In particular, two photon fluorescence microscopy, lifetime imaging, multispectral imaging, second harmonic generation microscopy principles will be described.
In the second part of the talk there will be an overview on the applications of these techniques in the field of biomedical imaging. In particular, tumor detection in tissue imaging applications will be shown in different fields, from urology to gastrointestinal surgery, dermatology and brain surgery.
Morpho-functional characterization of tissue pathologies will be displayed as an interesting tool for tumor early diagnosis. Other type of non tumor disease applications will be also shown together with the demonstration on using laser imaging for follow up of laser therapies.
In the last part of the talk, a fiber based endoscope based on multidimensional spectral detection (one photon fluorescence, lifetime and Raman detection) will be described with particular applications of tumor detection.

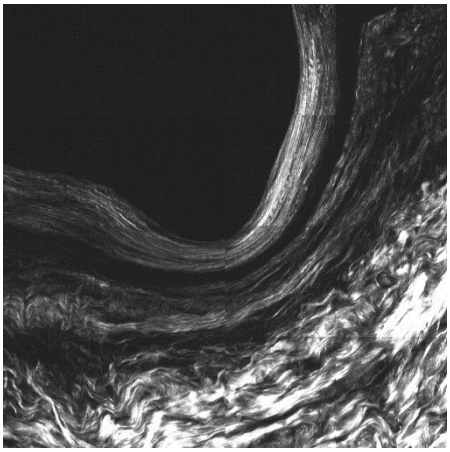

Figure 1: collagen fibers (second harmonic signal) and elastine fibers (two photon) in the stroma area of a tumor in a basal cell carcinoma

ORAL PRESENTATIONS

Allogeneic umbilical cord-derived mesenchymal stem cells as a potential source for cartilage and bone regeneration: an in vitro study

Marmotti A [a,b], Mangiavini L. [c], Mattia S [b], Castoldi F [a], Barbero A [d], Bonasia DE [a], Bruzzone M [a], Dettoni F [a], Scurati R [e] and Peretti GM [c,e]*

[a] University of Turin, Department of Orthopaedics and traumatology, Torino, Italy
[b] University of Turin Molecular Biotechnology Center, Torino, Italy
[c] IRCCS Istituto Ortopedico Galeazzi, Milano, Italy
[d] Department of Biomedicine, University of Basel, University Hospital of Basel, Switzerland
[e] Department of Biomedical Sciences for Health, University of Milan, Milano, Italy
e-mail: lauman82@hotmail.it

Keywords: Provide maximum 5 keywords (or one line)

Introduction
Umbilical cord (UC) may represent an attractive cell source for allogeneic mesenchymal stem cells (MSC) therapy [1-6]. These cells can be obtained through a simple and efficient protocol based on mincing the UC that simplifies any other enzymatic alternative, thus suggesting a potential widespread use. The aim of this in vitro study is to investigate UC-MSC stemness and, in particular, the chondrogenic and osteogenic potential of UC-MSCs grown onto tridimensional scaffolds, in order to identify a possible clinical relevance for an allogeneic use in cartilage and bone reconstructive surgery.

Methods
15 Fresh Umbilical cord (UC) samples from women with healthy pregnancies were retrieved at the end of caesarean deliveries. The UC samples were manually minced into very small fragments (less than 4 mm length) and cultured in MSC expansion medium. At day 14, the UC debris were removed and adherent cells were expanded for 2 additional weeks. At day 28, cells were collected and replated until confluence was reached (Passage 1 or P1).
i) For chondrogenic differentiation onto scaffold, Umbilical Cord-derived Mesenchymal Stem Cells (UC-MSCs) were loaded either on a hyaluronic-acid (HA) (Hyaff-11) or on a collagen-I/III membrane (Chondrogide), with a fibrin glue coating (Tisseel), and grown in Euroclone chondrogenic medium in normoxic or at low oxygen tension (8% O2) conditions. After 4 weeks, sections were stained with haematoxylin/eosin and Safranin-o. Expression of chondrocyte markers (sox-9, type II collagen) was assessed using immunofluorescence (IF). A percentage of cells was used for pellet culture to evaluate proteoglycan (PG) content by PG:DNA ratio.
ii) For osteogenic differentiation onto scaffold, UC-MSCs were loaded onto a bone-graft-substitute (Orthoss) and grown in Euroclone osteogenic medium. After 10, 20 or 30 days, sections were stained with haematoxylin/eosin and expression of osteocalcin and RUNX-2 was assessed using IF.
iii) UC-MSCs were also cultured in bone-graft-substitute in presence of Euroclone chondrogenic medium for 3 weeks and then in hypertrophic medium (DMEM, Linoleic acid, Dex, L-Thyroxine) for 2 weeks to assess their ability to differentiate into hypertrophic chondrocytes and, thus, to induce the endochondral process. The expression of Core-binding factor alpha(1) (CbFa1) was assessed by PCR analysis and compared to sox-9 expression.

Results
i) Chondrogenic differentiation on scaffolds was confirmed at 4 weeks by the expression of sox-9 and type II collagen; low oxygen tension improved the expression of these chondrogenic markers. A similar trend was observed in pellet culture in terms of matrix (proteoglycans) production.
ii) Osteogenic differentiation on bone-graft-substitute was also confirmed after 30 days of culture by the expression of osteocalcin and RUNX-2.
iii) Cells grown in hypertrophic medium showed at 5 weeks Safranin-o positive stain and an increased CbFa1 expression, confirming the ability of these cells to undergo hypertrophy.

Conclusions
These results suggest that the UC-MSCs isolated from minced umbilical cords may represent a valuable allogeneic cell population, which might have a potential for orthopaedic tissue engineering such as the on-demand cell delivery using chondrogenic, osteogenic and endochondral scaffold. The concept of this study may have a clinical relevance as a future hypothetical option for allogeneic single-stage cartilage repair and bone regeneration.

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Personalized regenerative medicine in spine surgery: stem cells from vertebral body

F. Salamanna[a], *G. Barbanti Brodano*[b*], *D. Contartese*[c], *C. Griffoni*[b], *A. Gasbarrini*[b], *M. Fini*[c]
[a]Laboratory of Biocompatibility, Technological Innovations and Advanced Therapies- Rizzoli RIT Department
[b]Department of Oncological and Degenerative Spine Surgery, Istituto Ortopedico Rizzoli- Via G.C. Pupilli, 1- 40136 Bologna, Italy
[c]Laboratory of Preclinical and Surgical Studies, Istituto Ortopedico Rizzoli, Via Di Barbiano 1/10- 40136 Bologna, Italy
**e-mail: giovanni@barbantibrodano.com*

Keywords: mesenchymal stem cells, bone marrow, spinal fusion, regenerative medicine, HOX and TALE genes

Introduction
The availability of tools and treatments that promote spinal fusion in patients undergoing surgery for degenerative spine disease is a relevant clinical need. Recently, our group has shown that mesenchymal stem cells (MSCs) derived from bone marrow can provide an alternative source of MSCs for tissue engineering applications in vertebral surgery (**Figure 1**). These cells have shown optimal biological characteristics and specific expression levels of HOX and TALE genes. Given the increase in average life, it is relevant to investigate the clinical efficacy of this application in spine surgery even in the presence of osteoporosis.
Cell proliferation, gene expression of main surface markers, osteogenic, adipogenic and chondrogenic potential and gene expression of the main HOX and TALE genes were evaluated and compared in MSCs derived from vertebral bone marrow of osteoporotic and non-osteoporotic patients.

Results and Discussion
Compared to MSCs derived from non-osteoporotic patients, MSCs from osteoporotic patients have altered proliferation, altered osteogenic activity and increased adipogenic activity. In addition, distinct and specific expression levels for HOX and TALE genes were found between MSCs derived from bone marrow of osteoporotic and non-osteoporotic patients.

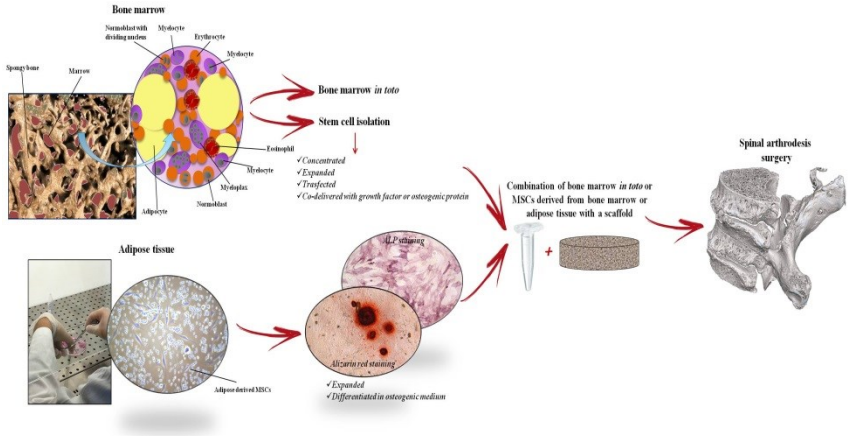

Figure 1: Mesenchymal stem cells and spinal arthrodesis

Conclusions
MSCs from osteoporotic patients have shown intrinsic functional alterations, emphasizing that the osteoporotic microenvironment of vertebral body bone marrow differs from the normal microenvironment as it presents an increase in pro-adipogenic and pro-inflammatory regulatory factors. However, preliminary data have shown that the use of the bone marrow aspirate clot can represent an alternative and effective biological approach for cell isolation even in osteoporotic patients.

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New composite membrane materials for hemodialysis

Iulian Vasile Antoniac[1*], *Aurora Antoniac*[1], *Stefan Ioan Voicu*[2, 3], *Andreea Madalina Pandele*[2, 3]

[1]University Politehnica of Bucharest, Faculty of Materials Science, Splaiul Independentei 313, Bucharest, Romania
[1]University Politehnica of Bucharest, Faculty of Applied Chemistry and Materials Science, Department of Analytical Chemistry and Environmental Engineering, Str. Gheorghe Polizu 1-7, Bucharest, Romania
[2]University Politehnica of Bucharest, Advanced Polymer Materials Group, Bucharest, Romania
e-mail: antoniac.iulian@gmail.com

Keywords: haemodialysis, modified graphene, composite membranes

Introduction

Polymeric membranes are widely used for various biomedical applications like proteins concentration [1], osseointegration [2] or substitutes as technological solutions for various organs, like artificial lung - oxygenator [3] or artificial kidney - haemodialysis [4]. This work presents the principle for synthesis of a new generation of composite polymeric membranes with functionalized graphene for haemodialysis. First, the amino functionalized graphene was modified with cyanuric chloride, giving the possibility for further functionalization with different molecules. Second, specific crown ethers, proteins, enzymes and vitamins were covalent immobilized on graphene in order to increase to selectivity and specificity for removing targeted compounds for specific medical conditions associated with chronic renal disease. In the third stage of synthesis, functionalized and derivatized graphene are used for obtaining composite polymeric membranes with controlled porosity for haemodialysis. Fully structural and morphological characterization of synthesized materials is presented and hydrodynamic and separation properties.

Results and Discussion

The versatility of method consist in the possibility to obtained functionalized graphene oxide with a wide range of specific molecules. This versatility is given by the presence of chlorine atom from cyanuric chloride, which can be involved in further reactions with amino groups. The reason for cyanuric chloride use is justified by the aromatic character of the molecule. After first substitution of a chlorine atom, the aromatic ring is deactivated, next substitution requiring higher temperature conditions. The reaction is schematically presented in Fig. 1.

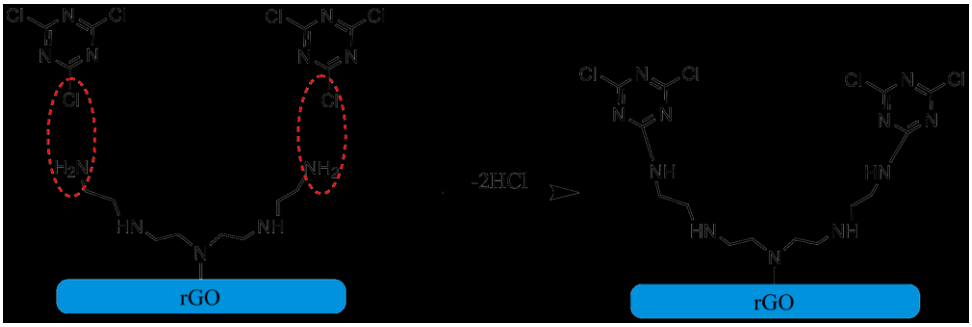

Fig. 1 Functionalization reaction between geduced graphene oxide and cyanuri chloride

All synthesized compounds were characterized by FT-IR, XPS, and thermal analysis. In the FT-IR spectra, the absorption band at 1440 cm^{-1} was attributed to vibration of C-N with C from aromatic ring. Also, the high resolution XPS spectra on C revealed the same bond at 285,2 eV. D and G band from Raman analysis are unmodified and not shifted which suggest that all modifications were performed at the aliphatic chain, graphene being unaltered during reaction steps. By increasing the reaction temperature, from 40 to 60 ^{o}C, another molecules were added with complex functions, like crown ethers (for the removing of heavy metals) or vitamins (for increasing the anticoagulant effect). Functionalized graphene were used for preparing polysulfone composite membranes for one day haemodialysis, by dispersing the nano species in polymer solution followed by the synthesis of the membrane by precipitation. Obtained composite membranes showed good results in the retention of heavy metals ions from synthetic solutions, simulating the retention of these ions in the case of intoxications. Lead and copper were investigated for retention, obtaining an efficiency of the process about 70%, respectively 82 % after 2 hours of recirculation through membrane. Simple polysulfone membrane retained in the same conditions 23, respectively 27% for same metal ions.

Conclusions and/or Outlook

A new principle for synthesizing one day haemodialysis membranes was presented. For this purpose, the amino functionalized graphene was modified with cyanuric chloride, giving the possibility for further functionalization with different molecules (like specific crown ethers, proteins, enzymes and vitamins which were covalent immobilized on graphene in order to increase

to selectivity and specificity for removing targeted compounds for specific medical conditions associated with chronic renal disease). Composite polysulfone-modified graphene were synthesied by dispersing the nano species in polymer solution followed by the synthesis of the membrane by precipitation.

Acknowledgement: This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS - UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0407 - Intelligent materials for medical applications, sub-project - New generation of hemodialysis composite membranes with derivatized graphene.

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Advances in laser processing for biomaterial applications

A. De Bonis[a], M. Curcio[a], R. Teghil[a], J.V. Rau[b]

[a] Dipartimento di Scienze, Università della Basilicata, Viale dell'Ateneo Lucano, 14 -85100, Potenza, Italy
[b] ISM-CNR, Via del Fosso del Cavaliere, 100-00133 Rome, Italy
**e-mail: angela.debonis@unibas.it*

Keywords: PLD, Laser ablation in liquid, Laser texturing

Introduction

In the last years there have been significant advances in the development of new technologies to meet the demand for low cost biomedical materials. In this contest laser assisted methodologies for the fabrication of biomaterials play a relevant role since it is possible to fine control composition and morphology of several materials with a high fabrication rate process. By tuning laser wavelenght, pulse duration and environment, laser ablation allows the selective removal of material [1]. Laser technologies have been used to deposit thin films of bioactive ceramics, glasses and metals, to prepare functional nanoparticles or to carefully create structured surfaces.

Results and Discussion

Pulsed laser deposition (PLD) has been widely used to deposit thin films of ceramic and glass biomaterials onto biocompatible and biodegradable substrates. Films deposited by nanosecond laser sources present morphology with micro and nanostructures that favour the interaction of surface with cells and proteins, respectively. It has been shown that bioglass with enhanced osteogenic and/or mechanical properties can be deposited, simply varying the target composition.

Laser ablation in liquid is a promising technique that allows to obtain bare nanoparticles whose composition, size and morphology can be tuned by varying target and liquid medium with a one-step and green approach that is desirable for biological and biomedical applications. In fact it does not require the use of reducing agents or precursor that can generate impurities. Moreover, the highly reactive prepared nanoparticles can be further processed with biological molecules to introduce specific functionalities [2].

The selective material removal can be used to modify material's properties in a confined region, by using a scanned laser beam. In particular using femtosecond laser sources it is possible to reduce thermal effects and the formation of debris, to finely create micro/nano structures on several substrates. This approach opens to the possibility to directly create a pattern on a tissue scaffold or 3D microfluidic channels.

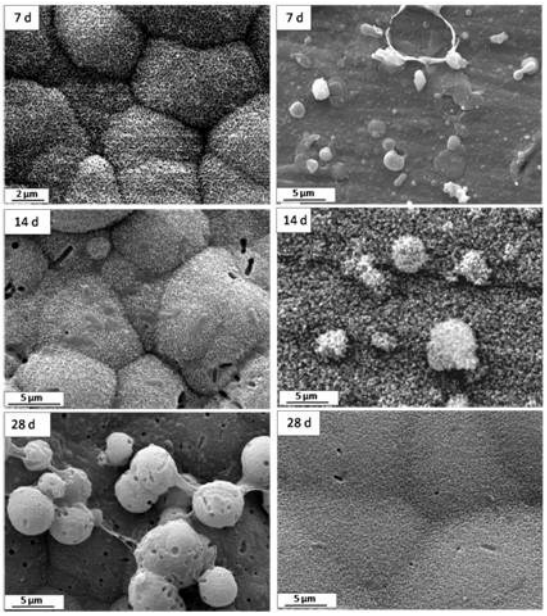

Figure 1: PLD bioglass coatings deposited at room temperature and 500°C after different immersion time in SBF.

Outlook

Laser fabrication techniques offer great advantages for fabrication of materials suitable for biomedical applications since they allow flexible, contactless, material independent, high precision processes. A multi-technique space resolved and surface sensitive approach is advised to fully characterize the properties of laser processed materials.

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Sound-induced fabrication of complex organoids network

*D. Pellicciott [a], R.G. Richards[a], M. Alini [a], D. Egli [a], T. Serra[a]**

[a]AO Research Institute Davos, Switzerland
**e-mail: tiziano.serra@aofoundation.org*

Keywords: 3D in vitro models, surface acoustic wave, organoids, cell patterning, biofabrication

Introduction

3D cell technologies are revolutionizing drug discovery and precision medicine. They can better recapitulate native physiological milieu in comparison to standard cell cultures and preclinical models. A wide range of 3D cell technology products are on the market. These include products to make organoids, gels embedding cells, synthetic scaffolds seeded with cells and the most advanced 3D-bioprinting technology to dispense cells and matrices. 3D-bioprinting tools allow precise dispensing of cell-loaded hydrogel and creation of complex 3D constructs. However, their main limitations are: a) lower cell viability, b) limited resolution, c) long processing time and d) high costs. Thus, an additive manufacturing process to create 3D models in a time-effective manner, with sufficient complexity, and retaining cell viability is crucially required. Here we report a proprietary 3D cell technology, named 3D Sound Induced Morphogenesis (3D-SIM), which allows producing hierarchically complex 3D organoids constructs[1]. This technology makes possible the generation of precise and reproducible patterns of particulates systems such as cells, spheroids, bioactive particles (tricalcium phosphate TCP, hydroxyapatite, etc.). 3D-SIM is based on an acoustic wave additive manufacturing technology. Acoustic waves move cells dispersed in a fluid. Depending on amplitude and frequency of the waves, multiple cell patterns are produced and stabilized through gelation of hydrogel precursors. Primary human mesenchymal stem cells (hMSCs) and human umbilical vein endothelial cells (hUVECs) were used to create complex 3D cell structures. For the patterns composed by calcium phosphate particles, three different sizes have been tested: 32-75 µm, 125-250 µm and 250-500 µm. As hydrogels: i) gelatin methacryloyl (GelMA, 5%w/v) / Irgacure 2959 solution in PBS and ii) fibrin gel (fibrinogen-thrombin, SIGMA) were used. Finite element analysis (FEA) was conducted to properly select cell pattern shapes, by using MATLAB software. Morphological analysis via optical and confocal microscope has been carried out.

Results and Discussion

We show that acoustic waves can move organoids/particulates systems dispersed in a fluid over an area of 28 cm^2 in less than 15s. The process is applicable to a wide range of off-the-shelf gelling biomaterial matrices. Layers composed by several combinations of hydrogel and cells/bioactive particles were generated and employed as matrices. As indicated in **Figure1**, particles patterns morphology confirmed FEA investigation. 3D constructs were created by staking layers of patterned cells embedded in hydrogel matrices. Biological evaluation confirmed that 3D-SIM is a mild fabrication process which does not affect cell viability.

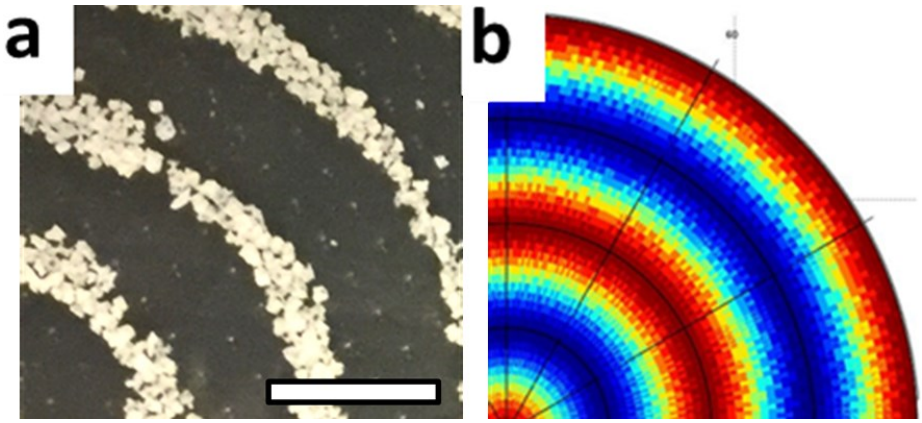

Figure 1: a) Tricalcium phosphate (TCP) microparticles embedded in a thin layer of gelatin methacryloyl hydrogel; b) FEA analysis visualization of the liquid wave formed within a circular chamber.

Conclusions/Outlook
We demonstrate that 3D-SIM is an affordable and user-friendly technology to create 3D *in vitro* organoids models in a time-effective manner, with sufficient spatial complexity, retaining cell viability.

Acknowledgments
The authors would like to thank BRIDGE programme (SNSF-Innosuisse) for providing financial support to this project (SNSF grant number: 20B1-1_178259).

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Biomaterials: a good alternative to autologous bone for spine fusion

G. Barbanti Brodano[*a], C. Griffoni[a], A. Nataloni[b], G. Giavaresi[c], S. Bandiera[a], S. Terzi[a], R. Ghermandi[a], G. Evangelisti[a], M. Girolami[a], V. Pipola[a], M. Fini[c], A. Gasbarrini[a]*

[a] Department of Oncological and Degenerative Spine Surgery, Istituto Ortopedico Rizzoli- Via G.C. Pupilli, 1- 40136 Bologna, Italy
[b]Finceramica Faenza SpA, Faenza (RA)- Italy
[c]Laboratory of Preclinical and Surgical Studies, Istituto Ortopedico Rizzoli, Via Di Barbiano 1/10- 40136 Bologna, Italy
**e-mail: giovanni@barbantibrodano.com*

Keywords: Biomaterials, Hydroxyapatite, Bone graft, Spinal fusion

Introduction

Spinal fusion is one of the most common surgical procedures in spine surgery, whose primary objective is the stabilization of the spine for the treatment of many degenerative, traumatic and oncological diseases of the spine. Autologous bone is still considered the "gold standard" technique for spinal fusion. However, biomaterials, in particular bioceramics, which are potentially osteogenic, osteoinductive and osteoconductive, can be used to increase the process of spinal fusion. During the last years we have studied in particular the use of ceramic biomaterials prepared from hydroxypatite (HA), starting from *in vitro* analysis, through an *in vivo* study on ovine animal model and a post-market surveillance analysis, to finally design and perform a clinical study, which is ongoing in our Department. In the first step, HA-derived biomaterials were tested *in vitro* in the presence of bone marrow-derived human mesenchymal stem cells (hMSCs) and evaluated for their ability to activate precursor cells. In the second step, the biomimetic bone graft substitute SintLife® putty (MgHA) was evaluated *in vivo*. A posterolateral fusion procedure was applied on 18 sheep, where a fusion level was treated with MgHA, while the other level was treated with autologous bone. Microtomography and histological/histomorphometric analysis were performed six months of after surgery. In the third step, we reported the results of a post-market surveillance study conducted on 4 independent cohorts of patients (total 115 patients), in which HA-derived biomaterials were used as bone graft substitutes or extenders. Finally, a clinical study has been designed and approved by the Ethics Committee of our Institute and is currently ongoing. This study aims to evaluate the efficacy of the ceramic biomaterial SintLife® putty for bone replacement in patients treated by posterolateral fusion for degenerative spine disorders (Figure 1).

Results and Discussion

HA biomaterials were effective in promoting the *in vitro* growth of hMSCs and their osteogenic differentiation. In the animal model, SintLife® putty has been effective in generating neo-formed bone tissue with morphological and structural features similar to those of the pre-existing bone. The post-market surveillance analysis has not reported any intra-operative nor early or late post-operative adverse events.

Fourteen patients are currently recruited for the clinical trial designed to evaluate Sintlife efficacy for spine fusion (FU range: 5-15 months). One adverse event has been recorded: one patient experienced an inflammatory reaction four days after the surgery, with fever and edema at the surgical site. Due to the persistence of the complication a debridement surgery was necessary and the graft was removed. CT analysis performed at 6 months follow up for all the patients showed an initial spinal fusion. Clinical outcomes, evaluated by patients' self-administered questionnaires for pain (VAS), functional ability (ODI) and quality of life (EQ-5D), significantly improved at 6 months FU. Only one patient reached 12 months follow up. The study is ongoing.

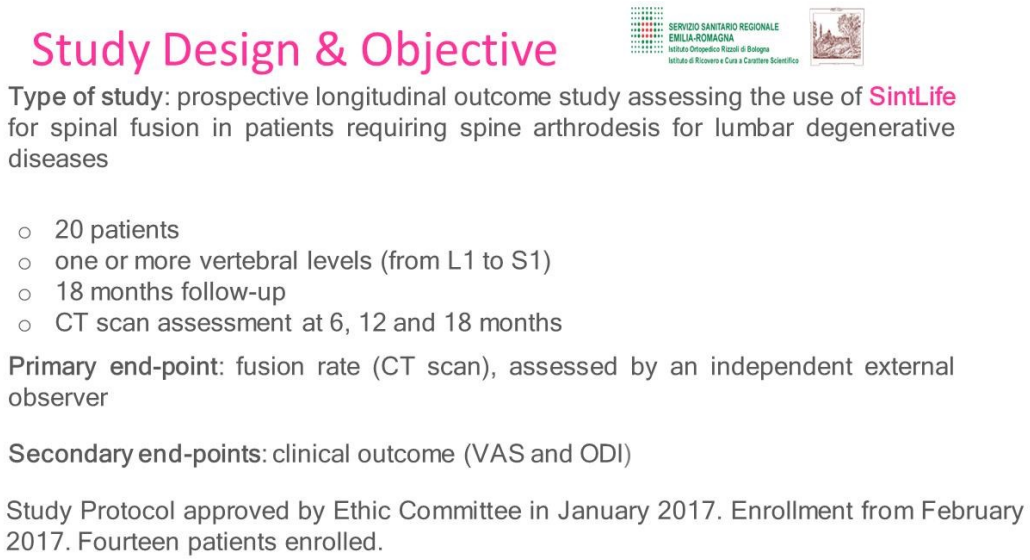

Figure 1. Clinical pilot study design

Conclusions

Our results, obtained from *in vitro*, preclinical and clinical studies, suggest that biomaterials derived from hydroxyapatite could be a valid alternative to autologous bone graft for vertebral fusion. This would potentially avoid or reduce the need of autologous bone harvesting and therefore, the risk of drawback-related side effects.

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Carbon-fiber-reinforced PEEK fixation system in the treatment of spine tumors

G. Tedesco[a], G. Barbanti Brodano[b], M. Girolami[b], V. Pipola[b], S. Bandiera[b], Silvia Terzi[b], R. Ghermandi[b], G. Evangelisti[b], T. Greggi[a], A. Gasbarrini[b]

[a] Deformity Spine Unit, Istituto Ortopedico Rizzoli- Via G.C. Pupilli, 1- 40136 Bologna, Italy
[b]Department of Oncological and Degenerative Spine Surgery, Istituto Ortopedico Rizzoli- Via G.C. Pupilli, 1- 40136 Bologna, Italy
e-mail: giovanni@barbantibrodano.com

Keywords: Carbon-fibre reinforced PEEK, Spine Tumour, Scattering effect

Introduction

Combination of surgery and radiotherapy is becoming more and more frequent in the protocols for the treatment of bone tumours of the spine [1].

Actually the metallic hardware interfere with the postoperative radiotherapy due to the artefacts on imaging and due to the scattering effects on treatment by ionizing radiations including accelerated particles. The risk of over irradiation of neighbouring structures limits the dose delivered making treatment less effective. Composite materials such as carbon-fibre-reinforced (CFR) polyethil-ether-ether-ketone (PEEK) have been used since many years for interbody and body replacement cages [2,3]. This material is biologically compatible and experimental works confirmed a strong effectiveness in promoting osteoblastic activity [4]. Moreover the modulus of elasticity of the composite material (13 GPa) is much closer to that of cortical bone (12 GPa) than is titanium (10 GPa). This might lower the risk for stress risers and secondary fractures, as well as to contribute to early fracture healing. [5-7] These cages are radiolucent at the standard radiograms, barely visible on TC scan and MRI, allowing easy planning CT scan [8], early detection of local recurrence and very useful to avoid any scattering effect during radiotherapy.

CFR-PEEK fixation systems are available as plates and nails since several years for long bones fixation [9,10]. A CFR-PEEK spine fixation system featuring an original rod/screw connection by impaction has been proposed few years ago.

We retrospective evaluate the first 60 consecutive tumor patients, treated in a single institution who underwent spinal surgery including a composite CFR-PEEK fixation system (CarboclearTM, produced by CarboFix Orthopedics® Ltd., Herziliya, IL).

Results and Discussion

There were 33 male and 27 female, mean age 58 years (range 18 - 78). 37 cases were primary tumour (24 recurrence) and 23 were metastases (13 recurrence). A separation surgery has been performed in 41 cases, a gross total excision in 11 cases and an en-block resection in 8 cases.

Only one intraoperative complication related to the implant occurred: a screw breakage during the third surgical procedure of the series. Weight-bearing was encouraged in the immediate post-operative course for all the patients without orthosis. No rod breakage, neither any screw/rod disconnection was found during the follow-up. One cases of loosening of sacral screws were found at 12 months in one patient submitted to previous surgery and revised with CFR-PEEK screws. This were a multi recurrent malignant tumour and loosening was found at the time of the local recurrence provoking instability of the construct. One case of screw mobilization with pull out of the distal screws at 6 months was recorded.

After the surgery in 41 patients a postoperative radiotherapy has been performed (23 cases with particle and 18 with photons).

The main advantage of the composite CFR-PEEK spine stabilization systems is its radiolucency that should produce low artefact level at the imaging. If compared to metal hardware, CFR-PEEK allows to analyse a large body segment and even to use particles for postoperative radiotherapy without any perturbation of the images and interference of the beams with metallic material.

Conclusions/Outlook

Data from this report show that CFR-PEEK fixation system is comparable to standard titanium system in term of intraoperative complications, stability at weight bearing and at functional recovery.

Thanks to radiolucency CFR-PEEK stabilization devices are more suitable in patients eligible for radiotherapy: the absence of image artefacts together with significantly less dose perturbation improve the treatment accuracy. Moreover the radiolucency is useful in the follow-up of patients thus allowing early detect of local recurrence.

The advantage of using CFR-PEEK composite implants in terms of overall results and patients' outcome needs to be prospectively defined with larger patient series and longer follow-up. In this perspective, even the final prognosis could be positively affected by combination of less aggressive surgery and appropriate courses of radiotherapy.

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Reverse Engineering of femoral condyles: shift and reduction of the tibiofemoral contact area after meniscectomy

R. De Santis[a], A. Gloria[a], S. Maietta[b], L. Ambrosio[a], D. Ronca[c]*

[a] Institute of Polymers, Composites and Biomaterials, Naples, Italy.
[b] Fraunhofer JL IDEAS, University of Naples "Federico II", Naples, Italy.
[c] Campania University Luigi Vanvitelli, Naples, Italy.
**e-mail: rosantis@unina.it*

Keywords: meniscus, 3D laser scanning, contact area, reverse engineering

Introduction

Several experimental tests and theoretical models have been carried out over the last half century for investigating the biomechanics of the knee. The research was also prompted by the compelling need to develop advanced surgical approaches for restoring or regenerating joint tissues through knee arthroplasty or through tissue engineering[1,2]. In the 70s Walker and Erkman3 used a self curing methylmethacrylate casting to detect the spatial location of tibial-femoral contact. Later, flexible polyester film sensors together with silicone rubber casting were used by Fukubayashi and Kurosawa[1] for measuring pressure and contact area, respectively. Indeed, the use of pressure sensitive films is the most popular choice, as they allow to simultaneously investigate pressure distribution and contact area. However, the shift in the contact area after meniscectomy, which is clearly evident since the late 70s, has been poorly described. Of course, sensitive films and strips need to be removed from the knee site for analysis purpose; this process prevents to determine the "exact" position of the contact area in a 3D reference system of the knee.

Meniscectomy significantly change the kinematics of the knee joint by reducing the contact area between femoral condyles and the tibial plateau, but the shift in the contact area has been poorly described. The aim of our investigation is to measure the shift of the tibiofemoral contact area occurring after meniscectomy.

Materials and Methods

Eight 4-month-old large white duroc race were sacrificed for commercial meat consumption at a local abattoir, and eight pairs of porcine knees were kindly offered for research purposes. A proximal femur osteotomy and a distal tibial osteotomy was performed. The extremities of the femoral and tibial diaphysis were cemented into square section tubes (30x30x50mm) using bone cement. An Instron 5566 testing machine equipped with a load cell of 1kN was used for compression tests. Two FlexiForce sensors were inserted in the medial and lateral compartments between the femoral condyle and tibial plateau. Two repetitive tests at a maximum load of 150 N were performed on each knee before meniscectomy. Contact areas in the lateral and medial compartments were detected during the second test by injecting eosin in the knee compressed at 150 N. Each knee was removed from the dynamometer and a bilateral meniscectomy was performed. The knee was again gripped to the dynamometer. Two repetitive compression tests at 150 N were performed again on each knee in order to measure local pressure. Contact areas were detected during the second test by injecting hematoxylin in the knee compressed at 150 N. The reverse engineering approach through the Cyberware Color 3D Digitizer was implemented for detecting contact area. Laser scan in conjunction with the optical surface texturing of the femoral condyles were elaborated through RapidForm and Rhinoceros software, and the centroid of the contact in the lateral and medial compartments was computed

Results and Discussion

In vitro experimental testing of the knee has been particularly active over the last half a century, it is still a basic research field motivated by at least three considerations: the first is to improve the knowledge on the biomechanics of this joint[1], the second is to provide experimental data for validating and calibrating 3D models derived from in vivo imaging through MRI or X-ray CT, the third is to design advanced biomaterials and surgical approaches for the knee arthroplasty[2].

The load-displacement curve shows a J-shaped profile. However, a more marked toe-region can be observed for the load-displacement curve before meniscectomy, as shown in Fig. 3(a). The stiffness of the knee joint before meniscectomy (357 N/mm) was significantly lower ($p=0.0012$) than that recorded after meniscectomy (640 N/mm), thus suggesting how menisci strongly affect the load-displacement behaviour. Fig. 3(b)-(c) show that in both the medial and lateral compartments pressure occurring at the femoral condyle-tibial plateau contact region after meniscectomy is significantly higher than that before meniscectomy ($p<0.0001$). At a load of 150 N, pressure values recorded after meniscectomy in the medial compartment (0.72±0.15 MPa) was higher than that in the lateral compartment (0.63±0.14 MPa), however this difference was not statistically significant. Similar observation was reported before meniscectomy, with maximum pressure values in the medial and lateral compartments of 0.25±0.05 MPa and 0.21 0.05 MPa, respectively.

Results suggest that laser scans combined to surface texturing is a powerful tool to investigate the 3D stained contours of contact area (fig. 1).

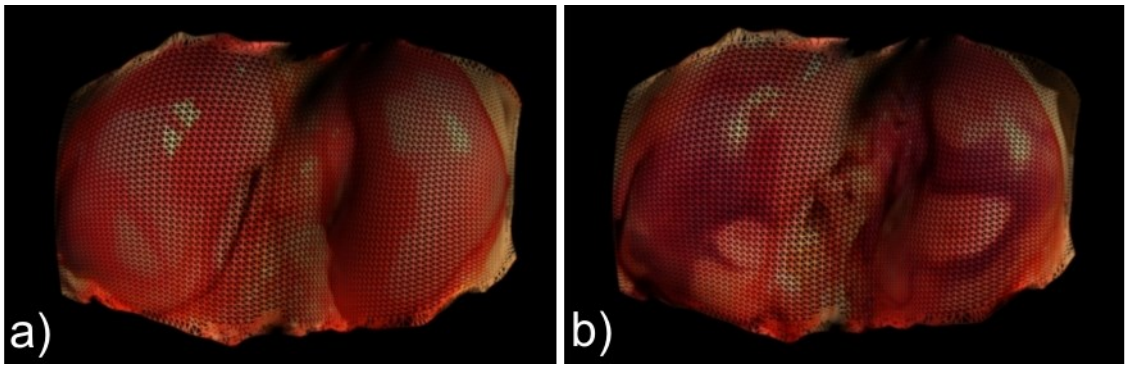

Figure 1: Surface texturing and polygonal mesh of the condyles stained with eosin before meniscectomy (a) and stained with haematoxylin after meniscectomy (b).

Beside the largely documented reduction of contact area and local pressure increase, a shift of the centroid of the contact area toward the intercondylar notch was measured after meniscectomy.

Conclusions and/or Outlook

In conclusion, laser scans combined to surface texturing is a powerful tool to investigate, in vitro, the 3D stained contours of the contact area between femoral condyles and tibial plateau, and this approach has been carried out, for the first time, on a porcine model.

Beside the largely documented reduction of contact area and local pressure increase, a shift of the centroid of the contact area toward the intercondylar notch was measured after meniscectomy. As a consequence, cartilage degeneration close to the intercondylar notch may occur as a consequence of the centroid shift.

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Hybrid Scaffold for Bone Reconstructive Surgery

G. Perale[*a,b], C. F. Grottoli [a], S. Molinari [a], G. Pertici [a], A. Bistolfi [c], R. Ferracini [d]

[a] Industrie Biomediche Insubri SA, via Cantonale 67, 6805 Mezzovico-Vira, Switzerland;
[b] University of applied sciences and technology SUPSI, Department of Innovative Technology, Galleria 2, Via Cantonale 2c, CH-6928 Manno, Switzerland;
[c] Orthopaedic Unit, A.O.U. Città della Salute e della Scienza, Turin, Italy
[d] Department of Surgical Sciences, Orthopaedic Clinic-IRCCS A.O.U. San Martino, 16132 Genova, Italy;
**e-mail: giuseppe@ibi-sa.com*

Keywords: Biomaterials, Bone regeneration, Reconstructive surgery, Bone remodelling

Introduction
Bone grafts for reconstructive surgery should ensure both volumetric stability and adequate strength. Moreover, their intimate structure should have an adequate open and interconnected porous network for cell migration and proliferation and vessel ingrowth, with a distributed pore size ranging between 50 to 350 μm, while also providing specific signals for bone regeneration and remodelling [1,2,3].

Materials
An innovative composite solution, inspired by natural bone architecture and bearing cues from both mineral components and polymeric ones, was here followed to develop a three-dimensional bone scaffold, SmartBone®: a bovine derived mineral matrix is used to provide adequate solid structure and porosity, while resorbable polymers are used to reinforce it. RGD-exposing collagen fragments are finally added to promote cell colonization and proliferation. Previously published clinical results indicate that SB is osteoconductive and osteoinductive, promoting remodelling to mature bone formation in about 8-12 months [4].

Results and Discussion
These composite bone substitutes have been successfully grafted onto more than 50'000 patients up-to-day: the high performances of this biomaterial allow its current use in different specialities, including orthopaedic reconstructive applications.

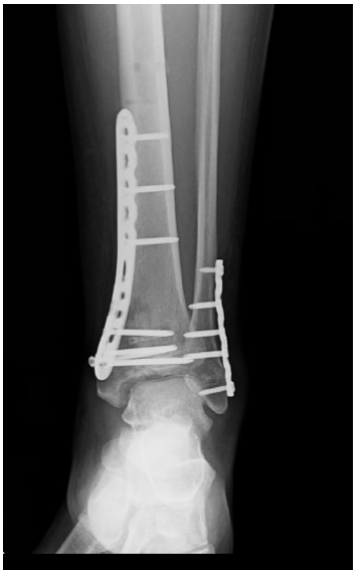

Figure 1

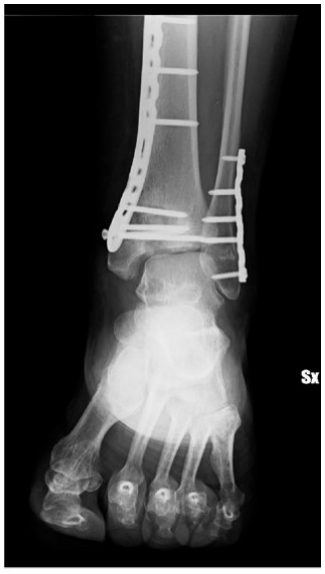

Figure 2

Figs. 1 and 2: SmartBone® blocks implanted during twin tibial and fibula traumatic injury stabilization surgery (fig.1) and at 6 months follow-up (fig.2), showing perfect integration, no volumetric resorption and already ongoing remodelling process.

Above pictures present an example of a reconstruction case: a twin tibial and fibula traumatic injury in an adult male. Capability to withstand heavy surgical manoeuvres, allowed SmartBone® blocks to be easily adapted to fit the residual defect and perfectly located inside the gap, being finally firmly fixed with osteosynthesis devices.
Surgery was fast and precise, allowing to obtain satisfactory results both in terms of anatomical reconstruction and functionality preservation. The post-operative follow-up recorded no issues of any kind and proceeded optimally, evidencing a

faster healing and rapidly decreasing patient pain together with mobility recovery: restored anatomy and functionality found confirmation from 6 months post-op the radiographic images, which showed complete volume stability and already ongoing graft remodelling process.

Conclusions

Radiologically evaluated bone density analysis indicates, in extremely good agreement with past histological studies, that SmartBone is osteoinductive and osteoconductive: it promotes fast bone regeneration, finally leading to mature bone formation in shorter time-windows with respect to alternative solutions such as synthetic materials and allografts, thus confirming the validity of the endogenous tissue restoration principle.

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Articular Cartilage Regeneration by tracheal chondrocytes in Equine Model

F. Canonici[a], A. Cersini[b], C. Cocumelli[b], E. Galvano[b], F. Di Egidio[b], A. Amaddeo[b], A. Fagiolo[b], C. Roncoroni[b], A. Altigeri[b], A. Zepparoni[b], P. Canonici[a], V. Antognetti[b], G. Pietrella[b], P. Ghisellini[c,d], C. Rando[c], G.P.M.Benedetti[e], S.L. Torchia[d], R. Eggenhöffner[c,d], K. Barbaro[b,d]*

[a]Equine Practice srl, Campagnano, Roma, Italy.
[b]Istituto Zooprofilattico Sperimentale Lazio e Toscana "M. Aleandri", Roma, Italy
[c]Università di Genova, Facoltà di Medicina, Dipartimento di Scienze Chirurgiche e Diagnostiche Integrate (DISC), Genova, Italy
[d]Consorzio Interuniversitario INBB - Viale delle Medaglie d'Oro, 305, 00136 Roma, Italy
[e]Studio Dentistico, Fiano Romano, Roma, Italy
**katia.barbaro@izslt.it*

Keywords: Stem cells, adipose tissue, bone regeneration

Introduction

The articular cartilage lesions are among the most common causes of lameness that compromises the quality of life of many animals, especially horses. The hyaline cartilage in the joints contains a low number of chondrocytes and is unvascularized. Thus, lesions in this tissue are generally unable to repair spontaneously and optimally [1]. For this reason, a lesion of the articular cartilage can easily evolve into arthritic degeneration. The different techniques commonly used to treat this type of injury are debridement, subchondral drilling, periosteal transplantation and perichondral and, more recently, transplantation of mesenchymal stem cells (MSC) from the bone marrow (BMSC) and adipose tissue (AMSC) [2,3].

Unfortunately, these methods have not proven to be particularly effective: in some cases, they lead to the formation of fibrocartilage with poorer biomechanical and functional properties than the hyaline cartilage.

Autologous chondrocytes isolated from articular cartilage have been used since very recently because they allow an optimal restoration exploiting the neo-synthesized cartilage of hyaline type. This method, however, has the drawback of subjecting the animal to a further arthroscopy for the levy of the articular cartilage fragment to use as a source of chondrocytes, and also it would require to subtract healthy tissue from a site already compromised.

The purpose of this project was to identify the anatomical region most suitable for collecting chondrocytes in the living animal, study replicative ability, multipotentiality and capacity to repair the hyaline cartilage in the osteochondral lesions by the chondrocyte population isolated.

To this purpose, an *in vitro* isolation and amplification protocol was developed to isolate chondrocytes from cartilage coming from different sites of origin (trachea, nose, ear, and knee) in order to establish the most convenient location for the levy of hyaline cartilage in the living animal.

Results and Discussion

It was thought to use hyaline cartilage by tracheal rings of the horse as a source of chondrocytes, as it is readily accessible, easy to levy (local anesthesia) thus avoiding to expose the animal to surgery with general anesthesia. The protocol developed has proved to be very efficient and has allowed isolating a homogeneous population of cells from all of the cartilaginous tissue samples collected (**Figure 1**).

The chondrocyte population, isolated from the trachea, was evaluated from multipotentiality by the study of their ability to differentiate *in vitro* in osteogenic lines, adipogenic and chondrogenic. The differentiative capacity of the chondrocyte population in the three lineages was highlighted, after appropriate stimulation, both by staining the monolayer cells or by the detection of specific RNA.

The chondrocytes stimulated by osteogenic differentiation showed a change of morphology, and extracellular matrix mineralization highlighted in red with the Alizarin S. color. The microRNAs detected and specific for osteogenesis in mammals are the ALP (alkaline phosphatase) and Runx2. In chondrogenic differentiation, deposits of intra- and extra-cellular glycosaminoglycans were observed, highlighted in blue with Alcian staining. MicroRNAs tested, measured and related to the chondrogenesis in mammals are AGG (aggrecan), Col-II (type II collagen) and Sox9 (sex determine region Y-box).

The adipogenic differentiation gives rise to rounded cells with lipid vesicles in the cytoplasm, highlighted in red by staining with Oil Red O. The tested microRNAs detected, and specific to adipogenesis in mammals is the PPARγ2 (peroxisome proliferator-activated receptor γ2). In order to evaluate the reparative ability of chondrocytes *in vivo* tracheal, a horse with a fracture of the carpal joint cartilage was selected for the study. A small cartilage fragment from the tracheal ring of the horse was drawn which the chondrocytes were isolated and grown from; subsequently, they were implanted into the lesion site arthroscopically.

The histological examination of the treated cartilage was performed at 8, 13 and 24 months after implantation. The histological sections were stained with hematoxylin-eosin, with toluidine blue, and with a polyclonal antibody to assess the COL2A1 available to the chondrocytes and the presence of type II collagen characteristic of hyaline cartilage.

In this project, we have shown that chondrocytes tracheal have the capability to de-differentiate after culturing and generate

chondrogenic progenitor cells (so with stem cell characteristics) with the reparative ability of articular cartilage when implanted. In fact, all of the differentiation *in vitro* (in the osteogenic lineage, adipogenic and chondrogenic) were positive with both histochemical and with the analysis of expression for specific genetic markers.

Therefore, this innovative approach for the treatment of articular cartilage lesions by the chondrocyte tracheal implant in the site of injury has led to excellent results in regenerative medicine. In fact, the images at the optical microscope relative to biopsies performed at the various sites of lesions at different times (8, 13 and 24 months after implantation) demonstrate the significant change in the neo-cartilage tissue. Biopsy investigations show the evolution from a disorganized and immature tissue (after 8 months from implantation); towards a much more organized one (after 13 months from implantation), almost as the natural hyaline cartilage. The appearance of a tissue perfectly comparable to native articular cartilage is detected 24 months after implantation.

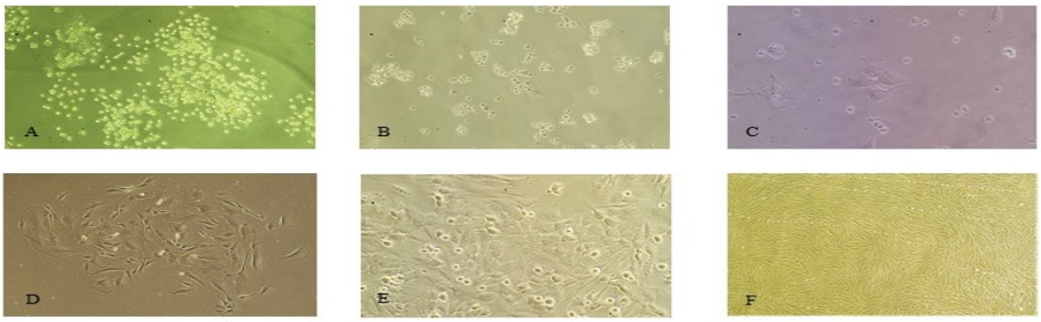

Figure 1: *In vitro* morphological changes of chondrocytes at inverted microscope at 10X. A) after cartilage tissue digestion, B) after three days of culture, C) after five days of culture, D) after seven days of culture, E) after ten days of culture, F) after fifteen days of culture.

Conclusions and/or Outlook

In conclusion, the innovative idea of using the tracheal cartilage of hyaline type as a valuable source of chondrocytes from cells to be de-differentiated *in vitro* into progenitor with stem cell characteristics, enabled to repair an articular lesions of the horse through the neo-synthesis of hyaline cartilage with characteristics identical to the articular cartilage of the surrounding skin.

Therefore, this method is characterized by effortlessness of removal of chondrocytes of the tissue source, the development of the technique of *in vitro* replication and the consequent plant is undoubtedly to date the most promising procedure for the optimal repair with *restitutio ad integrum* of cartilage lesions joint. In the near future, it may be the most effective solution for treating humans within the same protocol.

