BIOMATERIALS FOR BONE REGENERATION

PROCEEDINGS OF THE INNOVABONE CONFERENCE

INDEX

A radical innovation in biomaterials for orthopaedic applications

LEGEND

ELR	Elastin-like recombinamer
LCM	D,L-lactide, e-caprolactone, methacrylic acid

INNOVABONE

at the leading edge of regenerative medicine

Cellular and molecular biologists, immunologists, physicists, bioengineers and orthopaedic surgeons work together to develop a combination of novel bioactive biomaterials to tackle the morbidity associated with serious non-healing bone lesions.

The InnovaBone plan is to develop a prototype, upscale its production and go through all the steps required for future clinical phase trials and commercial exploitation.

This product will contribute to the EU competitive advantage in the biomaterials global market.

bone r

develop

bioactive

ensuring healthy

regeneration

oing bioinspired smart

biomaterials

INTRODUCTION TO THE INNOVABONE SCIENTIFIC CONFERENCE: BIOMATERIALS FOR BONE REGENERATION

Oskar Hoffmann

InnovaBone Project Coordinator
Department of Pharmacology and Toxicology , University of Vienna, Austria

On behalf of the InnovaBone consortium I would like to welcome you to the conference in Brussels. InnovaBone is a Large Collaborative FP7 Project with a strong multidisciplinary approach to develop smart bone biomaterials. 14 partners from 8 European countries were involved in the project.

The importance of developing novel approaches for bone repair is underscored by the heavy burden on health care costs and patient suffering caused by traumatic, osteoporotic and osteolytic metastatic bone lesions. To address these health challenges, InnovaBone is dedicated to the development of optimally performing bioinspired biomaterials mimicking the natural physiological processes underlying bone repair of non-healing bone lesions. Our ultimate aims are to ensure strong, healthy bone regeneration, reduce pain and suffering and to become a competitor in the biomaterials market of Europe. Using an extensive, state-of-the-art approach by the InnovaBone team of cellular and molecular biologists, immunologists, physicists, bioengineers, and orthopaedic surgeons, the aim is to tackle serious non-healing bone lesions. The overall approach of InnovaBone is to produce smart bioactive 3D scaffolds to fit within bone lesions, which will then be combined with functional, genetically-engineered self-solidifying elastin-like recombiners containing calcium phosphate nanoparticles. This combination of materials aims to encourage cells to attach within the scaffolded area, promote cell growth and ultimately start the bone regeneration process, which helps the body self repair. InnovaBone scientists established comprehensive production and testing platforms to obtain optimal products. A range of biomaterials was tested for their effects on bone cells, bone growth and healing, on immune and allergic cells with state-of-the-art *in vitro* and *in vivo* models, imaging technology, in addition to a thorough evaluation of physicochemical properties including strength, durability, elasticity, toxicity, sterilisation capacity, degradability, and more. Novel, automated 2-photon polymerization equipment and an innovative BioMEMs bioreactor system were developed during the project to upscale biomaterial production and to study the effect of the biomaterials under dynamic conditions. The InnovaBone multidisciplinary, multidimensional approach for the developments and preclinical assessment of bone biomaterials will be presented during this meeting. ■

THE INNOVABONE PRODUCT

A dual component product: 3D scaffold & bioactive gel

We combine bioinspired materials mimicking the natural physiological processes underlying bone repair. This pioneering strategy aims to accelerate bone healing and reduce adverse side effects with the currently employed biomaterials.

The scaffold and gel combination allows cells from the surrounding bone tissue to migrate into the scaffold and initiate bone repair by triggering new bone formation.

The Scaffold

Designed to fit within bone lesions, our three-dimensional scaffolds are made using biodegradable and biocompatible photopolymers. Upon implantation, they provide the necessary support for the bioactive gel and will be replaced by newly formed bone during bone regeneration.

The Gel

Our gel consists in genetically-engineered elastin-like polymers. Once the scaffold is in place, the liquid polymers are injected. The gel solidifies at body temperature and contains growth factors and hydroxyapatite nanoparticles required for new bone formation.

NEW DEVELOPMENTS IN BONE REGENERATION

Rainer Kluger

Sozialmedizinisches Zentrum Ost – Donauspital, Vienna

RAINER KLUGER, orthopedic surgeon from Sozialmedizinisches Zentrum Ost – Donauspital, Vienna will introduce the latest state-of-the-art therapeutic approaches for non-union bone lesions from a clinical perspective.