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New evidence of eco-friendly nanoparticles production and applications in biomedicine and food science

S. Cavalu[a], L. Fritea[a], V. Laslo[b],T. Costea[c], L. Moldovan[c], S. Vicas[b]*

[a*] University of Oradea, Faculty of Medicine and Pharmacy, P-ta 1 Decembrie 10, 410087 Oradea, Romania
[b] University of Oradea, Faculty of Environmental Protection, 26 Gen. Magheru St., 410048 Oradea, Romania
[c] Oradea, Faculty of Electrical Engineering and Information Technology, 1 University St., 410087, Oradea, Romania
**e-mail: simona.cavalu@gmail.com*

Keywords: nanoparticles, selenium, medical applications, food, health.

Introduction

Great attention is paid to the concept of "Green Chemistry", which aims at development of efficient methods for the synthesis of nanoparticles (NPs) in terms of the least possible impact on human life and environment.
Particularly, the green chemistry approach to synthesizing biocompatible selenium nanoparticles has gained attention in recent years. Plant extracts and other natural resources has been found to be an excellent alternative
methods for green synthesis, since this methods does not use any toxic chemicals and also have numerous benefits, including environmental friendliness, cost-effectiveness, and suitability for pharmaceutical and biomedical applications. However, these new synthesis routes should be optimized in terms of performance, cost, product quality (shape and size distribution) and scale-up capability. In the present work, the structure and morphology of synthesized Se nanoparticles were characterized using transmission electron microscopy (TEM), AFM (atomic force microscopy), UV/Visible and FTIR/FT Raman spectrophotometry, dynamic light scattering (DLS) and X-ray diffraction method (XRD). Possible applications of the prepared nanoparticles are also discussed such as improved nutrition and food functionality or nanostructured surface of medical implants for cranioplasty.

Results and Discussion

In this study, selenium nanoparticles are produced by two different methods, using biological synthesis and green chemistry. Biogenic selenium nanoparticles (SeNPs) were obtained via selenite (SeO_3^{2-}) reduction by *Lactobacilus casei* and *Lactobacilus fermentum* strains, aiming to demonstrate the protective effect of SeNPs against cadmium toxicity. Lactic acid bacteria have been reported to remove Cd and Pb from culture medium solutions and therefore represent a useful tool for decontamination of food and beverages from heavy metals. We have demonstrated that the protective mechanism of SeNPs-enriched probiotics against heavy metals is related to the mechanism of selenium ions reduction to elemental selenium nanoparticles and deposit them in intracellular spaces. Morphological characterisation of exogenous SeNPs was performed by UV/VIS spectroscopy, TEM/SEM (Electron Microscopy), DLS ((Dynamic Light Scattering) and Zeta Potential measurement, emphasizing the average size distribution of about 150 nm. FTIR and FT-Raman spectroscopy was apply in order to demonstrate the ability of *Lactobaccilus* strains enriched with nanoselenium particles to remove Cd from culture medium (Fig. 1).

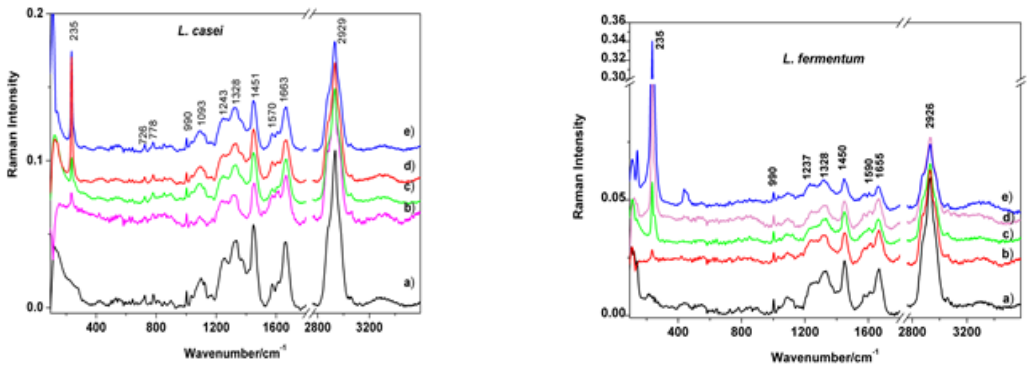

Figure 1: FT Raman spectra of *Lactobacilus casei* and *Lactobacilus fermentum* strains before and after different treatments: native strain, lyophilized (a), Cd treatment 20 ppm (b), concomitant Cd and SeNPs treatment (c), SeNPs treatment 60 ppm (d) and selenite treatment (e). Incubation time- 48 h.

In our experiment, the accumulation of selenium (either endogenous or exogenous) can be noticed in both *L. casei* and *L. fermentum* spectra, at the same wavenumber, 235 cm^{-1}, but the concentration is significantly higher in the case of L. fermentum strain.
On the other hand, an effective uptake of SeNps was demonstrated in plants, using Broccoli sprouts, aiming to develop novel functional food, enriched in selenium, for improved nutritional properties.
The green chemistry was also apply for synthesis of SeNps using starch (polysaccharide), glucose and galactose (mono-saccharides) as reducing agents and sodium hydrogen selenite ($NaHSeO_3$) as a starting selenium precursor. In this case, the size distribution of SeNps was lower, as demonstrated by DLS and TEM (Fig. 2).

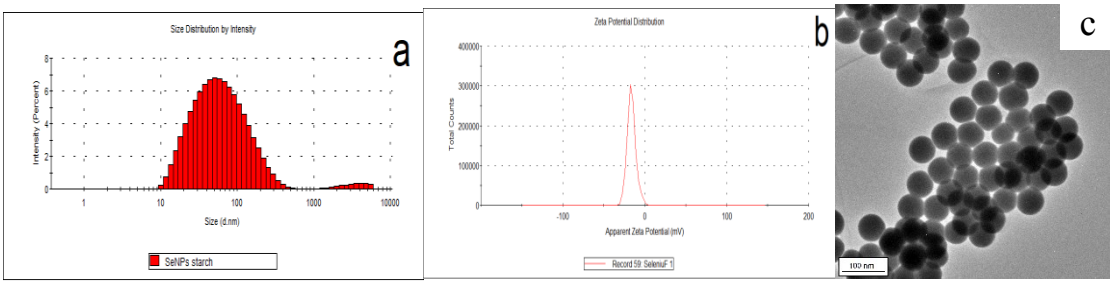

Figure 2: DLS analysis (size distribution and zeta potential) of selenium colloidal sol upon hydrothermal reaction of $NaHSeO_3$ precursor and starch reducing agent, along with the Zeta potential record and TEM image, showing spherical shape of SeNPs.

A nanostructured surface was created on commercial titanium mesh for cranioplasty, by adhesion of selenium nanoparticles (SeNPs) in situ, in a hydrothermal reaction, revealing that selenium nanoparticles adherence on titanium mesh surface had the best result in the case of starch-derived SeNPs, as demonstrated by SEM/EDX analysis: a uniform layer, nanostructured, preserving the spherical shape of selenium particles, without forming aggregates or clusters on the surface (Fig. 3 a,b). Moreover, fibroblast cells (HFL-1 cell line) on the surface of Ti/SeNPs specimen, after 7 hours incubation, showed typical elongated, fusiform shape and fibrous morphology, with good attachment to the surface and formation of filopodia (Fig. 3c). The proposed improvement of titanium surfaces for cranioplasty may offer important benefits in terms of osteointegration, without using additional screws for fixation and closure procedure. By tailoring the surface of titanium implants, our results may contribute to the general efforts dedicated to continuous improvement in the field on nano-bio-materials by opening new possibilities for long-time development and strategies in nanomedicine.

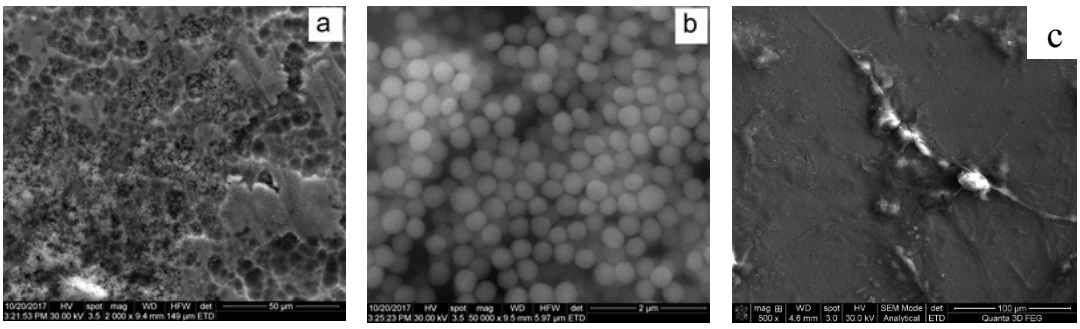

Figure 3: Surface morphology of Ti mesh (SEM), with different details and magnifications revealing selenium nanoparticles adhesion after hydrothermal reaction using starch as reducing agent (a,b), and fibroblasts attachment on the surface (c),

Conclusions. We have developed novel strategies for production of SeNps with different applications in food technology and regenerative medicine (nanostructured surface on Ti cranial implant). Nanoselenium-enriched probiotics may have an important role against toxicity with heavy metals, as they are able to remove Cd from the culture medium of *L. casei* and *L. fermentum* strains. On the other hand, nanostructured surfaces based on selenium nanoparticles, created using Ti mesh for cranioplasty as a substrate, demonstrated a good biocompatibility and accelerated bioactivity. So, important benefits can be achieved in terms of osteointegration, without using additional screws for fixation and closure surgical procedure.

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New ways to fight bacteria: towards the application of therapeutic ion release and polyester based biomaterials for antibiotic-free antibacterial devices

L. Gritsch[a,b], *C. Lovell*[b], *V. Mouriño*[c], *W.H. Goldmann*[d] *and A.R. Boccaccini*[a]

[a] Institute of Biomaterials, University of Erlangen-Nuremberg, Erlangen, Germany
[b] Lucideon Ltd., Stoke-on-Trent, United Kingdom
[c] Department of Pharmaceutical Technology, University of Buenos Aires, Argentina
[d] Department of Biophysics, University of Erlangen-Nuremberg, Erlangen, Germany
**e-mail:* lukas.*gritsch@fau.de*

Keywords: Antibacterial Resistance, Therapeutic ions, Chitosan, Coating, Composites

Introduction

Recent reports on the growth of antibacterial resistance gained worldwide resonance, as more and more authorities start recognizing superbugs as a major problem for the future of healthcare [1]. Antimicrobial drugs are progressively losing efficacy due to excessive use or misuse and the risk of regression to pre-antibiotic death-by-infection rates seems a close reality [2]. Together with optimal functional and biological properties, novel medical devices and scaffolds should also provide an inhibitory effect against bacteria, preferably avoiding the use of conventional antibiotics.

Therapeutic ions are a family of promising antibacterial agents that have already been incorporated both in polymers and ceramics for the development of new advanced biomaterials [3]. In particular, chitosan is known to be an ideal ion-carrying polymer thanks to its chelating ability. We investigated and demonstrated the complexation of chitosan with several biologically relevant ions, such as zinc, calcium and copper. Among these, we focused on copper. Copper acts as a quick inhibitor of bacterial growth and could be used to modulate angiogenesis in parallel. We incorporated it in chitosan and developed a novel antibacterial copper(II)-chitosan (C2C) biomaterial, which showed excellent antibacterial effect and cytocompatibility [4].

The application of antibacterial C2C as an active agent to be combined with medical grade polyesters was then explored in two prospectively relevant cases: (i) we prepared and characterized tissue engineering scaffolds using the freeze-drying and electrospinning techniques; also (ii) we developed a dip-coating procedure to cover resorbable sutures with a C2C layer as a possible alternative to the current main technology, based on triclosan, a probably toxic and resistant-triggering agent.

Experimental

Copper(II)-chitosan was prepared by *in-situ* precipitation. Copper ions are complexed with chitosan through chelation [4]. The morphology and homogeneity of the samples was investigated by optical and scanning electron microscopy. Energy dispersive X-ray and Fourier transform infrared spectroscopies verified the presence and complexation of copper, which introduces variations in characteristic peaks of the spectra of chitosan. Wettability tests (contact angle) were performed and demonstrated that the hydrophobicity of chitosan was maintained after the complexation. Copper release was then quantified by capillary electrophoresis following a previously published protocol [5]. Cell viability with mouse embryonic fibroblasts (MEFs) and direct contact killing assays with Gram-positive *Staphylococcus carnosus* and Gram-negative *Escherichia coli* were also performed. The combined analysis of cell and bacterial behavior, together with ion release, allowed the identification of an optimal threshold concentration of circa 10 to 30 ppm. When the release sits within this range our material shows outstanding antibacterial inhibition up to 85% compared to a pure chitosan control without significantly affecting the viability of fibroblasts (~70-90% of positive control). The capillary electrophoresis confirmed that the effects on cells in this study are consistent with the reported antimicrobial and cytotoxic concentrations of copper.

C2C was successfully combined with medical grade polyesters both in blends of benign solvents and composites, demonstrating its high versatility. Our proof-of-concept studies showed that chitosan and polyesters together could be useful in the design of composite films and coatings and electrospun tissue engineering scaffolds.

Subsequently, composite freeze-dried scaffolds with interconnected pores made of bioactive inorganic particles dispersed in a polymeric matrix were fabricated. A previously reported protocol [6] was adapted: copper(II)-chitosan and hydroxyapatite suspensions were frozen with liquid nitrogen and then lyophilized. Scanning electron microscopy and Barrett-Joyner-Halenda (BJH) analysis were performed to evaluate the morphology of the scaffolds and the pore size and distribution, respectively. Bioactivity, in terms of calcium phosphate deposition after immersion in simulated body fluid, was assessed and the scaffolds were proven able to trigger the deposition of amorphous calcium phosphates, a precursor of hydroxyapatite. Cell adhesion and proliferation on the scaffolds were also investigated: no significant variation in cell viability and cell morphology was observed, proving that C2C composite scaffolds are cytocompatible.

In parallel, the mechanical compliance of copper(II)-chitosan was optimized with plasticizing additives and the resulting material was used to coat polyester (polyglactin 910) surgical sutures by dip-coating. A design of experiment (DOE) approach was used to identify the best combination of solution concentration, type of solvent, dipping and retrieving speed, number of dips and post-processing to optimize the morphological and mechanical properties. The competitiveness of the construct was

then evaluated against a selected benchmark.

Concluding Remarks

The delivery of copper as well as of other therapeutic ions is a promising strategy in the development of novel approaches to simultaneously tackle bacteria and their resistance. Ion-chelating chitosan is an effective and versatile material thanks to its high availability and easy processing with FDA approved, medical grade polyesters. As our proof of concept applicative studies as tissue engineering scaffolds and surgical suture coating demonstrated, we believe that with further investigation and development, copper(II)-chitosan will soon find application in the medical field.

Acknowledgments

This work has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie (Grant Agreement No. 643050) and from the German Science Foundation (DFG). We thank Mr. L. Bullock, Ms. A. Grünewald, Ms. A Mainka and Mr M. Salt for their technical support.

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Antimicrobial hernia-repair meshes fabricated via laser-based methods

C.D. Alina, F.A.Gramaa, R. Papagheorghea, A. Palla-Papavlub, S. Brajnicovb, I.A. Paunb, M. Dinescu*b

[a] General Surgery Department, Coltea Hospital, Bucharest, Romania
[b] National Institute for Lasers, Plasma, and Radiation Physics, Lasers Department, Atomistilor 409, ZIP 077125, Magurele, Romania
*e-mail: dinescum@nipne.ro

Keywords: hernia-repair meshes, laser evaporation, MAPLE, thin films, gentamicin, vancomycin

Introduction.

Current biomaterial processing techniques aiming at the development of a new generation of multifunctional implants, i.e. easier to integrate into the body, with a minimum rejection reaction at the mesh-host tissue interface, thus reducing secondary fibrosis and allowing mesh integration without complications are of paramount importance. The surface properties of the material directly influence the type, concentration, and conformation of proteins adsorbed, being thus critical determinants for host tissue reaction, i.e. modulating the inflammatory cell adherence. Although it has been shown that proteins are adsorbed differently on different surfaces, resulting in various inflammatory responses, a detailed analysis of the adsorption of blood proteins to various biomaterials used in herniology has not yet been carried out [2]. However, approaches have been reported aiming to prepare materials surfaces with improved biocompatibility and decreased infections risks. Enrichment of meshes with various cells (cell-coated mesh) was carried out in order to make them "invisible" to the immune system, and thus favoring biocompatibility [3]. Following the incorporation of antibiotics, the local infection rate decreased, while the incorporation of cytokines or growth factors aimed at modifying scar tissue [4,5]. Meshes with antibiotic would ensure an increased level of antibiotic locally (superior to that provided by intravenous administration), particularly in open surgery procedures involving a larger area of contact with the outside world and hence an increased risk of infection [6]. This work aims at the elaboration of antimicrobial hernia-repair meshes by matrix assisted pulsed laser evaporation (MAPLE). In MAPLE the material to be deposited (polymer, protein, etc.) is suspended (1-3 %wt) in a solvent. The mixture solvent/material is then frozen and subjected to laser irradiation in a vacuum chamber. The laser radiation is absorbed by the solvent, (which is preponderant in the mixture) and the solvent evaporates. The material concentration in the solvent should be reduced due to the fact that any interaction with the laser beam should be avoided, as the laser can affect the chemical and physical integrity. The solvent vapors mechanically transport the material molecules to a substrate which is placed parallel with the frozen target and at a distance of several cm. This technique has many advantages in respect to other techniques: the used amount of material to be deposited is very small, (in respect to spin coating for example), which is important when expensive products are used; multilayers can be deposited without taking into account de compatibility of the solvent for the subsequent layer with the already deposited material, as they do not interact. "Targets" from material blends can be prepared by an appropriate choice of a common solvent, allowing thus the growth of polymer blends and polymer blends with different drugs. In addition, the deposition rate is small, which allows an accurate control of the layer thickness. In this work, primary hernia-repair meshes were chosen to be treated by MAPLE with polymers and polymer blends and further investigated in terms of their mechanical and biological properties, i.e. elasticity, low weight/light mesh, biologically inert, non-toxic, non-immunogenic, minimal surface area and stability, requirements for their usage in hernia surgery. Finally, the mesh samples were tested for stability and their antimicrobial properties were investigated. It has been found that the loaded meshes have demonstrated antimicrobial properties and further studies are being carried out, where the MAPLE loaded meshes are tested in clinical experiments.

Results and Discussion The aim of this work is to elaborate antimicrobial hernia-repair meshes by MAPLE, following a twofold strategy: i.e. to fabricate a mesh model preserving the mechanical and topographical properties required in hernia repair, to which antimicrobial properties were added. In order to achieve this goal, first, the primary meshes were selected, i.e. samples of meshes which are used in clinical practice and are made out of polypropylene. Second, different polymer: drug blends have been chosen to be deposited as thin layers onto the primary meshes, i.e. poly (ethylene oxide) (PEO) : single walled carbon nanotubes (SWCNT) which contain different concentrations of vancomycin (Van) or gentamicin (Gen). The laser-based method applied to deposit the thin layers is in agreement with the medical recommendations [1] to preserve the mechanical (elasticity, low weight/light mesh, biologically inert, non-toxic, non-immunogenic, minimal surface area and stability) requirements for use in hernia surgery. Finally, the hernia-repair mesh samples were tested for stability and antimicrobial properties. As an example, a scanning electron microscopy image of a primary hernia-repair mesh deposited by MAPLE with a thin layer of PEO:SWCNT: Gen is shown in Figure 1.

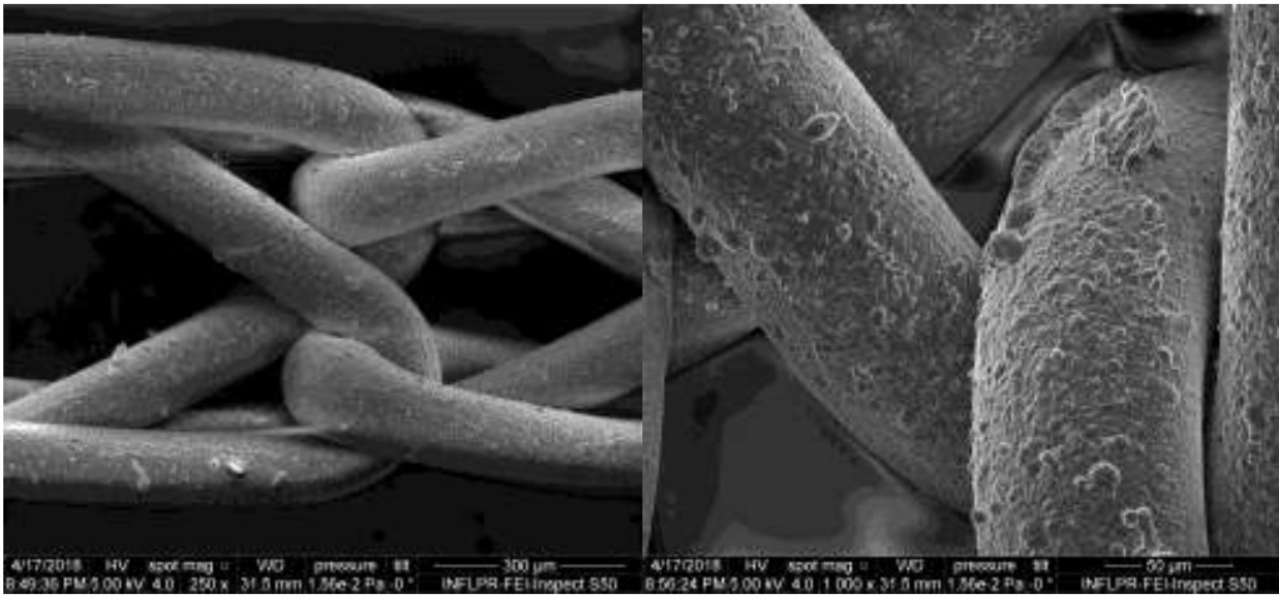

Figure 1: Primary hernia-repair meshes deposited with a thin layer of PEO:CNT:Gen

Conclusions and/or Outlook

Thin films of poly (ethylene oxide) have been deposited on polypropylene primary hernia repair meshes. The FTIR spectra indicate that following MAPLE deposition it is possible to obtain polymer thin films with unmodified chemical structure. The MAPLE deposited layers have a good adherence to the PP meshes. Furthermore, the ability to control the morphological and structural properties of the polymer: drug layers which cover the primary hernia repair meshes proves the fact that MAPLE is a powerful technique for the fabrication of systems with antimicrobial properties.

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Innovative micro- and nanostructured coatings for dental and bone implants

Angela De Bonis[b], Iulian Antoniac[c], Yurii P. Sharkeev[d], Mariangela Curcio[b], Marco Ortenzi[a], Roberto Teghil[b] and Julietta V. Rau[a]*

[a] Istituto di Struttura della Materia, Consiglio Nazionale delle Ricerche (ISM-CNR), Via del Fosso del Cavaliere, 100-00133 Rome, Italy
[b] Dipartimento di Scienze, Università della Basilicata, Viale dell'Ateneo Lucano, 14 -85100, Potenza, Italy
[c] University Politehnica of Bucharest, Splaiul Independentei 313, sector 6, 77206, Bucharest, Romania
[d] Institute of Strength Physics and Materials Science SB RAS, Russia, Tomsk
**e-mail: giulietta.rau@ism.cnr.it*

Keywords: Doped calcium phosphates, glass-ceramic materials, Titanium, Pulsed Laser Deposition

Titanium (Ti) and its alloys are extensively used nowadays for dental and orthopaedic implants due to their outstanding mechanical characteristics and acceptable biocompatibility. However, the properties of metal implant/native hard tissue interface can be substantially improved, if titanium is coated by bioactive materials, with the scope to optimise the long-term characteristics of implant, to elicit a required cellular response, and, as a final goal, to provide better performance and increased functionality of the implant.

As future implants, Magnesium (Mg) biodegradable alloys are very promising biocompatible materials for biodegradable biomedical implants, such as, for example, orthopaedic fracture fixation pins or screws. They decompose in the conditions of human body, releasing the Mg^{2+} ion, which has a functional role in many physiological processes. Moreover, Mg alloys have outstanding mechanical properties, similar to that of the natural bone tissue. However, the main problem in using Mg alloys for biomedical implants is their fast degradation in human body conditions, if compared to the native bone tissue growth. This drawback of Mg alloys can be improved by applying protective coatings of ceramic and composite materials, as prospective solution to control the *in vitro* degradation.

Doped (substituted) calcium phosphates and glass-ceramics are perspective materials for coatings on Ti and Mg alloys [1-9]. They provide superficial affinity, functional interface and improved osseointegration. Substituted calcium phosphates can endow coatings with a broad range of particular functional properties, from antibacterial to the magnetic one [1,2,4,5,8,9]. Whereas the focus point of bioactive glass-ceramic materials is their ability to continuously exchange ions with physiological liquids and to release appropriate active trace elements to stimulate cellular response aimed to activate genes responsible for osteogenesis and tissue regeneration [3,6].

In this work, we report the results on deposition of substituted hydroxyapatite (HA) implant coatings, such as Carbonate-HA [1], Fluorine (F)-HA [2], Silicate-HA [4], Iron (Fe)-HA [5], and Zink (Zn)-HA [8]. Furthermore, the results regarding several glass-ceramic composition coatings will be reported [3,6]. The bulk glass-ceramics materials for coating deposition were prepared by sol-gel method.

All the coatings were deposited using Pulsed Laser Deposition technique (PLD). It presents several advantages, compared to other deposition techniques, such as the congruent transfer of the target composition to the coating, the possibility to vary film's thickness, degree of crystallinity, adherence, surface morphology and topography, varying the experimental parameters, such as deposition temperature, time, laser pulse length and wavelength. It has been widely used to deposit thin films of ceramic and glass biomaterials onto biocompatible and biodegradable substrates. Films deposited by nanosecond laser sources present micro- and nanostructured morphology favouring the interaction of surface with cells. The properties of coatings were investigated by a number of physico-chemical techniques, such as X-Ray diffraction, FTIR spectroscopy, Raman spectroscopy, atomic force microscopy, scanning electron microscopy (SEM-EDS), and Vickers hardness. In vitro bioactivity and cell tests for the deposited coatings were performed.

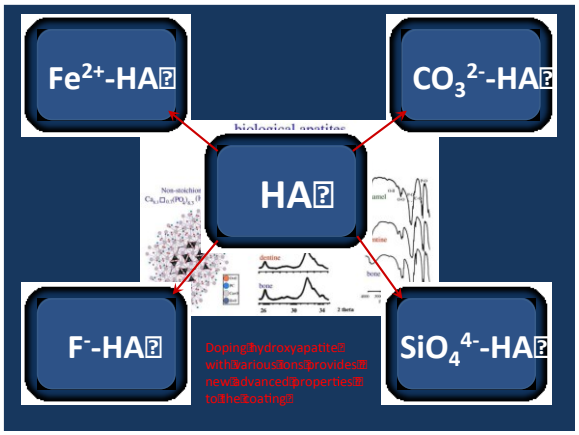

Figure 1: Substituted hydroxyapatite coatings deposited on titanium

The results of the present study suggest that novel nanostructured coatings of substituted calcium phosphates and glass-ceramics can be particularly relevant for dental and orthopaedic implants as new strategies in tissue regeneration and replacement, ensuring necessary structural, chemical, morphological and mechanical characteristics, and improving implant's osseointegration. Following the requirements of the modern medical technology, the novel research strategies in biomaterials field are nowadays directed towards biomaterials possessing characteristics suitable for drug delivery and for controlled release of active principles (especially against infections).

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Antibacterial Nanostructured Silver Coatings Deposited by Pulsed Electron Deposition

G.Graziani[a], S. Santi[b], A. Gambardella[a], M. Berni[a], A. Maso[b], E. Storni[b], M.Bianchi [b]*

[a] Rizzoli Orthopaedic Institute (IOR), NanoBiotechnology Laboratory (NaBi), Bologna, Italy
[b] National Research Council (CNR), Institute for Molecular Genetics, Bologna, Italy
[c] Rizzoli Orthopaedic Institute (IOR), Quality Control in GMP, Bologna, Italy
**e-mail: gabriela.graziani2@unibo.it*

Keywords: Infection, Inorganic antibacterial coatings, Bone Implants, Plasma assisted deposition, Thin Films

Introduction

Infections are among the main issues connected to implantation of medical devices, as they have significant incidence and can lead to severe complications (~4 million cases occur per year in Europe, resulting in a burden of ~37.000 deaths and a direct cost of ~7 billion Euros [1]). Upon bacterial colonization of the implant surface, biofilm formation may occur, which protects bacteria from the host immune system and from antibacterial compounds, while allowing colonization of other sites [2]. Biofilm formation makes infection hard to eradicate and frequently leads to the need for implant removal and revision surgeries [2]. Because eradication of infection is challenging, their prevention must be preferred over treatment. However, systemic antibiotic therapy (gold standard) presents issues connected to high systemic toxicity and development of resistant bacterial strains [2]. Hence, coatings capable of locally delivering the antibacterial compound, possibly inorganic, so as to prevent bacterial resistance, are desired [3].

Antibacterial silver coatings have been proposed and widely investigated to this aim, as silver is known to be active against several bacterial strains [4]. Silver coated prostheses are nowadays in the clinical practice and a variety of procedures have been proposed so far to deposit silver onto different devices (e.g. atomic layer deposition, photochemical deposition, electrodeposition, silver plasma immersion ion implantation, pulsed laser deposition etc.), also depending on their morphology and material [2,5]. The first methods consisted essentially in the immersion of the devices in liquid precursors. Despite this approach being currently still in use, novel procedures tend to focus on obtaining a higher adhesion and a better control over film thickness and silver release, so as to obtain suitable efficacy while preventing cytotoxicity.

Here, the deposition of nanostructured silver coatings is proposed by a novel modification of Pulsed Electron Deposition, namely Ionized Jet Deposition (IJD), which allows deposition on a variety of substrates (including heat sensitive ones) and to achieve highly adhesive nanostructured coatings, having sub-micrometric thickness. Low thickness and nanostructuration are expected to guarantee a fine control over film dissolution, and hence over silver release duration and extent. Deposition of silver coatings by IJD has been proposed for the first time by the Authors, for application on catheters, demonstrating the feasibility of the approach [6]. An extremely low toxicity has been found towards host cells.

Here, silver coatings are proposed for metallic implants and characterized in terms of composition (grazing incidence XRD), surface morphology (AFM, STM) and ion release (ICP). The coatings are deposited on TI6Al4V disks, directly from metallic silver targets (**Figure 1**). Deposition on silicon wafers and on glass microscope samples holders has been used for STM and confocal microscopy, respectively. Then, their antibacterial efficacy is validated *in vitro*, against relevant bacterial strains (gram+ *S. Aureus* and gram- *E. Coli*) by live/dead kit and confocal microscopy. For *S.Aureus,* super resolution imaging in the Structured Illumination Microscopy (SIM) setup was used to detect possible damage to the bacterial wall.

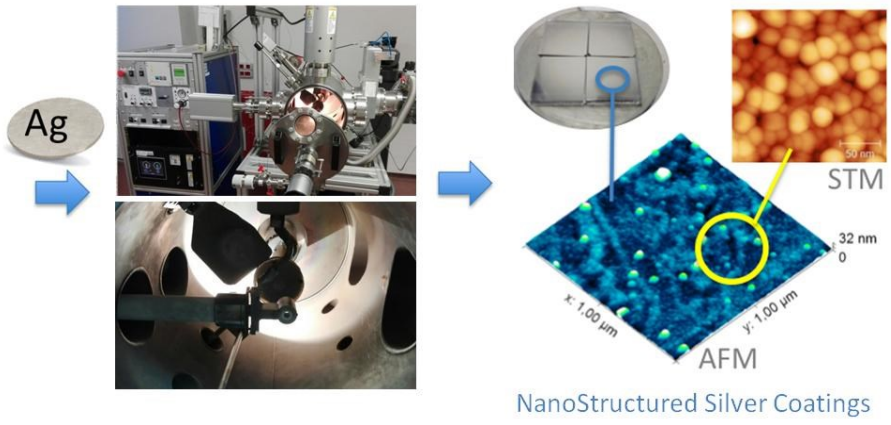

Figure 1: Deposition of nanostructured films and films surface morphology. Scale bar in STM image is 50 nm.

Results and Discussion

The coatings are composed by globular aggregates of 10 nm up to 40 nm, conferring them a nanostructured surface texture (**Figure 1**).

Interestingly, thanks to nanostructuration and because the coatings have submicrometric thickness, it is possible not to alter the micro- and macro-scale features of titanium implants, that are developed by the manufacturers to maximize primary stability (**Figure 2a**). To maintain these features, while guaranteeing a complete coverage of the substrate surface, a film thickness of 150 nm was selected. Film thickness is a key parameter, as it also influences nanostructuration of the surface and, hence, ion release and antibacterial efficacy. In terms of composition, the coatings are essentially composed by metallic silver, while no silver oxide peaks are detected by GI-XRD (**Figure 2b**).

The coatings exhibit high efficacy against *S.Aureus* and *E.Coli*, as can be seen in **Figure 2c**. Excellent efficacy was found on *E.Coli*, as essentially no alive bacteria were spotted on silver coated specimens. On *S. Aureus*, an increase in the dead/live bacteria from 23.7±8.6 % to 8.6±3.6 % was found. No significant damage to the bacterial wall was detected by SIM.

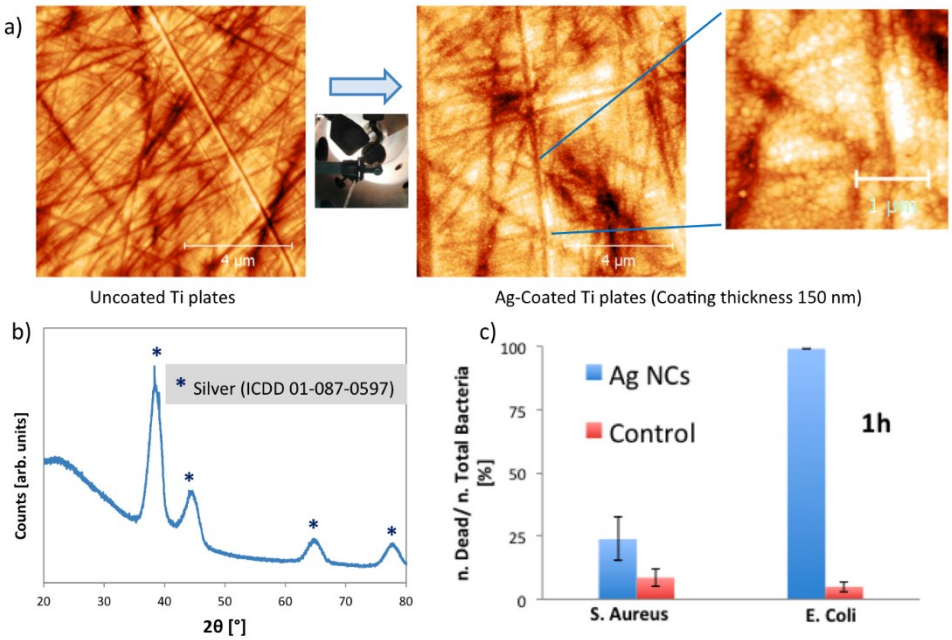

Figure 2: a) Surface morphology of uncoated and coated TI6Al4V disks, as determined by AFM; b) coatings composition by GI-XRD; c) Coatings efficacy on S.Aureus and E.Coli at 1 hour, expressed as the ratio between the number of dead and alive bacteria and as average of at least 8 measuring areas.

Conclusions /Outlook

Antibacterial, nanostructured silver coatings were successfully deposited by IJD. The coatings exhibit submicrometric thickness and nanorough surface morphology, as they are composed of nanosized silver aggregates. Thanks to these features, they are capable of preserving the micro- and macro- scale morphology of the implants. In addition, a perfect transfer of composition from the deposition target to the coating is achieved. Finally, remarkable efficacy is found against both gram+ *S. Aureus* and gram- *E.Coli*, indicating that the developed coatings are promising for antibacterial applications.

Further in vitro tests are in progress to evaluate efficacy on *S. Aureus* and *E. Coli* at different experimental times, including live microscopy. Finally, deposition of the coatings on substrates of complex shape, including highly porous metallic scaffolds, are in progress, to optimize clinical applicability.

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Antibacterial and osteotropic calcium phosphate coatings on metallic implants

M.B. Sedelnikova[a], E.G. Komarova[a], Yu.P. Sharkeev[a, b] T.V. Tolkacheva[a], V.V. Sheikin[c]*

[a] Institute of Strength Physics and Materials Science SB RAS, Russia, Tomsk
[b] National Research Tomsk Polytechnic University, Russia, Tomsk
[c] Siberian State Medical University, Russia, Tomsk
**e-mail: smasha5@yandex.ru*

Keywords: micro-arc oxidation, biocoating, microelement, calcium phosphate, titanium.

Introduction

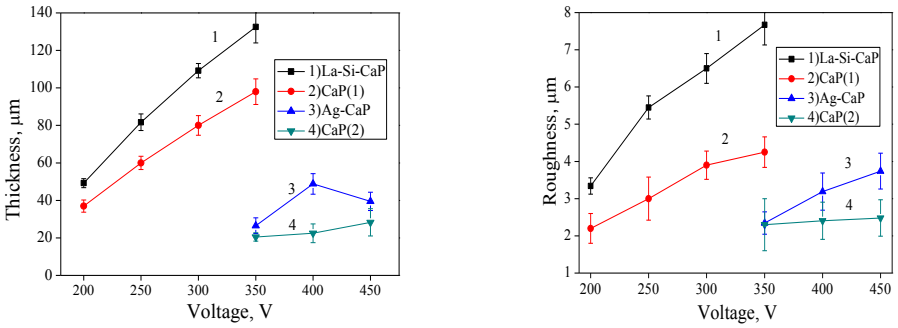
Promising tendency of the modern biomedical materials science is development of the biocomposites from bioinert metals or alloys with bioactive calcium phosphate (CaP) coating. Generally, hydroxyapatite (HA) coating is applied for medical implants from titanium and its alloy via plasma spraying technique to improve the implant bonding with bone tissue [1]. Introduction of the lanthanum (La), silicon (Si) or silver (Ag) microelement into the coatings provides the antibacterial and osteotropic properties. Ag ions and nanoparticles are extensively used for medical applications due to their well-known antibacterial action [1,2]. La restrains the Ca^{2+} pump of the human red cell and promotes the formation of osteoclast-like cells as was shown by authors [3]. Si, which is essential trace element in biological processes, stimulates intercellular responses and plays an important role in the bone tissue formation and collagen mineralization [4]. It is known, that hydrolysis and repolymerization of the Si-containing substance leads to the Si-OH silane bonds formation. These bonds stimulate sequential transformation of calcium phosphates as follows: octacalcium phosphate - amorphous CaP - crystalline HA.

The micro-arc oxidation (MAO) process combines the electrochemical oxidation and the high-voltage spark treatment in electrolyte, which also contains modifying elements in the form of dissolved salts to be incorporated into the resulting coatings. The addition of HA, tricalcium phosphate, silica or other bioactive powders may enrich the coatings [5].

Results and Discussion

In this paper, the results of the comparative investigations of the morphology, structure and physicochemical properties of CaP coatings deposited by the MAO method on titanium substrate were performed. Four types of the coatings such as CaP coatings without microelements (CaP(1) and CaP(2)), La-Si-containing CaP (La-Si-CaP) coating and Ag-containing CaP (Ag-CaP) coating were produced. All coatings were formed in the anodic potentiostatic regime for 5-10 min under the applied voltages of 200-450 V. To produce the first type of the coating CaP(1) the electrolyte No. 1 containing the 30% aqueous solution of H_3PO4, $CaCO_3$, and stoichiometric HA nano-powder was used. To synthesize the second type coating CaP(2) the electrolyte No. 2 containing Na_2HPO_4, NaOH and β-$Ca_3(PO_4)_2$ powder were used. The La-Si-CaP coatings were deposited at the electrolyte No. 1 incorporating the La-Si-substituted HA ($Ca_{9.5}La_{0.5}(PO_4)_{5.5}(SiO_4)_{0.5}(OH)_2$) instead the stoichiometric HA. To synthesize the Ag-CaP coatings the $AgNO_3$ was added into the electrolyte No. 2.

The influence of electrolyte composition and MAO voltage on morphology, thickness and roughness of the coatings was revealed. The thickness and roughness of CaP(1) and La-Si-CaP coatings increase intensively from 30 to 140 μm (Figure 1a, curves 1, 2) and from 2 to 8 μm (Figure 1b, curves 1, 2), respectively, when the applied voltage increases from 200 to 300 V.



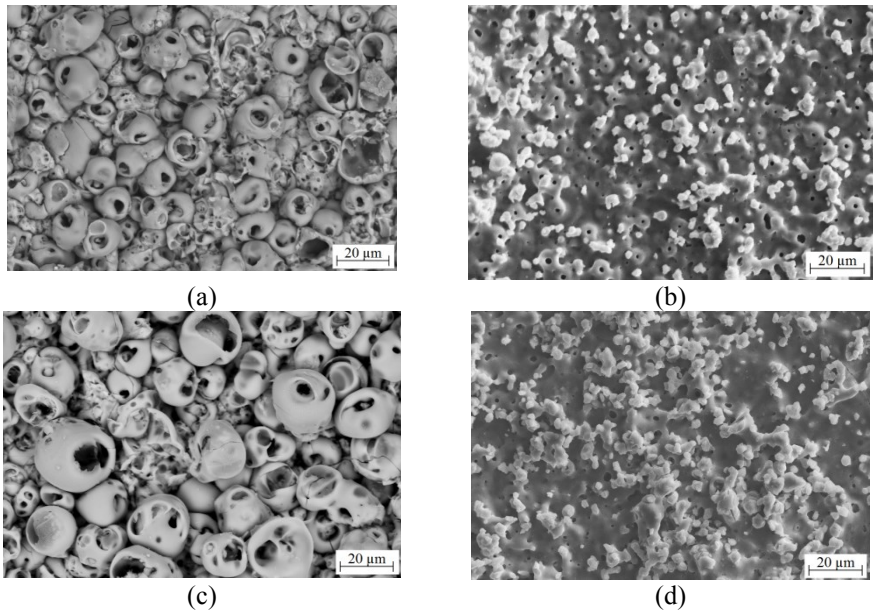
(a) (b)

Figure 1. Graphs of the coating thickness (a) and roughness *Ra* (b) against the MAO voltage for the coatings deposited under the different applied voltages

The thickness and roughness of the CaP(2) and Ag-CaP coatings increase from 20 to 50 μm (Figure 1a, curves 3, 4) and from 1.5 to 4.0 μm (Figure 1b, curves 3,4), respectively, with increasing of the MAO voltage from 350 to 450 V. It should be noted that the value of thickness and roughness for CaP(1) and CaP(2) coatings without microelements are lower than that for La-Si-CaP and Ag-CaP coatings. It can be assumed, that microelements participate in the plasma-chemical reactions during the MAO process and intensify them.

Figure 2 demonstrates SEM micrographs of the coatings deposited under MAO voltages of 200 and 350 V. The surface

morphology of the coatings formed in the identical electrolytes is similar. Spherical structural elements (spheres) with pores are formed on the CaP(1) and La-Si-CaP coating surface under the oxidation voltage of 200 V (Fig. 2 a,c). Absolutely different surface morphology is characterized for the coatings CaP(2) and Ag-CaP coatings: a lot of isometric particles with the sizes of 2-10 μm and pores with the average sizes of 1.5-5.5 μm are observed (Fig. 2 b,d). The size and shape of these particles are identical to that of β-$Ca_3(PO_4)_2$ particles contained in the electrolyte No. 2.



(a) (b)

(c) (d)

Figure 2: SEM images of the surface of the CaP(1) (a), CaP(2) (b), La-Si-CaP (c) and Ag-CaP (d) coatings deposited under different MAO voltages, V: 200 (a,b); 350 (c,d)

Conclusions

The comparative investigation of the CaP, La-Si-CaP, and Ag-CaP coatings deposited by the MAO method on the titanium substrate showed that the electrolyte composition influences considerably on the morphology, structure and properties of the coatings. In addition, the applied voltage effects significantly on the coating thickness and roughness. Introduction of La, Si and Ag microelements into the coatings can provide the formation of their osteotropic and antibacterial properties.

The work has been financially supported by the Fundamental Research Program of the State Academies of Sciences for 2013-2020, direction of research III.23.

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Antibacterial and bioactive thin RF magnetron hydroxyapatite-based coating: microstructure and properties s

K.A. Prosolov[a,b], Yu.P. Sharkeev[a, b], M.B. Sedelnikova [a], O.A. Belyavskaya [a]*

[a] Institute of Strength Physics and Materials Science SB RAS, Russia, Tomsk
[b] National Research Tomsk Polytechnic University, Russia, Tomsk
**e-mail: konstprosolov@gmail.com*

Keywords: radiofrequency magnetron, biocoatings, sputtering, calcium phosphate, titanium.

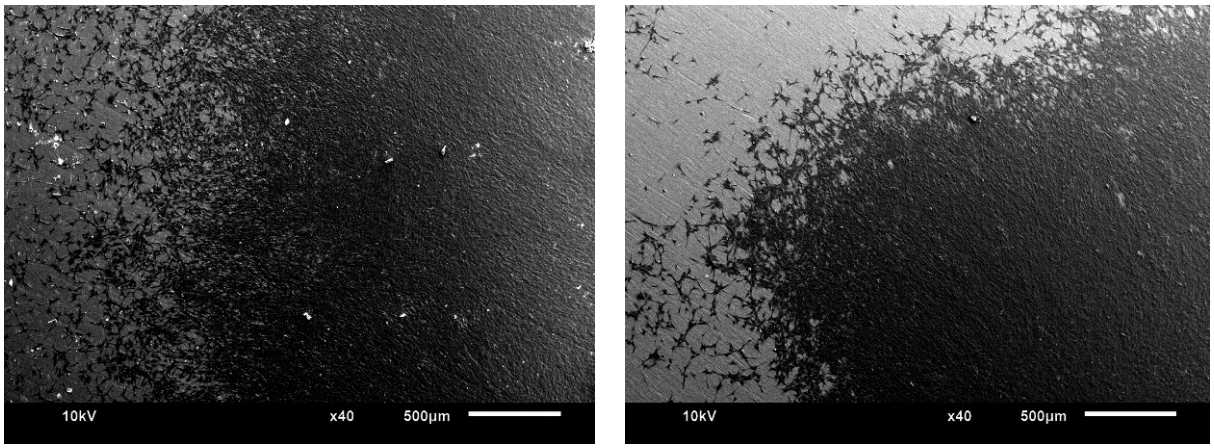
Introduction

The bone-implant interface is crucial for successful implant lifespan [1]. Calcium phosphate coatings are widely used in implantology because of the high biocompatibility and osteoconductive potential. However, the problem of infections related to unwanted bacteria spreading in the implantation site is still relevant for the healthcare [2]. It is mostly related to the nosocomial infection during a dental or endoprosthesis replacement. In some of the cases bacteria become antibiotic resistant or form a biofilm which is hard to treat and by that, a risk of failure of an implant is increased. Most researchers aim to functionalize hydroxyapatite coatings in order to increase their bioactivity. In order to overcome the problem of infection hydroxyapatite (HA) coating with antibacterial agents are suggested.

Zinc ions are well-known not only for its antibacterial properties but are known to play a crucial role in osteointegration processes. It was postulated that zinc-releasing coatings can serve as a support for cell culture and promote cell proliferation and osteointegration [3]. The radiofrequency (RF) magnetron sputtering method allows depositing not only amorphous but crystalline coatings. This fact is extremely relevant for the overall implant performance because the coatings' dissolution rate is mostly influenced by the crystallinity state. Therefore, Zn-substituted hydroxyapatite (HA-Zn) coatings in amorphous [4] and crystalline states which are represented by the equiaxed grain [5] and columnar structures are deposited on Ti substrates. Control over the coatings crystalline state will allow to manipulate the coatings biodegradation and can be used for different clinical cases.

Results and Discussion

Coatings' crystallinity state and cytotoxicity are described for thin RF-magnetron deposited hydroxyapatite films on Ti substrate. The vacuum installation with a planar magnetron operated at 13.56 MHz (ISPMS of SB RAS, Russia) was used. The magnetron sputtering targets which are made of the powder of HA-Zn and pure HA without substitution were used. The coatings were deposited by magnetron sputtering at RF-power value of 250 W in an Ar atmosphere. The deposition time was 3 h, and the target-to-substrate distance was 80 mm. During the coating deposition, the working pressure in the vacuum chamber was 0.7 Pa. In this case, coatings were represented by amorphous calcium phosphate for both HA and HA-Zn targets. Cytotoxicity tests for this coatings were performed using the mouse myoblasts C2C12 cell line. According to MTT test deposited coatings are proved to be not toxic. In **Figure 1** SEM images of HA and HA-Zn coatings with cells after 48 hours in culture are represented.



(a) (b)

Figure 1: SEM images of fixed C2C12 cells on the HA (a) and HA-Zn (b) coatings after 48 hours in culture.

Tests showed good cells spreading without signs of apoptosis. However, the proliferation of the cells was decreased in case of HA-Zn with the comparison to the same tests on the pure HA coatings. In order to manipulate coatings dissolution rate and possibly cell response coatings were deposited under elevated substrate temperature. We were able to produce crystalline coatings when the substrate temperature was set to 400°C during the deposition (**Figure 2**).

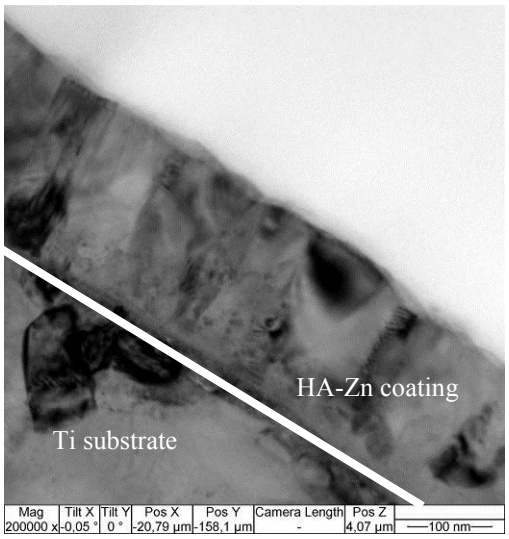


Figure 2: Cross-section TEM image of texturized HA-Zn coating represented by the columnar structure on Ti substrate

From the TEM image, polycrystalline nature of the coating is revealed. The texturization of the HA crystals with a preferential orientation perpendicular to the substrate is well seen.

Conclusions

Thin HA-Zn coatings were deposited on Ti substrate by the RF magnetron sputtering in different crystalline states. The absence of toxic effect of C2C12 cell line to the HA and HA-Zn coatings was shown. Additionally, we show that it is possible to deposit both amorphous and crystalline HA-Zn coatings which could be perspective for various medical applications.

The work has been financially supported by the Fundamental Research Program of the State Academies of Sciences for 2013-2020, the direction of research III.23.

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Next generation titanium implants: Vitronectin-derived retro-inverted peptides enhance h-osteoblast proliferation and gene-expression

A. Zamuner[a], *P. Brun*[b], *L. Stona*[a], *M. Dettin** [a]

[a] Department of Industrial Engineering, University of Padova, Padova, Italy
[b] Department of Molecular Medicine, University of Padova, Padova, Italy
**e-mail: monica.dettin@unipd.it*

Keywords: titanium, retro-inverted peptides, selective grafting, h-osteoblast, biomimetic.

Introduction

Nowadays titanium still represents the gold standard for dental and orthopaedic prostheses, though osseo-integration between the implant surface and the recipient's tissues could be further increased creating biomimetic surfaces. In previous works, we demonstrated that the (351-359) sequence of human Vitronectin (named HVP) enhances human osteoblasts adhesion *in vitro* [1] through an osteoblast-specific mechanism [2-3], and it is osteo-inductive and osteo-conductive *in vivo* [4]. However, this nonapeptide undergoes enzymatic degradation in serum solutions [5]. In this study, a new protease-resistant retro-inverted analogue of the HVP dimer (D-2HVP-F), a retro-inverted HVP with a spacer equal to its length (DHVP-G(7)3F), and a modified DHVP-G(7)3F sequence with all Arginine residues replaced with Alanine (named DHVP-G(7)3F/Ala and used as control peptide) were synthesized to functionalize silanized titanium disks with a chemistry different than our previously proposed one, which provided for the functionalization of the silanized titanium with a side-chain protected peptide, subsequently deprotected with trifluoroacetic acid [6]. In the present case, these peptides were synthesized with a C-terminal aldehyde group and conjugated to the surface amine groups in the presence of sodium cyanoborohydride. The strengths points of the chemistry used consist in the possibility to perform the reaction in aqueous environment and to obtain surfaces with a different degree of functionalization. The biological characterization of functionalised titanium surfaces was carried out *in vitro*. Adhesion and proliferation of human osteoblasts were evaluated at 2 and 24 hours; calcium levels were quantified at 7 days and gene expression of bone sialoprotein, osteopontin and vitronectin was measured at 48 hours from the seeding.

Results and Discussion

Titanium surfaces were preliminarly functionalised in water using three different D-2HVP-F concentrations: 1nM, 1µM and 1mM. In order to understand which concentration was proper to promote h-osteoblast viability, a MTT assay was carried out at 2 hours from the seeding ($3x10^5$ h-osteoblast/pt) on each functionalised sample and silanized titanium. Titanium functionalised with 1µM D-2HVP-F solution showed a significant increase in h-osteoblast adhesion compared to the control.
The biological characterization of the surfaces functionalized with the different peptides using 1µM concentration showed that the retro-inverted dimer significantly increased both adhesion and proliferation of h-osteoblasts at 2 and 24 hours, compared to the control (**Figure 1 a** and **b**).

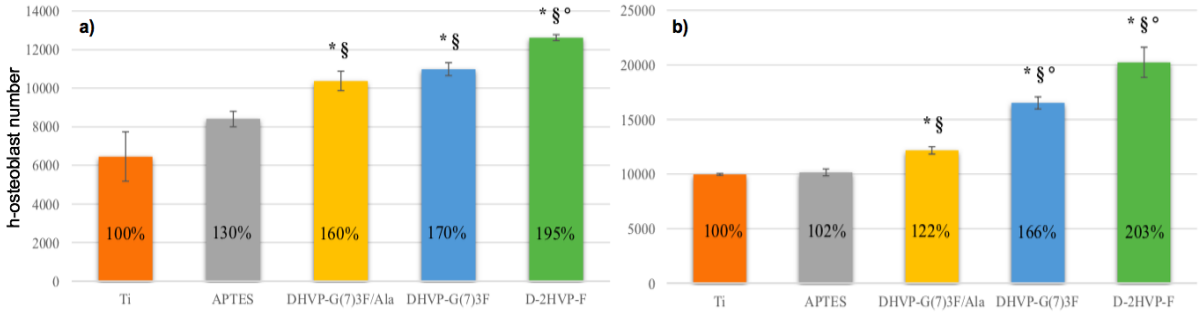

Figure 1: Histograms showing adhesion / proliferation results of MTT assays at 2h **a**) and 24h **b**) from the seeding. *=P<0.05 vs Ti, §=P<0.05 vs APTES, °=P<0.05 vs DHVP-G(7)3F/Ala.

H-osteoblast morphology was assessed at 24 hours from the seeding on glass coverslip functionalised using the same chemistry. As reported in **Figure 2**, cells seeded on DHVP-G(7)3F and D-2HVP-F show higher intensity of p-FAK-Alexa488 fluorescence, indicative of higher cellular interactions with the surface, and homogeneous adhesion regions that are necessary for the further development of focal adhesions with the substrate.

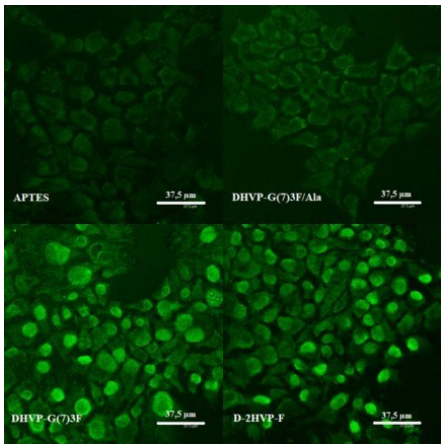

Figure 2: IHC Images of h-osteoblasts seeded on functionalised glass coverslips and marked for p-FAK-A488.

Furthermore, the selective functionalization with all peptides increased the calcium levels at 7 days (**Figure 3**) but the best result is obtained with D-2HVP-F. D-2HVP-F is the only sequence, between the tested peptides, able to promote the gene expression for bone sialoprotein, osteopontin and vitronectin.

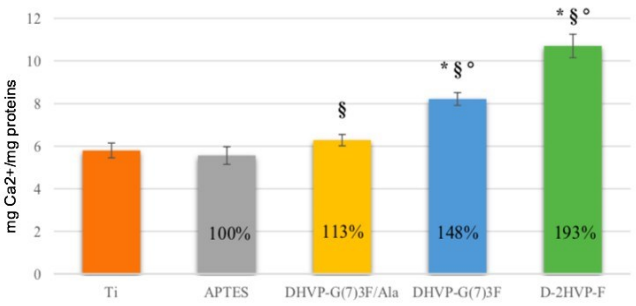

Figure 3: Histogram showing Calcium levels determined by o-CPC assay at 7 days from the seeding. *=P<0.05 vs Ti, §=P<0.05 vs APTES, °=P<0.05 vs DHVP-G(7)3F/Ala.

Conclusions and/or Outlook

In this study, we covalently and selectively functionalised Ti surfaces with proteolytic resistant adhesive peptide sequences from Vitronectin. This functionalization strategy is simple and allows to operate in aqueous solution and to obtain surfaces with different degree of functionalization. The biological characterization of the surfaces confirms that the retro-inverted dimer significantly increases the adhesion / proliferation of human osteoblasts both at 2 and 24 hours compared to the controls, stimulates cell spreading, increases the calcium levels at 7 days and promotes the gene expression for bone sialoprotein, osteopontin and vitronectin.

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Biocompatibility assessment of some experimental zirconia-based materials for dental prosthesis

Horia Manolea [a], *Nicoleta Cioateră* [b], *Laurentiu Mogoantă*[a], *Maria Magdalena Manolea**[a], *Mădălina Matei*[a], *Eduard Ciucă*[a], *Alex Sălan*[a]

[a] University of Medicine and Pharmacy Craiova, Romania
[b] INCESA, University of Craiova, Romania
**e-mail: manoleamaria@gmail.com*

Keywords: dental ceramics, yttria-stabilized tetragonal zirconia, histology

Introduction

Zirconia (ZrO2) is a ceramic material with adequate mechanical properties for manufacturing of medical devices. Zirconia stabilized with Y2O3 has the best properties for these applications. The mechanical properties of zirconia fixed partial dentures (FPDs) have proven to be superior to other ceramic/composite restorations [1]. Zirconia's biocompatibility has been studied in vivo, leading to the observation of no adverse response upon the insertion of ZrO2 samples into the bone or muscle. However, concern has been raised with regard to the low-temperature degradation of yttria-stabilized tetragonal zirconia polycrystalline for monolithic zirconia restorations [2]. Current state of the art shows the strong variability of zirconia sensitivity to in vivo degradation as a consequence of the strong influence of microstructure and process on low-temperature degradation . Zirconia-toughened alumina represents one trend followed by ceramic manufacturers to improve the resistance of zirconia-based ceramics to aging [3].
The aim of this study was to evaluate the biocompatibility of three experimental zirconia ceramics.

Materials and Methods

Three experimental zirconia ceramics (Zr0,88 Y0,12 O2; Zr0,85 Y0,12 Fe 0,03 O2; Zr0,85 Y0,12 Al 0,03 O2) were synthesized in the Laboratory of Physical-Chemical Characterisation and Materials Testing from INCESA Craiova.T en standard discs of 6 mm diameter and 1 mm thickness were prepared for each experimental zirconia ceramics.
The samples morphology was evidenced using a high resolution scanning electron microscope (Hitachi SU8010). The microscope was equipped with an EDXS detector from Oxford Instruments.
For this study we made three study groups, each of them consisting of five laboratory rats, a study group for each of the studied ceramics. The samples were subcutaneously implanted in the backs of male Wistar rats and submucosal in the oral cavity.
The animals were sacrificed after 6 weeks and the local tissular reaction was investigated first by a clinical examination and then samples and surrounding tissues were carefully excised and evaluated using an optical microscope. In the end the samples were prepared for a histological study, in order to obtain sections of 5 to 7 µm stained with hematoxylin and eosin and Masson trichrome colorations.

Results and Conclusions

The samples tend to be surrounded by fibrous connective tissue and usually there was no adverse reaction and only little evidence of any inflammatory response. In brief, the tested zirconia samples has been proved to be biocompatible, even the compositional changes may influence its soft tissue interactions. Their very good tolerance by the oral tissue, leading to the accumulation of collagen fibers in the area around the samples, make these materials promising candidates for their use in dental prosthetics.

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Porblem Solving in Endodontically Treated Teeth

Massimo Giovarriuscio

King's College London
giovarruscio@me.com

A percentage of endodontically treated teeth does not successfully respond to the therapy, developing an apical radiolucency after the intervention of the dentist. In presence of symptoms or periapical infection in an endodontically treated tooth with a iatrogenic damage (i.e. perforations, stripping, open apices, cracks), the dentist should think to the options he can give to the patient in order to solve the problem. Once assessed that the tooth has no vertical fractures and can be restored, the choice is between nonsurgical retreatment and endodontic surgery. Both options have vantage and disadvantage, and have precise indications. The clinician is supposed to know which is the best treatment, in order to give the patient the most suitable therapy. Non-surgical retreatment, in the last years, has broadened its field of employment and has remarkably increased its percentage of success. This is due to the new techniques and the novel equipment that help the dentist in solving complex clinical cases with multiple pathologies. The objective of retreatment is to eliminate the obstacles that make challenging the complete shaping, cleaning and filling of the root canal system. Thanks to ultrasonic tips and rotary dedicated instruments, emptying the root canal system is much easier, faster and safer than it was before. The cleaning action of the irrigating solution is extremely improved by he action of US-activated files, guaranteeing a deeper disinfection of the root canal system. The use of an operative microscope allows a better vision of the operative field, making possible to remove safely broken instruments, to go beyond ledges and to repair perforations or stripping of the root. The treatment of perforations has a better prognosis even thanks to the introduction of new biocompatible filling materials such as MTA or Bioceramics, characterized by the property of setting in a moist environment and sealing the endodontic space.

Aims
The aim of the lecture is to help the clinician in solving problems linked to the treatment of iatrogenic damages such as perforations, stripping, ledges and how to manage open apices teeth.

ObjectivesThe lecture will focus on the pre-operative analysis of the case and on the decisional process that brings to the choice of the best therapeutical option for the patient. Every technique will be described in its operative steps, focusing on advantage and disadvantage.

Novel composite systems based on natural compounds for periodontal tissue regeneration strategies

A.M. Stanciuc[a,b]*, A. Gaspar-Pintiliescu[a,b], A.M. Seciu, L. Moldovan [a,b], O. Craciunescu[a,b], O. Zarnescu[a]

[a] Faculty of Biology, University of Bucharest, Romania
[b] Department of Cellular and Molecular Biology, National Institute R&D for Biological Sciences, Bucharest, Romania
e-mail: annastanciuc@gmail.com

Keywords: biomaterials, collagen, cytotoxicity, periodontitis, cell carrier

Introduction

Periodontal diseases are associated with a large spectrum of inflammatory conditions that affect various tissues, e.g. gingival, alveolar bone, periodontal ligament or cementum, in severe cases leading to phonetic and aesthetic risks and up to tooth loss[1]. Progression of periodontitis from common gingivitis to severe advanced periodontitis is further contributing to systemic inflammation[2,3]. Tissue engineering approaches consist of natural or synthetic biomaterials used as scaffolds for controlled drug delivery or as cell carriers. This study reports a new strategy for the management of periodontal tissue regeneration by the development of novel composite systems based on natural compounds of the oral extracellular matrix, collagen (Col), chondroitin 4-sulfate (CS) and fibronectin (FN), which can serve as biocompatible carriers of bioactive factors and/or stem cells.

Results and Discussion

Col and CS were obtained by recycling bovine tissues from local slaughterhouse. Proteic-polysaccharidic mixtures of Col:CS 10:1 (w/w) - A, Col-CS-FN 10:1:0.001 (w/w/w) - B (fibronectin was purchased from Sigma-Aldrich) were prepared by vigorous vortexing, for 2h. They were conditioned as sponges by freeze-drying for 48h. The ultrastructure of the obtained bio-sponges was observed by scanning and transmission electron microscopy and their biologic activity was assessed in cell cultures.

SEM images showed networks of polymeric fibrils forming interconnected pores with sizes between 50-300 μm (Figure 1). TEM analysis revealed a heterogeneous aspect of the sponges, with loose and tight areas. Col fibers were disposed more orderly in samples containing less FN and CS appeared as electronodense deposits of different sizes.

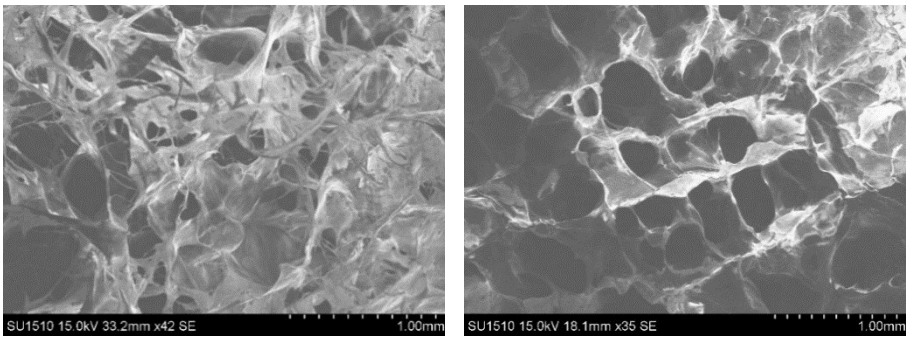

Figure 1 - SEM images of transversal sections of Col-CS-FN composite variants A and B

The biologic activity was assessed by *in vitro* evaluation of cytocompatibility on clinically relevant cell types. Primary cultures of human gingival fibroblasts were obtained from patients undergoing tooth removing surgery and murine osteoblast-like cells were obtained from foetal bones. Cell attachment and proliferation were evaluated by using Cell Counting Kit-8. Prior to quantitative measurements, cells were cultivated for 24h and 48h respectively onto the bio-sponges. Results showed a good *in vitro* biocompatibility for all the testes variants and improved cell proliferation on variant A. The immunomodulatory properties were measured as well, by quantifying the TNF-α level at early time points after direct seeding of the cells onto the scaffolds. Low levels of TNF-α were registered at all selected time points. Nevertheless, the human cells seemed to be more responsive in terms of immune molecules secretion.

Conclusions and/or Outlook

Col-CS-FN composite bio-sponges showed promising results with potential for applications in the field of new periodontal tissue engineering strategies development. The novel bio-composites developed here combine properties such as biocompatibility, suitability for multiple processing techniques including additive manufacturing and recycling of waste materials. The use of innovative biomaterials conditioned as 3D scaffolds suitable for drug delivery and/or cell carrier represent new emerging technologies in the field of periodontal tissue engineering.

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The LIFEDT concept in dentistry from fundamental researches to clinical applications

H Tassery

AMU University, Restorative department ; LBN4203 Montpellier University
e-mail: herve.tassery@univ-amu.fr

Keywords: fluorescence, enamel and dentine caries, Maillard reaction

Preservation of the natural tooth structures requires early detection of the carious lesion, associated with comprehensive patient dental care. Processes aiming to detect carious lesions in the initial stage with optimum efficiency employ a variety of technologies, including fluorescence detection tools which can discriminate between healthy and carious dental tissue. Based on the fluorescence principle, an LED camera (SoproLife®) was developed (Sopro-Acteon, La Ciotat, France) which combined magnification, fluorescence, pictures and video acquisitions and an innovative therapeutic concept called LIFEDT or light-induced fluorescence evaluator for diagnosis and treatment. This clinical concept based on 5 principles is consolidated by fundamental and clinical researches, providing evidence for daily clinical applications. This lecture with discussion is based on nearly 17 publications made step by step to validate the fluorescence concept. The goal of this lecture is to perform in 20mn a short summary of this amazing adventure. In the beginning the aim of the research was to evaluate the porphyrin and pentosidine involvement in the red fluorescence observed in enamel and dentin caries when illuminated with the Soprolife® camera and Vistacam® camera. Three techniques were used: single photon fluorescence spectroscopy, micro-Raman spectroscopy, and colour analysis with Image J software. Fluorescence spectroscopy and micro-Raman spectroscopy revealed the presence of Prot Porphyrin IX in carious enamel and dentin. Then few papers on Multiphoton confocal microscopy and nonlinear spectroscopy demonstrate that both 2 PEF(2 photons Emetting Fluorescence) and SHG (second Harmonic Generation) intensity of human dentin were strongly modified during the tooth caries process, and showed that the ratio between SHG and 2PEF signals is a reliable parameter to follow dental caries. Others publications focus on Raman imaging mapping of decay and sound dentin samples, through accurate analysis of the Raman band spectra variations of mineral and organic components. The correlation between the Raman signal and the signal of a fluorescent camera, by assaying the concentration of pentosidine and natural collagen fluorescent crosslink using reverse phase high-pressure liquid chromatography were also analyzed. The correlation with the Maillard reaction or brown reaction was demonstrated too. The spatial distribution of calcium phosphate crystallinity and the collagen crosslinks near DEJ were also studied using confocal Raman microscopy and calculated by different methods. To obtain collagen crosslinking, the ratio of two peaks 1660 cm−1 over 1690 cm−1 (amide I bands) was calculated and for crystallinity, the inverse full-width at half maximum of phosphate peak were used. Clinical validation was also performed thanks to a multi-centric study which was carried out recruiting 103 children, aged from five to 15 years, on 310 primary and 433 permanent posterior teeth. SE, SP and AUC were respectively of 88.50, 70.73 and 0.84. The validity was significantly higher for primary teeth (AUC=0.90) than for permanent teeth (0.80); the validity of the Soprolife® (0.84) was significantly higher than that of DIAGNOPen® (0.80).
Next steps will combine works on photoactivated disinfection, better knowing the end point of excavation and easier clinical applications. The LIFEDT concept has been fully validated and its clinical applications are numerous.

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Variations on concentration and application time of hydrofluoric acid, on ceramics' adhesive properties and tridimensional structural integrity

F. Murillo-Gómez[a], R.G. Palma-Dibb[b], M.F. De Goes[c]*

[a] Restorative Dentistry Department, Dental School-University of Costa Rica, San José, Costa Rica.
[b] Department of Restorative Dentistry, Ribeirão Preto Dental School-University of São Paulo, São Paulo, Brazil
[c] Dental Materials Division, Department of Restorative Dentistry, Piracicaba Dental School-University of Campinas, Piracicaba, São Paulo, Brazil
e-mail: fabian.murillogomez@ucr.ac.cr

Keywords: Ceramics, hydrofluoric acid, scanning electron microscopy, bond strength.

Introduction

Bonding glass-containing ceramics to resin cements is a tricky but fundamental procedure in indirect restorations' durability. The most accepted procedure to fulfil this task is the employment of hydrofluoric acid (HF) and silane coupling agents. However, HF concentration and application time are still controversial parameters. Bonding effectiveness derived from the modification of such application protocols has been well documented, pointing that the specific application time and HF concentration is material dependent, but generally largest etching times do not necessarily produce better bonding performance. Conversely, few studies have focused on the effect of HF etching on materials' integrity in a micromorphological approach. Thus, the objective of this in vitro study was to evaluate the structural integrity produced by different etching protocols on CAD/CAM glass-containing ceramic materials.

Results and Discussion

To evaluate structural integrity of three CAD/CAM materials (LEU: Leucite-based glass-ceramic, IPS empress CAD, Ivoclar-Vivadent; LDC: Lithium disilicate reinforced glass-ceramic, IPS e.max CAD, Ivoclar-Vivadent; and PIC: Polymer infiltrated glass-ceramic, VITA Enamic, Vita Zahnfabrik), tridimensional roughness mean (Sa) and etching depth evaluation, along with Scanning Electron Microscope (SEM) analysis were performed. The following experimental groups were assembled: C: no treatment, HF5%20s (5%HF application applied for 20 seconds), HF5%60s, HF10%20s, HF10%60s and MBEP (Monobond etch&prime®, Ivoclar-Vivadent).

For LEU, only HF10% treatments produced statistically different roughness values (20s: 0.55±0.04µm; 60s: 0.65±0.11µm) and Si/K ratios (only HF10%60s: 2.23±0.13) compared to C group (Ra: 0.44±0.02µm/ Si/K: 2.53±0.09). Regarding LDC and PIC, groups HF5%60s (LDC: 0.55±0.04µm/ PIC: 0.64±0.03µm) and HF10% (both: LDC-20s: 0.62±0.06µm; LDC-60s: 0.68±0.02µm / PIC-20s: 0.71±0.09µm; PIC-60s: 0.80±0.22µm) showed higher roughness values than C group (LDC: 0.35±0.08µm/ PIC: 0.54±0.05µm). In the case of PIC, all treatments (except MBEP) produced higher Si/C ratios than C group. All treatments (except MBEP) produced higher etching deepness values than C group for the three materials tested, being HF10%60s the highest (LEU:403.2±11.4µm; LDC:617.4±75.7; PIC:291.6±6.5µm). HF10% produced more aggressive etching morphology patterns on superior and lateral surfaces (SEM). Treatments MBEP and HF5%20s, produced the least aggressive structural alterations.

Conclusions

Acid etching produces in general tridimensional (surface/deepness) alterations on ceramics' structural configuration, which depends on acid type, concentration and application time. HF5%20s and MBEP demonstrated to be the best option to etch glass ceramic materials in terms of microstructure integrity.

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How can we see polymerization shrinkage stress?

Monika Lukomska-Szymanska[a*], Kinga Bociong[b], Agata Szczesio[b], Michał Krasowski[b], Jerzy Sokolowski[a]

[a] Department of General Dentistry, Medical University of Lodz, 251 Pomorska St., 92-213 Lodz, Poland
[b] University Laboratory of Materials Research, Medical University of Lodz, 251 Pomorska St., 92-213 Lodz, Poland
*e-mail: monika.lukomska-szymanska@umed.lodz.pl

Keywords: shrinkage stress, water sorption; photoelastic study, resin composite

Introduction
Polymerization shrinkage stress influences clinical performance of resin composite materials[1]. Restorative procedures are associated with stress formation in the tooth structure[2]. Moreover, chewing and biting loads are additional detrimental factors. These phenomena may result in damage of both tooth structure (deformation, cracks) and adhesive bond (secondary caries, post-operative sensitivity, and marginal discoloration)[3]. The dental restorations are surrounded by saliva leading to sorption and solubility[4]. The volume increase due to water intake should counteract or even exceed the amount of polymerization shrinkage[4,5]. The aim of this study was to estimate relationship between water sorption and polymerization shrinkage stress of resin-based dental materials.

Materials
The study tested five resin composites (Filtek BulkFill, Filtek BulkFill Flow, Revolution 2, X-flow), three resin cements, two giomers (Beautifil Bulk Flow, Beautifil Flow F02) and one compoglass (Compoglass).

Methods
Absorbency Dynamic Study was determined by means of procedure as described by Bociong et al.[6]. The samples were prepared according to ISO 4049[7]. Curing time was consistent with the manufacturer's instructions. Five samples were prepared for each tested material. The samples' weight was determined immediately after preparation and then for 30 consecutive days, and after 1344 h (56 days) and 2016 h (84 days). The absorbency was calculated according to the Equation (1)[8]:

$$A = \frac{m_i - m_0}{m_0} \cdot 100\% \quad (1)$$

where A is the absorbency of water, m_0 is the mass of the sample in dry condition, and m_i is the mass of the sample after storage in water for a specified (i) period of time.
Photoelastic Study shows the stress state (contraction or expansion) of resin materials. Photoelastically sensitive plates of epoxy resin with calibrated orifices were used. After selected period of time (30 min, 24 h, 72 h, 120 h, 168 h, 240 h, 336 h, 504 h, 672 h, 1344 h and 2016h) generated strains in the plates were visualized in circular transmission polariscope FL200 and photoelastic strain calculations were based on the Timoshenko equation[9].

Results and Discussion
All resin based materials exhibited shrinkage and the associated contraction stress during hardening process. The significant reduction in contraction stress was observed due to hygroscopic expansion of tested materials (Tab. 1). The overall results showed the development of the initial stress in the compressive direction during photopolymerization (Fig. 1, 2). The composition of resin materials influenced the sorption and solubility processes that in turn had an impact on the hygroscopic expansion. Thus, the compensatory effect was composition-dependent.

Table 1: Stress state, contraction stress drop, absorbency of tested materials.

Material	Stress state [MPa]		Absolute values of stress changes [MPa]	Contraction Stress Drop [%]	Absorbency [wt. %]
	0,5 h	2016 h			
Filtek BulkFill	8.9±0.9	3.1±0.2	5.8	65	1.0
Filtek BulkFill Flow	8.9±0.9	4.7±0.1	4.2	47	0.7
Revolution 2	7.8±0.0	4.7±0.2	3.1	40	0.5
X-flow	19.5±1.1	-3.1±0.1	22.6	116	3.1
Beautifil Bulk Flow	11.1±0.4	4.2±0.9	6.9	62	0.7
Beautifil Flow F02	16.7±0.9	-4.7±0.8	21.4	128	2.8
Compoglass Flow	10.4±0.9	-0.4±0.2	10.8	104	1.3

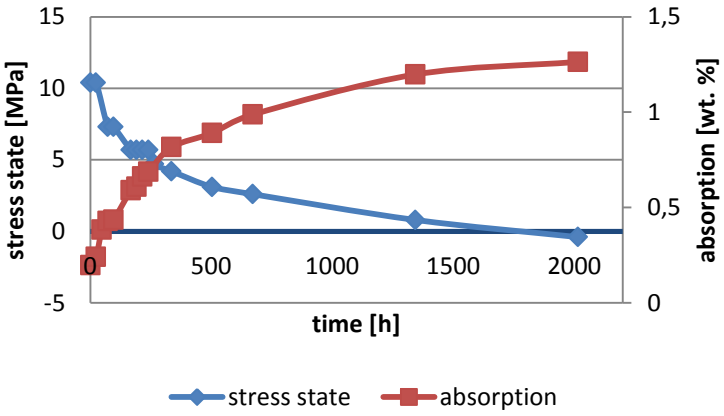
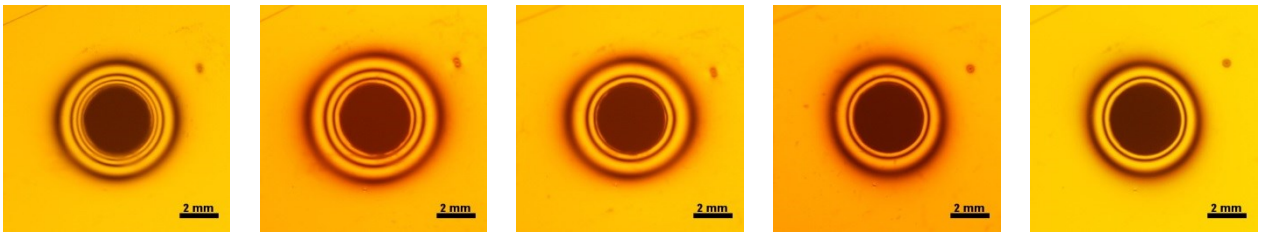


Figure 1: The influence of water sorption (84 days water immersion) on absorbency and contraction stress generated during polymerization of Compoglass Flow.



0.5 h 96 h 240 h 672 h 1344 h

Figure 2: Isochromes in epoxy plate around Revolution Formula 2 before and after water storage (0.5, 96, 240, 672 and 1344h).

Conclusions

The photoelastic method can be used to evaluate the contraction stress and to show the relationship between water sorption and stress value. Contraction stress, water absorbency, and stress were material-dependent values.

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Comparison through push-out test of two different techniques for the restoration of endodontically-treated teeth

A. Fabianelli[a], *F. Papacchini*[b], *A. Cerutti*[c]

[a]Lecturer, University of Sheffield, UK; Lecturer, University of Brescia, Italy; Private Practitioner, Cortona, Arezzo, Italy
[b]Private Practitioner, Viterbo, Italy
[c]Professor and Chair, University of Brescia, Italy

Abstract

Fiber-reinforced composite (FRC) posts are commonly used for the restoration of non-vital teeth with a significant loss of coronal tooth structure. To provide optimal retention and better stress distribution within the root, resin-based cements are used as luting agents. However, post debonding at the dentin/cement interface has emerged as the most frequent failure mode of post retained restorations in clinical trials. The extremely high configuration factor (C-factor) of the post space seems to prevent a complete stress relief of the curing resin cement, thus affecting the final bonding. A new short glass fiber-reinforced composite, Ever X Posterior (GC Corporation), has been introduced for bulk application with the claims of enhanced mechanical and curing stress behavior than traditional resin composites.

The purpose of this research is to assess by means of push-out test the retentive potential of a short glass fiber-reinforced composite when used as intracanal anchorage instead of traditional fiber posts.

Material and Methods. Forty-four single-rooted extracted human teeth were endodontically treated and randomly divided into two groups (n=22), depending on the materials used in the post space. In Group A (control), FRC Postec Plus (Ivoclar-Vivadent) posts were tested. In group B (test) no fiber posts were used: after acid etching and the application of the same adhesive system as in Group A, the short fiber-reinforced Ever X Posterior resin composite (GC Corporation) was used to fill the post space. In each group, three 1.5 mm-thick slices from each sample were obtained. Data obtained during the push-out strength test were statistically analyzed ($p < 0.05$).

Results. No significant difference as detected between the two groups. Conversely, within each group, intraradicular level was a significant factor for push-out strength ($p < 0.05$). In particular, for each material, significant higher push-out bond strengths were recorded at the coronal level than at the apical level. No significant difference was observed between the middle and the coronal/apical level in both groups.

Conclusions. When Ever X Posterior was used for intracanal anchorage, similar retentive strength was achieved when compared the traditional fiber post system, especially for intraradicular adhesion.

Clinical implications. Ever X Posterior, a short glass fiber-reinforced composite originally proposed for bulk filling of posterior restorations, might represent a viable and operatively simpler alternative to traditional fiber post adhesion in endodontically-treated teeth

Fluorescence in diagnosis- state of the art

L. Steier[a], *J.A.P. Figueiredo*[b]

[a] Dr.med.dent. The Royl College of Surgeons , Edinburgh
[b] BDS, MSc, PhD, Associate Professorof Oral Histology UFRGS, Porto Alegre, Brazil
email: lsteier@gmail.com

Keywords: fluorescence, Dentistry, diagnosis

Introduction
Fluorescence occurs naturally or can be intentionally triggered. Different mechanisms will be reviewed by the authors, focusing on the potential of UV Fluorescence application in Dentistry - from diagnosis to intervention.
Terminology is a pre-requisite for understanding and evaluation. Below, we point the main terms:
Emission of light is defined as **luminescence.**
Fluorescence is defined as the emission of light by a substance that has absorbed light.
Biofluorescence results from the absorption of electromagnetic radiation at one wavelength by an organism, followed by its reemission at a longer and lower energy wavelength, visually resulting in green, orange, and red emission coloration [1] .
Ultraviolet (UV) flourescence has been awarded a high importance in diagnosis.
UV wavelength are described from 10 nm to 400 nm.
UV Flourescence occurs in nature either as autoflourescence or as induced flourescence by adding physicochemical detectors, defined flourophores.
Fluorescent sensing can be successfully used to fulfill the described criteria.
The process can be intensity relevant or not due to a possible mix up of autoflourescence and flourophore results.

Dental Applications of Fluorescence
Fluorescence has found its application in visualization of biologic processes, in the detection of relevant species, their byproducts or induced processes.
Fluorescence sensing is a process initiated via different pathways, having at its base a UV light source, using intrinsic fluorescence or by application of add-ons (fluorophores).
Diagnosis via UV light biosensing
1. The **sensitive biologic element** ➔ tissue, microorganisms, etc.
2. Physico chemical detector ➔ autoflorescenece or via addition of a flourophore
3. The **biosensor reader** ➔ free eye via magnification
The review of current applications of flourescence in dentistry has identified the following potentials:

1. Detection of tooth demineralization as a result of initial caries attaque. 2. Differentiation of caries activity. 3. Detection of plaque. 4. Oral malodor. 5. Calculus detection. 6. Identification of inflamatory procceses. 7.Detection of precancerous lesions of oral mucosa. 8.Detection of viable bacterial flora at complition of chemical root canal disinfection

Outlook
The mechanisms leading to the above applications can be described in brief as following:
Ad 1. Enamel is autoflourescent. Demineralization due to caries attaque leads to loss of Ca ions and change in autoflourescence. Both processes are used to detect caries by different devices.
Ad 2. The flourescence potential of certain bacteria has been identified and correlated to caries activity.
Ad 3. Plaque can be detected either due to intrinsic red autoflourescence or by addition of plaque disclosing agent. While certainity exists on the methodology, there is controverse in regards to the discrimination of young or old plaque.
Ad 4. Oral malodor can be confirmed via autoflourescence visualization of the tongue.
Ad 5. Calculus detection
Ad 6. Identification of inflamatory procceses via Reactive Oxygen Species and flourescence.
Ad 7. Detection of precancerous lesions of oral mucosa.
Ad 8. Detection of viable bacterial flora at complition of chemical root canal disinfection by using the bioluminescence-based ATP assay.
Diagnosis is the base of any medical intervention. Speed, reliability, minimal invasiveness and simplicity are desired/required characteristics of a diagnostic process. Fluorescence can be successfully used to fulfill the mentioned requirements. It has found its application in visualization of biologic processes, in the detection of relevant species, their byproducts or induced processes.

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Root Canal Irrigation:state of the art

A. Luzi

University CEU Cardenal Herrera. Department of Dentistry.
e-mail: Arlinda.luzi@uhceu.es

Keywords: Root Canal Irrigants, sodium hypochlorite, surface-active agents, endo activator.

Introduction

The goal of all root canal procedures is to remove vital and necrotic pulp remnants, microorganisms, and microbial toxins primarily through mechanical preparation of the root canal, and chemical disinfection, by means of irrigation and locally applied medicaments[1] . Experimental animal studies as well as human studies have shown that without bacteria pulpal and periapical disease does not develop [2]. The challenge in achieving a successful outcome in endodontic treatment lies in the variety of factors that impact upon a clinician's ability to reduce the bacterial load inside the complex root canal system to a threshold beyond which the number of bacterial cells is insufficient to sustain or induce apical periodontitis[1,3]. Sjögren and Figdor in a study of 1997[4],using anaerobic culturing techniques with a sample group of 55 patients, obtained a complete periapical healing in 94% of patients where bacterial testing of the root canal after instrumentation and irrigation with 0.5% sodium hypochlorite yielded a negative culture before obturation, and healing in 68% of patients with a positive culture before obturation[4]. Studies have also tried to investigate the relationship between bacteria surviving in root canal treated teeth and persistent apical periodontitis. Nair et al [5], in a light and electron microscope study of teeth with persistent periapical lesions demonstrated the presence of bacteria in the unfilled voids and lateral canals in the apical part of root canals[5]. Summarizing complete debridement and disinfection of the root canal system are crucial for a successful root canal therapy. Both mechanical instrumentation and irrigating solutions cooperate in achieving this objective even if irrigation has been for many years one of the of the most neglected phases of root canal treatment especially if irrigation is considered of the most apical part of the root canal. In this literature review will focus on the importance of irrigation and on the mechanical and dynamic aspects of the irrigating procedures. The aim of this presentation is Demonstrate the importance of using irrigants with antimicrobic and tissue dissolving actions. Update the knowledges on the irrigating products available on the market in gel or in solution from the more traditional to the newest considering their advantages and drawbacks.

Results and discussion

To disinfect the root canal we use different solutions (Table 1). The most common method to introduce irrigant into the root canal has been through a needle connected to a syringe by a luer lock connection. Finger pressure is placed on the barrel of the syringe, which pushes irrigant through the needle and this is known as positive pressure irrigation (PPI). To overcome the limits of the Positive Pressure Irrigation such as the risk of extrusion of irrigants, difficulty in delivering and replenishing irrigant in the apical third of the root canal, due to limited space and gas entrapment (vapor lock), a new technique based on Negative Pressure Irrigation (NPI) have been introduced (Endovac -SybronEndo). In this technique, the irrigant is delivered into the root canal by tubing attached to a pump, positioned 12 mm from the root apex[1,6]. The Studies on antimicrobial efficacy of irrigation with the EndoVac system in comparison to syringe irrigation and supplementary sonic agitation techniques has shown that these techniques are comparable and highly effective in bacterial reduction[7]. However, when assessing the ability of different irrigation techniques to deliver irrigant into simulated lateral canals, ultrasonic activation and sonic activation were superior in this aspect in comparison to, EndoVac and syringe irrigation[8]. Various methods have been suggested and studied to energize the irrigating solution increasing their efficacy on debris and bacteria present in the lateral canals and other intricacies of the root canal system.

Table 1:Classification of the commonly used irrigating solutions.

All these methods transform the static PPI into a dynamic irrigation. We can use ultrasonics to activate the irrigating solutions

A) CHEMICAL AGENTS:
A. TISSUE DISSOLVING AGENTS: NAOCL
B. ANTIBACTERIAL AGENTS:
I. BACTERIOSTATIC: CHX, SOME ANTIBIOTICS
II. BACTERICIDAL: SOME ANTIBIOTICS, NAOCL
C. CHELATING AGENTS:
I. WEAK: HEBP (1-HYDROXYETHYLIDENE-1, 1-BISPHOSPHONATE) 18%
II. STRONG: EDTA
D. COMBINATION PRODUCTS (TISSUE DISSOLUTION & ANTIBACTERIAL EFFECT):
MTAD, QMIX, SMEARCLEAR, TETRACLEAN.
B) NATURAL AGENTS:

and laser activation of irrigating solutions using pips. A protocol of irrigation has to be used by the clinicians during the shaping of the root canal and another one before the obturation of the root canal. During the shaping of the root canal we have to use abundant 5-6% NaOCl, delivered through a gauge 27 or 30 side-vented needle (positive pressure irrigation). As an alternative is possible to introduce NaOCl using Endovac (negative pressure irrigation). Before obturation we have to irrigate the root canal 3-5 min with NaOCl 5-6%, 1 min with EDTA 17%, saline solution and 2-3 min of chlorhexidine 2%.

Conclusion

Sodium hypochlorite remains the most important irrigating solution during root canal treatment and should be used throughout the instrumentation. High concentrations of 5-6% kill bacteria and dissolve organic tissue better than low concentrations of 1-2%. After shaping, a more efficient irrigation can be performed since the ideal conditions for a deeper introduction of the irrigation needles, for a higher flow rate of the solutions and more space for a dynamic activation of irrigants using both ultrasonic or subsonic energy. The pre-obturation irrigation will be based on a 3-4 min. rinse with NaOCl followed by a 1 min. rinse with a decalcifying agent like EDTA. In severe infections a 2-3 min. final rinse with 2% Chlorhexidine, due to its substantivity, will create an antibacterial environment on condition that we will carefully avoid any interaction between CHX, Sodium Hypochlorite and EDTA. Instead of using EDTA and after CHX for the final rinse we may use one of the product (MTAD or QMix) where the decalcifying agent is premixed with an antiseptic agent and with a tensioactive.

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Cytotoxicity and odontoblast-initiated mineralization induced by sodium trimetaphosphate in vitro

P. H. P. D'Alpino[*a], M. R. Polassi[a], M. M. P. Alvarez[b], R. G. Carvalho[b], F. D. Nascimento[c], I. L. S. Tersariol[b,c]*

[a] Biotechnology and Innovation in Health Program, Universidade Anhanguera de São Paulo (UNIAN-SP), São Paulo, SP, Brazil
[b] Department of Biochemistry, Molecular Biology Division, Federal University of São Paulo, São Paulo, Brazil
[c] Interdisciplinary Center of Biochemistry Investigation (CIIB), University of Mogi das Cruzes, Mogi das Cruzes, SP, Brazil
e-mail: paulodalpino@gmail.com

Keywords: Odontoblast cells, Dentin-pulp complex, Dentinogenesis, Cytotoxicity, Sodium trimetaphosphate

Introduction

Sodium trimetaphosphate (STMP) is a cyclic polyphosphate that adsorbs to enamel surfaces, thereby reducing the enamel demineralization and changing the affinity between the enamel surface and salivary proteins. *In vitro*, *in situ*, and clinical studies have demonstrated that STMP-containing fluoridated dentifrices, gels, mouthrinses, and varnishes have a higher protective effect for both dental caries and erosion when compared with products without STMP. Despite the synergism of STMP/F, in a dose-dependent way, STMP interferes with F deposition on carbonated hydroxyapatite when highly concentrated. STMP has also been used as a reagent for phosphorylation, as it induces calcium phosphate precipitation and proliferation of osteoblasts on collagen, and improves the integration of polymeric biomaterials with natural hard tissues. STMP can also induce mineralization in dentin. In these cases, when hydrolysed, STMP provides phosphate to react with collagen, different from that when STMP is applied in topically applied dental products. In a previous study (Liu et al., 2011), it was found the STMP is a promising agent for acting as a biomimetic analogue of matrix phosphoproteins for remineralization of artificial caries-like dentin. Depending on the application form, STMP can reach the pulp via dentinal tubules in different concentrations. Currently, little is known about its cytotoxicity and its impact when in contact with odontoblasts and other pulp cells. Accordingly, all sorts of cell reactions might occur, potentially interfering in the biology and function of dental pulp cells. These reactions may vary from a slight inflammatory reaction that can lead to necrotic cell death, and to non-inflammatory apoptotic cell death. In this way, the purpose of the present study was to evaluate the effects of STMP on the inflammatory responses, odontogenic differentiation, and mineralization-associated proteins in odontoblast cells.

Results and Discussion

Calcium phosphate (Ca/P= 1.67) crystalline particles were prepared by the instantaneous addition of a phosphate solution to a gently stirred solution of calcium ions in the absence or in the presence of STMP (0.15 - 15 mM). The formation of crystalline material was monitored by measuring the radial size of the crystalline particles as a function of the reaction time with the aid of a light-scattering detector. The data showed that the size of crystalline particles grew with the time; the rate at which the crystalline material is formed can be described by a first-order law. Light scattering analysis demonstrated that the presence of 0.15 mM STMP greatly increased the rate of crystal growth at pH 7.4 at 25 ºC. In the absence of the STMP, the first-order rate of the crystal growth was Kobs = 0.54 ± 0.13 min-1 and in the presence of 0.15 mM STMP the rate was Kobs = 2.6 ± 0.3 min^{-1} ($P < 0.05$). As well as, the presence of the STMP altered significantly the average size of the crystalline particles, in the presence of STMP the size of the crystalline particles was 2000 ± 400 nm and in the absence of STMP was 534 ± 54 nm ($P < 0.05$). Data from the literature shows that the conversion of saturated solution of amorphous calcium phosphate (ACP) in hydroxyapatite (HAP) occurs in two distinct phases, the first phase is acid mediated and converts ACP into octacalcium phosphate (OCP), the second phase is base mediated and indicates the conversion of the OCP intermediary to hydroxyapatite. During the mineralization reaction in deionized water, 2.5 mM $CaCl_2$ and 1.5 mM K_2HPO_4 (Ca/P= 1.67), the pH was monitored as a function of time in the absence or in the presence of STMP (0.15 - 15 mM). The data shows that the presence of 0.15 mM STMP increased significantly the rate of conversion of ACP/OCP into HAP. In addition, the toxicity of STMP was evaluated in immortalized rat odontoblast MDPC-23 cells by MTT assays. It was observed that, up to 30 mM, STMP does not change the cell viability of MDPC-23 cells. On the other hand, STMP concentrations above 30 mM can cause cell death by loss of cell adhesion to the substrate.

Conclusions

Taken together, these data strongly suggest that STMP can organize the nucleation of HAP crystals *in vivo*.

Acknowledgements

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Computer simulation in dental research and dentistry

J. K. H. Tsoi

Dental Materials Science, Discipline of Applied Oral Sciences, Faculty of Dentistry,
The University of Hong Kong
**e-mail: jkhtsoi@hku.hk*

Keywords: Computer simulation, digital dentistry, dental research, finite element, artificial intelligence,

Computer simulation is one of the modern technologies to use a computer programme attempting to represent an abstract model of a particular system. It can be useful to gain insights if we cautiously used as a part of modelling in systems of science, engineering and social science. Modelling of these systems usually need to go through a mathematical process (i.e. an algorithm), such that an attempt is to be made to find a numerical solution for which no analytical solution is available. Thus, this enables the prediction of the behaviour of the system from a set of parameters and initial conditions. Dentistry examples for these are Monte Carlo (MC) and Molecular Dynamics (MD) simulations. On the other hand, for complex systems, exact analytical solutions are rare, and successive approximation might be necessary. Indeed, finite element (FE) method which is commonly used in dental metal and ceramics simulations belongs to this class. Nevertheless, close attention needs to be paid to such parametric matters such as sample size, program validation and verification of the simulation model. These contribute to the successfulness (e.g. closeness to reality) of computer simulation. Moreover, these are the key to excel in dental research and digital dentistry, utilizing some tactics such as artificial intelligence (AI) - as what currently our lab is working on in aspects of microbiology and dental automation.

Biological safety of resin composite

M. Daou

Assistant Professor, Member of the academy of dental materials, Member of the SFBD "Société Francophone de Biomatériaux Dentaires", Course Director - Biomaterials, St Joseph University Beirut, Lebanon, Pediatric Dentist - Private Practice, Lebanon
drmahadaou@gmail.com

Many patients have become concerned about the toxicity of resin composites because those materials may absorb water and, can release components, particularly TEGDMA, BPA, HEMA and, cause adverse biological reactions.

TEGDMA present in most of the composite resins is released more easily and more frequently and, represents therefore a high toxicity.

The aim of this presentation is to evaluate the risk arising from the elution of the TEGDMA, and also of BPA, and to give clinical recommendations to minimize biological risks including cavity preparation, monomers conversion, and selection of materials without TEGDMA

In vitro testing of biomechanical properties of different orthodontic composite resin

R. Condò [a], M.E Cataldi,[d], I. Frustaci[d],S. La Rocca[a], L. Pazzini[b], L. Cerronia, G. Pasquantonio[a], A. Notargiacomo[c], A. Convertino[b], V. Mussi[b], L. Maiolo[b]

[a] Department of Clinical Sciences and Translational Medicine, University of Rome "Tor Vergata", Rome, Italy
[b] Institute for Microelectronics and Microsystems-National Research Council (IMM–CNR), Rome, Italy
[c] Institute of Photonics and Nanotechnologies-National Research Council (IFN -CNR), Rome, Italy
[d] PhD in Materials for Health, Environment and Energy, Department of Science and Chemical Technology, University of Rome "Tor Vergata", Rome, Italy
e-mail address: roberta.condo@uniroma2.it

Key words: Bisco Ortho Bracket Paste LC; Light-Cure Orthodontic Paste; Transbond XT Adhesive Resin; chemical-physical comparison.

Introduction Orthodontic Composite Resins differ from Restorative bulk Composites in the increased proportion of co-monomer contained in the formulation, which reduces viscosity; therefore, they should be considered an adaptation of Composite Restorative materials [1]. A typical composition includes about 70-75% of Bisphenol A diglycidylether dimethacrylate (Bis-GMA) and 25-30% of triethylene glycidal dimethacrylate (TEGDMA) with other fillers used to improve physical properties of the resin such as some form of glass or ground quartz, pretreated with a silane coupling agent [2]. In Orthodontics, these materials are extensively employed as bonding system to guarantee an intimate and resistant connection between the base of the orthodontic bracket and the enamel surface of the tooth. Indeed, an ideal orthodontic adhesive should exhibit superior bonding properties to withstand masticatory and orthodontic forces and at the same time, it should allow easy debonding without impairing damage to the enamel at the end of the treatment [1].

Materials and Methods An extensive in vitro study on the chemical and physical properties of a novel material compared to two other orthodontic adhesive systems is presented. To investigate potential differences in the materials, Bisco Ortho Bracket Paste LC (Bisco, Schaumburg, Illinois, USA), Light-Cure Orthodontic Paste F3170-01 (Leone s.p.a., Sesto Fiorentino. FI, Italy) and Transbond XT Adhesive Resin (712-031, 712-036, 712-066) (3M Unitek, Monrovia, CA, USA) have been subjected to morphological, mechanical, optical and chemical characterization by Scanning Electron Microscopy and Focused Ion Beam analysis, UV-Vis and Raman spectroscopy, Shear Bond Strength tests and weight analysis both in saliva and in a sugary drink.

Results and Discussion Bisco Ortho Bracket Paste LC and Light-Cure Orthodontic Paste F3170-01 show a similar surface roughness respect Transbond XT Adhesive Resin samples. Moreover, Raman and UV-Vis Spectroscopy analyses highlight strong similarities between the first two resins respect the composition and the color of Transbond XT Adhesive Resin "**Figure"** 1.