INVITED SPEAKER

HIERARCHICALLY ORGANIZED POROUS DEVICES FOR BONE TISSUE ENGINEERING

João Mano

Universidad do Minho

Nanolayered films have been often fabricated using the layer-by-layer technology, where consecutive layers of macromolecules are assembled and stabilised by electrostatic interactions. Using adequate templates, non-flat coatings can be fabricated with tuned compositions. This enables the production of very well controlled multifunctional and structural devices using mild processing conditions that could be useful in biomedicine, including in bone tissue engineering. In such applications, where there is a direct interaction between the implant with tissues and cells, the biomaterials must exhibit adequate surface characteristics, both at the chemical and topographic points of view. Examples of structures having nano-stratified multilayered organizations as building-blocks are presented, based on the use of natural macromolecules. Functional and bio-instructive multilayers may be produced by introducing special chemical groups or bioactive agents in the assembly. Such elements may be then hierarchically organised into 3-dimensional systems for cell colonisation (e.g. capsules or scaffolds) with tuned structural and geometrical control. Adequate signals or cell sources may be used to direct the osteogenic route of the developed devices, to potentiate their bone regenerative capability. ■

DR JOÃO MANO

João F. Mano (CEng, PhD, DSc) received a PhD in chemistry from the Technical University of Lisbon (1996) and an habilitation on Tissue Engineering, Regenerative Medicine and Stem Cells from the University of Minho (2012). Is currently a Professor at the Polymer Engineering Department, School of Engineering, University of Minho, Portugal, a vice-director of the 3B's Research Group and a Member of the Governing Board of the PT Government Associate Laboratory ICVS/3B´s. His research interests include the development of new materials and multidisciplinary concepts for biomedical applications, especially aimed at being used in tissue engineering and in the controlled delivery of bioactive molecules. In particular, he has been developing bioinstructive materials, mainly derived from natural-based biodegradable polymers, or biomimetic and nanotechnology approaches applied to materials and surfaces, to be used in the biomedical area. He has published more than 480 research papers, and belongs to the editorial board of a series of scientific journals. He has been involved in numerous national and European research projects and participated in the organization of scientific events in the area of polymer/materials science, nanobiomaterials and biomaterials/tissue engineering. J.F. Mano awarded the Stimulus to Excellence by the Portuguese Minister for Science and Technology in 2005, the Materials Science and Technology Prize, attributed by the Federation of European Materials Societies in 2007 and the major BES innovation award in 2010. In 2015 he was awarded with a prestigious ERC Advanced grant by the European Research Council.

INVITED SPEAKER

BONE REGENERATION USING STEM CELLS AND BIOMATERIALS

Pierre Layrolle

University of Nantes

Bone is the most transplanted tissue in human with about 1 million procedures annually in Europe. Autologous bone graft is the gold standard in bone regeneration but it requires a second surgery, is limited in quantity and often associated with complications. Synthetic calcium phosphate biomaterial in association with mesenchymal stem cells is a potent alternative to autologous bone grafting. Starting from a bone marrow aspirate, several hundred millions of mesenchymal stem cells (MSC) are produced in 3 weeks in a culture medium containing human blood platelet lysate plasma. These cells are fixed on biphasic calcium phosphate (BCP) granules and then implanted in subcutis of nude mice where they produced mature bone tissue. The mixture of human mesenchymal stem cells and biomaterial is also effective in bone healing of critical size defects in calvaria and femurs of nude rats. The procedure has also proven efficacy in regenerating diaphyseal defects in metatarsis of sheep. In the European project REBORNE, bone regeneration was successfully achieved in 4 multi centre clinical trials. Patients suffering from non-union fractures, osteonecrosis of the femoral head, cleft palates or insufficient mandibular bone for dental implants were effectively treated with autologous stem cells and biomaterials. This presentation will give the latest pre-clinical and clinical results in bone regeneration. ■

DR PIERRE LAYROLLE

Pierre Layrolle has extensive experience in tissue engineering research both in industry and academia. He obtained his PhD in biomaterials in 1994 at the Polytechnic National Institute of Toulouse (FR) and his thesis was awarded the Leopold Escande prize. He completed his postdoctoral studies in Japan and later joined the tissue engineering company IsoTis (NL) prior to enter INSERM as Director of Research in Nantes. In 2007, he received the Jean Leray Award from the European Society for Biomaterials.

He is currently the coordinator of the FP7 REBORNE project, aimed at regenerating bone defects using a combination of stem cells and biomaterials. This project gathers 24 partners in 8 European countries, including research labs, hospitals, biomaterial companies and cell manufacturing facilities. His team has conducted a vast amount of pre-clinical studies and 4 clinical trials are currently underway which show excellent results. His work is routinely highlighted in national and international media.

Pierre Layrolle is inventor of 14 patents and co-founder of the spinoff company Biomedical Tissues that produces innovative medical devices based on biomimetic microfibrous polymer matrices. He has authored over 140 peer-reviewed publications (5955 citations, h-index 44) is a member of the Editorial board of several journals (Acta Biomaterialia, Biomedical Materials, J Mater Sci Mater Med, The Open bone journal) and regularly invited to present at international conferences. He has also organized several conferences such as the European Society for Biomaterials in 2006, Bioceramics 20 in 2007 and the European Orthopaedic Research Society conference in 2014.

ABOUT

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MATERIALS

ELASTIN-LIKE RECOMBINAMERS FOR INJECTABLE ECM ANALOGS

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Montequi Irene
Rodríguez-Cabello José Carlos

G.I.R. BIOFORGE - UVa- Universidad Valladolid, Valladolid, Spain

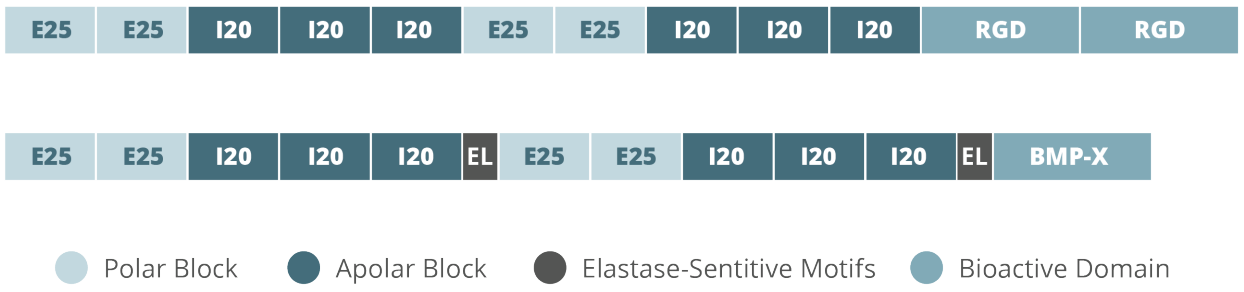

Figure 1 *Schematic representation of the ELR structures. The RGD-ELR does not contain elastase sensitive motifs, while those with BMP2 or BMP7 factors do.*

BIOFORGE´s main task has been the development and production of a gel matrix containing bioactive molecules which could be easily injected into the 2PP scaffolds and further solidifies at body temperature. To achieve such properties, BIOFORGE took advantage of its expertise in recombinant elastin-like thermoresponsive polymer gels and developed a biocompatible, smart and self-assembling high molecular weight polypeptide. This smart behavior arises from the elastin-like domains present in the elastin-like recombinamer (ELR) that make this material water soluble at 4ºC and to become gel-like at body temperature. Moreover, the recombinant origin of these ELRs allows BIOFORGE to incorporate bioactive domains such as cell adhesion sequence, extracellular matrix protease sensitive sequences and bone growth factors that enhance the microenvironment for regeneration and, as they are directly linked to the ELR they avoid their elution from the gel. Following this strategy, BIOFORGE has developed several ELRs based all of them in an amphiphilic structure schematically represented in *Figure 1* and with the incorporation of bioactive domains.

The final chosen bioactive domains were the αβ integrin binding domain RGD that enhances cell adhesion while migrating through the gel, the bone morphogenetic protein BMP2 (a FDA approved growth factor) and BMP7 to promote osteoblast growth and differentiation. Moreover, to facilitate cell migration through the gel and release of BMPs, elastase sensitive sequences were incorporated. The combination of bioactive domains directly linked to the ELR structure and the use of protease sensitive sequences allows the gel to constantly provide a homogenous density of bioactive domains as cells migrate through the scaffold.

Due to the limited stability of such structures for *in vitro* cell culture, 2 new approaches were developed in order to overcome this problem. First of all, ELRs

were re-designed in order to incorporate lysines which could be further modified to incorporate chemically crosslinkable motifs. This approach was discarded due to the difficulties of handling of the resulting materials. The problem was solved by incorporating *Bombix Mory* silk fibroin motifs, which undergo an unreversible physical crosslink that avoids dissolution of the ELR under excess of water.