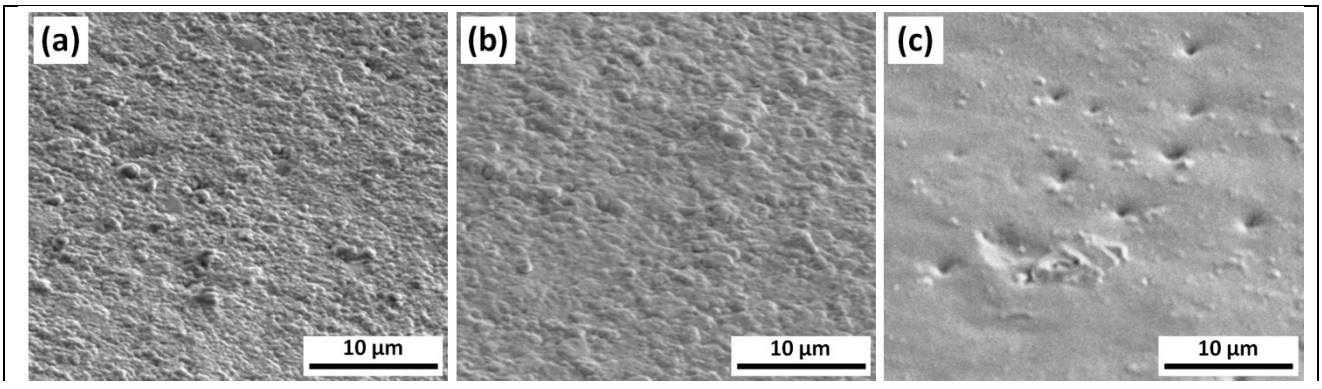

Figure 1: SEM images the surface of (a) Bisco, (b) Light-Cure and (c) Transbond XT samples.

Despite these morphological homogeneities, the milling rate of Bisco Ortho Bracket Paste LC performed by FIB is almost double respect the other two resins: this test reveals a peculiar intrinsic material composition that provides a higher level of elasticity for BISCO Bisco Ortho Bracket Paste LC "**Figure"** 2.

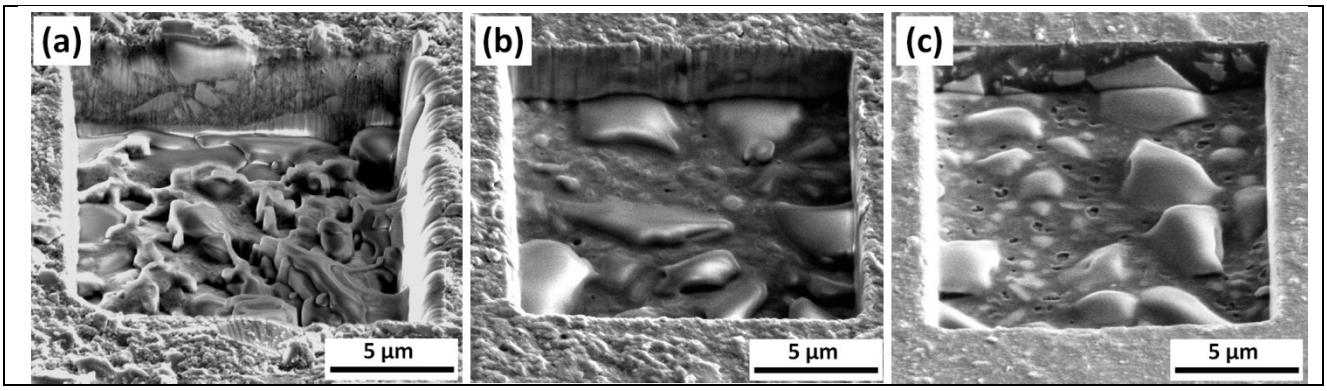

Figure 2: SEM images of hollows produced by milling test on (a) Bisco, (b) Light-Cure, and (c) Transbond. The milling rate is almost double in Bisco respect the other two samples.

No significant weight losses occurred, during the ageing in saliva and sugary drink up to one month, thus revealing that all the materials in exam are dimensionally stable and biocompatible. Conversely, a small weight increment can be detected in all the samples due to organic residues absorption. In particular, Raman spectroscopy analysis highlights that BISCO Bisco Ortho Bracket Paste LC surface is less affected by these residues in both cases.

Conclusions and Outlook All the samples do not show significant weight loss during the ageing in saliva and sugary drink up to one month, revealing a suitable stability for the patients. The analysis with the spectrophotometer suggests a similar absorption trend in the UV region, with the Transbond that exhibits a lower reflectivity as can be seen also from its greyish color.The milling tests conducted demonstrate a lower resistivity of Bisco respect the other two adhesives, probably due to a slight different composition of the materials, while at great magnification the structure of Transbond XT reveals a nanostructure with a lower density. No significant differences have been observed in SBS tests. These results demonstrate that all the three materials are suitable for their purpose, being all dimensionally stable, with Bisco that is probably more elastic and less hard respect the other two also if it has a structure very similar to Light-Cure. This in vitro study represents a starting point for even a more complete analysis that can include other parameters such as cytotoxicity, bacterial adhesion and clinical performance.

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Innovative Bone Substitute for Dental and Maxillo-Facial Applications

*G. Pertici**[a], C. F. Grottoli[a], S. Molinari[a], G. Perale[a,b]

[a] Industrie Biomediche Insubri SA, via Cantonale 67, 6805 Mezzovico-Vira, Switzerland
[b] University of applied sciences and technology SUPSI, Department of Innovative Technology, Galleria 2, Via Cantonale 2c, CH-6928 Manno, Switzerland
**e-mail: gianni@ibi-sa.com*

Keywords: Biomaterials, Bone regeneration, Oral surgery, Bone remodelling

Introduction
Scaffolds for bone tissue engineering should ensure both volumetric stability and adequate strength. Moreover, their intimate structure should have an interconnected porous network for cell migration and proliferation, while also providing specific signals for bone remodelling and regeneration [1,2].

Results and Discussion
An innovative composite solution, bearing cues from both mineral components and polymeric ones, was here followed to develop a new three-dimensional bone scaffold, SmartBone® (SB): a bovine derived mineral matrix is used to provide adequate solid structure and porosity, while resorbable polymers are used to reinforce it. RGD-exposing collagen fragments are finally added to promote cell colonization and proliferation. Previously published results indicate that SB is osteoconductive and osteoinductive, promoting remodelling to mature bone formation in about 8-12 months [3].
High performances of this biomaterial allowed developing custom-made products (a.k.a. SmartBone® On Demand™, SBoD), solving single specific cases of bone reconstruction: starting from CT scan, personalized grafts can be provided for every kind of defects.

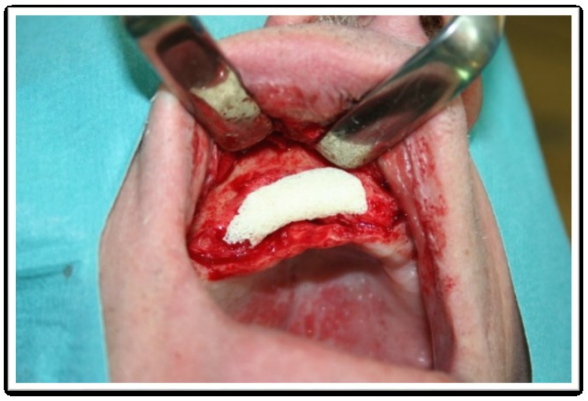

Figure 1

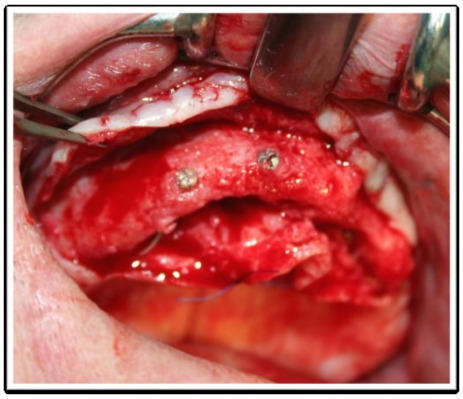

Figure 2

Figure 1 and 2: SBoD implantation at day 0 (fig.1) and at reopening after 8 months (fig.2), perfect integration and no volumetric resorption.

This technology was successfully applied around to 50.000 cases. In previous pictures a custom reconstruction of maxillary bone in a 70-years old male. During surgery, the piece was perfectly located inside the gap and firmly fixed with two osteosynthesis titanium screws. Surgery was fast (<2 hrs) and very precise, allowing to obtain very satisfactory results both in terms of anatomical reconstruction and functionality. The post-operative follow-up recorded no issues of any kind and proceeded optimally.

Conclusions
CT scan after 8 months showed impressive osteointegration and massive volume stability (>95%). SBoD custom made bone grafting technique allows complete restoration of wide defects. Histological analysis indicates that SmartBone is osteoconductive, promotes fast bone regeneration, leading to mature bone formation in about 8 months.

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Revealing Orthodontic Industrial Secrets with Neutron Spectroscopy

K.V. Tian[a,b], G. Festa[b,c], F. Basoli[d], G. Lagana[e], C.Andreani[b,f], S. Licoccia[a,b], R. Senesi*[bf], P. Cozza[b,e]

[a] Department of Chemical Science and Technologies, University of Rome Tor Vergata, Rome, Italy
[b] NAST centre, University of Rome Tor Vergata, Rome, Italy
[c] Centro Fermi-Museo storico della Fisica e Centro Studi e Ricerche "Enrico Fermi", Rome, Italy
[d] Department of Engineering, Campus Bio-Medico University of Rome, Rome, Italy
[e] Department of Clinical Sciences and Translational Medicine, University of Rome Tor Vergata, Rome, Italy
[f] Department of Physics and NAST centre, University of Rome Tor Vergata, Rome, Italy
e-mail: kun.tian@uniroma2.it, Roberto.senesi@unirma2.it

Keywords: Orthodontic archwire, Stainless steel, Martensite transformation, Neutron diffraction, PGAA

Introduction

Evolution of neutron instrumentation and scattering techniques has outpaced its broad application with respect to other spectroscopic determinations. Standardized translation of data from system-specific neutron measurements into useful information for the physical, chemical and indeed medical sciences remains a standing challenge. Following the direction of the encompassing successes of our developments relating to fracture toughness in biocements [1], we look to further bridge this gap relate atomic-scale structure and phenomena to bulk mechanical properties and material performance in clinical/practical settings.

Herein, we present a case study where, in an attempt to reveal orthodontic archwire alloy composition and manufacturing processes, which are usually kept as commercial secrets [2], simultaneous elemental analyses employing neutron resonance capture analysis (NRCA)/Prompt-gamma activation analysis (PGAA) and metallurgical analyses employing neutron diffraction were conducted at a pulsed neutron source (ISIS, RAL, UK) on two as-received commercial rectangular austenitic stainless steel orthodontic archwires, G&H and Azdent, 0.43×0.64 mm (0.017×0.025 inch). Standard PGAA measurement at a cold neutron source (BNC, Budapest, Hungary) was also done in parallel to provide benchmark and reference data [3,4].

Results and Discussion

The higher PGAA signal-to-background ratio (SBR) intrinsic to the pulsed nature at ISIS only allowed for qualitative analyses but the results are in line with those of the quantitative analyses performed at BNC. Fortunately, taking advantage of the time resolution afforded at ISIS we were able to carry out novel time-resolved T-PGAA analysis on the same samples through neutron energy selection. By applying the time-of-flight cut in the 200-700 μs range and selecting neutrons in the energy range of 5.55-67.94 eV, we accessed the epithermal neutron range (**Fig. 1a** red). In this range we detected the trace element ^{95}Mo in Azdent, which normally is masked by the more significant ^{55}Mn peak, even in the standard PGAA spectra. On the other hand, by applying the time-of-flight cut in the 3000-4000 μs range and selecting neutrons in the energy range of 0.17-0.30 eV, we accessed the thermal neutron range (**Fig. 1a** black). This peak interference of ^{95}Mo was eliminated and the SBR of ^{55}Mn was significantly increased, to an even higher value than that obtained with standard PGAA at ISIS (6.0 vs. 5.1), greatly increasing the potential of accurately quantifying ^{55}Mn content. The ability to detect Mo is of clinical importance because Mo is usually added to austenite SS to increase corrosion resistance.

NRCA detected minor amount of Co in Azdent (**Fig. 1b**), which was confirmed by the elemental composition quantified with standard PGAA at BNC. No Co was detected in G&H.

Metallurgical analyses revealed that both samples have a bi-phase structure containing martensitic phase (45.67% for G&H and 6.62% for Azdent) in addition to the expected metastable austenite (**Fig. 1c**). The former may be a strain-induced phase-transformation arising during the cold working process of wire fabrication. It can be concluded that the excessive cold-working and inadequate thermal treatment have resulted in higher degree of martensite transformation in G&H wires.

From the results, it could be predicted that with the higher Co content, Azdent may possess less heat hardenability and with the higher fraction of martensite, G&H may possess lower Young's modulus of elasticity, toughness and ductility, compared to Azdent. Corrosion and mechanical tests have been planned to test our prediction.

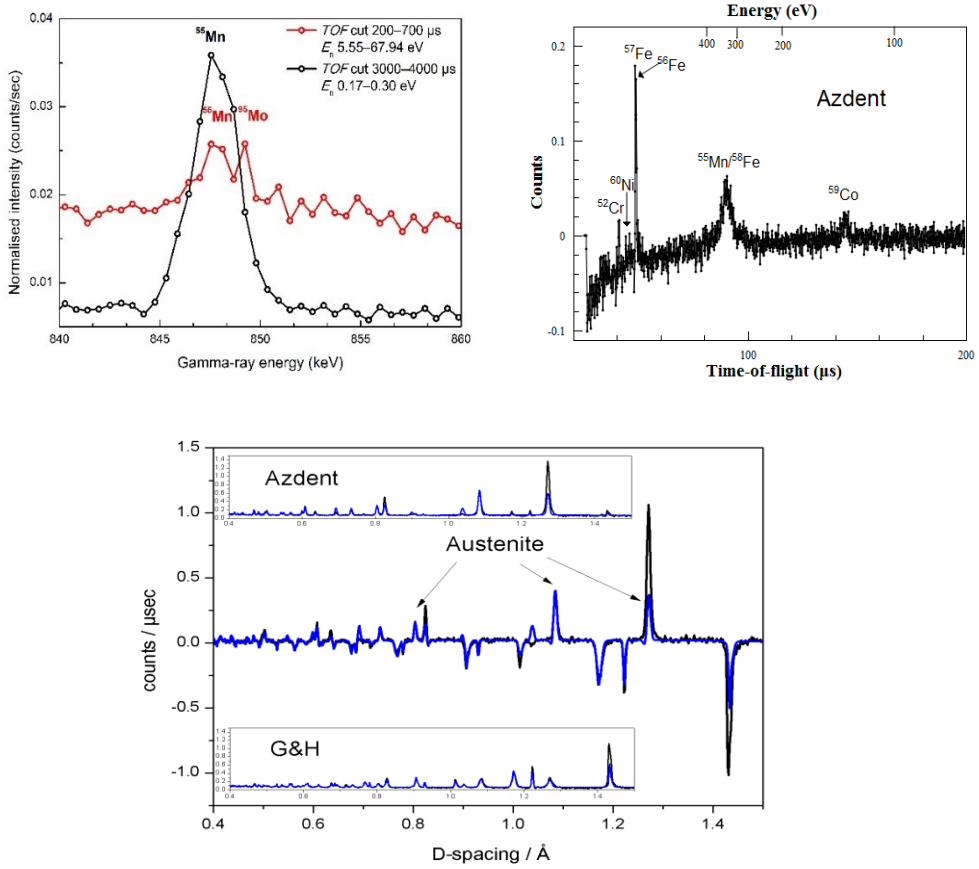

Figure 1:
Results obtained from the integrated neutron diffractometer and elemental analysis station. (a): The two T-PGAA spectra of Azdent obtained by the neutron time-of flight selection of 200-700 ms (red) and 3000-4000 ms (black), in the energy region of 840-860 keV. The gamma line from Mo in the red spectrum nearly diminishes in the black spectrum, in contrast with the significant increase of the line intensity of Mn; (b): NRCA spectrum of Azdent. The detected peaks are labelled with the elemental symbols; (c): The difference between Azdent and G&H diffraction patterns is plotted in black (normalized number of counts/µs as a function of d-spacing) and the difference of best fit of data obtained by Rietveld refinement is plotted in blue. The main austenite diffraction peaks at ~0.8, ~1.08 and ~1.27 Å are labelled to highlight these differences. Inset: diffractograms (black) of the two samples and their best fit of data (blue).

Conclusions and Outlook

The current study has successfully assisted in the elucidation of alloy composition and manufacturing processes. The obtained information has helped with prediction of clinical relevant material properties and guide future tests. This non-destructive technique, simultaneous elemental and metallurgical analyses using neutron scattering technique, may further be applied in the characterisation of other biomaterials made from metal alloys, directly related to industrial production controls under the CE-marking (Conformité Europeene, directive 93/68/EEC, 1993). The technique's transferability across a wide array of materials, accommodating diverse sample sizes and conditions, supports its strong potential in industrial forensics.

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Engineering 3D Human Vascularized Bone and Muscle Models as a Drug Screening Platform for Antimetastatic Drugs

M.V. Colombo[1], C. Arrigoni[1], S. Bersini[1], M. Gilardi[1], S. Lopa[1], M. Bongio[1], M. Moretti[1,2,3,4]

1. Cell and Tissue Engineering Laboratory, IRCCS Istituto Ortopedico Galeazzi, Milano (IT)
2. Regenerative Medicine Technologies Lab, Ente Ospedaliero Cantonale, Lugano (CH)
3. Swiss Institute of Regenerative Medicine, Lugano (CH)
4 Fondazione Cardiocentro Ticino, Lugano (CH)

**e-mail: matteo.moretti@eoc.ch*

Keywords: in vitro 3D models, bone metastasis, vascularization

Introduction

The discovery of new drugs against metastases is a very relevant goal for research and pharma industry, however, current in vitro models present an oversimplified microarchitecture as compared to native tissues. On the other hand, in vivo models, although widely used, suffer from species-specific differences in biological mechanisms [1]. Advanced 3D in vitro models, based on human cells, have been proposed by us and others as a promising tool to overcome these limitations [2]. In this context, our group has recently developed a mesoscale in vitro bone model containing endothelial cells (ECs), bone marrow mesenchymal stem cells and precursors of osteoblasts (OBs) and osteoclasts (OCs), based on collagen and fibrin hydrogels enriched with calcium phosphate nanoparticles (CaPn). We obtained cell differentiation towards osteoblastic lineage, as shown also by calcium deposition, associated to sustained TRAP activity, indicating the presence of bone remodeling activity [3]. Based on this combination, to better mimic the complex physiologic environment, we developed a new 3D mesoscale model of vascularized bone tissue including immune and cancer cells, thus replicating the microenvironment of early breast cancer metastases, in view of its potential use as a screening platform for anti-metastatic drugs.

Results and Discussion

Metastatic bone models were fabricated by embedding human bone cells (fully differentiated OBs and OCs), ECs, macrophages and breast cancer cells (bCCs) in a 3D fibrin gel. After 7 days of culture we observed the development of an interconnected microvascular network surrounding the OBs, while OCs remained round-shaped and were homogeneously distributed in the fibrin gel. In this system, bCCs co-localized with microvessels, mimicking bCCs extravasation in the target tissue (**Figure 1a**). Since it is known that bCCs are able to induce osteolytic metastases in bone, we focused also on the interaction between bCCs and OCs, finding that TRAP was expressed both by OCs and by bCCs. The induction of this osteoclast-like behaviour in bCCs (known as osteomimicry), was probably due to the presence of RANKL, secreted by OBs in the construct, which stimulated bCCs. Indeed, when bCCs were cultured alone and stimulated with RANKL they were able to resorb portions of calcium hydroxyapatite disks. Bone tissue hosts also specific populations of immune cells, such as macrophages. We thus included bone-resident osteomacs in the model, which promoted angiogenesis when co-cultured with ECs, OCs and OBs. In the presence of bCCs, macrophages tended to shift their polarization state from M0 towards M1 and M2. In particular, we observed a low ratio between M1 and M2 phenotypes, typical of the early metastatic breast cancer microenvironment [4]. To further validate the results obtained in our bone model, we compared the results to a 3D model of skeletal muscle tissue, which is not a target of bCCs metastasis, incorporating human skeletal muscle cells, muscle fibroblasts, ECs and bCCs in fibrin gel. In this muscle model the microvessels appeared less interconnected and bCCs proliferation was significantly lower as compared to the bone model (50% decrease, $p<0.05$) (**Figure 1b**).

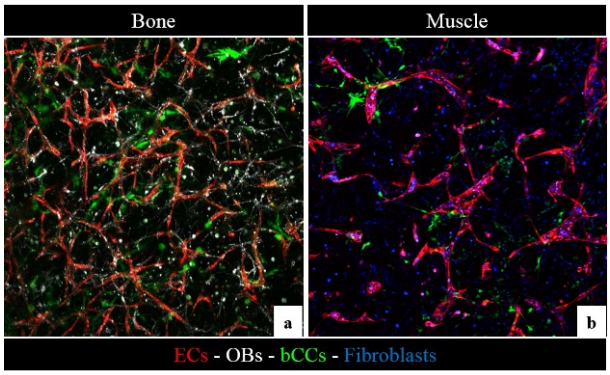

Figure 1: a) immunofluorescence of the bone model (red: HUVECs, green bCCs, white: osteoblasts), Osteoclasts and macrophages were not stained. b) immunofluorescence of the muscle model (red: HUVECs, green: bCCs, blue: muscle fibrobasts). Myoblasts were not stained.

Finally, we performed a proof of concept drug screening test, adding a known antimetastatic drug (rapamycin) to our metastatic bone model, in concentrations ranging between 2 and 20nM. We compared the response of our bone model to that of a simplified 3D model containing only bCCs embedded in a 3D fibrin gel. The results showed a different effect of rapamycin on bCCs proliferation and angiogenesis in our metastatic bone model, as compared to the simplified model, thus showing the importance and value of considering a more complex and physiological microenvironment for drug testing.

Conclusions

In conclusion, we implemented 3D human vascularized bone models embedding calcium phosphate nanoparticles and up to five different cell types, including tissue specific, immune and cancer cells, in a 3D fibrin gel and we performed analyses on known anti-tumour compounds. Results demonstrated the potential use of our 3D complex organotypic microenvironments as models for the screening of anti-tumour drugs.

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The technological variant of producing miniplates used in facial skeleton reconstruction

Eduard Ciucă[a], Horia Manolea[a],Maria Magdalena Manolea[a], Alex Sălan[a], Alexandra Ciucă[a], Marius Ciurea[a], Alexandru Grecu[a] Adrian Camen[a]*

[a] University of Medicine and Pharmacy Craiova, Romania
e-mail: alexsalan87@gmail.com

Keywords: biohpp, facial reconstruction, titanium miniplates

Introduction

In the reconstructive surgery of the facial cranium, the evolution of the materials used for osteosyntheses has known an extreme variety. [1,2] The development of miniplates represented a revolution in facial skeleton reconstruction. For the last four decades Titanium fulfilled most of the criteria. Within the OMF Surgery Clinic from Craiova we have conceived a new system of miniplates made from a new material, BioHPP (PEEK), which is already successfully used in orthopedics and neurosurgery.

Results and Discussion

BioHPP is a PEEK variant that has been optimized with a special ceramic filler. This ceramic filler has a grain size of 0.3 to 0.5 µm. Due to this very small grain size, constant homogeneity can be produced. The fine granularity of the filler is the basis for the extremely good polishing properties. This homogeneity is an important prerequisite for these outstanding material properties and forms the basis for consistent quality. In contrast to the framework materials previously used, BioHPP has an elasticity that is suited to the bone. Ceramics and NPM are approximately 20x as rigid as bone, and gold and titanium are 10x as rigid as bone. In vitro tests showed that the material doesn't produce injuries to the tissues. The E-modulus of BioHPP lies in the range of 4000 MPa, which very strongly resembles the elasticity of human bone (e.g. in the mandible). In the tests used to determine the resistance to fracture of BioHPP, values up to 1200 N were reached, in comparison to maximum chewing force of 500 N for a human bite.

Conclusions and/or Outlook

In conclusion, BioHPP plates are lighter than the titanium plates. Having the same structure and elasticity as the human bone these plates don't have to be preformatted preoperative. The plates adapt very easily to the facial bone borders. They proved efficiency regarding strength and contention of the bone fragments. There is no data regarding of the fact that the human body is not accepting the BioHPP plates. The plates are metal free, so they can be used to patients that are allergic to metal. They have a lower manufacturing price compared to the titanium plates. They be mold ended in any form and the time necessary to manufacture them is reduced.

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3D bioplotting for the recovery of cranial bone defects on the base of titanium and hydrogel composites

V. Bednarzig[a], A. M. A. Jaramillo[1], K. Teichmann[a], Dr. S. Gerth[b], J. Claußen[b], Prof. Dr. T. Steinberg[c], L. Volk[c], Prof. Dr. A. R. Boccaccini[a] & Dr. R. Detsch[a]

[a]Friedrich-Alexander-Universität Erlangen-Nürnberg; Lehrstuhl für Biomaterialien, Department Werkstoffwissenschaften
[b]Fraunhofer-Institut für Integrierte Schaltungen IIS; Anwendungsspezifische Methoden und Systeme
[c]Universitätsklinikum Freiburg; Abteilung Orale Biotechnologie

vera.bednarzig@fau.de
angela.m.arias@fau.de
karoline.teichmann@fau.de
stefan.gerth@iis.fraunhofer.de
joelle.claussen@iis.fraunhofer.de
thorsten.steinberg@uniklinik-freiburg.de
lukas.volk@uniklinik-freiburg.de
aldo.boccaccini@fau.de
rainer.detsch@fau.de

Keywords: Scaffold, Hydrogel, Hydroxyapatite, Alginate, Bioplotter, Composite, Cranial bone implant

Introduction

An array of people are affected by large craniofacial (CF) bone defects, which are a major challenge in surgeries. On average, 227.500 children are born annually with such defects, in the United States. These defects can be obtained, besides congenital nature, also through burns, caused by heat, electricity, chemicals, or through blunt trauma.[1,2]

To replace the defect, autologous and allogenous implants are usually applied. The aim of this project is to generate a functional bone implant, which consists of specific layers which differ in their composition, structure and properties. All these layers have dedicated functions, thereby a new bone structure can be build and more bone ingrowth should be found compared to existing implants. Hence, the implant has to fulfill several functions, like connectivity to the residual bone, the adherence of cells to the bone layer, whereas natural bone cells should infiltrate only special areas of the implant. To generate such a structure an additive manufacturing technique, 3D bioplotting has been applied. By means of this technique the layers with different compositions and structures can be generated.

Results and Discussion

The whole implant consists of three layers: the first one is a clinically used titanium (Ti) mesh which is responsible for the mechanical stability, acts as building platform for the entire construct and builds the part which can be fixed to the remaining bone using screws. On top and inside the free parts of the mesh, a pre-crosslinked sodium-alginate (Alg) layer is plotted. This soft and dense layer has to be inert for fibroblast cells to prevent cells from the surrounding soft tissue to invade the implant. On top of the dense alginate a second layer is plotted for the reconstruction of the hard tissue. This layer is plotted out of single struts lying upon each other to generate a macro-porosity. This layer is a question of the combination of diverse materials out of different material groups. It consists of a natural polymer, the hydrogel sodium-alginate (Alg), polyvenylalcohol (PVA) and the commercially available ceramic hydroxyapatite (HA).

Repeated plotting tests of the second layer showed that alginate dissolved in Dulbecco's Phosphate Buffered Saline (DPBS), showed a high shrinkage (up to 40 %) after the crosslinking with 0.1 M calcium-chloride ($CaCl_2$), and a poor shape stability. The post-crosslinking after the plotting leads to the deformation of its shape from a square shaped plotted to a circular structure. The lack of shape and size stability had to be improved through a pre-crosslinking procedure with calcium-carbonate ($CaCO_3$) and Glucono-delta-lactone (GDL) at 4 °C for 4 days. With this procedure the shrinkage was reduced to 20 % and the shape of the post-crosslinked structure shows a high exactness to the plotted one.

To confirm that the alginate is inert towards fibroblasts, these cells were seeded onto a 3D-plotted alginate layer *in vitro*. Light microscopy images show no cell adherence to this layer. However, cells grew on adjacent cell culture plastic surfaces, proving the non-toxic behaviour or any negative influence of the alginate material to the cells.

For optical tests, SEM images were recorded after 24 h of plotting. Images revealed a smooth and flat surface, as expected, this was also proven with light-microscopy images.

To create a macro-porous alginate-hydroxyapatite composite layer, the plotting properties on the shape followed by the behaviour of the printed struts are of great importance. Main factors are plotting speed, height of the needle and used pressure. Hence, the aim of this project was to create a composite material that can be printed in thin struts without filling the gaps in-between and without filling the gaps of the previous layer. So a 3D open porous structure was created. First *in-vitro* cell tests, like WST-8 and fluorescence-images, with the MC3T3-E1 cell line after 24 h show already promising results. SEM images, as shown in figure 1 left, prove a homogeneous distribution of the HA particles inside the hydrogel matrix and the shape stability of the printed struts in every single layer. Light microscopy images, shown below in figure 1 right, underline these and show

additionally the macro-porous structure with adjustable pore-size, depending on the space between the struts and the used plotting needle.

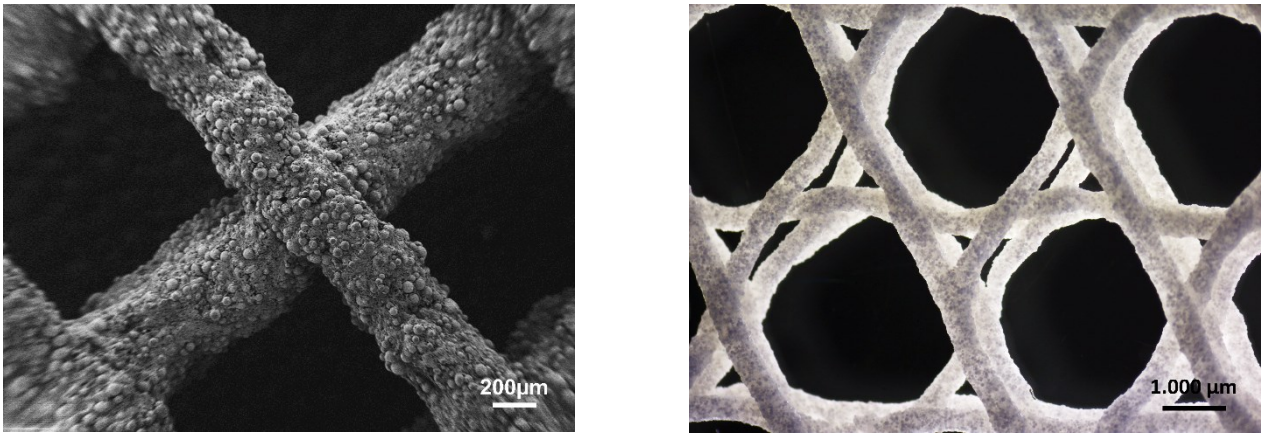

Figure 1: Images of a 3D plotted alginate hydroxyapatite composite material in a macro-porous shape. Left: SEM image, Right: Light microscopy image

Conclusions and Outlook

All experiments in this study showed promising results regarding printability, cell tests and manageability. The printed layers stick to each other with high stability and without coalesce. In future the composite material of the second layer has to be adapted by increasing the HA content to improve cell growth and proliferation. Additional materials should be added to the composite to improve cell binding properties, stability and to change the filler properties to adjust different capabilities.

Acknowledgement

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Update on custom made 3D printed titanium implants for anterior column reconstruction following en bloc resection for spinal tumours

M. Girolami[a], *G. Barbanti Brodano**[a], *S. Bandiera*[a], *S. Terzi*[a], *R. Ghermandi*[a], *G. Evangelisti*[a], *G. Tedesco*[b], *V. Pipola*[a], *A. Gasbarrini*[a]

[a] Department of Oncological and Degenerative Spine Surgery, Istituto Ortopedico Rizzoli- Via G.C. Pupilli, 1- 40136 Bologna, Italy

[b] Deformity Spine Unit, Istituto Ortopedico Rizzoli- Via G.C. Pupilli, 1- 40136 Bologna, Italy

**e-mail: giovanni@barbantibrodano.com*

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.

In 17 patients submitted to en bloc resection for spinal tumour between November 2015 and June 2018 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

At an average 18 months follow-up (range 1-28), 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.

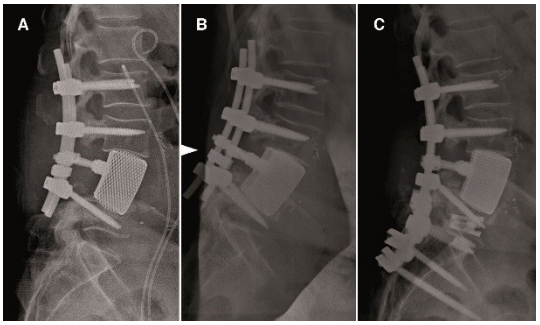

Figure 1. Lateral X-ray showing the anterior column reconstruction after L4 en bloc resection with a custom made 3D printed titanium implant: post-operative image (A), complication at 18 months FU (B), revision surgery (C).

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. Longer follow-up will be needed for fusion rates and long-term complication rates.

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Artificial 3D culture systems for T cell expansion

Eduardo Pérez del Río[a,b], *Marc Martinez Miguel*[a,b,c], *Jaume Veciana*[a,b], *Imma Ratera*[a,b]*, *Judith Guasch*[a,b,c,*]

[a] Institute of Materials Science of Barcelona (ICMAB-CSIC), Campus UAB, 08193, Bellaterra, Spain
[b] Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Campus UAB, 08193, Bellaterra, Spain
[c] Dynamic Biomaterials for Cancer Immunotherapy, Max Planck Partner Group, ICMAB-CSIC, Campus UAB, 08193, Bellaterra, Spain
e-mail: eperez2@icmab.es, jguasch@icmab.es

Keywords: T cell expansion, Adoptive cell therapy, scaffolds, 3D artificial structures

Adoptive cell therapy, which consists of the extraction, manipulation, and administration of ex vivo generated autologous T cells to patients, is an emerging alternative to regular procedures in cancer treatment. Nevertheless, these personalized treatments require laborious and expensive laboratory procedures that should be alleviated to enable their incorporation into the clinics. With the objective to improve the ex vivo expansion of large amounts of specific T cells, we used three-dimensional (3D) structures during their activation with artificial antigen presenting cells, thus resembling the natural environment of the secondary lymphoid organs. Thus, the activation, proliferation, and differentiation of T cells were analyzed when cultured in the presence of two 3D systems, Matrigel and a 3D polystyrene scaffold, showing an increase in cell proliferation compared to standard suspension systems.[1] Moreover, new synthetic biomaterials are being investigated with the same purpose.

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Electrospun PDLLA-Gelatin-RKKP tricomponent nanofibrous scaffold for bone tissue engineering

A. Pepe[a], B. Bochicchio[a], K. Barbaro[b], A. De Bonis[a], J. V Rau[c]*

[a] Dipartimento di Scienze, Università della Basilicata, Via dell'Ateneo Lucano 10, 85100 Potenza, Italy.
[b] Istituto Zooprofilattico Sperimentale Lazio e Toscana "M. Aleandri", Rome, Italy
[c]Istituto di Struttura della Materia (ISM-CNR), Via del Fosso del Cavaliere 100, 00133 Rome, Italy.
**e-mail: antonietta.pepe@unibas.it*

Keywords: Electrospun scaffold, RKKP Bioglass, PLA, gelatin, hydroxyapatite, mesenchymal stem cell differentiation.

Introduction

In the last decade, electrospinning emerged as a powerful technology for the rapid and cheap production of nano-fibrous scaffolds, able to mimic the morphology and structure of the ECM for tissue engineering (TE) applications. This technique has been extensively employed also to produce 3D scaffolds with nanofibrous, porous structure in bone TE[1]. According to the observation that bone is a highly complex inorganic - organic nanocomposite constituted mainly of nano-hydroxyapatite particles and collagen fibres, a biomimetic approach was applied in the production of a new tri-component electrospun scaffold composed of poly (D,L- lactic acid) (PDLLA), gelatin and RKKP bioglass microparticles. PDLLA is a widely used biomaterial for its good biocompatibility, biodegradation and good mechanical properties, however it has a poor cellular affinity. The introduction of gelatin within the PDLLA matrices is considered to improve the hydrophilicity and cellular affinity and thus to better allow its use as tissue regenerative matrices to direct and populate cells[2].
In the frame of a biomimetic strategies for the generation of regenerative bone scaffolds we have also incorporated inorganic RKKP bioglass microparticles. RKKP bioglass containing also La^{3+}/Ta^{5+} ions belongs to a new generation of bioglass with improved biological and cell-friendly properties able to influence scaffold strength and mechanical properties, enhance bioavailability/bioactivity and osteoconduction[3].

Results and Discussion

Blends of PDLLA/gelatin supplemented with different ratios (0-29%, w%) of RKKP bioglass microparticles were fabricated into fibrous membranes by electrospinning processes.
Scanning Electron Microscopy images show that the nanofibrous scaffolds were composed of bead-free and randomly arranged fibres, with similar morphology and similar fibre diameter distribution (**Figure 1A**). The presence of RKKP particles inside the nanofibres is confirmed by back- scattered electron microscopy (BSE) and Energy-Dispersive X-Ray Spectroscopy (EDS), highlighting an even distribution inside the scaffolds (**Figure 1B**).

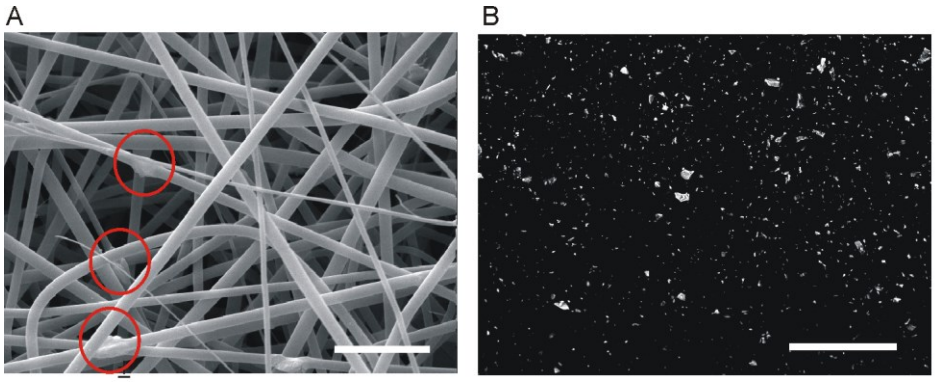

Figure 1: Morphology of electrospun scaffolds with RKKP microparticles (A). SEM images of an electrospun tricomponent scaffold; red circles highlight the fibres containing RKKP particles (Bar: 10 μm). (B) Back-scattered electron imaging showing an uniform distribution of the RKKP bioglass particles into the scaffold. (Bar: 200 μm)

The scaffolds were cross-linked and their bioactivity in terms of ability to induce mineralisation was tested *in vitro*. While the scaffold without RKKP bioglass particles did not form a mineralisation layer even after 28days of incubation in the simulated body fluid (SBF) buffer, the RKKP containing scaffolds show a significant mineralisation layer as observed by SEM images. The mineralisation layer was characterised by X-ray diffraction (XRD) measurements and Raman spectroscopy confirming the formation of hydroxyapatite crystals on the surface of the fibres.
All the scaffolds were tested for biocompatibility, proliferation and differentiation with canine Adipose Mesenchymal Stem Cell (AMSC) cultures. The scaffolds show a high viability (> 97%) by MTT colorimetric assay measured at 2 and 6 days of incubation. Differentiation of the AMSCs toward adipogenic, chondrogenic and osteogenic lineage was induced by appropriate conditioning medium. The level of differentiation was evaluated by analysing the expression of tissue-specific

genes by a quantitative "real time" reverse transcription/polymerase chain reaction technique. Our data indicate that the studied scaffolds were able to promote the osteogenic commitment and that supplementation of specific soluble factors was able to induce the differentiation of AMSCs in osteocytes as demonstrated by the appearance of bone distinctive markers.

Conclusions

Our result show that the electrospinning of a blend of synthetic and natural polymers in a single scaffold supplemented with an inorganic component constituted by RKKP microparticles is a suitable strategy to produce a biomimetic scaffold for bone tissue engineering.

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Past, Present, and Future of Bioactive Glass-ceramics

Maziar Montazerian[*]

Department of Materials Engineering (DEMa), Center for Research, Technology, and Education in Vitreous Materials (CeRTEV), Federal University of São Carlos (UFSCar), São Carlos, SP, 13.565-905, Brazil
e-mail: maziar_montaz@yahoo.com

Keywords: Glass-ceramic; Bioactivity; Mechanical Properties; Biomedical.

Introduction

In 1969, Prof. Larry Hench discovered the first man-made material which forms a chemical bond with bone and initiated a whole new field of bioactive glasses and glass-ceramics [1]. Later on, other bioactive glass-ceramics, such as Cerabone®, Bioverit®, and Biosilicate® were developed and commercialized [2]. Currently, there is an intense search for novel compositions and microstructural design of these materials. Additionally, the fracture toughness of these glass-ceramics (1-2 $MPa.m^{1/2}$) is still in the lower range compared to cortical bone (2-12 $MPa.m^{1/2}$). Bioactive glass-ceramics have been considered for low and medium load-bearing conditions, but their toughness (to $K_{IC} > 3$ $MPa.m^{1/2}$) and bioactivity should be promoted. 3D porous and mesoporous glass-ceramics for incorporation of biofactors, drugs, and cells are also promising for biomimetic regeneration of the complex structures of bone and teeth. Another potential application is hyperthermia treatment of cancer using magnetic bioactive glass-ceramics, and several other relevant examples could be given. Due to their inherent bioactivity and improved mechanical properties, bioactive glass-ceramics continue to be key candidates in the quest for adequate bone substitutes and scaffolds. There are clear signs that alone, or in combination with other materials, such as polymers, these materials will find a wealth of applications for bone therapy in our aging population [2], [3].

Results and Discussion

Glass-ceramics (GCs) are polycrystalline materials that contain one or more crystal phases embedded into a residual glass and are generally produced by two methods: melting and sol-gel followed by some heat treatment [2]. Figure 1 shows the main stages of glass-ceramic synthesis via these two methods [2], [3]. Furthermore, Figure 2 shows various bioactive glass-ceramics and respective future research areas.

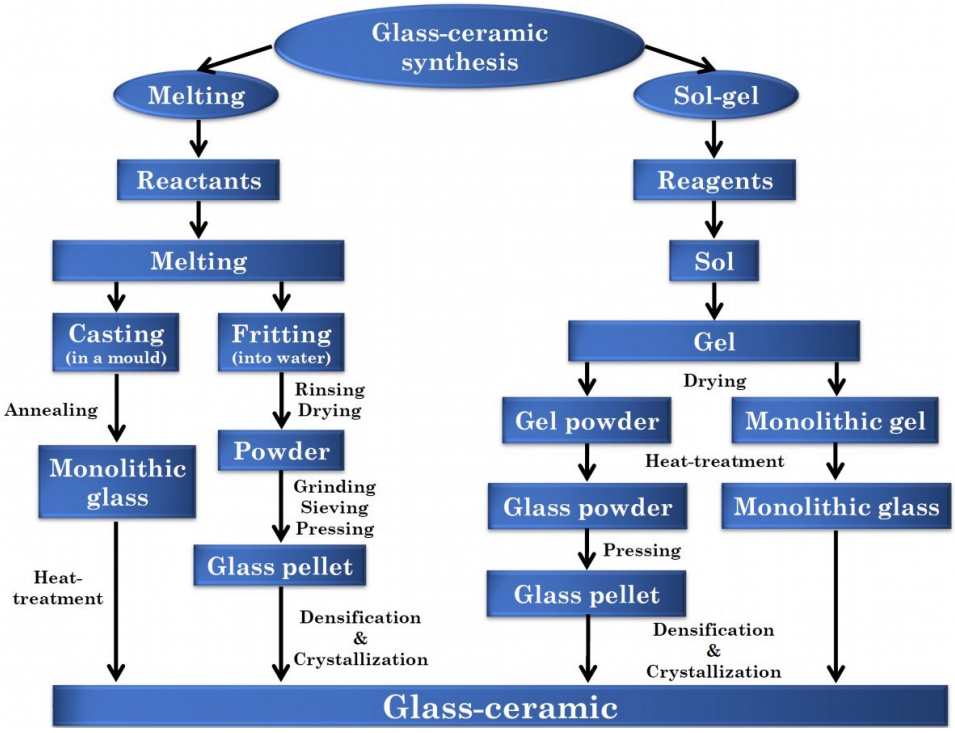

Figure 1: Schematic diagram of the main stages in the synthesis of glass-ceramics [2].

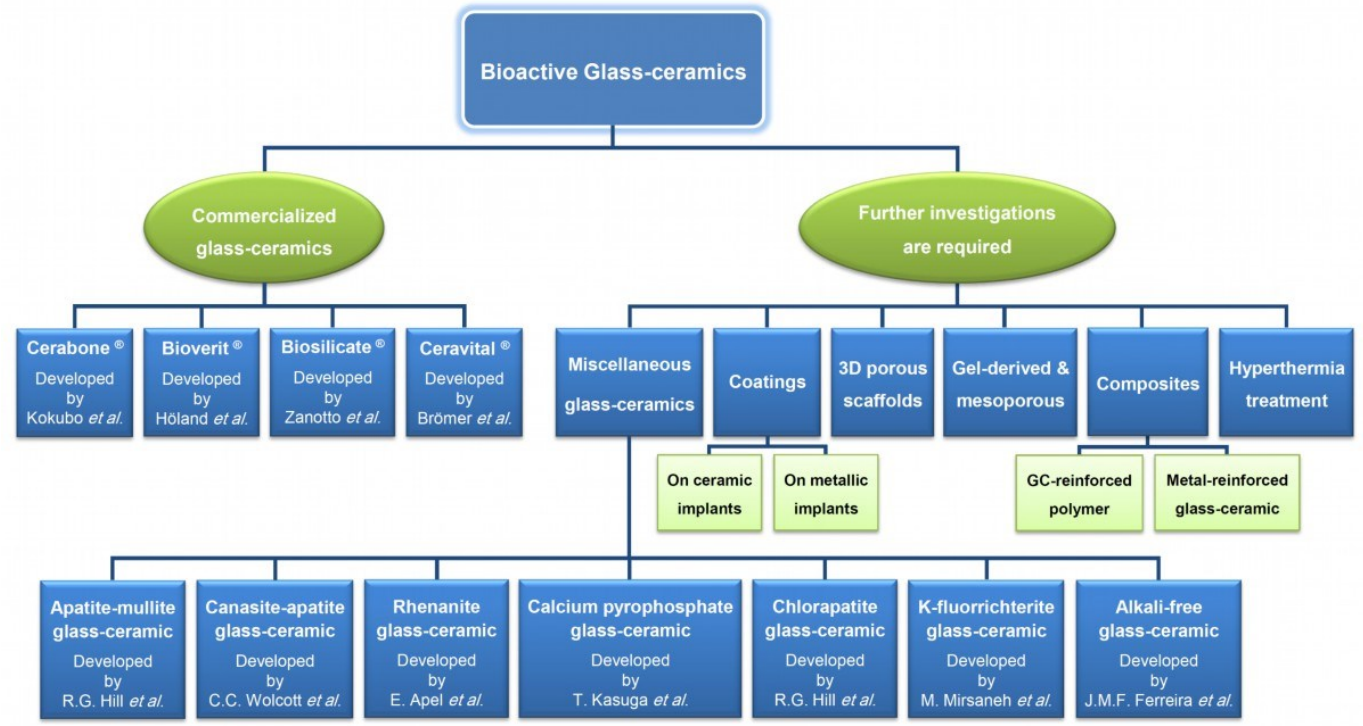

Figure 2: Various bioactive glass-ceramics and respective open research areas [3].

Acknowledgment

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Computational Modeling and Analysis of Knee Articulating Cartilage for Development of its Biodegradable Chitosan Tissue Scaffold

Nitin Sahai[a*], *Manashjit Gogoi*[a]

[a]Department of Biomedical Engineering, North Eastern Hill University, Shillong-793022
**e-mail: nitinbiomedical@gmail.com*

Keywords: Computer Aided Tissue Engineering, Cartilage, Scaffolds

Articulating cartilage plays an important role in smooth mobility and cushioning of bone joints. Loss/damage of cartilage by trauma or inflammation cause restriction in free mobility of joints, it naturally represents multiphasic and anisotropic material. Computer Aided Tissue Engineering plays a vital role in in-vitro regeneration of cartilage tissue on porous scaffolds, which can be a solution to replace the degenerated cartilage that can be possible by developing the biocompatible and biodegradable porous tissue scaffolds for cartilage regeneration. Biodegradable scaffolds are porous 3-Dimensional structures that are fabricated from biocompatible and biodegradable biomaterials like chitosan, polycarpolactum (PCL), hydroxyapatite (HAP) etc. This study is concentrated on the development and analysis of subject specific biocompatible scaffolds for knee cartilage. Recruitment of knee cartilage, computed tomographic (CT) image data in DICOM format of one human subject was performed on patient informed consent. Semi-automatic segmentation in conjunction with region growing algorithm was used to interpolate 2D-DICOM image data to reconstruct 3D knee cartilage model. Finite element analysis (FEA) was performed on developed cartilage to estimate the exact material strength and solid free form fabrication in the development of porous scaffolds. 3-D printing of designed models with CREO software is done with four different pore size and different shapes using PLA as a polymer.

Results and Discussion

The results showed that chitosan could be a better source of biomaterial for development of cartilage tissue scaffolds. Authors likewise discussed the importance of computational modeling and analysis in development of porous tissue scaffolds for cartilage development. The 3D printed PLA polymer having high mechanical strength.

Conclusions/Outlook

Chitosan, 1- 4 linked 2 - amino, 2 deoxy, β- D - glucan, is the only amino polysaccharide distributed in large amounts in nature. It is the deacetylated derivative of chitin, the most abundant natural polymer on earth after cellulose, obtained from crustaceans, such as shrimps, squids, and crabs can be promising polymer which will used for the fabrication of knee articulating cartilage using computer aided tissue engineering.

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3D Model Generation and 3D printing of Bone CT Image using Image processing Techniques

Deepshikha Shrivastava[a]*, *Arnab Kumar Maji*[a], *Debdatta Kandar*[a]

[a]Department of Information Technology, North Eastern Hill University, Shillong-793022
*e-mail: deepshikha.rke12@gmail.com

Keywords: - *3D modelling, Computed Tomography, 3D Printing*

Abstract
In the area of clinical research to enable the radiologist for accurate analysis of bone fracture, infections, arthritis and loss in bones there is need of accurate geometrical three-dimensional (3D) reconstruction of bone in today's era. Computed tomography (CT) images are used to obtain the reconstruction of bones. 3D model reconstruction of bone CT image will be very useful for setting up preoperative protocol and performing computer assisted surgery. The generation of 3D bone CT model is classified as 3D reconstruction from two dimensional (2D) CT bone images. The reconstruction of CT bone images can be achieved by large number of bone data set of single bone. In this paper we applied the image processing techniques and materialise's Interactive Medical Image Control System (MIMICS) to create a 3D reconstruction of lower limb from 2D CT image. For this work, bone CT image dataset in digital imaging and communication in medicine (DICOM) format of different age groups are taken from online resources. After pre-processing of CT image, thresholding is applied on the lower limb CT data set with deep learning for segmentation. The segmented lower limb image is reconstructed to 3D model in MIMICS in form of stereo lithography (STL) file format which can be further utilized for 3D printing for patient specific orthopedic intervention.