ELRs are bioproduced in *E.coli* under controlled conditions and further purified taking advantage of their smart behavior. Final product is checked to be pure and to match the design by different and complementary techniques such as SDS-PAGE electrofphoresis, MALDI-TOF mass spectrometry, HPLC analysis of their amino acid composition, H-NMR and FTIR spectral analysis and DSC in order to asses thermal behavior of the ELRs.

BIOFORGE has fulfilled partners' demands providing them with highly pure ELRs during the entire project. We have bioproduced, until now, more than 27 grams of ELRs for InnovaBone partners. ■

UVA
Universidad de Valladolid
G.I.R. BIOFORGE
Valladolid, Spain

CONSTANCIO GONZÁLEZ OBESO

He is a PhD student from the University of Valladolid (UVa). He holds a B.Sc. in Physics (2011) and a Master Degree in polymer chemistry and processing (2013). Since his last university years, he has been involved in different research groups: CELLMAT (UVa), 3B's Research Group (Universidade do Minho, Portugal), Biomaterials Group from the ITCP (CSIC) and BIOFORGE (UVa). His work has always been focused in biopolymers, from the development of superhydrophobic surfaces to obtaining and characterizing highly complex protein-based polymers.

DR MONTEQUI IRENE

She is holding a B.Sc. in Chemistry and a Ph.D. in Chemical Engineering from the University of Valladolid. She currently works as project manager in the Research Group BIOFORGE managing 12 active projects, five of them funded by the European Comission (one as coordinator partner) and seven funded by national and regional agencies. She contributed to the preparation of proposals which lead to a total funding from international and national projects of about 8 Million € through 7 years.

PROF RODRÍGUEZ-CABELLO JOSÉ CARLOS
LEADER

He is Full Professor in the University of Valladolid (UVa) and Director of BIOFORGE. He holds a B.Sc. in Chemistry and in Physics and a PhD in polymer physics (1994). His research focuses in the production of recombinant protein polymers and devices for biomedical applications. The output of his research is presented in 124 publications in peer-reviewed journals and a high number of invited lectures at international conferences. He has coordinated two EU projects and been involved in a number of nationally and internationally funded research projects.

ABOUT

TAILORING CELL INTERACTION THROUGH ION DOPING OF HYDROXYAPATITE NANOPARTICLES

Monserrat Espanol
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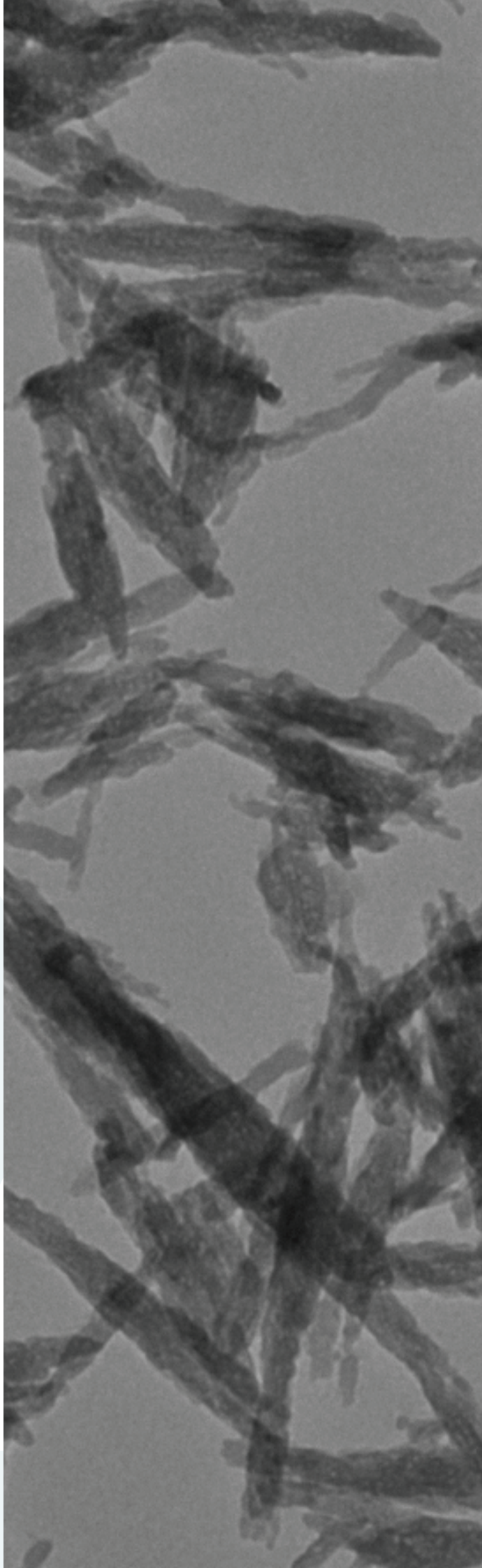
UPC - Universitat Politècnica de Catalunya, Biomaterials, Biomechanics and Tissue Engineering Group, Barcelona, Spain

INTRODUCTION

The use of nanoparticles is becoming a widely spread practice in biomedicine for many purposes ranging from imaging to cancer treatment, drug delivery and gene therapy. In any of these applications NPs should not be cytotoxic, must not degrade e.g. while carrying their cargo but should be degradable once they have had fulfilled their purpose. To find NPs sharing all these features is challenging yet, a strong candidate is hydroxyapatite (HA) NPs. HA, being the mineral phase of bone has widely been used in the bulk form for bone regeneration applications but is less investigated as NPs. One very interesting feature of HA is the fact that its crystal structure has great ability to incorporate foreign ions, a fact that is exploited in bone to concentrate traces of a wide range of ions imparting great impact on the biological performance of bone. The aim of this work is to assess if ion doping could also be used as a strategy to modulate NPs-cell interaction. For this purpose a series of different NPs doped with Mg, carbonate and a mixture of them will be made and their cytotoxity will be evaluated through cell culture testing using cancerous (MG63) and mesenchymal stem cells (rMSCs).

MATERIALS AND METHODS

HA-NPs were prepared by wet chemical precipitation via neutralization of calcium hydroxide with orthophosphoric acid. Doped NPs were prepared dissolving the appropriate amount of $MgCl_2$ or $NaHCO_3$ into the calcium hydroxide solution before acid addition. The obtained NPs were rinsed, freeze-dried and thoroughly characterized. Cell culture studies were performed using 100 ug·ml-1 of the various NPs with 10000 cells in 96 well/plate using MG63 and rMSCs in the following conditions: in the presence/absence of 10 v/v% foetal bovine serum and in the presence/absence of the dispersant sodium citrate. Cell viability was measured through lactate dehydrogenase quantification released by alive cells. NPs internalization was assessed through TEM imaging of cells upon embedding, sectioning and staining.