Results and Discussion:
The result shows the shorter time for 3D model creation and gives the effective result when projected to the 3D plateform. Output 3D model is validated with different validation technique which gives the effective results.

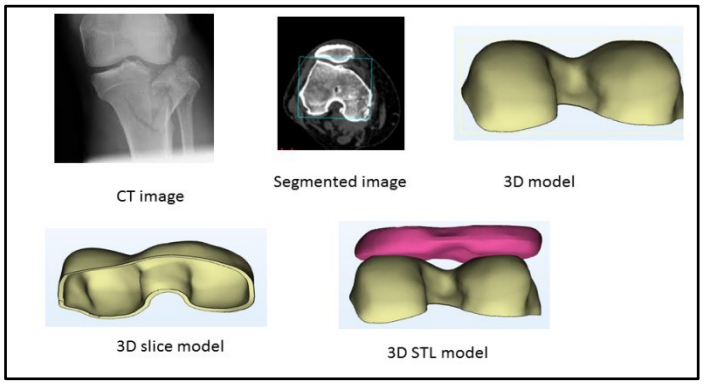

Figure1: 3D model of lower limb

Conclusions
In this work we demonstrate a robust, efficient and fast method to achieve reconstruction of modeling of human bones from 3D CT lower limb datasets that minimizes manual aspect. As a effective application, doctors can use these CT derived 3D models for setting up preoperative protocol and performing computer assisted surgery.

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Electroporation: novel scaffolds for in vitro tumor tissue models

E. Sieni[*a], M. Dettin[a], A. Zamuner[a], R. Marino[b], P. Sgarbossa[a], M. Lucibello[c], F. Keller[b], L.G. Campana[d], E. Signori[bc]

[a]University of Padova, Department of Industrial Engineering, Padova, Italy
[b] University Campus Bio-Medico, Laboratory of Developmental Neuroscience and Neural Plasticity, Roma, Italy
[c] CNR-IFT, Roma, Italy
[d]Sarcoma and Melanoma Unit, Veneto Institute of Oncology (IOV-IRCCS) and Department of Surgery Oncology and Gastroenterology, University of Padova, Padova, Italy
[*]*e-mail: elisabetta.sienin@unipd.it*

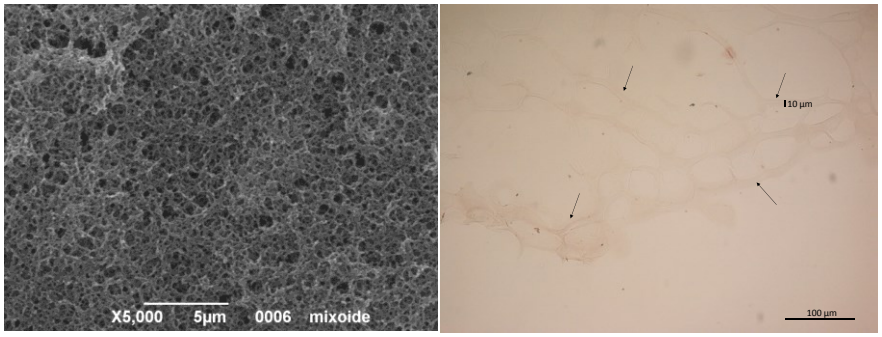
Keywords: Scaffold, electroporation, breast cancer cell line

Introduction

Electrochemotherapy employs cell electroporation to improve the drug uptake and potentiate the drug activity in tumor treatments [1]. Electroporation of different cell types needs the optimization of pulses protocols as well as the identification of suitable electric field intensity. Usually the parameter selection is determined in cell suspensions or monolayer cultures. Nevertheless, it is well known that cell suspensions and 2D cell cultures are not able to simulate the complex interactions between cells and the extracellular matrix (ECM). In electrochemotherapy (ECT) studies, spheroids and hydrogels have been recently proposed to better mimic the tissue complexity [2,3]. These models are easy to handle, but not all cell lines are able to form spheroids and cell sensitivity to electric field pulses depends on spheroid diameter [4-6]. In addition, spheroids are relevant for intercellular junctions, but they are limited in cell-ECM mimicry. Recently Ivey JW et al. [7] reported that cell-seeded 3D gelatin matrix is a good model for reproducing tumor tissue.
In the present work, we propose a 3D scaffold for cell culture with the aim to mimic the myxoid environment found in some tumors [8]. The scaffold is a crosslinked and lyophilized matrix based on hyaluronic acid and ionic-complementary self-assembling peptides condensed with IKVAV Laminin adhesion motif [9]. Different breast epithelial cancer lines (HCC1569 and MDA231) were seeded into the novel scaffold and allowed to grow for 7 days. MDA231 cells, cultured into the scaffold, were electroporated using Propidium Iodide to assess the electroporation efficiency.

Results and Discussion

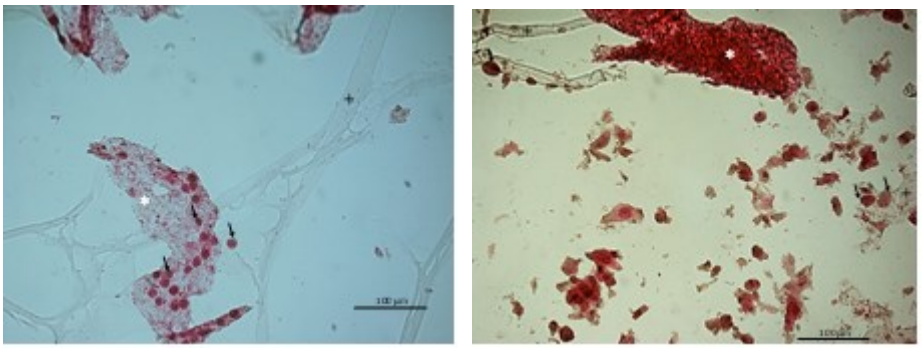
The designed scaffold is like a sponge as shown in SEM image (Fig. 1, a). Fig. 1,b shows the same matrix after 7 days complete DMEM treatment and Hematoxiline&Eosine (H&E) addition. The hydrogel is not dyed by H&E.



(a) **(b)**

Figure 1: SEM images of the dried scaffold (panel a, on the left side) and of the scaffold after 7 days complete DMEM treatment and Hematoxiline&Eosine (H&E) addition (panel b, on the right side).

Scaffold samples were pre-treated one hour with medium in order to hydrate the sponge before cell seeding. Sample were put in 24 wells not-tissue cell culture plates. For each matrix sample, $3 \cdot 10^5$ cells/well of HCC1569 or MDA231 in medium (RPMI for HCC1569 or DMEM added with 10% FBS,1% L-GLU and 1% P/S for MDA231) were seeded and cultured for 7 days in incubator at 37°C. After incubation, the samples were fixed in 2% Agar and sliced at crio-microtome for histological analysis. The slices were treated with Hematoxiline&Eosine to evidence cells and scaffold morphology. Fig. 2 shows the filaments of the hydrogel scaffold where cells are adhering to the scaffold and the ECM produced by cells, too. In this case, even if cells are both from breast cancer the structure of ECM is different as evidenced by stars in Fig. 2.



(a) **(b)**

Figure 2: (a) HCC1569 and (a) MDA231 MB cells cultured 7 days and dyed with H&E. Star shows ECM, arrow the cells and plus symbol the original fibres of the scaffold.

After 7 days MDA231 sample was electroporated. Before voltage pulse application, propidium iodide-enriched EP buffer was added to cell medium. Propidium iodide is a fluorescent dye able to cross the plasmatic membrane if the cell is electroporated [10]. The 8 voltage pulses (750 V at 5kHz, 100µs pulse length) were applied to the sample using 10 mm large plate electrodes with a gap of 7.5 mm. Finally, the samples were observed at fluorescence microscope: some nucleus in the acquired layer appear red (Fig. 3) confirming electroporation effectiveness.

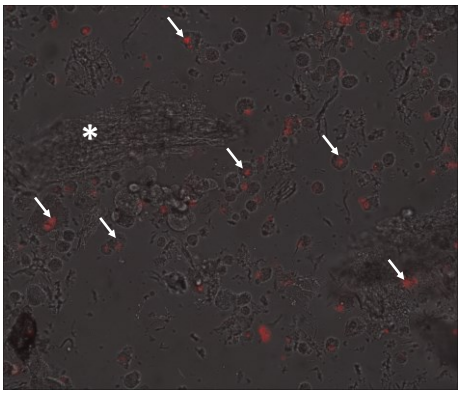


Figure 3: Superposition of bright field and red images (magnification 20x). Star shows ECM and arrow the cells.

Conclusions

The proposed 3D myxoid-mimetic scaffolds can promote both cell-cell and cell-matrix 3D interactions: the detected cell morphology is very similar to histology of biopsy samples; cells appear round-shape and not elongated. After adhesion, the cells produced their proper ECM. Electroporation procedure was performed and gave the expected results.

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Biofabrication with Alginate di-Aldehyde/Gelatine (ADA/Gel) Hydrogels: Progress, Applications and Challenges

*T. Distler, F. Ruther, R. Detsch, A.R. Boccaccini **

Institute of Biomaterials, Department of Materials Science and Engineering
University of Erlangen-Nuremberg, 91058 Erlangen, Germany
**e-mail: aldo.boccaccini@ww.uni-erlangen.de*

Keywords: hydrogels, biofabrication, bioplotting, alginate di-aldehyde, gelatine

Introduction

The investigation of suitable biomaterials for 3D-printing based regenerative approaches (biofabrication) has been an uprising field for the last decade [1, 2]. The alginate di-aldehyde / gelatine (ADA/Gel) hydrogel has been extensively researched due to its combination of high biocompatibility [3] paired with printability [4, 5] and tailorable physico-chemical properties [6, 7], providing a versatile material platform for applications in tissue engineering [5, 8].

We present the current advances in the development of ADA/Gel matrix for biofabrication including applications in bone, cartilage, muscle, and blood-vessel tissue engineering. Alterations in ADA/Gel formulations have been implemented, allowing different printability depending on the field of interest and the process requirements behind the fabrication of different cell-laden tissue constructs.

Results and Discussion

ADA/Gel has successfully been fabricated in tubular, vessel-like structures. Based on the general applicability of alginate based hydrogels for vascular tissue engineering [9], cell laden ADA/Gel was investigated as a potential hydrogel platform for developing tubular structures by extrusion using double-nozzle approach. The process allowed the formation of hydrogel vessels of different diameters, dependent on the double needle system employed. The technique also indicated the possibility of a cellular polarization in the longitudinal direction of the bioplotted hydrogel vessels and the stiffness of the hydrogel vessel constructs was tuned by utilizing either Ca^{2+} or Ba^{2+} ions.

Concerning the aim to investigate the potential fabrication of other tissue types requiring layer-by-layer deposition of cell-laden hydrogel, an improved printability of ADA/Gel was achieved by tuning the chemical composition. It was found that by a refined gelatine preparation protocol, porous hydrogel scaffolds with a height stability of up to 1 cm could be fabricated. The hierarchical bioplotting of ADA/Gel for application in cartilage tissue engineering was investigated to mimic the native complex structure of hyaline cartilage. It was possible to achieve different layer-by-layer composed ADA/Gel scaffolds with graded macro-porosity.

Through the bioplotting technique utilized, different parameters are prone to influence cells encapsulated inside the bioink. For example in the context of cardiac tissue engineering, mouse myoblast muscle cells (C2C12) were embedded in ADA/Gel formulations to investigate how bioplotting parameters, namely scaffold geometry, pressure, cell density and nozzle size, influence the formation of myotubes of muscle cells.

Conclusions and Outlook

The ADA/Gel hydrogel system has been proven to be a high versatility matrix that can be applied in several tissue engineering branches, such as bone, vascular, cartilage or muscle tissue engineering. Due to its two main components, ADA and gelatine, two main gelation mechanisms exist. The ionical crosslinking of ADA and the thermal gelation of gelatine allow to tune the viscoelastic properties of the bioink towards high printability as well as to tailor the final hydrogel stiffness to match that of the target tissue (soft tissue, hard tissue). ADA/Gel is thus a versatile hydrogel platform with unique capacity of altering properties (mechanical properties, degradation behaviour) yet not compromising biocompatibility. Future research will involve biofabricating cell-laden ADA/Gel constructs and investigating their long-term behaviour in realistic in vitro and in vivo conditions.

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Osteoconductive hydrogels filled with calcium phosphates for bone grafting

N. Orlov[a,d], *A. Tikhonov*[a], *E. Klimashina*[a,b], *P. Evdokimov*[a,b], *V. Putlayev*[a,b], *I. Scherbackov*[c], *V. Dubrov*[c]

[a] Department of Materials Science, Lomonosov Moscow State University, Russia
[b] Department of Chemistry, Lomonosov Moscow State University, Russia
[c] Faculty of Fundamental Medicine, Lomonosov Moscow State University, Russia
[d] Federal Institute for Materials Research and Testing (BAM), Berlin, Germany

Keywords: hydrogels, octacalcium phosphate, stereolithography, osteoconductivity, permeability, simulation

Introduction

Implants based on biocompatible materials can be used for restoring the functions of damaged bone tissue and replacing the lost bones. When a polymer with viscoelastic mechanical properties is introduced into the materials, it is possible to create a composite material that could be reversibly deformed over wide range without significant stressing surroundings. In addition to the similarity of their chemical composition to that one of native bone, architecture of the material should facilitate the flow of biological fluxes, bone ingrowth (i.e., osteoconductive properties) and determine the required mechanical characteristics (strength/ stiffness).

This work was aimed at the development of osteoconductive composite materials with viscoelastic properties based on calcium phosphate-filled hydrogels for their use in the reconstruction of bone tissue. The following tasks were accomplished:

a) fabrication of layered calcium phosphates (brushite $CaHPO_4 \cdot 2H_2O$ and octacalcium phosphate (OCP) $Ca_8(HPO_4)_2(PO_4)_4 \cdot 5H_2O$);

b) design of osteoconductive architecture of implants in sense of reaching high permeability and low stiffness;

c) testing the ways of uniform filling of hydrogels by calcium phosphates;

d) search for optimal parameters of stereolithography 3D-printing of composite hydrogel/calcium phosphate implats, viz., type and concentration of photoinitiator, the degree of dilution of the monomer, the degree of loading the gel with calcium-phosphate powder;

e) carrying out the rheological, mechanical and biology tests of the biocomposites.

Results and Discussion

In the course of this work, hydrogels based on polyethylene glycol diacrylate (PEG-DA), which are mostly used for the regeneration of the soft tissues, were studied. To create the biocomposite, a slurry, based on aqueous solution of biocompatible PEG-DA (M_w = 700 Da), and containing photoinitiator (PI) Irgacure®819 (phenyl *bis* (2,4,6-trimethylbenzoyl) phosphine oxide), quinoline yellow dye (maximum extinction at 405 nm), and the filler (either a) synthesized powders of brushite and OCP, or b) salts of $CaCl_2$ with a mixture of NaH_2PO_4 and Na_2HPO_4), was prepared. The composites were fabricated by means of stereolithography as a result of slurry photopolymerization under the irradiation (ca. at 390-440 nm) by LED source of DLP-projector of the 3D-printer Ember (Autodesk, USA).

Flow of water and uniaxial and unilateral loading of different architectures, including open-cell and triply periodic minimal surfaces (TPMS) collections, were FEA-simulated to determine the most permeable and the least rigid samples. It was shown that the Kelvin structures (from open-cell collection) and the "gyroid" type (from TPMS-collection, fig. 1) with 70% pore fraction are the most permeable and mild. For suspensions of different compositions, photosensitivity and critical polymerization energy were determined depending on the concentration of the photoinitiator, the dye and the reinforcing filler. When the suspension was filled with powder (up to 10% by weight), it was possible to obtain the composites with a uniform distribution of the filler over the volume of the hydrogel. The mechanical characteristics of unfilled hydrogels and the composites were determined by rheometry oscillation tests. Elastic shear modulus ranged from 40 kPa to 100 kPa depending on the composition of the slurry, viscous shear modulus - from 1 kPa to 6 kPa, which corresponds to the loss factor - at 2-8 °, were acquired.

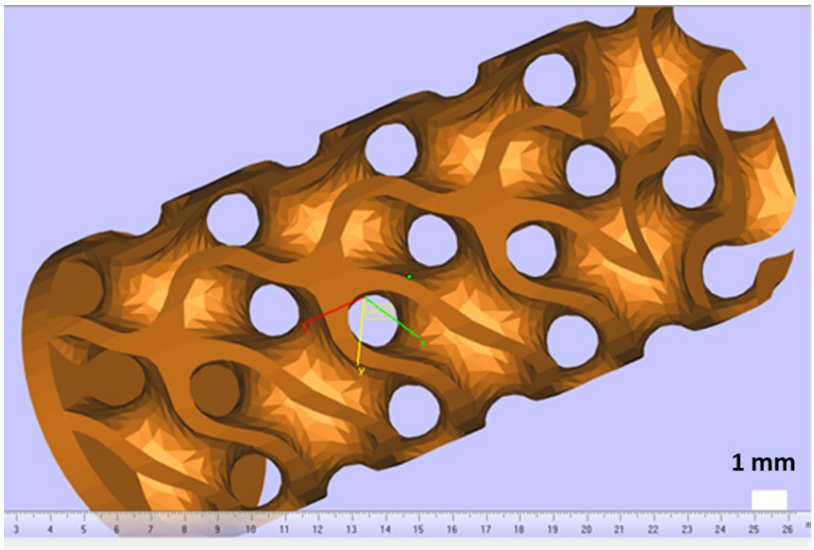

Figure 1. 3D-model of biocomposite implant (hydrogel/brushite) in the form of a cylinder (diameter 6mm, height 10-12 mm) with a "gyroid"-type architecture.

Preliminary *in vivo* study on the model of monocortical rat defect was done. To develop options for creating a bone defect experiments, a computer microtomography (microCT) was performed using a computer X-ray microtomograph of rat femur to create a three-dimensional model. To create full-scale models of phantoms of the femoral femur, a stereolithographic 3D-printer Form2 (Formlabs, USA) was used.

Conclusions

1) Study of the optical properties of suspensions revealed the main problem of stereolithographic printing of hydrogels due to the high photosensitivity of an optically transparent medium. It was shown that the addition of a dye, a calcium phosphate filler with different size of the particles, as well as variation of the photoinitiator content, allow to reduce the photosensitivity and achieve an acceptable printing resolution along z-axis (up to 100 μm).

2) Uniform filling of the hydrogel (up to 10 wt.%) was succeed only by introducing the pre-synthesized crystals of brushite or OCP into green PEG-DA monomeric solution. In case of one-sided diffusion of Ca^{2+} or HPO_4^{2-} ions from a salt solution into the polymerized hydrogel, phosphate crystals were formed predominantly on the surface of the hydrogel contacting with the solution.

3) Rheological, mechanical and toxicological tests of calcium hydrogel/calcium phosphate composites have been carried out, and the process of their swelling in water has been studied depending on the composition of the photosuspension (molar mass of the monomer, water fraction, PI and filler). It was shown that the introduction of calcium phosphate filler reduced the degree of conversion of the C = C into ordinary bonds under hydrogel cross-linking, increased the contribution from the viscous element in the rheological description of the material, as well increased of its compressive strength.

4) Within the framework of the proposed *a priori* approach to the analysis of the architecture of osteoconductive implants, it was shown that Kelvin and “gyroid” structures have the greatest flexibility and permeability. The permeability of such implants with 70% porosity are close to that one of cancellous bone tissue (about 1000 darcy, for water flux).

5) Surgical system for forming, filling and fixing rat femur defect was proposed, which makes it possible to study new bone-substituting materials properties *in vivo* with a high degree of reliability. Diaphysial defects were chosen as a potential model, since defects in the metaphyseal zone in rat bone are very small and cannot be fully considered as critical ones. Rat femur was chosen as largest in the animal body and allowing variation the size of the defect. As options, the final defect obtained by drilling the diaphysis through, a monocortical defect and a complete defect was considered. Finally, the monocortical bone defect was selected as a basic model.

Thus, the biocomposite material with “gyroid” structure based on PEG-DA hydrogel that is similar in chemical composition and structure to native bone tissue has been developed, which possesses viscoelastic mechanical properties and reversibly deforms up to 20% and allows full filling of defects of complex shape.

Acknowledgements

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The Condorscan technology from fundamental researches to clinical applications

B. Pelissier[a], *e. Duret*[b], *s. Caserta*[b], *F. Duret*[b]

[a]Professor and past head of restorative department of Montpellier dental school of Montpellier University, France
[b]Château de Tarailhan

The aim of this lecture is to present the only second-generation camera based on multiple fine-tuning research and through clinical cases. The condor camera looks like a small camera the size of a turbine weighing in at a hundred grams.
It is characterized by the presence of two cameras in its tip. It is therefore very similar to our current, intra-oral, 2D video cameras except that it provides us with a 3D image containing the measurements of the objects it observes and thus allows for a double application; scanning to make digital impressions and 3D color visualization for diagnostic purposes.
This second function pushed its designers to make it a totally open system. It was therefore necessary to have a system capable of sending files to any CAD/CAM equipped laboratory where the dental technician would be able to use them with the devices already equipping his laboratory but also files that could, easily, be incorporated into a telemedicine chain.
This, ambitious, goal was achieved by this small intra-oral scanner. All digital impression enthusiasts know that the first technology used in this field was based on the projection of a grid onto the teeth. The deformation of these straight grids allowed us, the possibility, of calculating the objects' geometry and its dimensions.
True dental version of a cubic painter like Vasarely , introduced for the first time in the dental offices during the Eighties by Prof Duret and a French company, Hennson SA. The chosen technology was emulated by many resulting in no less than 20 intra-oral scanners based on the concept. This new camera, called Condor, turned its back on this proven technology, first because it was expensive and second because it often needed the teeth to be powdered, depriving it from full color analysis and finally because it made scanners fragile. To solve these complex problems and meet the objectives set, Aabam (French SAS) first collaborated with the space industry (CNES) before developing a, more and more powerful software, specific for the dental community.
It was no longer a question of projecting deformable grids on the teeth complicating the scanners, but of finding in the image itself the information necessary for dimensional reconstruction. As a result, it is no longer the grid and its deformation that is measured but the object itself, as it is seen. Whereas color was the main problem in grid deformation analysis, in this innovative technology it becomes its ally in finding the details necessary for recognition. The Condor's two eyes, I should say the two cameras, do the rest. The on-board and dynamic stereoscopic scanning method allows the measurement of dimensions and the filtering of information, based on complex calculations and filters. We apply in a dedicated and medicalized way, the topographic scanning methodology, by cameras embedded in satellites. Condor bases its software, and therefore its measurements, on pure passive stereoscopic techniques that capture its information in the oral environment without any other artifice.
As far as hardware is concerned, electronics and the miniaturization of its components; mainly the ones accompanying mobile telephony, have enabled us to build a compact, precise and light scanner. As for data transmission, it is based on a completely open, standard, language because we did not want to lock the clinician into any type of CAD/CAM hard- nor software. It is a standard STL (or PLY) that can be read by any design software or by any unit based on additive manufacture (rapid prototyping) or subtractive manufacturing (milling machine) as long as it does not impose add-ons that force the user to buy licenses. It goes without saying that if the data can be sent to a laboratory, it can also serve a direct CAD/CAM system integrated into the dental practice. More and more CAD/CAM software (like ExoCAD) have understood this. They can receive standard STL files allowing the dentist to work on his temporaries or send his data to his dental technicians. The same applies to rapid prototyping machines. A dentist using condor can equip himself to make models or temporary crowns in on straight flow.
For its development, numerous tests were carried out in vitro and in vivo. Comparing the CondorScan with a high-precision 5-micron laboratory scanner has allowed us to conclude that the Condor's accuracy is between 30 and 50 microns. For Full Arcades, comparisons show an accuracy between 20 and 50 µm between the reference pieces and the dental preparations

Mean	accuracy tests v3.3.0 in vitro	accuracy tests v3.3.0 in vivo
Single preparations	34µm	43µm
Ful arches	79µm	96µm

Full arch time v3.3.0 in vivo	
scan	**reconstruction**
2min 23	**1 min 52**

Very beautiful images can be even obtained by zooming characterizing in a real way dental, gingival tissues and dental lesions. The clinical results are very good qualities with a remarkable precision as well as files STL and PLY obtained and passed on in

laboratories; the plans of the lines of finish are very easy to realize so that the occlusion and its transfer of data in laboratories. The chain CAD-CAM was validated by numerous tests and clinical cases. The optical camera Condor is ready.

The Possibility of Dentin Regeneration with Currently available Commercial Products

John C Comisi

Assistant Professor - Oral Rehabilitation, Medical University of South Carolina.
email: comisi@musc.edu

Keywords: Dentin remineralization, bioactivity, biomineralization

1. Introduction

Dentin is a complex and unique substance in the human body. It has been defined as "the most voluminous structural component of human tooth. Dentin protects pulp tissue from microbial and other noxious stimuli. It also provides essential support to enamel and enables highly mineralized and thus fragile enamel to withstand occlusal and masticatory forces without fracturing. Furthermore, it is the first vital tissue to meet external irritation, and instead of being merely a passive mechanical barrier, dentin may in many ways participate in dentin-pulp complex defensive reactions."[1] This defensive action is all predicated as a reaction to an acid attack on the dentin. It is the acids from the advancing bacterial invasion into the dentin. This acid attack creates a change in the overall composition of the dentin. Fusayama[2] described the two types of carious dentin, infected and affected, with the infected dentin being a denatured structure that has lost its mineralization characteristics via the loss of its calcium and phosphate composition. Goldberg, et al[3] in their work have stated that there is a cascade of events that lead to the mineralization of collagen based tissue including the interaction of calcium ions with the acidic residues from the demineralization process. This complex then combines with phosphates to initiate a nucleation process. The collagen matrix provides a spatial template upon which the mineral crystals can deposit. And this entire process of collagen synthesis is controlled by the odontoblasts.

But how can we in dentistry assist this process? We have attempted to do so with "dentin replacements" such as: calcium hydroxide, glass ionomers, composites and calcium silicates. Calcium hydroxide (CaOH) has been considered the gold standard in this process. Its effectiveness is based on the ability of it to act with moisture to release the calcium from the material. If resin is introduced, there is a loss of this release since most resins used in our current dental materials are hydrophobic. Calcium silicates have been effective in that they too can release calcium from their matrices as well. However, rather than the calcium dissolving from the material as with calcium hydroxide, the calcium leaches out as CaO+ and then reacts with water (H_20) to form Ca^{+2} and OH-. This leads us to the subject of bioactivity and biomineralization.

2. Discussion:

The concept of bioactive materials was first introduced in 1969 and later defined as a "material that elicits a specific biological response at the interface of the material which results in the formation of a bond between tissues and the material."[4] A new definition has been suggested stating, "a bioactive material is one that forms a surface layer of an apatite-like material in the presence of saliva or a saliva substitute"[5]. As described here these materials deliver minerals that are beneficial to the tooth structure and facilitate and that stimulate mineralization and the formation of chemical bonds that help to seal the tooth and prevent microleakage[6]. These materials are active, not passive, play a dynamic role and perform favorably the oral environment. They have the potential to reduce sensitivity[7], reduce marginal leakage and marginal caries[8] and can be significantly less technique sensitive[9].

Biomineralization is defined as how living organisms secrete inorganic materials in an organized manner[10]. It is how the formation, structure and properties of inorganic solids are deposited in biological systems. This occurs via the selective extraction and uptake of elements from the local environment and how they are incorporated into functional structures under strict biologic control. The formation of biologic apatite is a biologically controlled process and requires nucleation sites to be present. Nucleation is the initial process of crystalline formation; it requires calcium and phosphates to be present to create a complex from which there can be a continuous growth of crystalline structures. If this is accurate, then simple crystalline formation does equate to biomineralization. Camilleri[11] has stated that although materials form crystals, it doesn't mean it is doing what is needed. Crystals larger then 40nm will not aid in remineralization of dentin, as shown by Hench and Greenspan[12] and Sauro and Pashley[13] since they may not "fit" into demineralized dentin. These minerals should also be in an amorphous state of calcium phosphate to enter into the collagen fibril in a stabilized "fluidic" state. The hierarchal nano-apatite assembly mechanism associated with collagen mineralization is a precisely controlled process that recapitulates the gap and overlap of collagen molecules[14]. It possible to pre-fabricate intermediate precursors that can enable intrafibrillar remineralization in novel restorative materials[15,16]. It has been suggested that this remineralization process is influenced by the Gibbs-Donnan effect is that more water moves into the extrafibrillar compartment than would be predicted on the basis of oncotic pressure of poly(allylamine) hydrochloride (PAH) molecules alone. This works provides insights to the driving forces for infiltration of polyelectrolyte-stabilized prenucleation clusters into the water compartments of collagen to initiate intrafibrillar mineralization[17].

3. Conclusion/Outlook:

The formation of a crystalline structure at the restorative/dentin interface is very attractive as a means of overcoming the challenges manifested in resin-bonded adhesives. First, consider that hydration and activity of materials in vivo may not be similar to those displayed in vitro because of insufficient fluid available in contact with dentin[18]. Second, crystalline formation of our materials must be associated with water and osmotic forces, which allow the transmission of the necessary ions (Ca and P) to act as nucleation sites and the effective penetration of these ions into the fibrillar and intrafibrillar collagen. The presence of water is critical, so that the demineralized collagen structure can accept the needed ions to rebuild. This cannot be accomplished with traditional hydrophobic materials and methods. Crystal formation must also be of the correct size and in the correct proportions since crystals too large may not be usable by the tooth structure and too much apatite may be more problematic and prevent true biomineralization.

New materials are needed to restore the dentition, which have; appropriate physical properties to withstand the hostile forces of the mouth and the essential ability to enable the tooth to "breath" in the oral environment; create a bioactive/biomineralizing interface to allow Ca/P ion release to "feed" mineral starved tooth structure via nucleation enabling appropriate biologic biomimetic remineralization processes so that the elastic modulus, structure and hardness of collagen fibrils can be completely recovered. We must embrace water. Water is not the enemy that it has been portrayed. It is the enemy of hybridization, which interestingly can't occur without water, yet also can't be done well with water. We have also learned that in order to biomineralize collagen and tooth structure, water is essential for the osmotic gradient to set up and enable expansion of the collagen to enable ion transfer and preserves proteins in that structure. The presence of water enables the nucleation of calcium and phosphates into the collagen. The development of commercial materials, such as Novamin, MTA, Biodentine, and the Activa family of products are leading the way in this arena. However, there is a need for further development and commercialization of other restorative materials that can predictably be used each day in clinical practice which will work within the moist oral environment, protect the tooth surfaces they are applied to and enable long term success in the hostile oral environment.

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Endosseous Implants Surface Modifications

Georgios A. Katikarides

Dentist, Periodontics Department 251 General Airforce Hospital, Athens
e-mail: bio.katik@gmail.com

Keywords: *Endosseous implants coatings, wettability, Nanoroughness, bioactivity, tissue engineering, hybrid implants*

Introduction
Endosseous implants already count five decades of application on reconstructive dento-maxilloficial surgery. Continuous research has broaden our knowledge regarding tissue - implant interactions highlighting the importance of implants physical and chemical attributes in the success of the implantation protocols.

Results and discussion
Early research by pioneers on this field, such as Branemark and Mitch focused on implants geometry, a crucial factor on stress distribution, and the bulk material of implants which implied its mechanical properties. A Ti- alloy (cTi) was established as industry standard due to its relatively bioinert nature and isotropic behavior with human bone in compliance with Frosts' mechanostat theory of bone metabolism. Thereupon scientists' attention was switched on bone - implant interface which is greatly affected by surface characteristics of the implant. It was found that surface energy, wettability and micro roughness greatly affect the development of the integration bond both qualitative and quantitative. Consequently new techniques of thermal, electrical and chemical modifications were developed, leading to a new generation of implants with greater functional contact surface and initial stability, leading to a better healing and weight bearing ability. However clinical experience shows that its usage is still a sensitive procedure on mechanical and microbiological parameters and there is plethora of clinical conditions where implantation remains challenging. Nowadays our knowledge of tissue specific wound healing cascade provides a better understanding of the importance of the implants' surface Microorganisms' adhesion and biofilm formation remains a challenge in the oral environment that cannot be overlooked , while initial blood clot stability, osteoblastic cell proliferation and adhesion, protein adsorbance and neoangiogennesis as well as tight epithelial attachment are crucial factors for a successful Osseointegration. . It is clear that different surfaces promote osseous healing while others promote soft tissue attachment and thus hybrid systems should ideally be constructed. For these reasons, a new generation of coated implants is being developed, introducing revolutionary, novel properties to the implant systems, aiming in the manipulation of tissue healing. From biologically inert HAp to bioactive TCP and other bioactive inorganic and organic molecules there are two greater families of activated implant surfaces. On one hand some techniques aim on strong physical or chemical bonding on implants surface of bioactive molecules with direct deposition, altering their topography and chemical behavior, while on the other hand there are techniques of coating biodegradable films, with indirect deposition. Those films contain bioactive substances on polymeric scaffolds and can lead to controlled release of bioactive chemical profiles under predefined stimuli and thus modulating the tissue response on a biological level. Moreover there is enough evidence that living stem cells can be incorporated in such implants matrixes thus manipulation of healing sequence can be achieved. These hierarchically structured surfaces can incorporate a series of bioactive molecules as shown in *figure 1*. While they offer a potential for patient and tissue specific implants, the complexity of their chemical composition, sensitivity of their manufacturing process, and lack of decontamination and surgery protocols shows that there is need for extended research on this field in the years to come , before clinical trials is possible. Moreover we are yet far from understanding the biological responses in vivo that many times are not in compliance with in vitro testing.

Conclusion: Since the first implant surgery we have come a long way on understanding how Osseointegration is possible. New findings suggest that implants surface chemical and topographical aspect play a crucial role as far as the tissue response is concerned, especially on the first stages of the healing cascade.

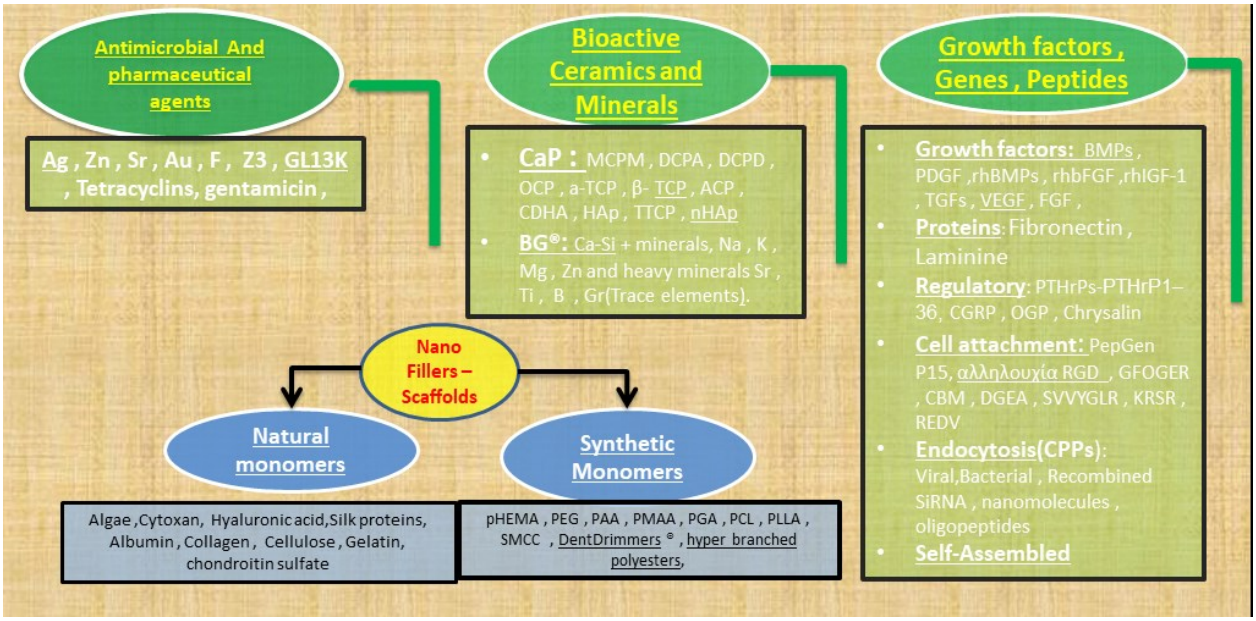

Figure 1 Bioactive molecules that can be incorporated on implant systems and monomers that build scaffolds.

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The Restoration Guided Creeping Attachment (RGCA) technique for the treatment of non-carious cervical lesion combined defects

G. Derchi[a], *V. Borgia* [b], *V. Marchio* [c], *U. Covani* [d], *A. Barone* [e]

a Researcher, University of Pisa, Italy
b Private Practice, Lecce, Italy
c Private Practice, Lucca, Italy
d Full Professor, Department of Surgical Pathology, Medicine, Molecular and Critical Area, University of Pisa; Tuscan Stomatologic Institute, Lido di Camaiore (LU), Italy
eProfessor and Chairman, Unit of Oral Surgery and Implantology, Department of Surgery, University of Geneva, Switzerland
e-mail: gnolo78@gmail.com

Keywords: Creeping attachment ; Gingival Recession ; RGCA ; Healing ; Restoration

Introduction

The management of marginal tissues nowadays has seen a rapid evolution regarding the techniques, surgical and non surgical, aimed towards obtaining optimal health and form.

The objective of this presentation is to present a novel non surgical technique called Restoration Guided Creeping Attachment (RGCA), capable of treating Miller Class I and Miller Class II defects associated to non- carious cervical lesions.

The gingival margin is a line positioned 1 to 2 mm coronal to the CEJ, following its scalloped profile[1] and a gingival recession occurs when the gingival margin is displaced apically and root surface is exposed[1].

These gingival recessions were divided in four classes by Miller in 1985[2]: the classification is based on the degree of apicocoronal extension of the recession, its relationship with the mucogingival junction and the loss of interdental hard and soft tissue.

A combination between non-carious cervical lesions and gingival recessions is a common clinical finding: these lesions are presented as a loss of hard tissue at the cementoenamel junction (CEJ) of a tooth and have been shown to be caused by a combination of erosion, abrasion and/or attrition[3].

Surgical treatment of these lesions can be made difficult because of the absence of a CEJ caused by a non- carious cervical lesion[4] and an exclusively restorative approach for root coverage can result in aesthetic disharmony. Using a combined surgical and restorative approach, however, can improve both dentin hypersensitivity and the final aesthetic result.[4]. As of today, however, the surgical treatment remains the gold standard.

RGCA is a technique aimed at forming a blood clot under the marginal gingiva and protecting it with a provisional restorative composite margin. This is inspired from the Biologically Oriented Preparation technique (BOPT) and is based on the modelling and recontouring of a class V composite resin restoration to obtain progressive repositioning of the gingival margin and obtain a creeping attachment.[5]

Results and Discussion

The coronally advanced flap, with or without a connective tissue graft, is deemed as an effective surgical technique: its disadvantages, however, have pushed clinicians and researchers to find alternative methods.[6] Using RGCA, as of today, a repositioning of the gingival margin in a coronal direction was observed: it was not possible, however, to detect the nature of the tissues present at the margin of the gingiva after the procedure.

Even if the gingival margin is very close to the restoration, no significant signs of inflammations were detected. An interesting phenomenon called “creeping attachment” could be related to the results obtained using this technique. It was described in the 1970s as the “postoperative migration of the gingival marginal tissue in a coronal direction over portions of a previously denuded root”[7] and some Authors observed that after a free gingival graft for the treatment of gingival recessions, a certain amount of creeping attachment could be detected over a period of 2 years[8] and that several factors could influence the phenomenon such as recession width, position of the tooth and the hygiene habits of the patient[8].

Conclusions and/or Outlook

The authors hypothesize that creeping attachment, a phenomenon that has been observed in periodontology for the past 50 years, can in some way be stimulated and guided to repair the aesthetic appearance of marginal tissues and resolve moderate gingival recession. However, it is important to note that a greater number of patients, different radiologic and histologic examinations and longer follow-ups are needed to assess the reliability of this technique and to help assess the clinical situation in which it could be used: the histologic investigation could help to explain and support the clinical observations establishing the nature of the soft tissue modified by RGCA and its reliability in long-term results.

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How Can a Resin be Bioactive?

L. Clark

Director of Clinical Affairs and Marketing
Pulpdent Corporation
**e-mail: larry@pulpdent.com*

Keywords: Bioactive, Composites, Diffusion, Nucleation, Apatite

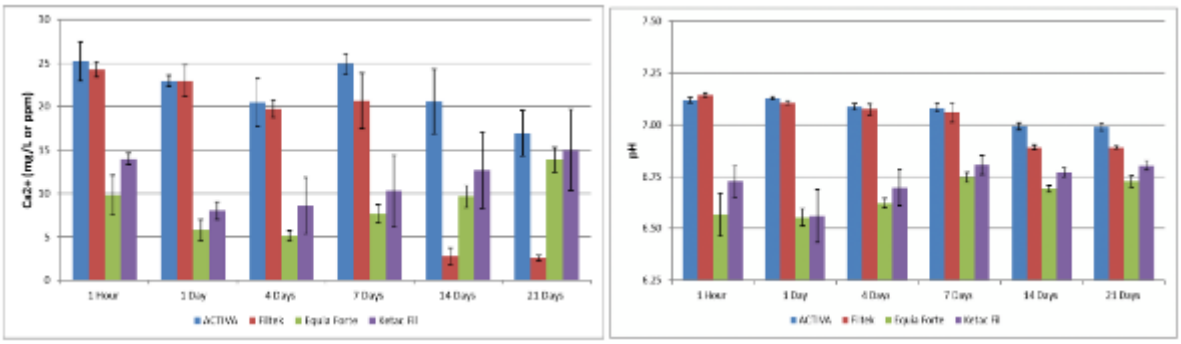
Introduction

The desire to have an aesthetic and bioactive material has only recently been explored. The benefits could be many including the ionic seal of the restorations, reduction of demineralization, and the limiting of biofilm activity. L. Hench defined bioactive material as one that elicits a specific biologic response at the interface of the material, which results in the bond between the tissues and the material. Several aspects have hindered the manufacture of a dental composite resins to be bioactive. To date, most dental composite resins have been hydrophobic in nature. Hydrophobic resins inhibit the exchange of ions with the surrounding dentition and reduce intimate association to moist dentition. With the development of a hydrophilic resin, it is possible to introduce minerals in ionic form such as calcium, phosphate and fluoride. These ions now have the opportunity to diffuse, release and recharge with pH adjustment. In addition, to have hydroxy apatite forming potential, functional biomimetic analogs are also desired. The idea of a composite resin with bioactive potential may be possible.

Results and Discussion

Results: In-vitro testing has been performed to show ion release of calcium, phosphate and fluoride. One in-vitro experiment has been performed to show surface deposition of apatite. One in-vitro experiment has been performed to evaluate dentin integration. Two abstracts have been presented to show the influence on enzymatic activity, MMP's. One study has been presented to show the release and recharge of a bioactive resin. One study has been published showing the bioactive nature using human stem cells. One abstract has been presented showing ion release with dental adhesives. One study and abstract has been presented to show biocompatibility in the bone of rats. Two peer-reviewed articles have been published illustrating in-vivo clinical results.

Discussion: The bioactive resin has been shown to release and recharge essential minerals in-vitro. This exchange creates the possibility for interaction with tooth-structure as well as saliva.



The release of these Ca ions may be a possible reason for buffering of the acid response preventing enzymatic degradation within the bonded dentin interface.

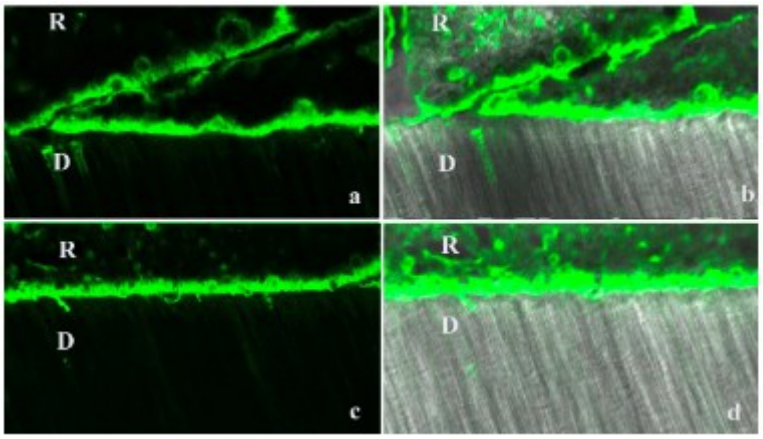


Figure 2. Resin-bonded dentin interfaces prepared with Activa Restorative, incubated with quenched fluorescein-labeled gelatin. D = Dentin; HL = Hybrid Layer; R = Restorative Material. **(a,c)** Images acquired in green channel showing fluorescence of the samples. **(b,d)** Image acquired as optical microscope showing the morphology of the sample.

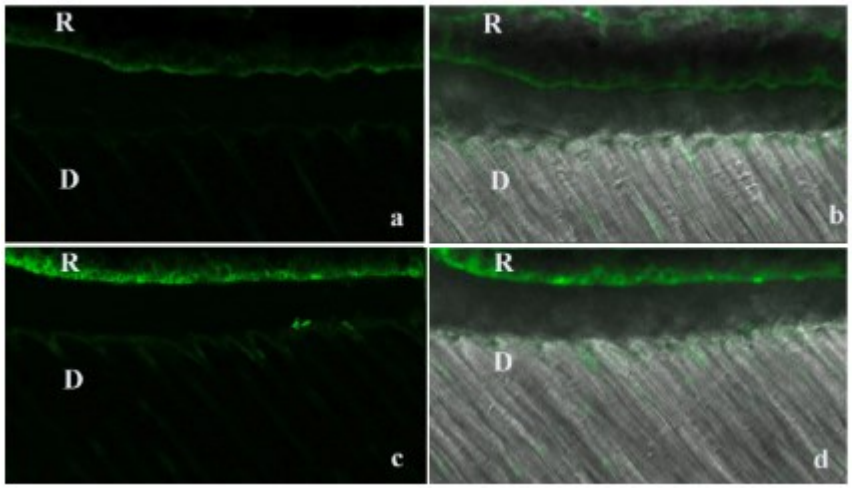


Figure 4. Resin-bonded dentin interfaces of 1-month-aged samples prepared with Activa Restorative, incubated with quenched fluorescein-labeled gelatin. D = Dentin; HL = Hybrid Layer; R = Restorative Material. **(a,c)** Images acquired in green channel showing fluorescence of the samples. **(b,d)** Image acquired as optical microscope showing the morphology of the sample.

Conclusions and/or Outlook

Today, it now possible to use clinically, resin base restoratives materials that are able to release ions and recharge with pH changes. In addition, such innovative materials can stimulate hydroxyapatite formation in dentin and enamel, as well as reduce the enzymatic degradation within the bonded/dentin interface

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New possibilities of using bioactive resins in dental surgery and endodontics

M. Maciak

Alfa Dental - Private Healthcare Institution, Kraków
martamaciak2012@gmail.com

Keywords: bioactive resin, bioactivity, bone regeneration

Introduction

Modern approaches to dentistry focus on the preservation of tooth structure, while with the introduction of bioactive materials a potential breakthrough in dental treatment can be achieved. The use of bioactive materials in dentistry has significantly evolved over the last decade. One of the advantages of bioactive material is its ability to elicit a response from tissues by releasing calcium and phosphate ions leading to remineralisation and the formation of apatite. These "smart materials" can also react to pH changes in the oral environment to elicit changes in the material as well as the tooth.

Results and Discussion

Activa BioActive-Restorative and Activa BioActive-Base/Liner (Pulpdent Corp. USA) has been shown to exhibit bioactive properties based on the definition mentioned above of bioactivity. Initial research on Activa's bioactive resin showed that the material imitates nature, plays a dynamic role in the mouth, responds to pH cycles, and delivers minerals in the natural way of remineralisation by releasing calcium and phosphate ions, which are the essential minerals that are present in teeth.

There are bioactive materials designed for special endodontic procedures, such as a root-end filling after apicoectomy, obturation of internal and external root resorption, pulp capping, closing perforations, and apexification. Whereas in the USA the FDA allowed the bioactivity claim for Activa, being the first bioactive resin material, and based on its strength and durability due to a patented rubberised-resin molecule that absorbs stress and resists fracture, the author has used Activa BioActive in lieu of Mineral Trioxide Aggregate (MTA) and Biodentine for selected endodontics procedures. These procedures were not listed in the company's Indications For Use and carried out at the author's discretion after explaining the possible benefits to the patient and a signed written consent.