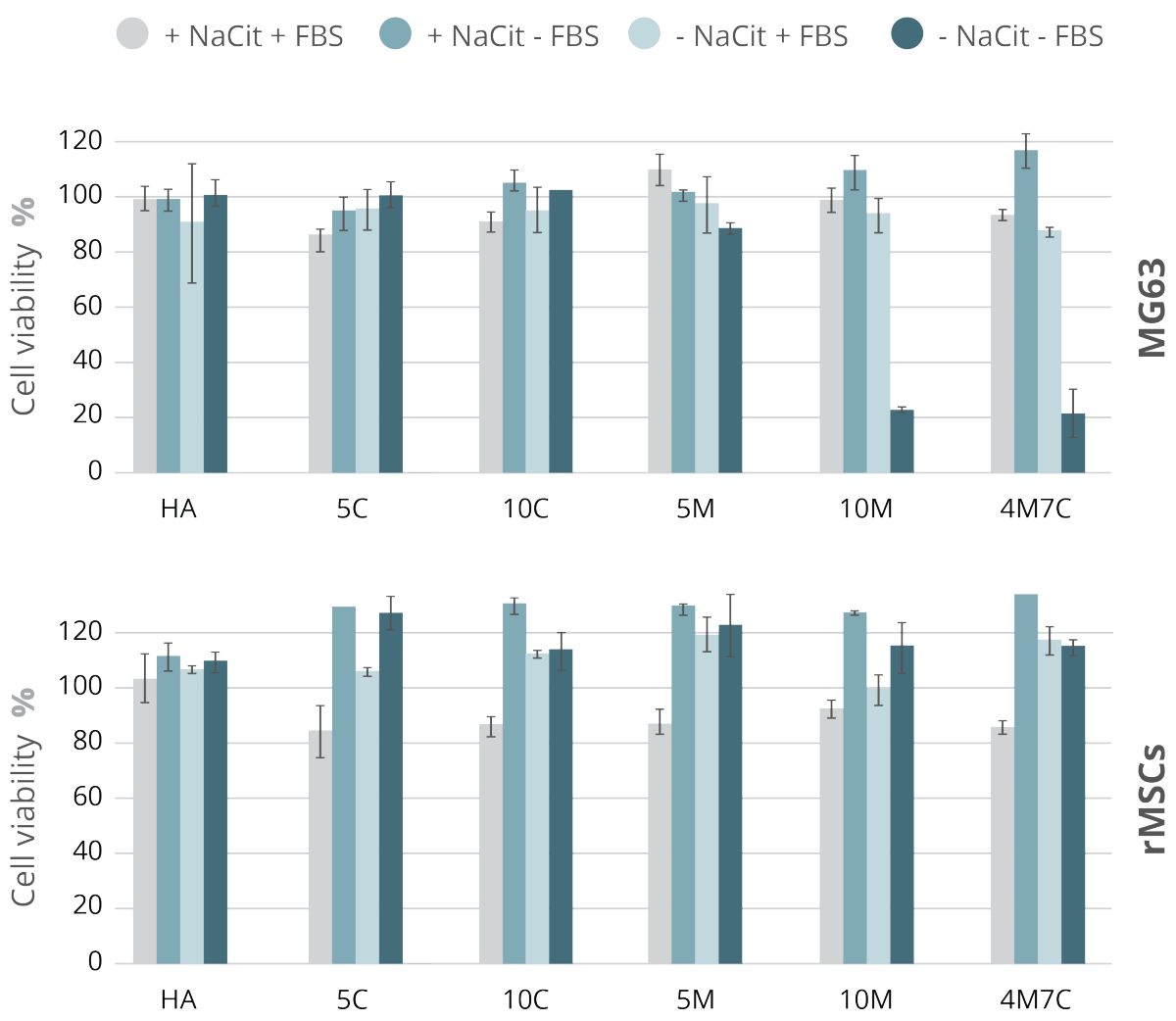


Figure 1 *Viability of MG63 and rMSCs cells exposed to the various NPs in the presence and absence of FBS and sodium citrate.*

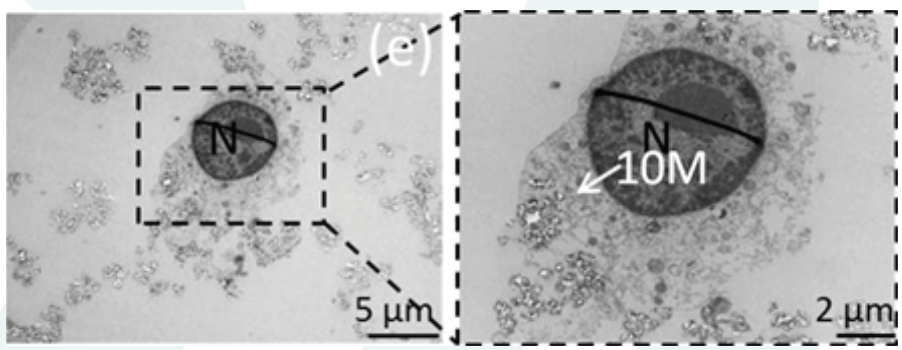


Figure 2 *TEM section of a stained MG63 cell proving NPs internalization of Mg doped NPs (10M). Extensive cell damage is readily visible.*

RESULTS & DISCUSSION

The graphs in *Figure 1* shows that Mg-doped NPs could selectively kill MG63 cells but not rMSCs provided FBS and sodium citrate were excluded during cell culture. These results put forward two aspects: on the one hand they show that cells can sense the composition of the bare NPs and on the other hand they demonstrate that adsorption of negatively charged molecules on the NPs prevents their internalization in MG63 cells. These findings have important implications as points that through simple incorporation of non-toxic ions we could selectively kill osteosarcoma cells without the need of using current toxic drugs and at the same time proves that ion-doping can be used as a strategy to modulate cell behaviour. ■

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DR MONTSERRAT ESPANOL

Montserrat Español Pons is a researcher at the Department of Materials Science and Metallurgy at the Technical University of Catalonia (UPC). She obtained her degree in chemistry at the University of Barcelona and received her PhD degree in Materials Science at the Nanyang Technical Univeristy (NTU). She later worked as research fellow in NTU and then joined UPC as Juan de la Cierva researcher. Her research focuses on the investigation of calcium phosphates for biomedical applications.