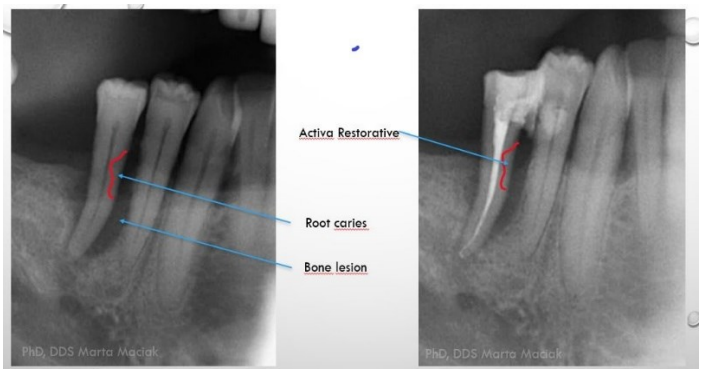

Figure 1: Initial situation

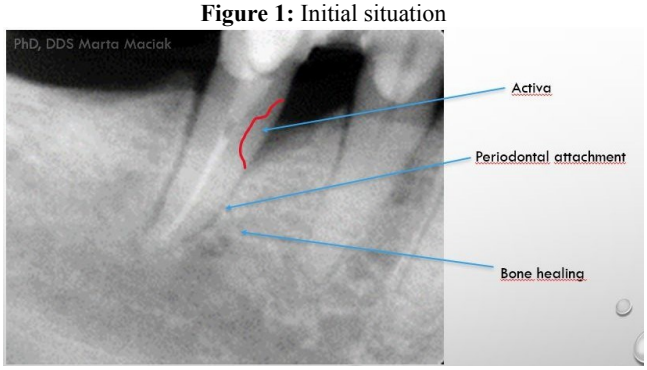

Figure 2: Three months after treatment

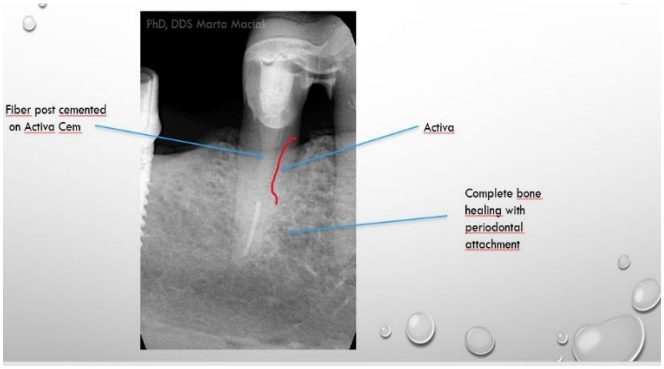

Figure 3: Three years follow up

Conclusions

Based on available published research and after early favourable results had established the effectiveness of Activa BioActive materials, and based on the pH, release of calcium and phosphate ions and apatite formation in the presence of saliva, the decision was made to move forward and expand the number of suitable cases. Although a favourable outcome could not be guaranteed, clinical cases followed over a period of three and more years presented with positive outcomes and provided evidence that the bioactive properties of Activa BioActive materials through its ability to stimulate apatite formation was a viable treatment option. The evidence is herewith presented with X-rays and in CBCT showing new bone formation. Although histopathological evidence is not provided, a periodontal evaluation demonstrated a periodontal attachment in the cases presented here.

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Film properties of dental universal adhesives

D. Papadogiannis[a]*, M. Dimitriadi[a], G Eliades[a]

[a] *Department of Biomaterials, School of Dentistry, National and KApodistrian University of Athens, Greece*
**e-mail: deminho@gmail.com*

Keywords: hardness, adhesion, oxygen inhibition, dental adhesive, degree of cure

Introduction

While dental adhesion has evolved in the past decades, the weakest area in esthetic restorations and the main reason of failures is still the interface between hard dental issues and the restorative material[1]. The most recent advancement in the field of dental adhesives is the introduction of the so called universal adhesives[2]. The manufacturers claim that these materials can be used in different application modes such as self-etch, etch and rinse or selective enamel etching enamel, with all modes of polymerization, for direct and indirect restorations.

Moreover, universal adhesives were introduced for bonding resins to other substrates (alloys, polycrystallic ceramics), while the addition of silane, in some of them, provides an additional bonding capacity to glass-ceramic materials.

The aim of this study was to investigate the performance of five commercial universal adhesives regarding their film setting characteristics. The null hypothesis was that there would be no differences between the materials in the different properties tested.

The universal adhesives used in this study were: All-Bond Universal (ABU, Bisco, Schaumburg IL,USA), Adhese Universal (ADU, Ivoclar Vivadent, Schaan, FL), Clearfil Universal Bond (CUB, Kuraray Medical, Okayama, JPN), Prelude One (PRO, Danville Materials, S. Ramon, CA, USA), Scotchbond Universal (SBU, 3M ESPE, Seefeld, D). The following tests were performed:

a) Degree of C=C conversion (DC%)

Specimens (Ø=7 mm, h=1 mm, glass-molds covered with matrix strips, n=5/product) were light-cured for 10 s with a LED curing unit (Bluephase G2, Ivoclar Vivadent, FL, 1,2 W/cm^2 intensity, at standard mode) and stored at 37^{o}C (dark/dry) for 10 min. The directly irradiated surfaces were used to measure the DC% by attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR). Spectra were acquired under the following conditions: 2000-600 cm^{-1} wavenumber range, 4 cm^{-1} resolution, 40 scans co-addition, 2 μm depth of analysis at 1000 cm^{-1}. Spectra were also acquired from unpolymerized materials which served as a reference. The DC% defined as the amount of C=C bonds converted into C-C, was estimated for each specimen on a relative percentage basis with the two-band method and the tangent baseline technique.

b) Hardness

Specimens prepared as before wee used for hardness measurements, performed after 24 h of storage (37^{o}C, dark/dry). The samples were transferred to the reading table of a microhardness tester (HV 2000, Shimadzu, Tokyo, JPN), equipped with a Vickers indenter. Three indentations in triangular mode were performed on the directly irradiated surface of each specimen under 0.2 kp load and 10 s contact period and averaged. The results were registered in VHN (0.2kp).

c) Extent of oxygen inhibition

In order to measure the extent of oxygen inhibition the microscopic method was used. The materials were irradiated for 10 s with the LED curing unit, as before. The extent of the oxygen inhibited zone, oriented from the difference in the refractive index between set and unset materials, was photographed under standard magnification (200X) at three different areas, 10 min and 24h after light curing and the mean value was used as representative of each specimen (n=5/product).

Statistical analysis of the results was performed by one-way ANOVA plus Tukey multiple comparison test (α: 0.05).

Results and Discussion

The results of the experiments are shown in Table 1. The DC% values of the tested materials ranged between 77.7% - 81.5% and there were no statistically significant differences among the universal adhesives.

Table 1. Results of DC%, O_2 inhibition and VHN of the adhesives (means and standard deviations). Same letters show mean values with no statistically significant differences per test.

PRODUCT	DC%	VHN $_{(0.2kp)}$	O_2 Inhibition (μm)
ABU	77.7 (2.9)[a]	3.2 (0.1)	233.1 (8.0)[a]
ADU	78.7 (3.7)[a]	5.3 (0.2)	9.7 (0.3)[b]
CUB	77.9 (1.9)[a]	1.7 (0.3)	6.1 (0.3)[c]
PRO	81.5 (3.9)[a]	1.1 (0.1)	5.6 (0.1)[c]
SBU	81.5 (3.3)[a]	6.6 (0.3)	77.7 (6.4)[d]

Despite the fact that all materials contained high molecular weight aromatic dimethacrylates, the values obtained were high. The inclusion of 2-HEMA which may copolymerize with the pendant C=C groups of the bulky monomers seems to improve the overall conversion.
The hardness values ranged between 1.1-5.3 VHN with SBU showing significantly higher values. However, all values measured were low. Thus, questions may be raised regarding the cohesive strength of these films when subjected to shrinkage stresses of the main restorative material
The O2-inhibition measurements showed very high values in ABU and SBU. The ranking of the statistically significant differences was ABU>SBU>ADU>CUB, PRO. These differences may anticipate variations in the minimum film thickness capable of setting under atmospheric conditions

Conclusions
The universal adhesives tested exhibited satisfactory and uniform curing efficiency, showing relatively high DC% values. On the other hand, based on the oxygen inhibition results, there are significant differences in the thickness of the material that may not set after thin film application. The low hardness found is a concern for their clinical effectiveness and further evaluation should take place regarding their mechanical performance.

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New generation restorative dental biomaterials that modulate biofilm formation

Andrei C. Ionescu[a]*, E. Brambilla[a]

[a] Oral Microbiology Laboratory, IRCCS Galeazzi Orthopedic Institute, Department of Biomedical, Surgical and Dental Sciences, University of Milan, via Pascal, 36, 20133 Milan, Italy
e-mail: andrei.ionescu@unimi.it

Keywords: biomaterials, biofilm, bioreactors, nanoparticles, bioactive

Introduction

The main reason for failure of current dental restorative materials is the development of secondary caries. This process is driven by the activity of a dysbiotic, pathogenic biofilm. A huge importance in causing the dysbiotic shift of an oral biofilm growing on dental materials can be given to a high fermentable sugar intake, and to the lack of buffering effect from the surface of "inert" restorative materials, such as resin-based composites[1,2]. Bioactive dental materials are built on purpose to elicit a response both from the host and from the biofilm permanently colonizing its surfaces. Biomaterials can be designed to feature many properties, such as remineralization, antimicrobial or anti-adhesive activities. It is very difficult, however, to synthesize materials showing these activities and maintaining them over time[3,4]. It is even harder to design bioactive materials that can additionally satisfy strength and durability requirements in a demanding environment such as the oral one. Biofilms can even modify that interface by progressively modifying materials' surfaces or release kinetics[5].

Results and Discussion

The lecture presents the results obtained in this sense by previous studies[3,4,5,6] as well as a study on the clinical performances of a kind of dental biomaterials, that is high-viscosity glassionomer cements (HVGIC) for permanent restorations[7].

The lecture will also disclose the results of unpublished data on the biological and microbiological mechanism of action of HVGIC, such as, for instance, their remineralizing effect on dental hard tissues, and their resistance to secondary caries (Figure

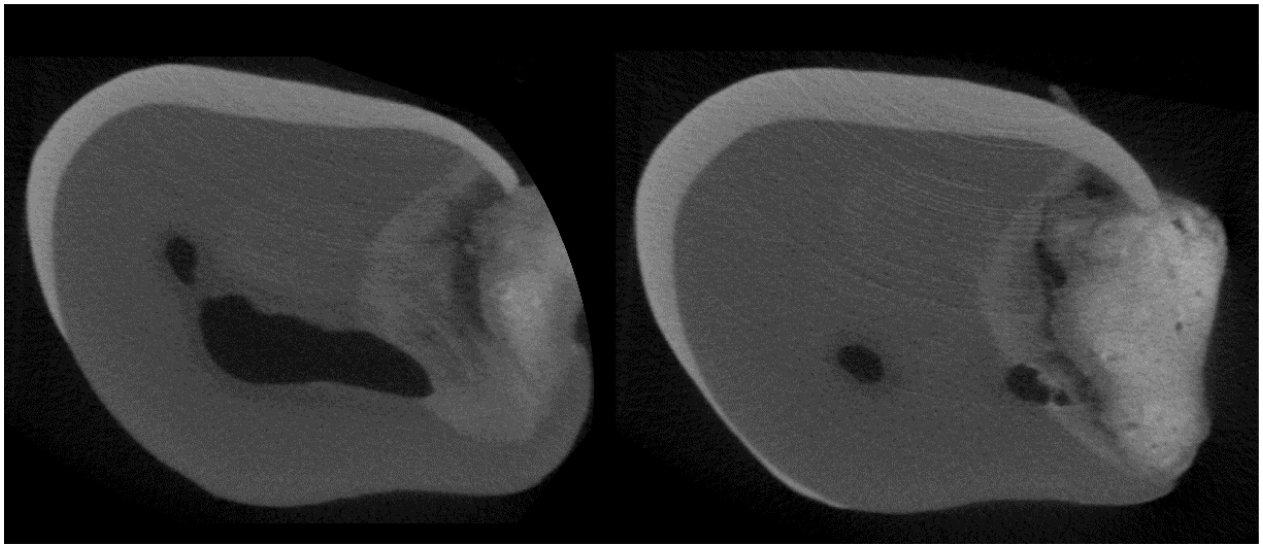

Figure 1: Micro-CT sections at two different levels of an upper third molar, 2 years after application of a bioactive restoration. Hyper-mineralized dentin is clearly detectable as a consequence of ions release from the HVGIC material. The material itself presents a layer with lower density in proximity to tooth structures, as a consequence of the ions release. This remineralizing activity was however not sufficient to prevent secondary caries occurrence, which eventually jeopardized tooth vitality and led to failure of the restorative treatment.

Conclusions/Outlook

The key for obtaining successful dental materials able to positively replace and repair damaged tissues, and to prevent diseases, likely consists in a better understanding of the interactions taking place between materials and biofilms. Next generations of dental materials will be designed considering interactions both with biofilms and the host and will likely be built to show a defined activity over an estimated amount of time, rather than trying to indefinitely maintain their initial properties.

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Optical Nanospectroscopy for Tissue Imaging and Early Cancer Diagnostics

A. Cricenti

Istituto di Struttura della Materia (ISM-CNR), via del Fosso del Cavaliere 100, Rome, 00133, Italy
e-mail: antonio.cricenti@ism.cnr.it

Keywords: (Raman, SNOM, IR, Cancer Cells, ALS)

Carcinomas are complex biochemical systems and in the past their diagnosis was based on morphological differences between malignant cells and their benign counterpart. Recently the paradigm has changed and great interest is focused now on the biochemical profile of tumours in view of the availability of new drugs that specifically target neoplastic cells. This new paradigm requires biochemical analysis of each tumour in order to establish the correct personalized oncological "target therapy". Understanding the mechanism of molecular alterations of a specific tumour is a critical issue to predict the response to personalized therapy. This is important not only for discrimination between healthy and pathological tissues, but also for pre-cancerous tissue state earlier detection and understanding.
The potential of infrared [1] and Raman spectroscopy [2] to characterise cancerous tissues has long been recognised and studies of various cancers by many groups have established that regions of malignant tissue can be easily identified on the basis of its optical spectrum. Early diagnosis of cancer requires an instrument providing specific chemical images at sub-cellular level and the development of diagnostic imaging. Infrared Scanning Near-field Optical Microscopy (IR-SNOM) and micro-Raman set-up meet these requirements provided that SNOM can been coupled with an appropriate infrared light source, that can be based on Free Electron Laser, femtosecond laser or quantum cascade laser.
We present IR-SNOM and micro-Raman in their spectroscopic mode, that is related to the local chemical composition and, thus, to the biological properties of the sample, for tissue imaging and early cancer diagnostics. Applications in the case of Oesophagous [3] and Cervical Cancer [4] as well as in the progression of Amyotrophic Lateral Sclerosis (ALS) will be presented.

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Hybrid nanoplasmonic fibrous biocomposite for surface-enhanced Raman spectroscopy platform

R.V. Chernozem[a,b], *B. Parakhonskiy*[a], *M.A. Surmeneva*[b], *R.A. Surmenev*[b], *A. Skirtach**[a]

[a] Ghent University, 9000 Ghent, Belgium
[b] Tomsk Polytechnic University, 634034 Tomsk, Russia
**e-mail: Andre.Skirtach@ugent.be*

Keywords: scaffolds, biocomposite, SERS, calcium carbonate, Ag nanoparticles

Introduction

Detection of pharmaceutically important molecules in tiny amounts is one of actual task for bioanalytical science. Methods of detection of analytes that are based on chromatographic or spectrophotometric approaches have limits in sensitivity, time and problems with in vivo-detection[1]. Whereas, a very versatile and powerful technique for rapid identication and sensing of different analytes, such as drugs, pollutants, biomolecules, microorganisms, and cancerous cells is surface enhanced Raman scattering (SERS) [2]. A number of plasmonic nanostructures have been designed by the deposition of gold and silver nanoparticles on glass, quartz, and silicon substrates [3]. However, an elaboration of the SERS sensors, which can be applied outside specialized laboratory, is required [3]. Flexible nanoplasmonic platforms (e.g. polymer fibers and cellulose paper) are a new class materials with low cost for fast diagnostics [4]. Flexible SERS platform can be adaptable to a rough substrate in terms of wrapping and bending in comparison with rigid-substrate [3]. Electrospinning (ES) allows to prepare flexible polymer scaffolds with the porous structure that can be effective for the capture of an analyte. Polyhydroxybutyrate (PHB) is a nature origin polymer, which is intrinsically biodegradable and piezoelectric polymer. Whereas, PHB often uses for the ES preparation of the scaffolds. It is well-known fact that the polymer scaffolds possess the hydrophobic surface. Therefore, for deposition of the plasmonic nanoparticles, the pretreatment is required. There are plenty of methods for the polymer surface modification. However, the $CaCO_3$ mineralization is one of the most prospective due to that that $CaCO_3$ particles allow to increase the relative surface area of polymer and can possess porous structure, which can increase an amount of nanoplasmonic particles on the surface of scaffolds. Also, $CaCO_3$ mineralization remain unchanged polymer fiber structure compared to plasma treatment. Thus, the aim of the present study is to fabricate and investigate Raman signal of an analyte on the surface of hybrid nanoplasmonic fibrous biocomposite based on PHB, $CaCO_3$ and Ag nanoparticles by means of the SERS.

Results and Discussion

Figure 1 represents SEM images of the prepared biocomposites in the present work. An average diameter of fibres of PHB scaffolds was 5.2±0.8 μm (**Figure 1A**) Analysis of the SEM image of PHB scaffolds after two cycles of the ultrasound (US) treatment in the solution of Na_2CO_3 (1M) and CaCl (1M) revealed that the surface of the fibers undergoes changes after the modification, as shown in **Figure 1B**. The authors of the paper [5] showed that two cycles of US treatment in solution of Na_2CO_3 (1M) and CaCl (1M) allow to obtain uniform coating polymer fibers of the scaffolds. The similar result was observed in the present work. To functionalize $CaCO_3$-covered PHB fibers with silver nanoparticles, we applied the silver mirror reaction as reported in [6]. First mineralized scaffolds are saturated with Ag ions from the solution of $AgNO_3$ (0.5 M) and ammonium hydroxide (0.5 M). In the second stage, reduction of Ag ions to the metallic nanoparticles is achieved by injection of D-glucose (40 wt%) solution. The alteration of the fiber surface after silver mirror reaction can be seen more clearly on the SEM image obtained at the backscattered electron mode (Figure 1C). The observed alteration corresponds to Ag nanoparticles coated the $CaCO_3$-covered PHB fibers, as reports in [3, 6].

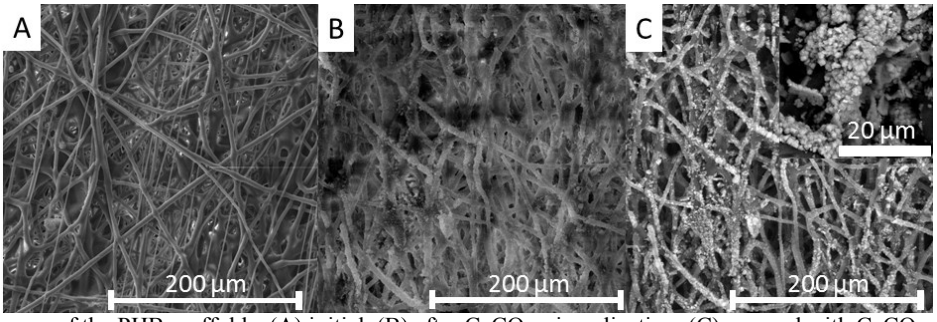

Figure 1. SEM figures of the PHB scaffolds: (A) initial, (B) after $CaCO_3$ mineralization, (C) covered with $CaCO_3$ and plasmonic Ag nanoparticles

Subsequently, Ag nanoparticle functionalized $CaCO_3$-coated PHB fibrous scaffolds were used for label-free detection of molecule, namely Rhodamine 6G. Rhodamine 6G is a fluorescent marker often used for the first testing of SERS platforms [3, 7]. To estimate an enhancement of the Raman signal on the fabricated plasmonic substrates, first, Rhodamine 6G spectrum was

collected from the surface of the quartz glass at the concentration of 10^{-3} and laser power of 167 mW and presented in **Figure 2.** All typical peaks of Rhodamine 6G were observed [3]. All these peaks remain unchanged on the fabricated SERS platform. To calculate enhancement factor (EF) for Rhodamine B the following equation was used (1):

$$E_f = \frac{I_{SERS}}{I_R}\frac{P_R}{P_{SERS}}\frac{C_R}{C_{SERS}} \quad (1)$$

where I, P, and C are intensity, laser power and concentration of an analyte, respectively. The indexes correspond to normal (peak height at 1368 cm^{-1} from stretching of arom C−C) Raman and SERS intensities. Thus, taking the molecule of Rhodamine 6G on the surface of Ag nanoparticles, the average enhancement factor is estimated to be 118196, i.e. 1.2×10^5. It can be seen that the obtained enhancement is higher that for another method of preparing fibrous SERS substrates recently reported in [7].

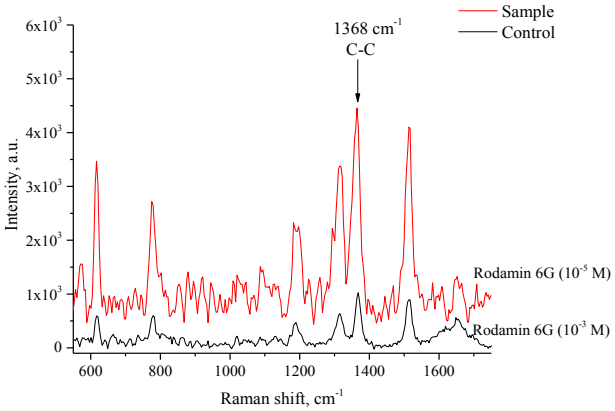

Figure 2. Raman spectra of Rodamin 6G on the glass (as a control) at the concertation of 10^{-3} M and laser power of 167 mW and on the plasmonic fibrous biocomposite at the analyte concentration of of 10^{-5} M and laser power of 0.5 mW

Conclusions

In the present study, fibrous scaffolds based on PHB were fabricated via electrospinning technique. To improve wettability of the hydrophobic PHB surface and increase the relative surface area, PHB fibers were completely coated with $CaCO_3$ particles by means of US treatment. Afterwards, plasmonic Ag nanoparticles were grown on the mineralized biocomposite using Ag mirror reaction. SEM analysis confirmed the presence of $CaCO_3$ and Ag nanoparticles on the PHB fibers. The SERS performance is examined by applying confocal Raman imaging on the plasmonic fibrous biocomposites. This plasmonic fibrous substrate displays effective SERS detection of the standard Rodamin 6G of a concentration less than 10^{-5} M. A calculated enhancement factor was larger than 1×10^5 for the most intensive peak of stretching of arom C−C at the 1368 cm^{-1}. Thus, prepared plasmonic fibrous biocomposites can be applied for effective detection of analytes with high sensitivity.

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Disposable Elastomeric Caps for In Liquid SERS Detection

C. Credi[ab], *R. Cicchi*[ac], *F.S. Pavone*[acd]

[a] European Laboratory for Non-Linear Spectroscopy, Sesto Fiorentino, Italy
[b] Department of Informatics, University of Florence, Firenze, Italy
[c] National Institute of Optics, National Research Council, Sesto Fiorentino, Italy
[d] Department of Physics, University of Florence, Sesto Fiorentino, Italy
**e-mail: credi@lens.unifi.it*

Keywords: Soft-lithography, SERS, fiber-based spectroscopy, optical sensors

Introduction

Label-free optical detection techniques based on surface enhanced Raman spectroscopy (SERS) and capable of providing relevant information on molecular and protein composition of biological samples, are gaining rising attention in clinical research as alternatives to traditional assay detection technologies. Due to the recent technological advances in compact instrumentation as well as in nanofabrication processes, SERS techniques based on guided-wave systems such as optical fibers, have been realized thus providing better accessibility into complex environment and enabling for real-time detection of low concentrated target analytes dispersed within fluids of both environmental and biological nature. Moreover, the surface chemistry of SERS substrates could be properly modified by covalently binding various molecules acting as baits to selectively fish target analytes within heterogeneous samples (e.g. blood) thereby increasing the specificity of SERS signals.
In the present study, low-cost nanofabrication processes (e.g. soft-lithography) were combined with standard surface functionalization strategies for the time-saving fabrication of disposable SERS-active caps, engineered to tightly but reversibly fit the distal end of a portable Raman instrument. Caps SERS-functionality was achieved either by gold sputtering or by immobilization of gold nanoparticles whose size and shape directly affect the plasmonic properties of the substrates thus playing a key role in Raman signal enhancement. Optimal SERS conditions were considered those that upon matching with the Raman set-up were able to provide the highest signal when performing real-time measurements against standard raman reporter solutions. Finally, these optical spectroscopic devices represent a rapid and low-cost sensor for in liquid SERS detection that could be potentially implemented for the early diagnosis of widespread pathologies by real-time analysis of the molecular composition of biological fluid aimed at detecting specific biomarkers with high specificity and sensitivity.

Results and Discussion
The distal end of the portable raman instrument employed in the present study is constituted by a bundle of 25 100-um optical fibers, one for light delivering the others for signal collecting, gathered together with a 2.1 mm metallic jacket. A low-cost reproducible replica molding process was exploited to fabricate disposable caps that could be easily inserted/removed from the raman probe while guaranteeing no leakage. Polydimethylsiloxane (PDMS) was selected due to its optical and mechanical properties, perfectly addressing requirements for the present application. Subsequently, the strategies employed for the fabrication of SERS-active elastomeric caps can be differentiated into bottom-up and top down approaches. Specifically, in the first case, SERS platforms were obtained by immobilization of gold nanoparticles (NPs) on flat PDMS surfaces. Negatively charged citrate-capped nanospheres with diameter of 10 nm, 60 nm, and 100 nm were used. To strengthen NPs-PDMS binding and maximizing surface coverage, both amine ($-NH_3^+$) and thiol (SH)-terminals were introduced onto inert PDMS surfaces by silanization with aminosilane (APTES) and mercaptosilane (MPTMS), respectively. Energy dispersive X-rays spectroscopy (EDS) and scanning electron microscope (SEM) analysis (**Figure 1a**) confirmed that PDMS surfaces were successfully modified. Therefore, the thiol groups in MPTMS should conjugate with NPs via Au-S covalent bonding, while the amine groups of APTES should capture NPs via weaker Au-N bonds. UV-vis absorption spectra clearly demonstrated that the optical responses of PDMS-NPs substrates can be spectrally tuned by changing the diameter of the spheres and that efficient particles deposition was achieved for APTES-modified PDMS substrates. On the other hand, the poor deposition observed for MPTMS-modified substrates (data not shown) could be ascribed to the charge repulsion between the net surface charge of NPs and the deprotonated -SH groups, being both negative.

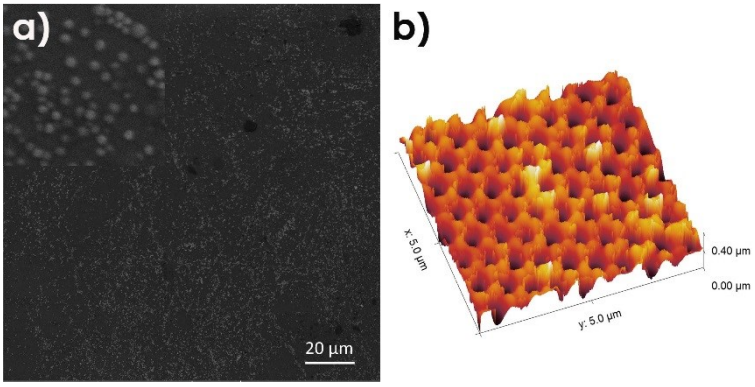

Figure 1: a) SEM (a) and AFM (b) images of plasmonic substrates obtained by bottom-up and top-down approaches, respectively.

For top-down approaches, PDMS-substrates were previously nanostructured by implementing standard nanospheres lithography (NSL) process to realize micro-structured moulds and homogeneously pattern the active surface of the PDMS cap that were subsequently gold sputtered to realize plasmonic platforms. AFM measurements (**Figure 1b**) confirmed the periodicity of patterned substrates but further experimental conditions optimization are needed to increase the overall patterned area to dimensions comparable to the diameter of the probe (2.1 mm). Finally, preliminary in liquid real-time SERS measurements performed by dipping the probe-cap system in rhodamine (Rh6G) solution, without preliminary incubation, confirmed UV-vis results, with higher raman signal enhancement obtained for substrates characterized by higher absorbance at 785 nm (excitation laser wavelength).

Conclusions and/or Outlook

Bottom up and top-down functionalization strategies were combined with standard soft-lithographic processes to fabricate SERS-active disposable caps that upon perfectly fitting the distal end of a portable raman probe can be employed for real-time in liquid SERS detection of diluted analytes. Further work will be focused on the functionalization of the plasmonic surfaces with biologically relevant molecules aiming at realize clinically meaningful biosensors potentially implementable for the early diagnosis of pathologies by real-time analysis of the molecular composition of biological fluids.

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Combined THz -NIR spectroscopic imaging for Bioclinical recognition

Annalisa D'Arco (Italy)

Italian National Institute for Nuclear Physics -INFN, Rome1 section

The demand for advanced biomedical and clinical detections is continuously increasing, promoted by their significant benefits in these fields, and in particular in cancer diagnosis field. Currently, cancer diagnosis relies on the availability of qualified pathologists. Thus, the diagnosis workflow and accuracy could benefit from the introduction of automated visualization techniques, which could ensure fast, non-invasive and early recognition. Therefore, it would be an advantage in the diagnosis and prevention to access to label-free and non-invasive techniques, which simultaneously allow to have morphological and chemical information relating to the biological sample. Combining the advantages of using THz spectroscopic imaging and NIR spectroscopy, we propose a methodology for bioclinical recognition.

In comparison with electromagnetic radiation, THz radiation is far less energetic than those of x-rays and therefore it is non ionizing radiation, secondly, the scattering in heterogeneous biological material is many orders of magnitude less s for the THz band than for the infrared or visible regions. Thirdly, many biological molecules exhibit vibrational and rotational modes at THz frequencies which may provide characteristic fingerprints to differentiate biological tissues in a region the spectrum not previously explored for medical use. Finally, polar molecules, such as water, exhibit strong absorption in the THz range; normal tissues and cancerous ones could be accurately differentiated for their different water contents. On the other hand, NIR spectroscopic investigation ensures the chemical mapping, relating to the characteristic biosignatures.

We propose and illustrate the advantages of combined THz-NIR spectroscopic imaging for bioclinical recognition. Instead, we 'll outline the application in the preclinical field and the technological development of an imaging method, not commercially available, based on their combination and our preliminary studies.

Non-Invasive Monitoring of Stem Cells and Regenerative Medicine Processes Using Multimodal Nonlinear Optical Microscopy

Leonardo Mortati[1], Carla Divieto[1], Lisa Accomasso[2], Monica Boffito[3], Andrea Lapini[1], Massimo Zucco[1], Valeria Poli[4], Gianluca Ciardelli[3], Caterina Cristallini[5], Claudia Giachino[2], Maria Paola Sassi[1]

[1] Istituto Nazionale di Ricerca Metrologica – INRIM, Strada delle Cacce 91, 10135 Torino, Italy.
[2] Department of Clinical and Biological Sciences, Università di Torino, Regione Gonzole 10, Orbassano, 10043 Torino, Italy
[3] Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Corso Duca degli Abruzzi 24, 10129 Torino, Italy
[4] Department of Molecular Biotechnology and Health Sciences; Molecular Biotechnology Center; Università di Torino, Italy
[5] Institute for Chemical and Physical Processes, IPCF-CNR, Pisa, c/o Largo Lucio Lazzarino, 56126 Pisa, Italy

Stem cell therapies are rapidly progressing gaining unprecedented opportunities based on recent advancements in stem cell and biomaterials research. However, the behavior and function of transplanted stem cells in vivo in combination with biomaterial delivery systems are key questions that still need to be fully addressed. Particularly, the interactions between cells and their microenvironment in a living cell-scaffold construct is a critical factor to study to improve and further develop novel therapeutic approaches.

Nonlinear optical imaging techniques are fundamental for these purposes being able to provide real-time non-invasive visualization of stem cells and scaffolds, and they're gradually becoming a really precious and essential tool for the regenerative medicine research. A simultaneous multimodal imaging of the different components of regenerative processes, such as cells, scaffold and de novo ECM synthesis, would be ideal.

In this work five dimension (5D) multimodal nonlinear optical imaging is applied to noninvasive monitoring of cell-scaffold constructs mimicking three different tissues: connective, cardiac and mammary gland tissues.

We demonstrate how simultaneously monitoring the morphological structures of labeled (by two photon excitation fluorescence) and non-labeled (by coherent anti-Stokes Raman scattering and second harmonic generation) biological samples we're able to provide images that can be related to the cell/scaffold interaction, cell function and cell behavior. At the same time, we provide also the characterization (dimension and morphology) of biological scaffolds specifically developed for regenerative medicine applications.

Photoluminescence of radiation-induced defects in lithium fluoride for dosemeters in radiotherapy

*M. A. Vincenti**[a], M. Leoncini[b], F. Bonfigli[a], E. Nichelatti[c], M. Piccinini[a], A. Ampollini[a], L. Picardi[a], C. Ronsivalle[a], R. M. Montereali[a]

[a] Fusion and Technologies for Nuclear Safety and Security Department, ENEA C.R. Frascati, Via E. Fermi 45, 00044 Frascati, Rome, Italy
[b] ENEA guest
[c] Fusion and Technologies for Nuclear Safety and Security Department, ENEA C.R. Casaccia, Via Anguillarese 301, 00123 Rome, Italy
*e-mail: aurora.vincenti@enea.it

Keywords: lithium fluoride, thin films, photoluminescence, colour centres, dosemeters

Introduction

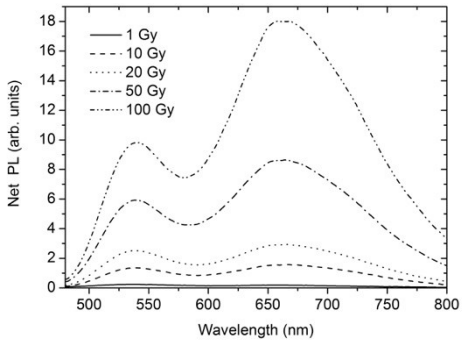
Among alkali halides, lithium fluoride (LiF) occupies a special place because of its peculiar physical-chemical and optical properties, which make this material widely used in photonics [1] as well as in radiation dosimetry [2]. Irradiation of LiF by ionising radiation induces the stable formation of electronic defects, known as colour centres (CCs). The primary F center (an anionic vacancy occupied by an electron) has an absorption band peaked at a wavelength of 248 nm. Up to now, the photoluminescence (PL) originating from this centre in LiF, theoretically expected at ~900 nm, has not been detected unambiguosly. The aggregate laser-active F_2 and F_3^+ CCs (two electrons bound to two and three anion vacancies, respectively) possess almost overlapping broad absorption bands peaked at the wavelengths of 444 and 448 nm, respectively, which together form the so called M band. Under optical pumping in the M band, they simultaneously emit broad PL bands peaked at 678 nm and 541 nm for F_2 and F_3^+ defects, respectively. In the last decade, LiF crystals and thin films were proposed for novel solid state imaging detectors based on F_2 and F_3^+ CCs PL for soft X-rays up to energies of 80 keV [3]. Recently, their use was extended to advanced proton beam diagnostics [4]. Among their main advantages there are very high intrinsic spatial resolution on a large field of view and a wide dynamic range. Their reading technique is based on the detection of the visible PL signal emitted by radiation-induced F_2 and F_3^+ CCs under simultaneous excitation at 450 nm. Due to the stability of aggregate CCs at room temperature (RT), they can be handled in air and in normal illumination conditions, and can be read many times. Due to the LiF tissue equivalent characteristics, it was proposed to test the feasibility of LiF crystals for clinical dosemeters based on CCs visible PL [5].

In this work, the preliminary experimental results about the response of radiation-induced CCs in nominally pure LiF crystals irradiated by 6 MV X-rays in the clinically relevant dose range of 1-100 Gy are presented together with the enhancement of the F_2 and F_3^+ CCs emission properties in LiF film-based detectors irradiated by 3 MeV proton beams at doses higher than 10^5 Gy. LiF films assure great versatility as they can be grown by thermal evaporation on different substrates with the desired geometry, size and thickness. On the other hand, their use as sensors of ionising radiation at clinical irradiation doses is difficult because of the low PL intensity emitted. A systematic investigation is under way to obtain high substrate-enhanced PL intensities thus improving the PL response of such LiF film-based detectors.

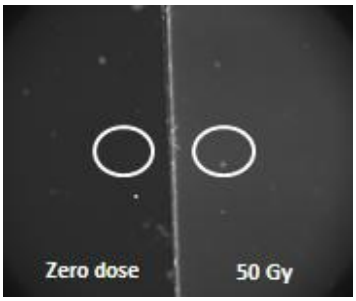
Results and Discussion

Nominally pure commercially-available LiF crystals, dimensions (5×5×0.5) mm^3, polished on both sides, were irradiated under full electronic equilibrium conditions using 6 MV X-rays produced by a clinical linear accelerator at the Tom Baker Cancer Centre, Calgary. The irradiations were set to 1, 10, 20, 50 and 100 Gy; all the doses refer to doses to water. The PL emission, excited at a wavelength of 457.9 nm, was spectrally filtered in the 480-800 nm range by a monochromator and detected by a photomultiplier by using a lock-in technique. The integrated PL signal associated with each PL spectrum, defined as the sum of the areas of the two F_2 and F_3^+ Gaussian bands, was also measured using a conventional wide-field optical microscope Nikon Eclipse 80i in fluorescence mode. Figure 1 shows the PL spectra of the irradiated LiF crystals (a) and a fluorescence image of two LiF crystals, one not irradiated and the other irradiated at 50 Gy (b). Each emission spectrum consists of the superposition of two broad emission bands due to F_2 and F_3^+ CCs. The integrated PL signal as a function of the irradiation dose (not shown here) shows a linear behaviour in the investigated dose range.

Polycrystalline LiF thin films, about 850 nm thick, were deposited on glass and Si(100) substrates, in controlled conditions, at the Solid State Lasers Laboratory in ENEA C.R Frascati. They were irradiated by proton beams of nominal energy of 3 MeV, produced by the modular linear accelerator for protontherapy under development at ENEA C.R. Frascati (TOP-IMPLART project). The irradiations were performed at several doses, in the range between 10^5 and 10^7 Gy. PL and Photoexcitation (PLE) spectra were acquired by a Horiba Scientific Fluorolog-3 spectrofluorimeter Model FL3-11 adopting a front-face detecting geometry.



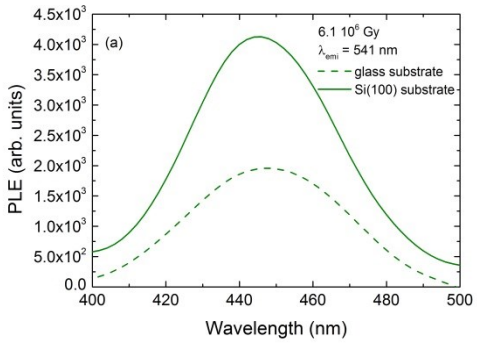
(a)



(b)

Figure 1: Laser-induced (457.9 nm) PL spectra of 6MV X-rays irradiated LiF crystals. The Net PL signal is obtained by subtracting the PL background signal (given by an unirradiated "zero dose" LiF crystal) to the corresponding PL signal for every irradiated LiF crystal (a). Fluorescence microscope image of a blank (zero dose), left, and the 50 Gy 6 MV X-rays irradiated LiF crystal, right (b). The two white circles show the regions selected for the measurement of the integrated PL.

Figure 2 shows the PLE spectra of two LiF films grown on glass and silicon substrate in the same deposition run, irradiated at a dose of 6.1×10^6 Gy, acquired at the emission wavelengths of 541 nm (a) and 678 nm (b). The PLE signal is higher for the LiF film grown on Si(100) substrate than on glass one. An enhancement of almost 100% is observed on the band peaks. This PL enhancement is due to the high reflectivity of silicon at the optical wavelengths where the exciting light and the F_2 and F_3^+ PL are located.



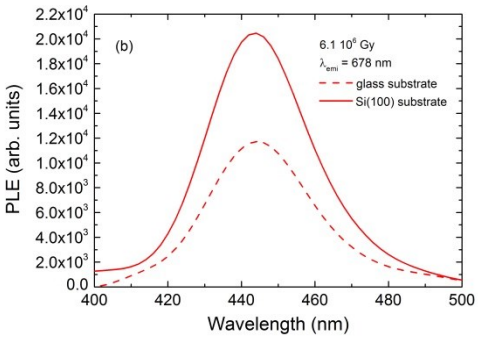


Figure 2: PLE spectra of two LiF films thermally evaporated on glass and Si(100) substrates, irradiated by 3 MeV proton beams at 6.1×10^6 Gy, acquired at the emission wavelengths of 541 nm (a) and 678 nm (b).

Conclusions

The suitability of pure LiF crystals as dosemeters based on optical reading of F_2 and F_3^+ visible PL in the clinically relevant dose range (up to 100 Gy) has been investigated for clinical 6 MV X-rays. The linearity of the integrated PL response due to F_2 and F_3^+ electronic defects as a function of irradiation dose, which is a very desirable feature of any radiation detector, was obtained. PL and PLE spectra of F_2 and F_3^+ electronic defects induced by irradiation with 3 MeV proton beams, in the dose range from 10^5 to 10^7 Gy, were measured and analysed in LiF thin films thermally evaporated on glass and silicon substrates. Enhancement up to almost 100% of the PL signal, due to the presence of the reflective silicon substrate, was observed.
Further work is in progress to improve the performance of the radiation detectors based on LiF films and investigate the potential role of dopants.

Acknowledgements

The authors are indebted with J. E. Villarreal-Barajas for stimulating discussions and suggestions. Part of this research is carried out within the TOP-IMPLART (Oncological Therapy with Protons – Intensity Modulated Proton Linear Accelerator for Radio-Therapy) project, founded by Regione Lazio, Italy.

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POSTER PRESENTATIONS

Bioceramics from Ca3(PO4)2 - CaKPO4 - CaNaPO4 system for bone replacement and grafting

N. Orlov[a, c], P. Milkin[a], P. Evdokimov[a, b], V. Putlayev[a,b], J. Günster[c], D. Nicolaides[c]*

[a] Department of Materials Science, Lomonosov Moscow State University, Moscow, Russia, 119991
[b] Department of Chemistry, Lomonosov Moscow State University, Moscow, Russia, 119991
[c] Federal Institute for Materials Research and Testing (BAM), Berlin, Germany, 12203
e-mail: nicolasorlov174@gmail.com

Keywords: bone grafts, calcium phosphates, ceramics, resorption, phase diagram, sintering, solubility

Introduction

Biomaterials for bone replacement and grafting should possess sufficient strength, be bioresorbable and demonstrate osteoconductivity/osteoinductivity. Nowadays, hydroxyapatite (HA) and tricalcium phosphate (TCP) are the most widespread ceramics for bone grafting at the market, however, their resorption is reported, in some cases, to be not enough. This is why the search for more soluble ceramics compared to HA and TCP looks rather viable.

A possible way to increase ceramics solubility leads to partial substitution of Ca^{2+}-ions in $Ca_3(PO_4)_2$ by alkali cations, like Na^+ or/and K^+. Improvement of solubility stems from decreasing lattice energy of a substituted phase, as well as increase in hydration energy of the ions releasing from the phase to ambient solution. From this viewpoint, bioceramics based on compositions from $Ca_3(PO_4)_2$ - $CaKPO_4$ - $CaNaPO_4$ ternary system seems to be prospective for bone replacement and grafting in sense of resorption properties. At the same time, one should bear in mind that solubility level (resorbability) is governed not only by reduction of lattice energy, but also by microstructure features. Grain sizes and porosity contribute much to dissolution rate making study of sintering of aforementioned ceramics highly important.

Results and Discussion

In this work, an isothermal section for phase diagram of $Ca_3(PO_4)_2$ - $CaKPO_4$ - $CaNaPO_4$ ternary system is studied with several techniques. According to XRD of quenched samples, this phase triangle has four single-phase areas at 1200˚C (**Figure 1**). Upper temperature thresholds of these phase areas were determined by DTA.

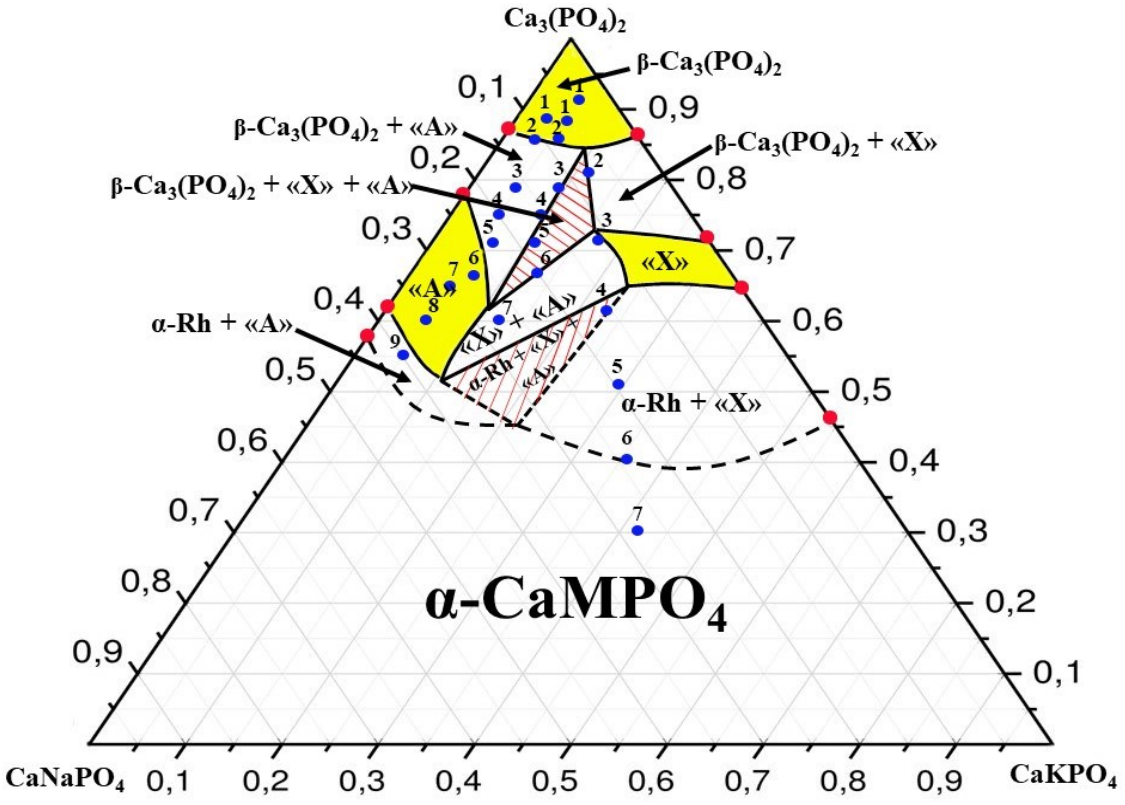

Figure 1. Section of $Ca_3(PO_4)_2$ - $CaKPO_4$ - $CaNaPO_4$ ternary phase diagram at 1200˚C

It was shown that single-phase $CaK_{0.6}Na_{0.4}PO_4$ cannot be sintered to full-dense ceramics by conventional sintering regardless

time-temperature schedule. Two-step sintering technique, beneficial in the case of HA-ceramics, was unsuccessful in all cases of calcium-alkali phosphate compositions. Large grains and a big amount of intragrain pores was a common feature of microstructure of the ceramics under study. This fact was explained by sufficient impact of grain growth phenomenon over densification at sintering temperatures leading to easier separation of grain boundaries from pores. However, field-assisted sintering techniques like, e.g. Spark Plasma Sintering (SPS), can overcome this problem due to significant impact on grain boundary diffusion. In connection with this fact, grains grow much more slower retaining sintering procces in a pore control regime. In this work $CaK_{0.6}Na_{0.4}PO_4$ low-porous ceramics was also fabricated by SPS.

Strength properties were evaluated by B3B-testing, micro- and nanoindentation techniques. The results showed an increase of strength with K-content increasing in $CaK_xNa_{1-x}PO_4$ ceramics. Fracture toughness becomes also higher with potassium content increase, guiding porosity level. High fraction of plastic deformation within the work of nanoindentation, rising with K-content increase, was found for calcium-alkali phosphate based ceramics.

According to thermodynamics evaluation, all $Ca_{3-x}(K_xNa_{1-x})_{2x}(PO_4)_2$ compounds are soluble; this was proved by special solubility experiments in model solutions of citric acid at constant level of pH. It is worth to note that dual- and triple-phase samples with overall composition $Ca_{3-x}(K_xNa_{1-x})_{2x}(PO_4)_2$ showed uniform solubility rate during experiments in model solutions with pH=5 (**Figure 2, 3**).

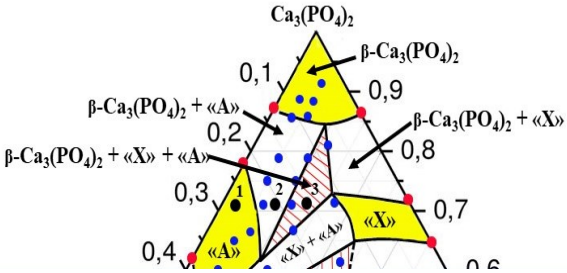

Figure 2. Compositions chosen for solubility experiments (black dots 1, 2, 3)

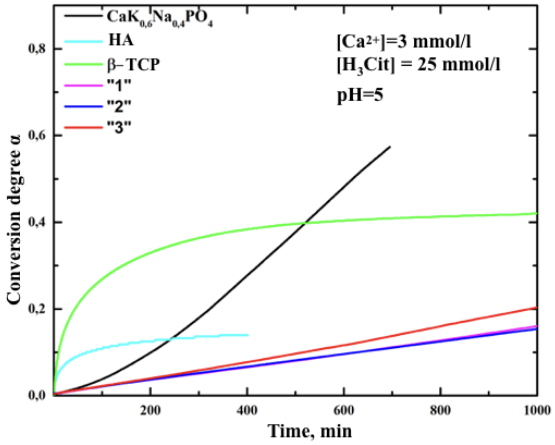

Figure 3. Kinetic curves of dissolution compositions 1, 2, 3 slow cooled from sintering temperature.

Conclusions

In this work, a section of $Ca_3(PO_4)_2$ - $CaKPO_4$ - $CaNaPO_4$ ternary phase diagram at 1200˚C were constructed for the first time. The section was used to choose prospective resorbable compositions of bioceramics. Sintering behavior of $Ca_{3-x}(K_xNa_{1-x})_{2x}(PO_4)_2$ ceramics was assessed on $CaK_{0.6}Na_{0.4}PO_4$ composition as a model one. It was found that potassium has beneficial effect on strength and fracture toughness of $CaK_xNa_{1-x}PO_4$ ceramics. Solubility of $Ca_{3-x}(K_xNa_{1-x})_{2x}(PO_4)_2$ ceramics was found to be much higher than that one for HA and TCP, demonstrating constant dissolution rate.

Acknowledgements

Studying phase diagram, samples behavior during conventional sintering, mechanical properties and solubility experiments were supported by RSF, grant #14-19-00752. SPS experiments were conducted within RFBR project № 18-33-00974. The help of Dr. A. Tyablikov in SPS is greatly appreciated.

Crystal-chemical investigation of rare earths tricalcium phosphates

F. Capitelli

Istituto di Cristallografia - CNR, Via Salaria Km 29.300, 00016 Monterotondo (Rome), Italy
e-mail: francesco.capitelli@ic.cnr.it

Keywords: TCP, rare earth elements, powder X-ray structure refinement, vibrational spectroscopy

Introduction

β - tricalcium phosphate $Ca_3(PO_4)_2$ (β - TCP) compounds, considered as one of the most important biomaterials owing to its ability to reproduce human bone density have recently been tested in biological imaging as materials able to store charges and emitting fluorescence, to be thus used as red long-lasting phosphors for optical markers [1]. β-TCP doped with Eu^{3+} has been proven to be a good candidate for these applications [2], thus β-TCPs doped with other rare earths from La (Z=57) up to Lu (Z=71), also called lanthanides, are worth to be investigated for similar properties, considering that compounds with substituting RE^{3+} cations typically show to be isostructural owing to the so-called *lanthanide contraction* and similar physical properties are then expected.

Although β-$Ca_3(PO_4)_2$ has been widely investigated and recognized isostructural with the mineral whitlockite $Ca_{18}Mg_2(PO_4)_{12}(PO_3OH)_2$, detailed studies on *RE*-doped β-TCP materials are lacking, due to the difficulties to obtain single crystals suitable for X-ray diffraction studies.

The aim of the present contribute is to fill this gap by providing a multi-methodological characterization of a set of new polycrystalline $Ca_9RE(PO_4)_7$ (*RE* = La, Pr, Nd, Eu, Gd, Dy, Tm, Yb, Lu) TCP materials obtained from solid state reaction (T=1200°), by combining SEM-EDS morphology analysis, X-ray powder structure refinements via Rietveld method, and Fourier Transform InfraRed (FTIR) and Raman spectroscopies. This work also represents a further contribution in the context of a crystal chemical survey on inorganic phosphates that the authors are carrying out in the last years [3-4].

Keywords: TCP, rare earth elements, powder X-ray structure refinement, vibrational spectroscopy

Results and Discussion

TCP materials, chemically $Ca_9RE(PO_4)_7$ (*RE* = La, Pr, Nd, Eu, Gd, Dy, Tm, Yb, Lu) were synthesized by solid-state reaction at T = 1200 °C. *RE*-TCP powder phases were studied by a combination of SEM-EDS microscopy, XRD, FTIR and Raman spectroscopies. SEM morphological analyses revealed the presence of sub spherical micro crystalline aggregates, while EDS semi-quantitative analyses confirmed the nominal *RE*/Ca composition for all the phases. Structural studies by X-ray powder diffraction data, showed that all phases in the series crystallize in a whitlockite-type structure (rhombohedral *R*3*c)*; unit cell constants are a linear function of the dimension of the substituting *RE* element, and range from $a = b = 10.4695(3)$ Å, $c = 37.500(3)$ Å and V=3559.7(2) Å^3 (La) up to $a = b = 10.4164(1)$ Å, $c = 37.302(1)$ Å, $V = 3505.1(1)$ Å^3 (Lu). The distribution of *RE* within the available structural sites is discussed according to the results of the Rietveld refinement. The FTIR and Raman spectra show slight band shifts of the phosphate modes correlated to the evolving size of the *RE* element.

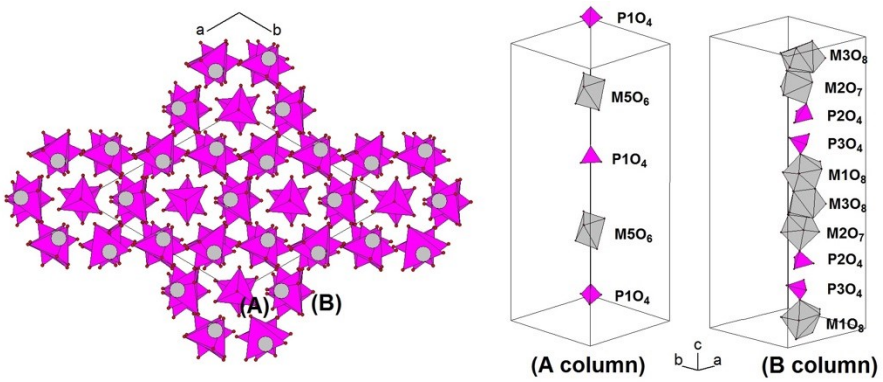

Figure 1: Structure of present $Ca_9RE(PO_4)_7$ phosphates*:* view down c, and details of A and B columns.

Conclusions

$Ca_9RE(PO_4)_7$ (*RE* = La, Pr, Nd, Eu, Gd, Dy, Tm, Yb) TCP compounds were investigated by means of SEM-EDS, XRD, FTIR and Raman techniques, highlighting how their whitlockite-type structure (rhombohedral *R*3*c)*, owing to the contribute of rare earths cations, can be applied for different biomaterial applications.

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Extraction and characterization of hydroxyapatite from Madeira Island fish transforming industry by-products

Pedro Ideia[a], André Marques[b], Joana Pinto[a], Rui Ferreira[a], Leandro Figueiredo[a], Carmo Barreto[b], Michael Caldeira[b], Helena Tomás[a], Miguel Cardoso[b] and Paula C. Castilho[a]*

[a] CQM-Centro de Química da Madeira, Universidade da Madeira, Campus da Penteada, 9020-105 Funchal, Portugal
[b] MadeBiotech, Zona Franca Industrial Plataforma 28, Pavilhão Industrial K- Módulo 2, 9200-047 Caniçal, Portugal
**pcastilho@staff.uma.pt*

Keywords: Fish transforming industry, by-products, hydroxyapatite, calcination, biomedical applications.

Introduction

Fish transforming companies generate large amounts of fish waste every year, such as heads, bones, scales and viscera. Bones and scales are especially important for extraction of hydroxyapatite (HAp) and/or organic moieties (i.e. collagen). HAp has many useful characteristics, including ion-exchange and sorption capacity. It is also biocompatible, bioactive, non-toxic, non-inflammatory and non-immunogenic. For this reason, HAp has also been studied for medical applications [1]. Tricalcium phosphate (TCP) is another calcium phosphate employed in the medical field. Despite being less biocompatible, it has better resorbability than HAp. Therefore, biphasic mixtures of HAp and TCP can be also considered as potentially interesting materials for biomedical applications. Calcination is the most common method to obtain HAp for both fish bones and scales [2].

Fishery and fish transforming industries are quite relevant in Madeira Island's economy. Tuna fish, black scabbard fish, rockfish, whiting and mackerel are five of the most important species in the regional fishing sector. By-products from these species were used for the experiments.

Results

Around 40 experiments of calcination of fish by-products were performed, under different conditions of time and temperature, with or without pretreatment. Results of characterization by spectroscopic methods (namely FTIR and XRD) show that, in some cases, extracted HAp had a high purity grade, by comparison with a commercial standard. In other cases, biphasic materials have been obtained. Moreover, for the same conditions, different species seem to lead to different products.

The work was developed under the project *MarineBlueRefine*, in a consorcium with a local company (Madebiotech). For that reason, not all the extraction details should be mentioned in the poster.

Acknowledgements

The authors are thankful to FCT for Project PEst-OE/QUI/UI0674/2013; the work was performed in the frame of project **MarineBlueRefine** PROCiência2020 (Portaria nº 371/2015, de 16/12), M1420-01-0247-FEDER-000006; Pedro Ideia is the recipient of a PHD grant under the project M1420-09-5369-FSE-000001.

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From waste towards valuable applications- marine residues as collagen natural sources.

Joana Pinto[a], *Leandro Figueiredo*[a], *Pedro Ideia*[a], *Rui Ferreira*[a] *and Paula Castilho**[a]

[a] CQM-Centro de Química da Madeira, Universidade da Madeira, Campus da Penteada, 9020-105 Funchal, Portugal
**pcastilho@staff.uma.pt*

Keywords: Collagen, fish residues, extraction, characterization, biomedical applications

Introduction

Collagen is the foremost constituent of extracelular matrix [1]. This protein has been considered as an excellent for cosmeceuticals [1], biomaterial applications [2] and tissue engineering [3], such as: artificial skin and scaffolding materia1.

Until now most of the collagen used in industrial products is obtained from mammalian sources, typically bovine and pig skins. However, due to cultural and health-related concerns the use of animal by-products as sources of collagen have now been avoided. In that manner marine residues have emerged as promissing natural sources for collagen [4].

Fish transforming industry (FTI) generates a considerable amount of discarded products (such as skin, scales, bones, viscera and swim bladder). Following the major efforts that have been done to deal with these residues, researchers have drawn their attention to the recovery and purification of high added value compounds like collagen [5].

This work focus on discards from FTI and compares the quality of collagen from different sources, including different species, body parts and methods of extraction.

The present work results from a consortium between university and local company which implies that some information remains confidential.

Results and Discussion

Viscosity, stability and thermal stability of protein structure are mandatory properties to characterize extracted collagen.

The FT-IR spectra dof each extract was assessed and compared with the ones from mammalian sources. The degree of triple helix preservation was evaluated through the absorption ratio of amide III to 1450 cm^{-1} band (A_{III}/A_{1450}). Results show a slightly different FT-IR spectra for the collagen derived from different extraction procedures. Despite this it is confirmed that triple helix structure was mantained on collagen obtained from fish residues.

Secondary structures of collagen obtained from fish residues were determined by XRD. Samples showed characterisitc peaks of collagen indicating that the structure of this protein remained stable which was in agreement with FT-IR analysis.

SDS-PAGE was used to determine molecular weight and to identify collagen type. SDS-PAGE suggests that the collagen contained two distincts α-chains.

Denaturation temperature was an important parameter to compare the thermal stability of extracted collagen with other collagen sources (bovine, porcine).

Conclusions and/or Outlook

Collagen was succesfully extracted from fish residues. The obtained collagen is affected by several factors wich are dependent on the raw material, specie, morphological parts, living habitat and processing conditions.

Future work: determine the potential of these materials as an alternative to mammalian-derived collagen in several apllications, such as in biomedical field.

Acknowledgements

This work was performed in the frame of project ***MarineBlueRefine*** PROCiência2020 (Portaria nº 371/2015, de 16/12), M1420-01-0247-FEDER-000006 and also sponsored by FCT (Project PEst-OE/QUI/UI0674/2013, CQM, Portuguese Government funds).

The authors are also thankful to MadeBiotech for the supplying of samples.

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Alpha-Tricalcium Phosphate Based Brushite Cement For Osteoplastics - Some Physicochemical And Biological Characteristics In Vitro

A.S. Fomin[a], *I.V. Fadeeva*[*a], *N.S. Sergeeva*[b], *I.K. Sviridova*[b], *S.A.Akhmedova*[b], *S.M. Barinov*[a], *J.V. Rau*[c], *A.V. Knotko*[d]

[a]A.A.Baikov IMET RAS, Leninsky avenue, 49, Moscow, 119334, Russia
[b]Moscow P.A. Gerzen Research Oncological Institute- branch of the Federal State Budgetary Scientific Institution "National Medical Research Center of Radiology" Ministry of Healthcare of the Russian Federation, 2-nd Botkinsky pass., 3, Moscow, 125284, Russia;
[c]ISM-CNR Istituto di Struttura della Materia, Consiglio Nazionale delle Ricerche, Via del Fosso del Cavaliere, 100, 00133 Roma, Italy
[d]Department of chemistry, Lomonosov MSU, Vorobievi Gory, 1, Moscow, 119334, Russia
**e-mail: fadeeva_inna@mail.ru*

Keywords: calcium phosphates, brushite bone cements, microstructure evaluation, in vitro investigation.

Introduction

Calcium phosphate cements (CPCs) are advanced materials to fill bone tissue defects due to the complex of benefits [1, 2]. CPCs have a simple protocol of preparation and application, set under physiological conditions and exhibit osteoconductive properties. The bioresorption rate of brushite cements (BCs) is close to growth rate of bone *de novo*. The main lack of BCs is poor mechanical properties and acidosis of surrounding tissues during setting and hardening. Carbonated hydroxyapatite ceramics granules can be used to enhance mechanical characteristics of BCs and to stabilize pH values close to neutral of the BCs surrounding media, simultaneously. Moreover, to prevent deformations of BCs scaffolds, porous polylactide (PL) framework with Kelvin structure can be applied as reinforcement.

Current investigation is focused on *in vitro* study of the BCs with the addition of carbonated hydroxyapatite (CHA) granules and reinforced by the porous polylactide frame.

Results and Discussion

The porous PL frame was used to increase BCs compressive strength. Earlier we developed bone cement based on α-tricalcium phosphate (α-TCP) with the addition of dense ceramic granules of carbonated hydroxyapatite [3]. CHA granules were obtained by crushing of dense CHA ceramics [4]. The 30% water solution of magnesium dihydrophosphate was used as a hardening liquid. The setting time of BCs modified with sodium hexametaphosphate was extended to 8-10 min. Addition of CHA granules decreased BCs acidity and, at the same time, increased the mechanical strength of the investigated cements. It was found that addition of 10% of CHA granules made it possible to obtain the BCs with pH 6,9-7,2 of their aqueous extract and compressive strength of about 20 MPa. The reinforcing of such BC with PL frame allows to reach the compressive strength value up to 35-40 MPa.

In vitro experiments to study the cytocompatibility of CPCs samples were performed on two cell lines: the immortalized human fibroblasts (hF, clone 1608 hTERT, Engelhardt Institute of Molecular Biology, Moscow, Russia) and the human osteosarcoma cell line MG-63 (Russian Collection of Cell Cultures, Institute of Cytology, St. Petersburg, Russia)). The cells ($2x10^4$ per well) were seeded on the sterile (γ-ray sterilization ,15 kGr) CPCs samples into 96 well plates for cultivation (Corning Costar, USA) into triplets with one plate per each incubation period and covered with complete growth medium (DMEM medium (PanEko, Russia), 10% fetal bovine serum (PAA, Austria), glutamine (0.65 mg/mL, PanEko, Russia), gentamycin (50 μg/mL, PanEko, Russia), into triplets with one plate per each incubation period. The dynamic of cell population growth in the presence of samples and control (cells on the cultural plastic polystyrene) was studied at 1, 4, 7, 11 and 14 days of incubation by MTT assay [5]. It was shown that the hF and MG-63 cells were attached to the surface of the samples and began to proliferate actively. At the same time, the rate of cellular expansion on CPCs was higher than in the control during cultivation on polystyrene. As a result, the largest increase in the hF pool (446-466% vs 265% in the control) on the fourteenth day in relation to the first days of observation was obtained for samples of BC and a bit less (342 %) was established for a sample of BC reinforced with a 3D PL framework. Similar results were obtained on the MG63 cell line.

In general, the results indicate that the developed CPC has good cytocompatibility to support the cells to attach and proliferate on their surface.

Conclusions

The developed CPC formulation is perspective for osteoplastic surgery, accounting for its acidity, compressive strength and biological *in vitro* properties.

Acknowledgements

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An in-vitro Comparative Study of Shear Bond Strength of New Developed Hydraulic Calcium-Silicate Versus Conventional Cements

*M Leo** [a] , *B Ranjkesh* [b], *G Pasquantonio* [a], *R Condo* [a], *H. Løvschall* [b]

[a] Department of Clinical Sciences and Translational Medicine, University of Rome Tor Vergata
[b] Institute of Dentistry, Health, Aarhus University, Aarhus C, Denmark
**email: m.leo@med.uniroma2.it*

Keywords: shear-peel strength, calcium-silicate cements, orthodontic bands

Objectives
The aim in this study in vitro was to compare the enamel bonding strength of the routinely used luting cements including Glass ionomer and Zinc phosphate cement versus novel developed fast-setting calcium silicate cement for 1 month in phosphate buffering solution (PBS).

Materials and methods
95 extracted human molars were used in this study; the teeth were divided in three groups. Each tooth was molded in acryl from the apical part to the cervical part of the tooth. An orthodontic band was fitted on every tooth and cemented with dental cement.
3 different cements were used; novel Calcium Silicate (Protooth), Glass ionomer (Orthocem) and Zinc phosphate (DeTrey Zinc) were mixed according to the manufacturers instructions. Each group of the teeth (n>30) was cemented with one of the cements. Samples were stored at 37ºC in a humid chamber for 24 h, and after 1 month in phosphate buffering solution (PBS).
Standardized pull-off test was applied by Instron machine, to measure individual stress-strain curve obtained and shear peel strength of the orthodontic band for all the teeth.
Data were analyzed using non parametric Mann-Witney test at the significant level of 0.05 using IBM SPSS, statistical software

Results and Discussion
The results of the test for the three different cements showed that, both novel Calcium Silicate (Protooth) and Zinc phosphate (DeTrey Zinc) cement were significantly higher (p>0.05) than Glass ionomer (Orthocem) cement when looking for the force (N) and the shear stress (MPa). In the same time, the values for novel calcium silicate (Protooth) cement were higher than Zinc phosphate (DeTrey Zinc) cement, but not with statistical significance. Novel calcium silicate cements with fluoride release called 'Protooth' has interestingly demonstrated apatite-forming capability *in vitro* with improved mechanical properties. Novel calcium silicate cement, with suitable creamy consistency and handability as a candidate for cementation, revealed identical bond strength similar to zinc phosphate cement and superior to glass ionomer cement.

Conclusion
Stable attachment of orthodontic molar bands for arch wires play an important role in successful orthodontic treatment. This study on luting of orthodontic bands demonstrated that bond strength of novel calcium silicate (Protooth) can match and improve the bond strength of orthodontic bands compared to zinc phosphate (DeTrey) and glass ionomer cement (Orthocem). In conclusion, the shear peel strength of novel calcium silicate cement with fluoride seem relevant for band cementation. The problem of demineralization and caries in relation to orthodontic appliance is of great concern to orthodontic clinicians. Interestingly, novel calcium silicate cement with fluoride and strength improvement has been developed for practical caries treatment in the crown.

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Citrate-stabilized amorphous calcium phosphate doped with fluoride ions: a new biomimetic nanomaterial in dentistry

L. Degli Esposti[*a], F. Carella[a], J. Gomez Moralez[b], E. Brambilla[c], A. C. Ionescu[c], G. B. Ramirez-Rodriguez[d], J. M. Delgado-Lopéz[d], A. Tampieri[a], M. Iafisco[a]

[a] Institute of Science and Technology for Ceramics (ISTEC), National Research Council (CNR), Faenza (Italy)
[b] Laboratorio de Estudios Cristalográficos, Instituto Andaluz de Ciencias de la Tierra, IACT (CSIC-UGR) Armilla (Spain)
[c] Oral Microbiology Laboratory, IRCCS Galeazzi Orthopedic Institute, Department of Biomedical, Surgical and Dental sciences, University of Milan, Milan (Italy)
[d] Department of Inorganic Chemistry, University of Granada, Granada (Spain)
e-mail: lorenzo.degliesposti@istec.cnr.it

Keywords: Amorphous calcium phosphate, nanoparticles, remineralization, dentine desensitization, fluoride.

Introduction

The demineralization of dental hard tissues (enamel and dentin) is the main cause of dental caries and dentin hypersensitivity[1]. Several factors lead to the generation of a low pH environment in the mouth, thus triggering the dissolution of hydroxyapatite (HA), the main component of enamel and dentinal tissues. However, demineralization is, in its initial steps, a reversible process and the tissues are naturally remineralised by the growth of new HA crystals, since the saliva provides the necessary Ca^{2+} and PO_4^{3-} ions[1, 2]. Nevertheless, remineralization of enamel by saliva alone is seldom completely achieved, and the use of an external source of ions is required to induce the mineral precipitation[1]. Various form of calcium phosphates has been proposed for dental hard tissue remineralization as restorative materials. The research on these materials was focused on nanostructured crystalline calcium phosphates, thanks to their biomimetism, high surface area, and their ability to adhere and penetrate into the hard tissues' lesions[2-4]. However, these crystalline materials do not act as source of Ca^{2+} and PO_4^{3-} ions, and the newly formed mineral phase might be not as resistant as the original one. Amorphous calcium phosphate (ACP) is a nanostructured calcium phosphate compound that has all these characteristics. Synthetic ACP is a biomimetic nanomaterial that has the ability to quickly release a significant amount of Ca^{2+} and PO_4^{3-} ions compared to crystalline counterparts, and, at the same time, it can attach to enamel and dentine lesions and convert to HA crystals[5]. The major hindrance to the use of ACP in preventive dentistry is that this material converts spontaneously to crystalline phases even when stored dry, and therefore its use and handling is difficult. Several additives and ions were studied to stabilize ACP[6, 7], but most of these processes are difficult to be scaled up. We have developed a biomimetic citrate-stabilized ACP that is simple to scale up and (i) delivers efficiently Ca^{2+} and PO_4^{3-} ions, (ii) occludes the dentinal tubules and (iii) induces enamel remineralization. In addition to that, we have doped ACP with fluoride ions to generate a material with a potentially enhanced anti-caries and remineralizing properties. Moreover, our citrate stabilized ACP can act as substrate for the growth of microarrays of highly ordered HA crystals, with a nano-to-microstructure similar to prismatic enamel.

Results and Discussion

Citrate stabilized ACP was synthesized by fast precipitation in aqueous solution in presence of citrate ions[8]. Fluoride-doped ACP (FACP) was prepared with the same method but in presence of F^- ions, at two different concentrations. The citrate/calcium molar ratio of the reagents was set to 4, 2 or 1, producing three different ACP/FACP compounds. All the ACP samples were proved to be spherical nanoparticles around 50 nm of diameter, and their amorphous nature was preserved up to one year at room temperature, thanks to the presence of citrate ions. ACP was successfully doped with fluoride ions up to 1 wt.% without altering their amorphous nature, nor their morphology. Changing the initial citrate/calcium molar ratio did not alter the morphological, structural and compositional features as well as the long-term stability of ACP/FACP. However, the specific surface area of the materials was influenced by the citrate/calcium ratio, achieving a high mesoporosity value that was not yet reported for calcium phosphate-based materials. All the ACP/FACP samples acted as effective source of Ca^{2+} and PO_4^{3-} (and F^-) ions, giving a sustained ion release when put in acidic artificial saliva. The citrate/calcium ratio proved to be a method to control the ion release kinetics. A preliminary *in vitro* experiment on samples of human enamel and dentin showed, as reported in **Figure** 1, that ACP/FACP successfully occluded the dentinal tubules and, at the same time, was able to reminalize enamel.

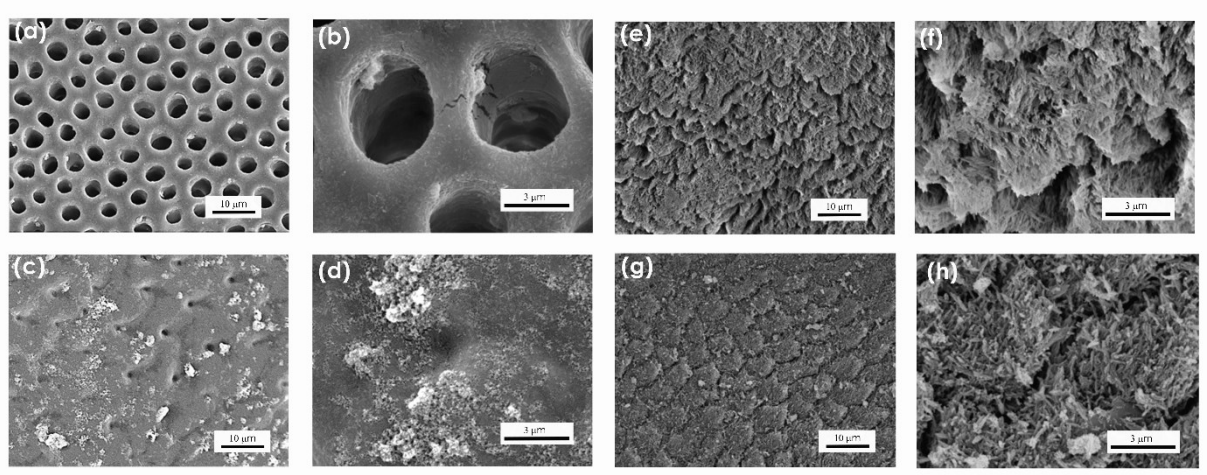

Figure 1: SEM micrographs of (a, b) demineralized dentin, (c, d) demineralized dentin treated with ACP, (e, f) demineralized enamel and (g, h) demineralized enamel treated with ACP at two different magnifications.

Additionally, compression moulded ACP acted as substrate for the growth of HA microcrystals when in presence of Ca^{2+}, PO_4^{3-} and F^- ions. These microcrystals formed a highly ordered array bearing a close resemblance to human prismatic enamel.

Conclusions and Outlook

ACP can be a promising material for prevention and treatment of demineralization, since it can be used as localised source of Ca^{2+} and PO_4^{3-} ions with a sustained release. In addition to that, it can be considered a biomimetic compound since it was able to attach to the damaged crystals, converting to HA and forming a new crystalline phase in continuity with the biological one. However, the issues regarding ACP stability and production scalability have led insofar to a very limited number of products on the market. On the other hand, our citrate-stabilized ACP proved to be stable for a long period of time and to be easily produced. It could be doped with fluoride ions and this feature, together with the citrate/calcium ratio, can be used to fine-tune its physicochemical behaviour and its effect on the dental hard tissues. These results suggest that our ACP/FACP can have excellent applications in formulations directly applied on tooth surfaces for treating dentin hypersensitivity and tooth demineralization. Moreover, these compounds could also be used to grow highly oriented HA crystals, forming microarrays that may have interesting features such as enhanced cell adhesion, nanoinjection, and drug delivery.

Aknowledgements

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Preparation and characterization of poly(ε-caprolactone) microparticles containing antimicrobial peptides for treatment of local infections

U. Piotrowska[*ab], *M. Sobczak* [ab]

[a] Department of Biomaterials Chemistry, Chair of Analytical Chemistry and Biomaterials, Faculty of Pharmacy with the Laboratory Medicine Division, Medical University of Warsaw, Banacha 1 St., 02-097 Warsaw, Poland
[b] Department of Organic Chemistry and Biochemistry, Faculty of Materials Science and Design, Kazimierz Pulaski University of Technology and Humanities in Radom, 27 Chrobrego St., 26-600 Radom, Poland
e-mail: upiotrowska@wum.edu.pl

Keywords: antimicrobial peptides; drug release; microparticles; poly(ε-caprolactone)

Introduction

Despite the rapid development of innovative therapeutic methods and improvement in the quality of life achieved with better specialist healthcare, infections with antibiotic-resistant bacteria continue to pose a major challenge to modern medicine [1]. Infections by multidrug-resistant bacteria are estimated to cause 25,000 deaths in the European Union every year. The increasing appearance of multidrug-resistant bacteria has created an urgent need for suitable alternatives to current antibiotics.

Antimicrobial peptides (AMPs), also called host defense peptides, are a diverse class of active substances, exhibiting a broad spectrum of biological activity[2]. In addition to their antibacterial effects, AMPs have been shown to bind and neutralize bacterial endotoxins and possess immunomodulatory, anti-inflammatory, angiogenic and anticancer properties. Unlike conventional antibiotics, which have very specific molecular targets, AMPs show complex and very rapid mechanisms of action based on electrostatic interactions between the cationic amphipathic peptide molecule and negatively charged microbial cell membrane lipids. However, the use of peptides has certain clinical limitations. While penetrating through healthy tissues to the infection site, cationic AMPs may bind to anionic serum proteins, which accelerates their elimination from the bloodstream. In addition, they are exposed to the activity of hydrolytic enzymes. At high concentrations, they may also be toxic to blood cells [3].

The main purpose of this study was to develop and characterized polymeric micropartices (MPs) of AMPs: citropin 1.1, temporin A, CAMEL and LL-37. Polymeric MPs are able to improve bioavailability, as well as provide precise distribution and suitable pharmacokinetics with the improved toxicological safety of the peptides. AMPs-loaded PCLs MPs were successfully obtained using the double emulsion solvent evaporation method. Their morphology (SEM) and physicochemical properties (particle size, ζ potential, encapsulation efficiency) were assessed. The influence of topology of poly(ε-caprolactone) matrices on kinetics of the peptide release in PBS (pH 7.4) at 37°C were also investigated.

Results and Discussion

The results of the SEM investigations revealed that spherical and non-porous PCL MPs were successfully obtained (**Figure 1**).

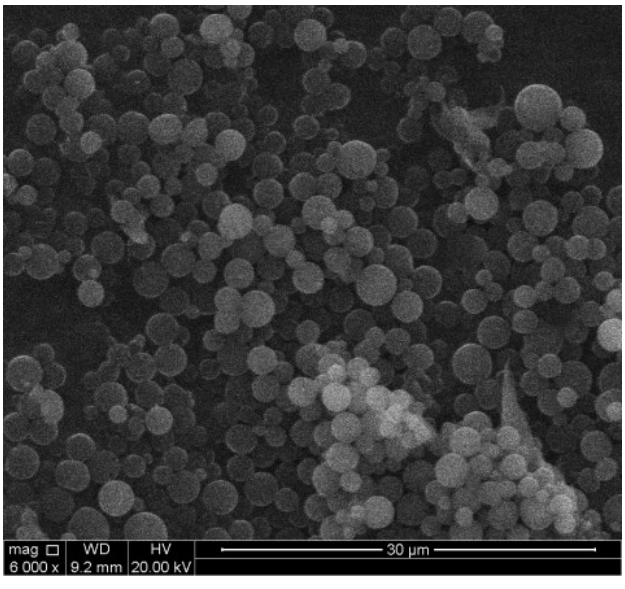

Figure 1: SEM micrographs of CIT-loaded PCLs MPs.

Peptide net charge and polymer microstructures (the content of negative charged, linear PCLs chains and non-charged cyclic PCLs) affect on peptide encapsulation efficiency (*EE*). Moreover, the crystallinity of polymer and hydrophilic-hydrophobic properties of AMPs affect on kinetics of peptide release from new systems [4-6]. Peptides release occurs mainly by the by erosion of the polymer matrix. The peptides were released from the obtained MPs with controlled kinetics and exhibited a near-zero-order or near-first-order profile [4]. Moreover, it is possible to reach the therapeutic concentration of the AMPs within the first hour of release.

Conclusions

The results of the study show that PCL MPs could be a promising peptide delivery system for the treatment of local infections.

Acknowledgments

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Synthesis and Characterization of Graphene Oxide Reinforced Injectable Bone Substitutes

Ö. D. Oğuz[a*], *D. Ege*[a]

[a] Boğaziçi University, Institute of Biomedical Engineering, Istanbul, 34684, Turkey
e-mail: oznur.demir@boun.edu.tr

Keywords: calcium phosphate cement, graphene oxide, methylcellulose, injectable bone substitutes

Introduction

Calcium phosphate cements (CPCs) are used as promising injectable bone substitute materials but their injection into the body is limited due to their relatively low strength and their brittleness [1]. To this end, an injectable and thermoresponsive polymer, methylcellulose (MC), was combined with CPCs to overcome limitations of CPCs and to improve their mechanical strength, viscosity, bio-absorbability, and injectability. Also, gelatin was incorporated together with MC and CPCs to lower the phase transition temperature of the bone substitutes and to enhance cellular adhesion via RGD sequence within its structure. However, our previous study [2] showed that synthesized injectable bone substitute (IBS) had low mechanical properties which requires to be improved. Therefore, graphene oxide (GO) which has excellent mechanical properties [3] was incorporated into IBS as a reinforcement material. The aim of the research was to produce a novel IBS with optimum mechanical properties, based on the reaction of used bioceramic powders (tetra calcium phosphate, dicalcium phosphate dihydrate, calcium sulfate dihydrate) to hydroxyapatite and also with the superior mechanical properties of GO as reinforcement. In this study, the physical properties of produced IBS were analysed by means of setting time and temperature, injectability, morphology, viscosity and mechanical properties.

Results and Discussion

Initially, weight fraction (wt%) of GO incorporation was optimized by using test tube inversion technique. The setting time and temperature were recorded accordingly and the three different wt% of GO, 0.2, 0.6 and 1.0 were used. The addition of 1wt% GO reduced the gelation time to 5min. The gelation temperature was also achieved at approximately 37°C. The injectability was qualitatively determined by measuring the mass % of extruded IBS from syringe manually from a 10 ml syringe with 18 gauge needle. The L/P ratio was kept constant at 1 g/g to observe the effect of GO addition. The injectability was varied between 93-97% and it gradually decreased by further addition of GO. Time and temperature dependent variations of loss (G'') and storage (G') modulus and complex viscosity (Pa.s) of hydrogels were investigated by using oscillation rheometer. The sharp increase of G' and Pa.s indicates the gelation temperature and gelation time. According to the results, gelation temperature and time were decreased effectively with the addition of GO. The compressive strength of the GO added IBS samples were increased with the increase of wt% of GO. The compressive strength of 1wt% GO added IBS was found to be 103.94kPa at day 7 which was 10 times higher than the compressive strength on day 1 after being incubated at 37°C under 97% humidity. X-ray diffraction analysis (XRD) of IBS showed the peaks for the crystalline phases of the samples which were for TTCP, DCPD, and CSD. Scanning Electron Microscopy (SEM) analysis showed that GO added IBS samples had a denser microstructure. As an ongoing study, prepared samples were incubated in simulated body fluid for bioactivity measurements. Overall, the addition of GO as a reinforcement has a positive effect to improve the mechanical properties and handling of injectable bone substitutes.

Conclusions/Outlook

In this study, different wt% of GO were used as a reinforcement material to enhance the mechanical properties and handling of injectable bone substitutes. Although the increase of wt% of GO reduced the injectability. The mechanical and rheological properties of GO added IBS samples were significantly improved. Bioactivity and biocompatibility studies are carried out with regarding to promising results of optimization studies.

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Development and Characterization of Enstatite-Zirconia Glass-ceramic

*Maziar Montazerian**

Department of Materials Engineering (DEMa), Center for Research, Technology, and Education in Vitreous Materials (CeRTEV), Federal University of São Carlos (UFSCar), São Carlos, SP, Brazil
**e-mail: maziar_montaz@yahoo.com*

Keywords: Glass-ceramics; Crystallization; Enstatite; Biomedical.

Introduction

Glass-ceramics are highly attractive because they are processable by fast (glass making) methods, and can have outstanding aesthetics, translucency, low thermal conductivity, high strength, high chemical durability, biocompatibility, wear resistance and hardness similar to that of natural teeth, and, in certain cases, they are ***bioactive*** [1]. Our thorough literature review [2]-[4] reveals that, despite all the numerous developments in the past 45 years, the fracture toughness of commercial glass-ceramics (1.0 - 2.8 $MPa.m^{1/2}$) should be improved. As all possible toughening mechanisms of glass-ceramics have not been fully explored; there are still some toughening routes, such as transformation toughening, bridging, microcracking and pull out, which can be stimulated by controlled crystallization of different crystals with a variety of morphologies and structures. For instance, chain silicate crystals, such as wollastonite, diopside, canasite, etc., have an elongated, needle-like morphology that could reinforce glass matrices. Other phases, such as enstatite could, in principle, undergo transformation toughening. Therefore, glass-ceramics containing such crystals may have high fracture strength and toughness.

Results and Discussion

In this work, we present preliminary results of a work aiming at developing and characterizing a tough and strong enstatite-zirconia glass-ceramic. A preliminary composition in the SiO_2-MgO-NaO_2-TiO_2-ZrO_2 system demonstrated to undergo internal crystal nucleation when properly heated. The nucleation temperature was optimized near the glass transition, T_g (680-700 °C), and crystal growth was stimulated below and above the DSC crystallization peak (1000 °C). In one of the many compositions tested we found copious nucleation that results in the crystallization of enstatite-zirconia. ***Interlocked rod-like crystals of clino/proto-enstatite*** and fine zirconia particles were detected by X-ray diffraction, XRD, (**Figure 1**) and scanning electron microscopy, SEM, energy dispersive spectroscopy, EDS (**Figure 2**).

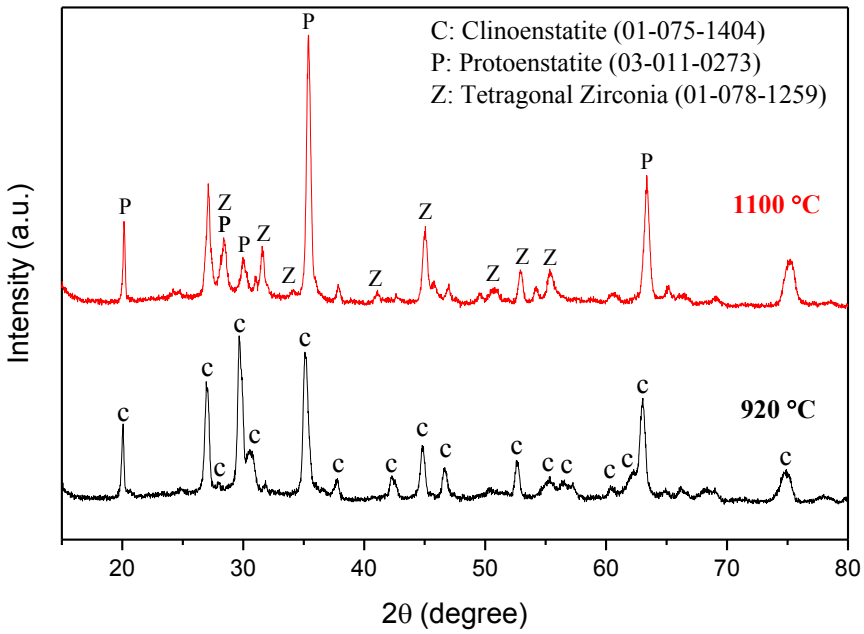

Figure 1: XRD pattern of enstatite-zirconia glass-ceramic heat-treated at 700 °C for 12 h and then 920 and 1100 °C for 1 h.

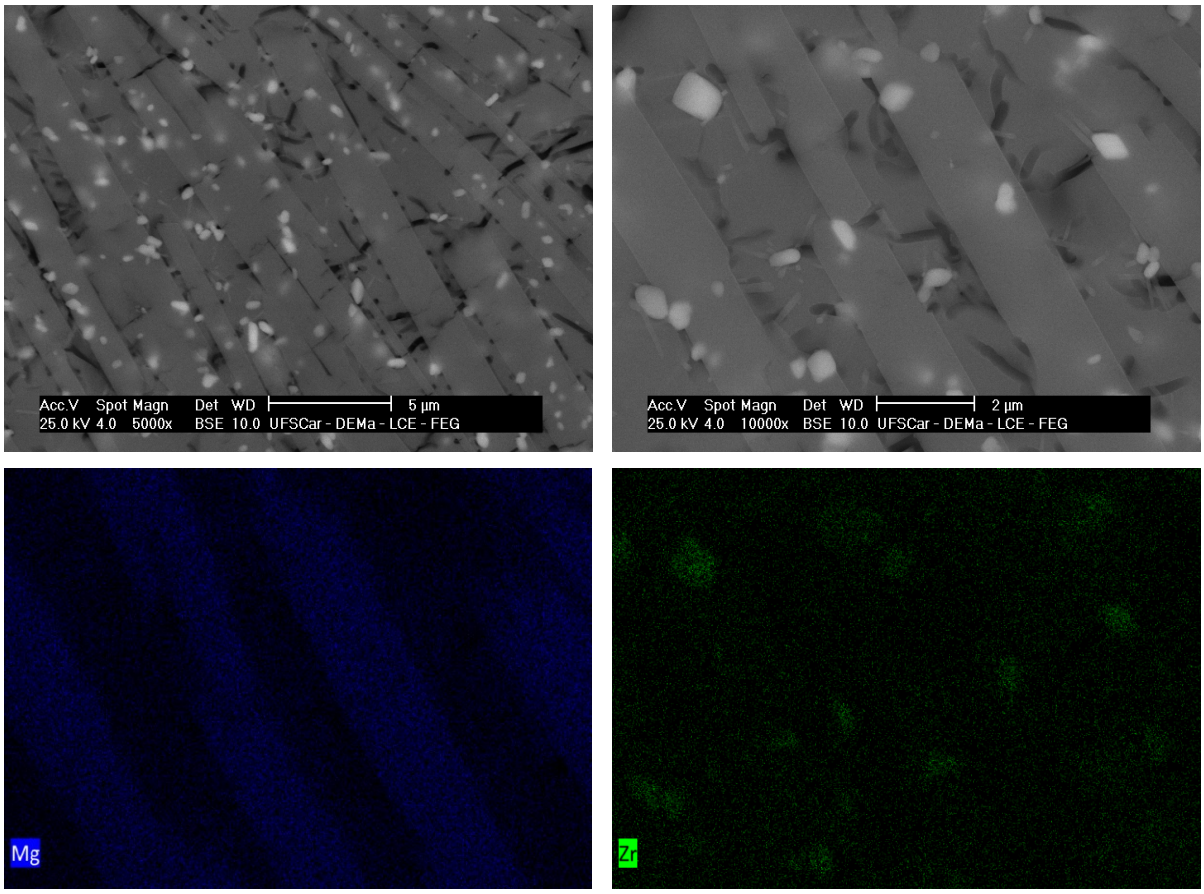

Figure 2: SEM and EDS-MAP from enstatite-zirconia glass-ceramic heat-treated at 700 °C for 12 h and 1100 °C for 1 h. Rod-like enstatite crystals and bright particles of zirconia are rich in Mg and Zr, respectively.

Conclusions and Outlook

Glass-ceramics showing internal crystallization, containing clinoenstatite, protoenstatite, zirconia potentially have high strength and toughness. A tough glass-ceramic of this kind would have numerous applications as an electronic substrate, refractory, dental restorative, and domestic products. Therefore, we are planning to investigate its mechanical properties thoroughly.

Acknowledgment

The author is grateful to the São Paulo Research Foundation (FAPESP) for financial support of this work and the post-doctoral fellowship granted (# 2015/13314-9).

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Glass-ceramic materials of R2O-RO-CaF2-P2O5-Al2O3-SiO2 composition for biomedical implant applications

M. Kh. Aripova[*a], *J. V. Rau*[b], *T. O. Nam*[c], *J. D. Aripov*[d]

[a] Tashkent Chemical Technological Institute, 32, Navoiy street, 100011, Tashkent, Uzbekistan
[b] Istituto di Struttura della Materia, Consiglio Nazionale delle Ricerche, Via del Fosso del Cavaliere 100, 00133 Rome, Italy
[c] Tashkent Chemical Technological Institute, 32, Navoiy street, 100011, Tashkent, Uzbekistan
[d] Tashkent Chemical Technological Institute, 32, Navoiy street, 100011, Tashkent, Uzbekistan
**e-mail:* aripova1957@yandex.ru

Keywords: materials science, bioactive glasses, filling materials, implants, crystallisation capacity.

Introduction

Bioactive glasses are used as a base materials for creating biocompatible implants, fixing bones, and also as filling materials accordingly in modern traumatology, orthopaedics and dentistry. Nowadays the creation of such materials in medical materials science is an actual problem.

The aim of the scientific work is the synthesis of bioactive glasses in the Na_2O-CaO-MgO-CaF_2-P_2O_5-Al_2O_3-SiO_2 system, the study of their physic-chemical properties, biocompatibility to living tissue.

The object and subject of the study are physicochemical regularities between the components of the state diagrams in the Na_2O-CaO-MgO-CaF_2-P_2O_5-Al_2O_3-SiO_2 system, as well as the body's reaction to the introduced materials. Methods of research - high-temperature microscopy, X-ray diffraction, optical microscopy.

Results and discussion

Glasses were synthesized on the basis of the Na_2O-CaO-MgO-CaF_2-P_2O_5-Al_2O_3-SiO_2 system. In laboratory conditions, the heat treatment was carried out at the temperature of 1400 °C with an exposure time of 1 hour in a silicate furnace. Synthesized glasses were cooled suddenly, draining into a container with cold water.

The regularities of the change in the physicochemical properties of synthesized glasses, such as density, the coefficient of linear thermal expansion, the refractive index depending on the composition, are established.

The crystallization ability of the synthesized glasses is investigated. It turned out that synthesized glasses are characterized by bulk crystallization. The compositions of glasses characterized by segregation were determined[1].

The structures of synthesized glasses were learned by using scanning electron microscopy method. The studies of synthesized glasses using raster microscopy showed that the access glass is a complex heterogeneous structure associated with the formation of embryonic or formed crystalline phases, as well as microheterogeneous glass stratification. The presence of nanoscale heterogeneities is established.

It is determined that the IR spectra of synthesized glasses differ from each other in the number and intensity of the absorption bands, which is due to the difference in their chemical composition and the different degree of structural perfection of the vitreous matrix [2]. Toxicological studies were carried out on animals, particular on rats, which showed positive results.

The effect of copper oxide additives from 0,1% to 1% on the properties of bioactive glass was studied. Experimental data have shown that copper oxide from the region of crystallization of glass and the formation of crystalline phases, and also increases strength and microhardness.

Conclusions and Outlook

First of all, there was investigated the system Na_2O-CaO-MgO-CaF_2-P_2O_5-Al_2O_3-SiO_2 and learned the dependence of the properties of the system. The diagrams "composition-thermal expansion coefficient", "compound density", "composition-index of refraction" were studied.

The represented work is devoted to the study of the properties and the establishment of the dependence in the Na_2O-CaO-MgO-CaF_2-P_2O_5-Al_2O_3-SiO_2 glass system, which are necessary for selecting the optimal composition and conducting further studies in this area in order to obtain bioactive materials that meets international requirements. The designed material will find application in dental practice, orthopaedics, traumatology and oncology.

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The effect of bioactive particles on mechanical properties of PMMA based cements for cranioplasty

R. De Santis[a]*, *A. Gloria*[a], *G. Millauro*[b], *L.Ambrosio*[a], *J.V. Rau*[c]

[a] IPCB-CNR Institute of Polymers, Composites and Biomaterials, Naples, Italy.
[b] BIOCAD Srl Cranioplastiche su misura, Rome, Italy
[c] ISM-CNR Istituto di Struttura della Materia, Rome, Italy
e-mail: rosantis@unina.it

Keywords: PMMA, cranioplasty, bioactive particles, mechanical properties

Introduction

Over the half past century several autogenus and alloplastic materials have been proposed for bone reconstruction of the skull. Poly(-methylmethacrylate) (PMMA), also known as bone cement, is the most adopted alloplastic material for cranioplasty. As large defects of the skull are concerned, PMMA reconstructions are preformed based on the reverse engineering approach: a 3D model of the defect is obtained through imaging, a virtual model of the reconstruction is realised. A mould reproducing the negative shape of the skull defect is manufactured through conventional or additive manufacturing approaches, and a solid model of the PMMA reconstruction is obtained by pouring the PMMA solution before setting.

The main functions of the PMMA implant is to protect the brain, to provide customized shaped support for soft tissue and to promote bone integration/regeneration. From a mechanical point of view, skull implants are considered non-load bearing. However, due to the trauma that often occur in the head, mechanical strength is the most relevant biomechanical feature for the material used in cranioplasty.

Modification of PMMA with bioactive particles is a common approach to improve biological properties of bone cements. We modified PMMA bone cements using bioactive glass particles and tricalcium phosphate particles.

Mechanical properties and morphological investigations have been performed in order to assess the effect of bioactive particles on PMMA based materials for cranioplasty.

Materials and Methods

PMMA bone cement (Palacos, Heraeus Germany) was modified bioactive glass particles and tricalcium phosphate particles. Cu^{2+}-substituted TCP powders were obtained using the precipitation technique[1]: 0.5 mol/L solution of $Ca(NO_3)_2$ were mixed with $Cu(NO_3)_2$ and $(NH_4)_2HPO_4$ solution was added dropwise to the solution. The pH was kept at 6.5-6.9 level by the addition of ammonia solution. After 30 min, the precipitate was filtered, washed with distilled water, dried at 80 °C, and calcined at 900 °C to form the whitlockite structure.

Bioactive particles were incorporated into PMMA. Briefly, 10% by weight of bioactive particles were dispersed in the solid PMMA phase using ultrasonic dispersion. The liquid MMA phase was added and hand mixed to the solid phase, hence the paste was poured into moulds in order to obtain prismatic specimens suitable for three point bending tests.

Mechanical testing were performed after 10 days since specimen preparation using the Instron 5566 equipped with a load cell of 1kN. Bending tests were carried out at a speed of 2 mm/min.

Results and Discussion

Modification of PMMA based cements is a common strategy to overcome drawbacks of bone cements[2].

The effect of bioactive particles on mechanical properties (e.g. maximum strength and Young's modulus) of PMMA cement adopted for cranioplasty is reported in Figure 1.

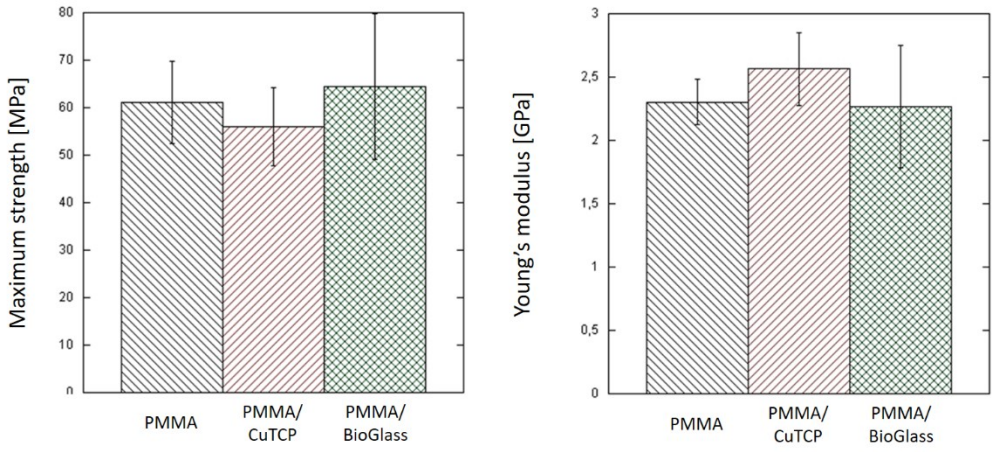

Figure 1: Maximum strength and Young's modulus of modified PMMA cements for cranioplasty.

No statistical significant difference was observed among PMMA cement and modified cements. A slight increase in the stiffness of PMMA/CuTCP was measured, but a slight decrease in the strength was also detected.

Conclusions

Within the limitations of this investigation and according to the amount of nanoparticles used to modify PMMA based bone cement for cranioplasty, it can be concluded that the incorporation of bioactive particles (i.e. CuTCP and Bioglass particles) into PMMA based do not alter mechanical properties in bending. Further research is needed to determine the effect of bioactive nanoparticles on bioactivity of PMMA cements.

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Biodegradation evaluation of some experimental magnesium alloys

Alex Sălan[a], Mădălina Matei[a], Laurentiu Mogoantă[a], Iulian Antoniac[b], Maria Magdalena Manolea[a], Eduard Ciucă[a], Horia Manolea[a]*

[a] University of Medicine and Pharmacy Craiova, Romania
[b] University of Politehnica București, Romania
e-mail: ciucaeduard@yahoo.com

Keywords: magnesium alloys, biodegradation, rat model, histology

Introduction

Magnesium alloys are very good candidates biodegradable implants for healthcare [1]. Unlike ceramics or polymeric materials, which are the alternatives, metallic Magnesium materials are more suitable for load-bearing applications due to their combination of mechanical strength and resistance to fracture [2]. Despite favorable mechanical properties, a magnesium alloy can only be considered suitable as a biomaterial, if the released components during the degradation of an implant are biocompatible [3].

Materials and Methods

In this study we tried to evaluate the host reactions of four experimental magnesium-based alloys. The alloys used were created at the Faculty of Materials Science and Engineering, University Politehnica of Bucharest. These are:

- *MgCa;*
- *MgCaZr;*
- *MgCaZrAg;*
- *MgCaZnAg.*

Their microstructure was analyzed using optical and electron microscopy. The chemical structure was analyzed by energy dispersive and X-ray diffraction analysis.

For this study we have used 20 Whistar laboratory rats. They were divided in 4 experimental groups and one control group. On the calvaria of this animals experimental grooves were carried out in order to receive samples of appropriate size.

The animals were sacrificed after 6 weeks and the local tissular reaction was investigated first by a clinical examination. Then samples and surrounding tissues were carefully excised and evaluated using optical microscope. In the end the samples were prepared for a histological study. Sections of 5 to 7 μm were obtained and stained with hematoxylin and eosin and Masson trichrome colorations.

Results and Conclusions

The alloys samples recovered after the 6-weeks biological insertion were again subjected to a structural analyse using scanning electron microscopy and X-ray diffraction analysis.

The histological sections showed various inflammatory answers, fibrous reactions in he soft tissues, but the most important it allowed us to observe the pattern of bone remodeling with the presence of numerous osteoblasts and osteoclasts, while the XRD analyses reveal the pattern of the corrosion process.

The key to developing a biodegradable Magnesium alloy seems to remain to adjust their corrosion rate and biomechanical integrity in the human physiological environment [4].

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The effects of different framework designs and different materials on fracture load of implant-supported single crowns after aging

Adnan Kose[1], *Pinar Gultekin*[1], *Baris Yavas*[2], *Gultekin Goller*[2], *Deniz Sen*[1]
*
Department of Prosthodontics, Faculty of Dentistry, Istanbul University, Istanbul, Turkey.
†Department of Metallurgical Materials and Engineering, Istanbul Technical University, Istanbul, Turkey.
*Department of Prosthodontics, Faculty of Dentistry, Istanbul University, Istanbul, Turkey.
†Department of Metallurgical Materials and Engineering, Istanbul Technical University, Istanbul, Turkey.
*Department of Prosthodontics, Faculty of Dentistry, Istanbul University, Istanbul, Turkey.
†Department of Metallurgical Materials and Engineering, Istanbul Technical University, Istanbul, Turkey.
[1]Department of Prosthodontics, Faculty of Dentistry, Istanbul University, Istanbul, Turkey
[2]Department of Metallurgical and Materials Engineering, Istanbul Technical University, Istanbul, Turkey

Replacing a missing tooth by a single implant-supported crown has increasingly gained popularity by both the clinicians and the patients. Long-term clinical studies have shown excellent survival rates of single implant tooth replacement. However, the success of an implant treatment does not only depend on the successful osseointegration of the implant, but also the prosthetic design i.e abutment-crown complexes materials and designs. Zirconium dioxide (zirconia) is the strongest and toughest of all dental ceramics and meets the mechanical requirements for high-stress-bearing posterior restorations. The purpose of this study is to evaluate the effects of abutment and framework design, different layering materials (feldspathic porcelain, indirect composite material) and monolithic restorations on the fracture load of implant-supported zirconia-based single crowns after thermocycling.

Seventy ti-base abutments (Conelog 4.3-0.8mm, Camlog® Implant) were screwed onto dental implants (Conelog 4.3-11mm, Camlog® Implant). Abutment-implant complexes were randomly divided into seven groups (n = 10) according to the design of the zirconia abutment and framework (VITA YZ T, VITA Zahnfabrik) as follows: uniform-thickness zirconia abutment and uniform thickness zirconia framework layered with feldspathic porcelain (Group 1); layered with indirect composite material (Group 2); uniform-thickness zirconia abutment and anatomic design zirconia framework layered with feldspathic porcelain (Group 3); layered with indirect composite material (Group 4); anatomic design zirconia abutment and anatomic design zirconia framework layered with feldspathic porcelain (Group 5); layered with indirect composite material (Group 6); uniform-thickness zirconia abutment and monolithic zirconia crown (Group 7). All fabricated zirconia abutments were cemented on ti-base abutments, then all crowns were cemented on ti-base-zirconia abutment complexes. All specimens were exposed to 10.000 thermal cycles between 5°C and 55°C and then tested for fracture load.

The data were analyzed statistically by SPSS software. The data were analyzed by 3-way ANOVA to test the effect of abutment design, framework design, two different layering material and their interaction on the fracture load. Post-hoc assessment was performed using Independent sample t-test and Bonferroni corrected tests ($p \leq 0.05$). After fracture load testing, randomly selected specimens were sputtered with platin, for 120 s and observed with a scanning electron microscope (SEM) (JSM 7000 F, JEOL) at an original magnification of x10, and fracture mode classified as veneer fracture or framework fracture.

Anatomical abutment and framework designs showed significantly higher load compared to uniform abutment and framework designs regardless layering material ($p<0.05$). Porcelain layered groups performed significantly higher fracture load values in comparison to the groups layered with indirect composite ($p<0.05$). Monolithic zirconia crowns cemented onto ti-base abutments exhibited highest fracture load values ($p<0.001$).

Within the limitations of this in vitro study, it is concluded that uniformly thick layering material (anatomic abutment/framework design) of zirconia frameworks improve fracture load of implant-supported zirconia-based prostheses after thermocycling. The fracture loads for flat abutment/anatomic framework and anatomic abutment/anatomic framework designs in the layering feldspathic porcelain groups were significantly higher than those in the layering indirect composite groups. All types of implant-supported zirconia-based prostheses tested after thermocycling have the potential to withstand clinical chewing forces in posterior applications.

Effects of Different Sintering Times and Temperatures on Strength and Adhesion of Zirconia Ceramic

Eray Bal[a], *Baris Yavas*[b], *Gultekin Goller*[b], *Deniz Sen*[a]

[a]Department of Prosthodontics, Faculty of Dentistry, Istanbul University, Istanbul, Turkey.
[b]Department of Metallurgical Materials and Engineering, Istanbul Technical University, Istanbul, Turkey.

Keywords: zirconia, shear strength, adhesion

Introduction

Nowadays, zirconia-based restoration is widely used as a promising dental ceramic due to its excellent biocompatibility, high mechanical properties, good esthetics, and long- term stability.[1-4] The most frequently used type of dental zirconia is yttria tetragonal zirconia polycrystalline (Y-TZP). The mechanical properties of zirconia were proved to be higher than all other dental ceramics. Zirconia coping for crown or bridge framework requires the application of veneering ceramic for excellent esthetic. Studies showed the high success rates on zirconia crowns as 96% and 97% on tooth- supported and implant-supported. However, clinical studies in long-span FPDs reports approximately 27% of veneering ceramic chipping, and also founds framework fractures at 7% of FPDs for five years. Studies have shown less problem of zirconia core fracture, but higher rate of veneering ceramic chipping. There are a lot of factors such as coloring, sintering temperature, phase transformation, grain size which effects the mechanical properties of zirconia and acts on strength and adhesion of the material. Studies showed that increased sintering temperatures decreases the porosity of the material resulting in increased translucency and strength. Also change in sintering temperature effects the grain size and wetting properties of the zirconia which has an impact on adhesion with the ceramic superstructure. The aim of this study was to investigate the effects of different sintering times and temperatures on strength and adhesion of zirconia ceramic by mechanical tests and SEM evaluation.

Results and Discussion

The results of the BFS values could be divided into two significant groups. The lowest group was group 6, followed by the group 5. According to the results from the three tests made, no statistical difference was found between analyzed 6 groups ($p>0,05$). SEM evaluation of biaxiel bending test specimens showed larger grain size in higher temperatures. Microtensile and shear-bond test specimens showed cohesive and combined fractures on ceramic superstructures.

One test specimen from each group of biaxiel test was taken for SEM analysis. Grain sizes of zirconia substructures were measured on magnification of x20.000. With respect to the heat increase of the specimens grain size was increased (Fig. 1,2,3,4,5,6).

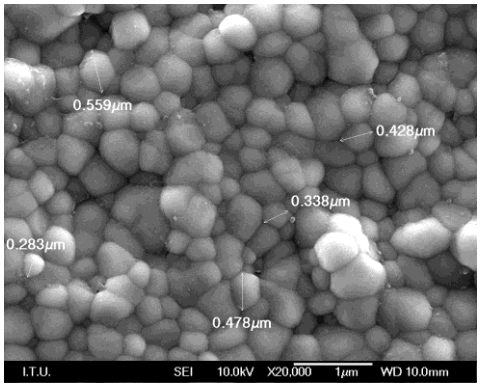

Fig 1. Group 1 SEM image

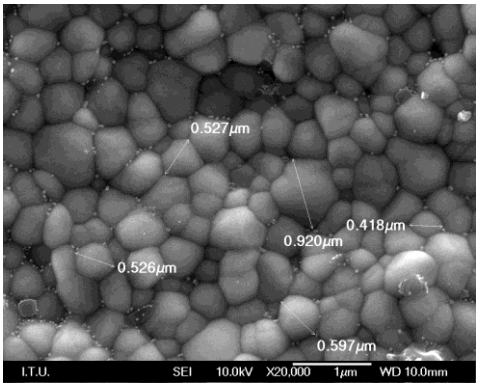

Fig 2. Group 2 SEM image

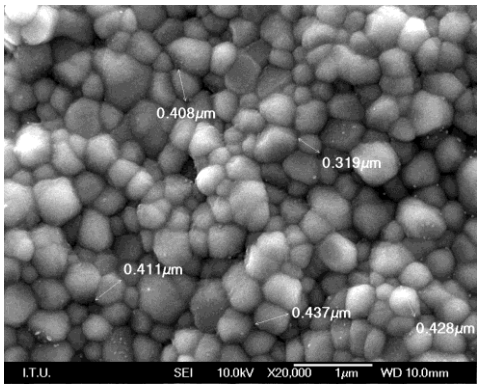

Fig 3. : Group 3 SEM image

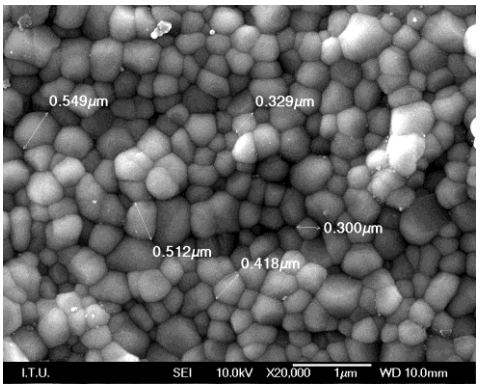

Fig 4. Group 4 SEM image

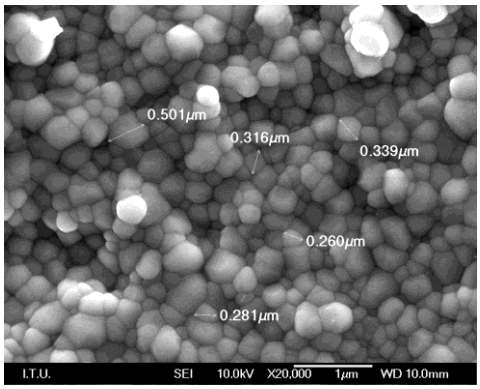

Fig 5. Group 5 SEM image

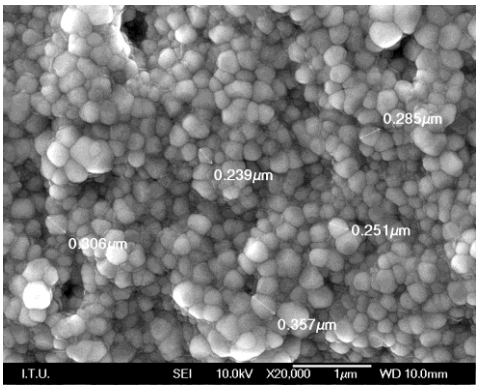

Fig 6 Group 6 SEM image

During our study biaxiel tests (3 ball on piston) was performed to evaluate strength, microtensile and shear bond tests was applied for bonding strength. XRD and SEM analysis was mad effort phase change and grain size. According to the test results groups 1530°C de 2 hour 1530°C 1 hour 1450°C de 2 hour and 1450°C 1 hour there were no significant difference but in the groups 1350°C de 2 hour and 1350°C de 1 hour sintering decreased tensile strength. When the sintering times was fixed for two hours and the sintering temperatures was chancing between the groups 1530°C and 1450°C there was no statistical difference but with the heat 1350°C there was a decrease. Same results was seen when sintering time was fixed for one hour at 1530°C and 14 50°C there was no statistical difference but at 1350°C there was an decrease. These test results show that sintering temperature under 1350°C decreases the strength. On a study about effect of coloring and sintering on translusensy Sen et al. changed temperatures between 1350°C, 1450°C and 1600°C. Through the study they tested two different zirconia substructures with one sintering time, biaxiel fracture test results showed a decrease at 1350° C. Their results shows parallel out comes as our study.

Results;

- No significant difference on strength with heat change
- Critical temperature for sintering is 1350°C and below
- Shear bond tests results shows no effect with time and heat change
- Microtensile test results shows no effect with time and heat change
- SEM evaluation shows increase on grain size with increased heat
- XRD analysis showed similar monolitic fase results

The present study suggests that lowering the sintering temperature until the threshold value of 1350°C has no effect on the BFS and has no effect on the shear bond values. For further results lowering the sintering temperature might be the key factor and should be taken into consideration. The dental laboratories should concern the factors mentioned above to for proper veneering ceramic adhesion and for the BFS of zirconia core material.

Conslusions

Within limitations of this study, the BFS of the zirconia did not effected from the change of the sintering time and heat. Shear bond modulus showed no difference between the groups. Grain size is effected by the heat change. Further studies of interfacial bonding strength and sintering factors should be investigated to obtain for the optimal strength and shear of bilayered ceramic.

Nanostructured Biomimetic Coatings for Orthopaedic Implants

G. Graziani[*a], M. De Carolis[a], A. Gambardella[a], M.Boi[a], D. Dallari[b], L.Vivarelli[b], M. Govoni[b], M. Berni[a], M.C. Maltarello[c], M.Bianchi[a]*

[a] Rizzoli Orthopaedic Institute (IOR), NanoBiotechnology Laboratory (NaBi), Bologna, Italy
[b] Rizzoli Orthopaedic Institute (IOR), Reconstructive Orthopaedic Surgery Innovative Techniques - Musculoskeletal Tissue Bank, Bologna, Italy
[c] Rizzoli Orthopaedic Institute (IOR), Laboratory of Musculoskeletal cell biology, Bologna, Italy
**e-mail: gabriela.graziani2@unibo.it*

Keywords: Bone implants, Biogenic apatite, Nanostructured coatings, Ion-substituted hydroxyapatite

Introduction

Calcium phosphates-based (CaP) coatings have been widely studied to confer osseoinductive properties to bio-inert orthopaedic implants and favour a firm bonding between the implant and the host bone [1]. However, traditional, thick plasma sprayed hydroxyapatite coatings (thickness >30 micron), exhibit severe drawbacks that limited their efficacy, linked to scarce homogeneity, tendency to cracking and delamination and/or insufficient control over coatings composition [1,2]. As a consequence, several techniques have been proposed to obtain CaP thin films (thickness below 1 μm) [1], that could overcome these issues and could have a nanostructured surface texture capable of boosting platelet, proteins and cells adhesion [3]. In addition, an increasing similarity to bone apatite is being pursued to boost host cells response; to this aim, both deposition of synthetic multi-substituted hydroxyapatites and deposition from biogenic apatites have been proposed.

Biomimetic bone apatite coatings have been recently proposed by the Authors [4], manufactured by a novel modification of Pulsed Electron Deposition technique, namely Ionized Jet Deposition (IJD). To maximize similarity to the host bone, deproteinized bone apatite was used as a deposition target. The coatings have shown promising results, as they were capable to boost host cells adhesion and to promote their osteogenic commitment, compared to conventional hydroxyapatite [4]. However, because the coatings are of very recent introduction, several parameters need to be investigated and optimized to ensure optimal efficacy.

Here, biomimetic (~ 450 nm thick) are deposited by IJD and different precursors and post deposition treatments are compared to find the best conditions to be applied. First, because the technique allows for a fine control over the coating composition, including trace elements, different bone apatite sources (bovine, equine, ovine and porcine bone) were compared in terms of composition and morphology (XRD, FT-IR, SEM/EDS), to be possibly used as deposition targets. All specimens were deproteinized by bleaching for 2 weeks in NaOCl, to remove organic components. Interestingly, the differences between these apatite sources can be of interest for xenografts, bone cements, granulates and other biomaterials where biological apatite finds application. In addition, after deposition, several annealing conditions are tested and compared, to obtain maximum adhesion and a crystallinity as similar as possible as that of bone, while preventing cracks formation and not altering coatings composition. A temperature range between 350 and 500°C is examined, as a significant transition in film crystallinity is expected around 350-400°C, based on literature data [5]. Heating duration (1h and 6h) is also taken into exam. Morphology (SEM/AFM), composition (grazing incidence XRD, FT-IR, EDS), crystallinity (XRD) and adhesion (micro-scratch) of the coatings are compared to determine the best conditions to be applied.

Results and Discussion

All biological samples are essentially composed by hydroxyapatite and carbonated hydroxyapatite, and minor differences are evidenced in their composition, mainly regarding the content of carbonates and magnesium, while essentially no differences are assessed in terms of crystallinity. Bovine bone was selected as a precursor, based on the higher content of Mg, that has significant biological role, high availability and easiness in shaping to obtain the targets.

All the obtained coatings exhibit a nanostructured surface morphology, as they are composed of granular aggregates having mean size ranging from few tens of nanometres up to a few microns. Surface morphology does not undergo significant modifications as a result of post-deposition annealing at 425°C and below. Instead, all films heated at 450°C and above experience cracking, so they were discarded and not subjected to further testing. Because of the nanostructuration and sub-micrometric thickness of the coatings, machining features at the micro- and macro- scale are not covered, which is desired as they are generally designed by the producers to promote implant stability (**Figure 1**).

Coatings composition perfectly resembles that of the deposition target, also for what concerns trace ions magnesium and strontium: 0.2 wt% Mg, 1 wt% Na and 0.2 wt% Mg, 0.8 wt% Na are detected by EDS for bone targets and for the deposited coatings, respectively. Films composition is scarcely influenced by annealing (**Figure 2a,b**), but a general decrease in the content of carbonates is registered for longer heating times (see bands at 1465, 1429, and 870 cm^{-1} in the FT-IR spectra). Crystallinity, instead is influenced by both annealing temperature and duration, as a significant transition in crystallinity of the films, from highly amorphous to partially crystalline, is assessed above 350°, also depending on heating duration (**Figure 2**).

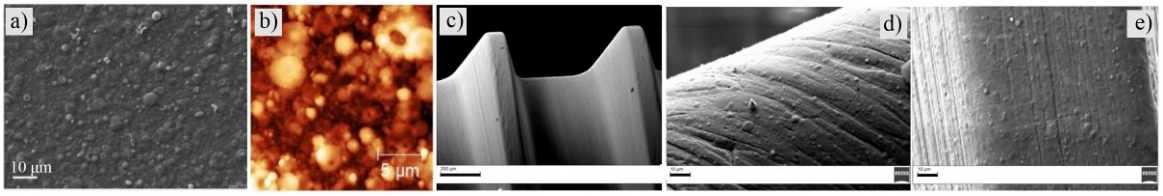

Figure 1: Nanostructured films morphology, as displayed by SEM (a) and AFM (b). In (d), (e) and (f) coatings are deposited on a dental screw and their morphology is examined by SEM. Scale bars in d) and e, f) are 100 µm and 20 µm, respectively.

As-deposited films exhibit scarce adhesion to the titanium substrate, that experience modifications as a consequence of annealing. More in detail, annealing at 350°C for 1h causes a slight increase in adhesion, while significant increases are registered when heating time is prolonged to 6h or the temperature is raised. These results (**Figure 2c**) closely match data obtained by FT-IR and XRD concerning films crystallinity, that is very scarce for as-deposited films, slightly increases for heating at 350°C for 1h and is remarkably higher for the other heating conditions. Films annealed at 350°C, for which a difference in crystallinity exists depending on heating duration, are the only ones to exhibit relevant differences in adhesion.

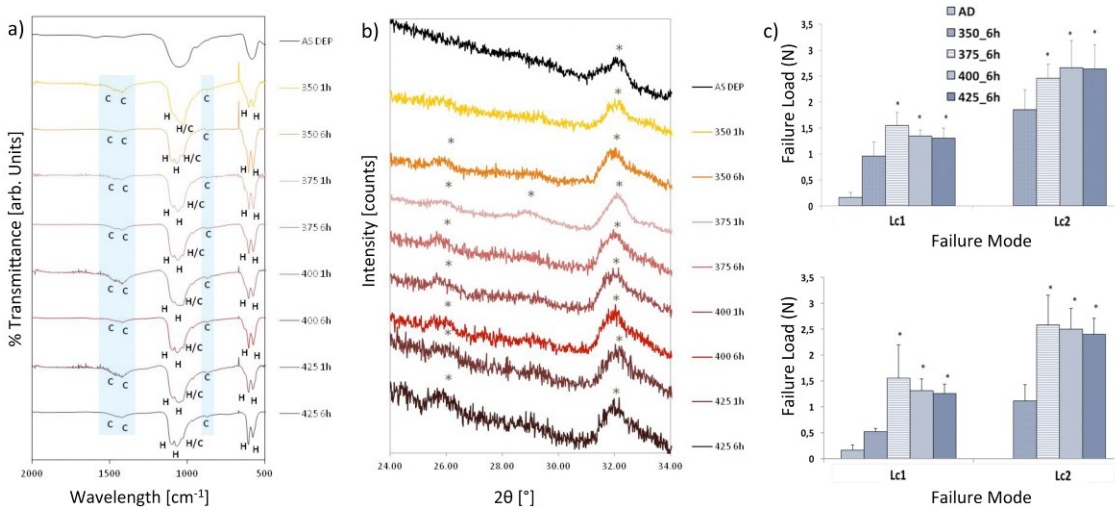

Figure 2: a) FT-IR and b) GI-XRD spectra of as deposited and annealed coatings. In Figure H and * stand for hydroxyapatite bands and peaks, respectively. c) Micro-scratch test on as-deposited and annealed coatings.

Based on these data, heating at 400°C for 1h was selected as the most promising condition, in view of the following considerations: (i) as-deposited films and films annealed at 350°C exhibited scarce adhesion to the substrate; (ii) heating for 6h causes a decrease in the content of carbonates, thus decreasing the similarity to the deposition target and, hence, to real bone. This similarity, instead, is expected to boost biological behaviour of the coatings, based on the biomimetic principle; (iii) no significant differences are assessed between the films annealed at temperatures above 400°C, so heating at the lowest temperature is preferred.

Conclusions

Biomimetic coatings were successfully deposited by IJD, having nanostructured surface texture and a composition perfectly resembling the deposition target (bone apatite). Upon annealing at 400°C, a crystallinity similar to that of bone is achieved and adhesion to the substrate is significantly increased, both characteristics being known to have a strong positive impact on promoting host cells attachment, proliferation and differentiation.

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Comparative Study of Histologic Responses to Pulpectomy with Metapex, Metapaste, and Vitapex in Dogs' Teeth

Je Seon Song,[a,b] *Byurira Kim*,[a] *Yooseok Shin*[c]

a Department of Pediatric Dentistry, College of Dentistry, Yonsei University, Seoul, Republic of Korea
b Oral Science Research Center, College of Dentistry, Yonsei University, Seoul, Republic of Korea
c Department of Conservative Dentistry, College of Dentistry, Yonsei University, Seoul, Republic of Korea
*e-mail: densys@yuhs.ac

Keywords: pulpectomy, vitapex, metapex, metapaste, histology

Introduction

The aim of the present study was to evaluate and compare in vivo response of apical tissues to Metapex(Meta Biomed Co., Ltd., South Korea), Metapaste(Meta Biomed Co.), and Vitapex(Neo Dental Chemical Products Co., Ltd., Tokyo, Japan) in dog pulpectomy models. Twelve beagle dogs aged 6 months were selected for the experiment. Pulpectomy was performed in 132 teeth (incisors and premolars) and periapical radiographies were carried out before and after experiment. The root canals were randomly filled with either Metapex(n=44), Metapaste(n=44), or Vitapex(n=44). All teeth were sealed with a layer of glass ionomer cement (KetacMolar, EPSE, Seefeld, Germany). After 4 or 13 weeks, respectively after operation, the dogs were sacrificed, and then longitudinal paraffin sections were made for histologic investigation. The following qualitative observations were recorded: the presence of polymorphonuclear neutrophils, lymphocytes and plasma cells, macrophages and/or giant cells, necrosis, neovascularization, fibrous condensation, fatty infiltrate and abscesses. Inflammatory reaction was evaluated and scored in a blind manner. The Fisher's exact test and Kruskal-Wallis test were used to compare three test groups (the significance level, < 0.05).

Results and Discussion

In 4-week-group, all inflammatory responses were in normal range except for fibrosis. Analyzing the mean scores for fibrosis, there was no statistically significant difference among three materials (Fisher's exact test, $p=0.91$). In 13-week-group, increased response to various inflammation reaction was shown compared to the 4-week-group. The presence of polymorphonuclear neutrophils, lymphocytes, macrophages, neovascularization, fibrosis, and abscesses were found. However, there were no statically significant differences between the tested substances in all inflammatory reaction. (Kruskal-Wallis test, $p>0.05$)

Conclusions

There were no significant differences among three materials in responses of apical tissues.

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Regeneration of Large Articular Subchondral defects using Adipose-derived Stem Cells and Platelet-Rich Plasma

F. Canonici[a], A. Zepparoni[b], A. Altigeri[b], A. Cersini[b], C. Cocumelli[b], E. Galvano[b], A. Fagiolo[b], C. Roncoroni[b], V. Antognetti[b], G. Pietrella[b], P. Ghisellini[c,d], C. Rando[c], G.P.M.Benedetti[e], S.L. Torchia[d], P. Canonici[a], F. Di Egidio[b], A. Amaddeo[b], R. Eggenhöffner[c,d], K. Barbaro[b,d]*

[a]Equine Practice srl, Campagnano, Roma, Italy.
[b]Istituto Zooprofilattico Sperimentale Lazio e Toscana "M. Aleandri", Roma, Italy
[c]Università di Genova, Facoltà di Medicina, Dipartimento di Scienze Chirurgiche e Diagnostiche Integrate (DISC), Genova, Italy
[d]Consorzio Interuniversitario INBB - Viale delle Medaglie d'Oro, 305, 00136 Roma, Italy
[e]Studio Dentistico, Fiano Romano, Roma, Italy
**katia.barbaro@izslt.it*

Keywords: Stem cells, adipose tissue, bone regeneration

Introduction

Multipotency of mesenchymal stem cells (MSC) derived from bone marrow (BMSC) as well as from adipose tissue (AMSC) has been broadly demonstrated. However, compared with BMSC, use of AMSC is advantageous regarding their major concentration in the tissue of origin, the practicability of the fat collection, more straightforward cell isolation and enrichment.
In this study, we investigated the feasibility of using autologous AMSC for the bone regeneration in repairing a case of subchondral bone cyst of the medial femoral condyle of a thoroughbred horse. *In vitro* and *in vivo* characterization and verification of differentiation capacity into osteogenic and adipogenic lineage was carried out. A platelet gel was used not only as a scaffold but, more importantly, to guarantee the supply of cell growth factors.
The efficacy of treatment, based on the radiographic and clinical follow up, was conducted over 20-month period assessing bone growth and functional recovery following implantation.
BMSC, in the presence of adequate growth factors, are capable of multiplying and differentiating into mesenchymal cellular lineage lines (myocytes, osteocytes, chondrocytes, neurons, adipocytes, fibroblasts, hepatocytes, and so forth) AMSC [1] can be multiplied *in vitro* and differentiated from those of bone marrow [2, 3, 4]. Considering the various advantages - including ease of isolation, rapidity of expansion, multipotentiality and the number of cells present up to 50 times higher [5, 6], AMSC can be an alternative protocol concerning BMSC. The aim of the work was to characterize the differentiation potential of equine AMSC, *in vitro* and *in vivo*, in the osteogenic and adipogenic sense and to define an experimental protocol for their therapeutic use in the treatment of the subchondral cyst of the medial femoral condyle of the horse.

Results and Discussion

AMSC cells isolated from the subject under treatment reached confluence since four-five days after the beginning of their isolation. The cell population shows an elongated fibroblastoid morphology, rather homogeneous since the first passage.

The *in vitro* osteogenic differentiation of AMSC resulted already at the end of the first week of stimulation with induction effects, as highlighted both by the presence of abundant calcium deposits, coloring with Alizarin S (**Figure 1a**).

In vitro adipogenic differentiation was instead considered after the second week by specific stimuli. It was made detectable by the positiveness of some adipocytic colonies to the coloration with Oil Red (**Figure 1b**).

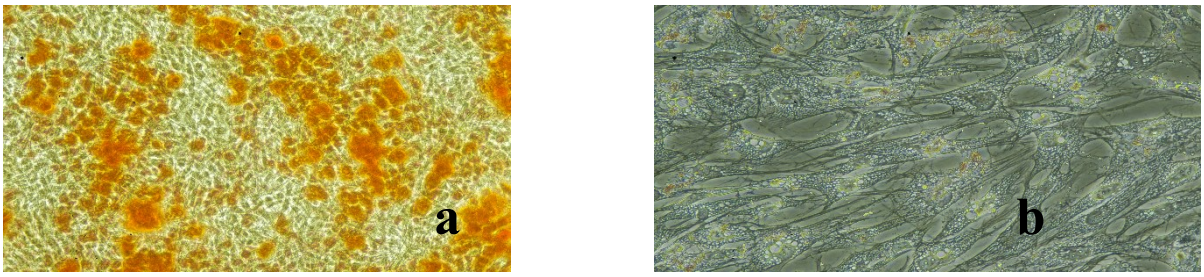

Figure 1: *In vitro* (a) osteogenic and (b) adipogenic differentiation.

The control radiographs carried out after 3, 5 and 10 months after insertion of the AMSC-containing platelet gel, showed steady and progressive improvement with an increase in thickness of the subchondral plate and filling of the cyst cavity until it almost disappeared. From a functional point of view, the lameness has disappeared from the second month after the intervention, the subject has resumed training from the fourth month and started the competitive activity between the tenth and the eleventh month.

Conclusions and/or Outlook The AMSC implant in platelet gel has provided encouraging results and could therefore also find useful applications for other bone diseases of non-critical dimensions resistant to conventional orthopedic therapies.
In particular, with the use of AMSC and autologous gels, every problem related to rejection is overcome. Adipose tissue can, therefore, be considered an elective substrate for obtaining mesenchymal progenitor cells. Given the minimally invasive sampling techniques, the abundance regarding a biological matrix, concentration, and observed growth characteristics, the present approach can be considered more widely applicable besides the regenerative medicine described in the present work.

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Does the harvesting site influence the osteogenic potential of MSCs?

Thi Van Nguyen [a], *Irene Tessaro* [a], *Giuseppe M. Peretti* [a,b], *Laura Mangiavini* [a]
[a]

[a] IRCCS Istituto Ortopedico Galeazzi, Milano, Italy
[b] Department of Biomedical Sciences for Health, University of Milan, Milano, Italy
**e-mail: lauman82@hotmail.it*

Keywords: Mesenchymal cells, bone, bone stock, in vitro

Introduction
Hip arthroplasty represents one of the most common surgery in the orthopaedic field and it represents the definitive treatment for osteoarthritis or hip dysplasia. The osteointegration between the implant and the surrounding bone is crucial for a successful surgery: osteointegration is mainly due to new bone formation between the prosthesis and the bone (bone ingrowth). Thus, the quality of bone (bone-stock) at the level of the proximal femur and the acetabulum is essential to obtain an adequate osteointegration. In some cases of first implant, bone-stock is insufficient due to comorbidities (i.e. osteoporosis); whereas bone-stock is often scarce in case of revision arthroplasties due to aseptic mobilization of the implant. Gold standard techniques to improve bone-stock require the use of autologous bone grafts [1-7]. However, scientific evidences on the effect of bone harvesting site on the osteogenic potential of multipotent mesenchymal cells are still lacking. Thus, the aim of our project is to characterize and compare mesenchymal stem cells harvested from the subchondral bone of acetabulum, from the trabecular bone of the femoral head or from the bone marrow of the proximal femur.

Methods
Cells were harvested from 13 patients undergoing hip arthroplasty. The percentage of MSCs were evaluated by FACS analysis and CFU assay. Then, cells were induced to differentiate into the adipogenic, osteogenic and chondrogenic lineage and they were compared.

Results
Preliminary data by FACS analysis demonstrated the positivity for mesenchymal markers for all the cell populations. Moreover, CFU assay show that there is an increase in Colony Forming Units in cells harvested from the acetabulum. These results need to be further confirmed.

Conclusions
This study will allow us to identify differences in the osteogenic potential between MSCs harvested from different site and, therefore, we will be able to choose the best harvesting site to improve the bone-stock during hip arthroplasty.

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New medical device for a rapid and benchless methodology for batteriuria screening

E. Nicolai[*a], S. Frattegiani, G. Motolese[b], C. D'Agostini[c], C. Favalli[c], N. Rosato[a]

[a] Department of Experimental Medicine and Surgery, University of Rome Tor Vergata, Rome, Italy
[b] ASI-Apparecchiature Scientifiche Innovative, srl Milan, Italy
[c] Clinical Micorbiology Laboratories, Tor Vergata Policlinic Foundation, Rome, Italy
e-mail: nicolai@med.uniroma2.it

Keywords: urinary tract infection, fluorescence assay, bacteriuria screening, nucleic probe.

Introduction

Urinary tract infection (UTI) is one of the most common infections, in both hospitalized and community patients and its diagnosis requires symptoms, signs and the urinoculture results. These last data are obtained in the clinical microbiology laboratory. For most patients the disease of the infection is minimal but for particular subpopulations may develop serious complications. Urine culture is the standard for diagnosing urinary tract infections, even if it is a laborious procedure and produces more than 60% of negative results. A fast screening method able to reduce the urine culture will have a deep impact on laboratory in term of workload and cost for a clinical analysis. Moreover the exclusion of bacteria infection through a fast test may help to reduce unnecessary prescriptions and usage of antibiotics. The increased usage of antibiotics indeed has consequences, such as prolonged infections, that may dramatically increase recovery time, hospital stays and health care costs. Furthermore it may also determine the growth of resistant micro-organisms which do not respond to conventional treatment, resulting in prolonged illness and greater risk for health.

We evaluated the detection of bacteria by Bioscreen, an instrument developed by ASI (Milan, Italy) based on a technology patented in collaboration with the University of Urbana-Champaign, IL, USA.

Results and Discussion

We compared the detection of bacteria of Bioscreen to the standard methodology adopted by the clinical microbiology laboratory of Polyclinic Tor Vergata. A total of 962 urine specimens were collected from both inpatients and outpatients of the Tor Vergata Policlinic (PTV), University hospital of Rome Tor Vergata. The microbiology screening was performed using the HB&L-Uro4 system by the hospital Clinical Microbiology Laboratory, and using the Bioscreen system by the department of Experimental Medicine and Surgery of the University of Rome Tor Vergata.

The data analysis performed to evaluate the BiesseBioscreen, was done by calculating sensitivity, specificity, LR+, LR-, DOR, positive predicted value (PPV), negative predicted value (NPV), false-negative rate (FNR), and false positive rate (FPR) according to European Committee on Antimicrobial Susceptibility Testing. The results are reported in table1 and table 2.

Data analysis of Bioscreen performance in bacteria screening showed very good testing results, demonstrating the equivalence of this system versus the traditional approach.

Table1:

number of samples	Sensitivity [a] (%)	Specificity [b] (%)	PPV [c] (%)	NPV [d] (%)	FNR [e] (%)	FPR [f] (%)
962	99.0	77.6	54.0	99.7	0.99	22.4

[a] Sensitivity = number of true positives/(number of true positives + number of false negatives)
[b] Specificity = number of true negatives/(number of false positives + number of true negatives)
[c] PPV= positive predicted value
[d] NPV= negative predicted value
[e] FNR= false negative rate
[f] FPR= false positive rate

Table 2:

number of samples	LR+ [a]	LR- [b]	DOR [c]
962	4.426	0.0128	345.781

[a] LV+= positive likelihood ratio
[b] LV- = negative likelihood ratio
[c] DOR = diagnostic odd ratio

Conclusions/Outlook

Overall the BiesseBioscreen system showed an excellent diagnostic accuracy, compared with the standard HB&L-Uro4 analysis, in terms of all statistical parameters. The high sensitivity and the high NPV obtained demonstrate that BiesseBioscreen analysis can be used to identify negative samples, which do not need further culture testing. In particular the 45% of positive samples obtained with this methodology implies that 530/962 (55%) samples could be excluded from urine culture after BiesseBioscreen analysis. This reduction of avoidable urine culture sample examinations could lead to a decrease of laboratory analysis costs. Furthermore the short time (less than ten minutes) needed to identify a negative sample could save workload and unnecessary antibiotic therapy. With our industry partners (ASI), we could also develop an automated and portable instrument that is validated and ready for commercialization, to increase a widespread use in a point-of-care setting.

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New Technologies for Aseptic Production of Tissue Engineering Products

M. Fadda[a], *G. Guidi*[a], *M. Cesarini*[a]

[a] COMECER spa, Castel Bolognese, RA
**e-mail: mfadda@comecer.com*

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Introduction

The interest in advanced therapy medicinal products (ATPMs) - or CGTs (cell and gene therapies) - for human use continues to grow due to the promising therapeutic potential of these drugs. Never have approvals of such products from the European Medicine Agency (EMA) or the US Food and Drug Administration (FDA) been so popular as in 2017 and 2018. Consequently, a growing demand has been generated, which will suddenly lead to requirements for mass production of these biological products.
These products are classified into three main areas: gene, which work by inserting/substituting recombinant genes into the body to treat genetic disorders or any other gene-bounded diseases; somatic-cell, which contain cells or tissues that have been manipulated to change their biological characteristics to cure, diagnose or prevent diseases; and tissue-engineered, modified to repair, regenerate or replace degenerated or injured human tissue. These advanced therapies are subject to legislation and must comply with current good manufacturing practices (cGMP), Annex 1 and 2, to allow commercialization; production is assimilated to traditional sterile pharmaceutical production.
There are different possible manufacturing scenarios. When we deal with the treatment of rare diseases, the quantities of cell cultures required are limited. On the other hand, when considering the cell therapy or tissue-engineered products necessary for diseases that are spread across a large population, the quantity of cells required is incredibly high: thus more and bigger cell factories are necessary.
Initial formulation and doses ATMPs usually start in a laboratory approach. It is not uncommon to find that all the preclinical and Phase I clinical studies run according to protocols prepared in this way.
A laboratory approach is obviously distant from the principles set in cGMP. However, there has been success in single procedures for the manufacture of these cell products. But transition from an individual culture system to mass production, requires additional classified spaces, process scalability (to increase production capacity without losing quality), extra attention on integrity and cross-contamination issues (when facing multi-patient jobs) and an overall increased quality outlook.
Expanding a cleanroom facility is a costly and challenging task; there are considerations about footprint, connections with the existing facilities and loss of continuity in production; it also implies arduous logistical considerations in managing construction work inside an environment that should be aseptic.

Results and Discussion

Some of the issues mentioned above can be addressed by moving from an "all-in-one room" solution, typical of the current cleanroom approach, to an integrated modular system, benefitting from isolation technology advantages and capabilities offered by sterile connections.
Following these guidelines, we have designed and built a Grade A modular incubation system that we call FlexyCult^{TM}, with all the characteristics of a laboratory incubator (**Figure** 1): temperature, relative humidity and carbon dioxide are controlled and maintained within given boundaries. The air flow is a HEPA filtered closed air loop and the system kept in overpressure. The innovation is that the incubator itself is stowed in a docking station where all the utilities are concentrated and shared with the incubations modules in a 3-, 6- or 9- module configuration. Single incubators can then couple with an isolator through a dedicated rapid transfer port (RTP) - i.e. an aseptic connection. Cells undergoing cultivation are then transferred to the isolator and processed according to the procedural needs. The isolator is equipped with all the necessary laboratory tools for cell processing and can be personalized to customer's requirements. The equipment is a closed system and can be located in a Grade D area, according to regulations [1].
The proposed approach meets expectations of the cell culture environment for several reasons: the sterility is superior due to the segregated environments in which the cells are manipulated; the cells are also entirely separated from the operator; never exposed to direct contact with humans; cells are always manipulated under Grade A conditions. Vaporized hydrogen peroxide (H_2O_2) sterilization can be carried out in the incubator when it is empty, as well as in the isolator when not in use between different production cycles. The footprint of the FlexyCult^{TM} system (incubators and isolator) is smaller than that of a Class B cleanroom required to perform comparable activities. The energy requirement is lower, and so it is the volume of air exchanged. This reflects in operational costs which are much lower in terms of power consumption and environmental monitoring

Figure 1 The Flexycult incubator

requirements; savings are estimated at around 60% of the yearly costs of the standard cleanroom approach. Operators can also work without special sterile gowning, saving costs in disposable wearables and time for gowning/de-gowning, which definitely translates into a productivity gain.

Conclusions and Outlook

The COMECER approach to cell culture applications has been chosen by CO.DON AG, a German biotech company that already produces well-established autologous cell therapy for tissue regeneration. The procedure enables the regeneration of high-quality, biomechanical load-bearing and pressure-resistant cartilage tissue. By using the potential of autologous cells, joint cartilage defects can be treated successfully. The three-dimensional structure of autologous cultured chondrocytes and autologous self-constructed matrices leads to the reconstruction of cartilage-specific functional properties [2].

The procedure has been already successfully used in more than 12,000 patients. The product was approved by the CAT committee of the European Medicine Agency in July 2017 for commercialisation within the European Union, and the company has started a project for improving its production capacity, with the intent to reach approximatively 4,500 manufactured batches per year.

To accomplish these goals a total of 16 docking stations, each composed of 9 FlexyCultTM incubators, 3 FlexyCultTM Isolators and an accompanying H2O2 sterilisation stations have been arranged to fit into a brand new dedicated laboratory (see photo on p17).

Isolators are managed by operators in a Class D environment while incubators can be located in a non-classified environment. Extensive use of software controls allows easy management of incubator retrieval from the docking system and automatic guided vehicles run into the lab transporting appropriate elements according to operator instructions.

With this level of automation, operators are fully concentrated on the critical operations, minimising their involvement in tasks not strictly linked to their specific competency.

Controls, cross-checks, tracking and logging of the operations in each incubator are demanded to software and massive use of barcodes and radio-frequency identification (RFID) technology. These tools and its design make the system robust, easy to use and to the highest regulatory standards.

Respect for quality requirements is a key point in any drug production process. Robust manufacturing makes the production independent from interruptions and ensures consistency of the result over time.

When dealing with ATMPs, living cells used in these products must be viable, pure and, most importantly, sterile. Containment of the product is crucial as the process is patient specific and involves highly qualified operators. Very often, we do not have a second manufacturing chance, so requirements are even more stringent. Larger quantities need to be produced without compromising on quality and the price needs to be feasible so that healthcare organisations can afford the treatments.

The proposal of a modular, partially-automated manufacturing system for ATMPs based on isolation technology makes possible its use to reduce the production costs of the drugs. Moreover, the system is designed to be expandable allowing extensions of the production in successive steps.

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Nanofibrous matrices for contaminated wounds management: Sustained antibiotic release via surface corrosion

M. Rysová[a,b], *H. Tománková*[a], *D. Kadlečík*[a], *T. Zajíc*[c], *J. Maryška*[a,b]

[a] Institute for Nanomaterials, Novel Technologies and Innovation, Technical university of Liberec, CZ
[b] Institute of New Technologies and Applied Informatics, Technical university of Liberec, CZ
[c] Centre for Laboratory Medicine, Regional Hospital Liberec, CZ
**e-mail: miroslava.rysova@tul.cz*

Keywords: Nanofibres, silica, drug delivery, wound healing, degradation

Introduction

Development of contaminated non-healing wounds, ulcers and decubitus ulcers represents one of serious issues related to deceases of affluence - diabetes mellitus primarily. According to statistics, the risk for developing foot ulcers is 25% high in patients with diabetes and it is also reported that every 30 seconds, one lower limb amputation in diabetes patients occurred around the world. It is estimated in diabetic patients that of all amputations, 85% are contributed by foot ulceration which further deteriorates to chronic infection and severe forms of gangrene. Overall, the prevalence rate of diabetic foot ulcers is estimated to 5.1% in Europe with cost demand increasing every year. [1] In 2005, for instance, the average cost of contaminated foot ulcer treatment in Germany was $ 2,957. [2] This cost doesn´t involve the social impact or any emotional effects on the patient.

In such as complex issue, if the ulceration formation can´t be avoided, the most suitable therapy is demanded to prevent the amputation and minimize the long term impact on patient. The in situ therapy based on the individual wound bacterial profile seems to be the most effective and the most sensitive treatment and application of a nanosized dosage form may bring further benefits in form of modification of the wound environment and increasing the bioavailability of the released drug. From this point of view, silica nanofibres represent a very promising material for this application, combining traditional nanofibres´ properties such as small fibre diameter and high porosity and unique properties given by their inorganic nature, specific degradation profile, stability in various solvents and modular functionality of their surface. In this study, we present a short overview of their properties and kinetics of the antibiotic released.

Silica nanofibres prepared by sol-gel method and subsequent needleless electrospinning, confirmed to be biocompatible in our previous work [3], were thermally stabilized under specific conditions (<200°C) and studied in terms of morphology, degradation *in vitro* and drug release kinetics. As a model drug, tetracycline was used due to its easy visualization and wide-spectrum antibacterial effect. The drug release profile was compared in pristine and nanofibres amino-functionalized via silanization process.

Results and Discussion

The morphology evaluation performed on pristine silica nanofibres after stabilization and the same matter after 72 hours of degradation shown (a) the original fibrous morphology obtained by electrospinning and (b) revealed the degradation character of the silica nanofibres (see Figure 1). The corrosive degradation character, typical for the so called Stöber silica, based on surface corrosion of the outer layers without swelling or loss of the fibrous structure, was observed in pristine and amino-functionalized nanofibres. Moreover, the degradation of the functionalized nanofibres was found to be slowed down within first 96 hours. This was found to be beneficial for drug release.

Both, the functionalized and non-functionalized nanofibres exhibited strong adsorption capacity for antibiotic and sustained release for 96 hours. The sustained antibacterial activity was demonstrated on *E. Coli* as a model bacterial strain. As shown on Figure 2, even after 72 hours of exposure, an evident diffusion zone was observed, although the functionalized nanofibres produced more stable and extensive zone over the tested time course (see Figure 2c). There results can be attributed to the synergic effect of the positive charge of the surface of functionalized nanofibres and prolonged degradation enabling sustained and stable release over 72 hours.

Conclusions and outlook

The performed research confirmed the silica nanofibres an interesting candidate for antibiotic adsorption and release in the wound site. Their degradation character, keeping the porous structure opened without swelling, was altered by sustained release, achieved by the functional groups grafting. This behaviour is advantageous over the conventional biodegradable nanofibrous structures, as the degradation process of these usually leads to swelling and subsequent closure of the interfibrous pores and formation of impermeable film, limiting the gas exchange between wound and outer environment. We believe that the synergic effect of the degradation and surface properties causes the sustained release of the antibiotic, stable for over 72 hours, which is clinical practice common dressage interval. These results imply the applicability of this system in modern wound therapy.

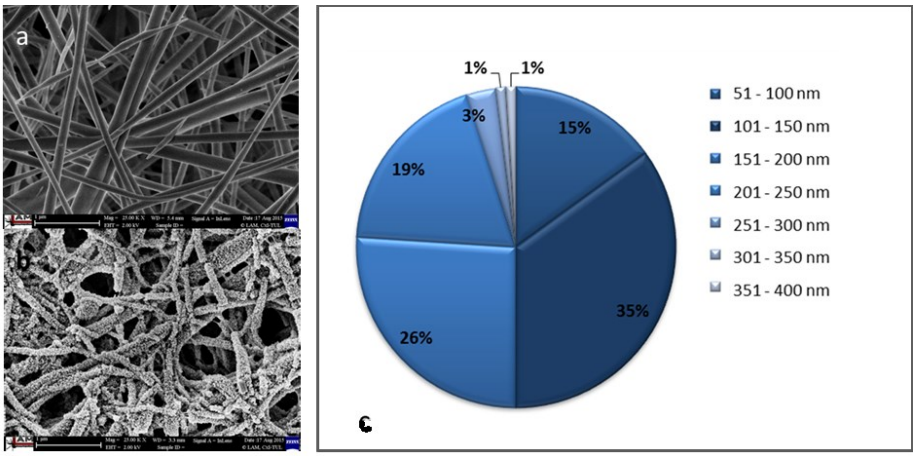

Figure 1: Morphological characterization of the (a) pristine silica nanofibres and (b) silica nanofibres after 72 hours of degradation in SBF. (c) The size distribution of pristine silica nanofibres – fibre diameter classes.

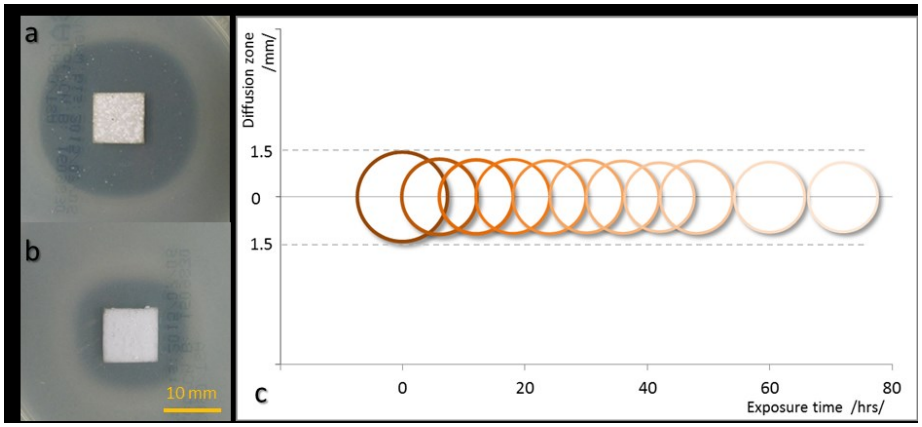

Figure 2: Antimicrobial effect of the silica nanofibres with antibiotic. Diffusion zone on *E. Coli* culture after 72 hours induced by antibiotic adsorbed on (a) functionalized nanofibres and (b) pristine nanofibres. (c) Size of the diffusion zone induced by antibiotic released from the amino-functionalized silica nanofibres over time.

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