ZHITONG ZHAO

Zhitong Zhao is a PhD Student at the Department of Materials Science and Metallurgy at the Technical University of Catalonia (UPC). He obtained his MSc in Physics at Sichuan University in China. His thesis topic focuses on the synthesis and characterization of calcium phosphate nanoparticles and the synthesis of biohybrid materials.

DR JORDI GUILLEM-MARTI

Jordi Guillem Martí is a researcher at the Department of Materials Science and Metallurgy at the Technical University of Catalonia (UPC). He obtained his degrees in biochemistry and biology at the University of Barcelona and received his PhD degree at Vall d'Hebron Research Institute (VHIR). Then he joined the Biomaterials, Biomechanics and Tissue Engineering group (UPC) where is holding a junior postoctoral fellowship. His research focuses on the investigation of cell-biomaterial interactions.

DOREEN KEMPF

Doreen Kempf received her International Bachelor's degree in Materials Science and Engineering at the Saarland University (Saarbrücken, Germany) and has recently finished her International Master's degree in Materials Science and Engineering at the Saarland University and the European School for Materials Science and Engineering ("École Européenne d'Ingénieurs en Génie des Matériaux, EEIGM") in Nancy, France.

PROF MARIA-PAU GINEBRA — LEADER

Maria-Pau Ginebra is Full Professor at the Department of Materials Science and Metallurgy at the Technical University of Catalonia and co-founder of the spin□off company Subtilis Biomaterials. She has received various awards (ICREA Academia Prize, the Narcis Monturiol Medal and the Racquel LeGeros Award) for her contributions in the biomaterials field. Her research interests include the design and development of new biomaterials and the fundamental study of biological mechanisms between biomaterials and cells/tissues

ABOUT

APPLICATION OF 2-PHOTON-POLYMERIZATION (2PP) FOR THE ESTABLISHMENT OF BIOCOMPATIBLE SCAFFOLDS FOR TISSUE ENGINEERING

Nicole Hauptmann
Leander Poocza
Gerhard Hildebrand
Klaus Liefeith

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The generation of bio-degradable and porous scaffold materials is a prerequisite for the development of the final implant architecture, as it is the supporting material for the injection of the bioactive hydrogel. For the generation of these complex structures with high µ-resolution, we focused on direct laser writing techniques in particular two-photon polymerization (2PP), to avoid the necessity of photomasks when using traditional lithography methods. Another advantage of this approach is the long wavelength of the NIR, which allows a deeper penetration of NIR radiation into the material and thus the capability of structuring in millimeter dimension was attained in the first place [1-5]. 3D scaffolds based on poly(D,L-lactide-co-ε-caprolactone) (pLC) copolymer with different compositions were manufactured by 2PP in a variety of dimensions tailored for different biomedical applications and related testing routines. For 2PP, only optical transparent materials are suitable, since all materials show an increasing opacity with increasing caprolactone content, lactide-rich variations were explored with LA:CL ratio of 90% (LC 18:2) or 80% (LC 16:4). In addition, the number of total monomer units added at 90% LA content was halved from 20 units to 10 units (18:2 to LC 9:1 respectively) to reduce the chain length and the resulting mesh sizes in the structure.

The reactivity of the resulting three LC variants was examined by photo-DSC at three different temperatures. The material could be classified in terms of reactivity by the time to maximal heat generation tmax, resulting in following reactivity order LC 18:2<16:4<9:1. The materials were further investigated in terms of cytotoxicity with a special focus on the initiator systems, which revealed no toxicity as a first estimation.

The 3D structure was based on a Schwarz Primitive (P) minimal surface derived unit cells, which was obtained by 2-Photon direct laser writing and validated by SEM and µ-CT. By combining the unit cells in x, y and z, a tailor-made scaffold with controlled pore size, porosity and distinct dimensions was generated. The pore sizes for all LC scaffolds were approx. 300 µm and throat sizes varied from 152 to 177 µm. Due to the load bearing capacity of the structure it was possible to generate a material which has a high compressibility and recovers its shape even after larger stress loadings. So it is possible to handle it in clinical bone defect models, by simply pushing it into the defect site to recover there and stay in place. Overall the material has the potential for the use as a supporting material in tissue engineering applications such as bone and cartilage repair. ■

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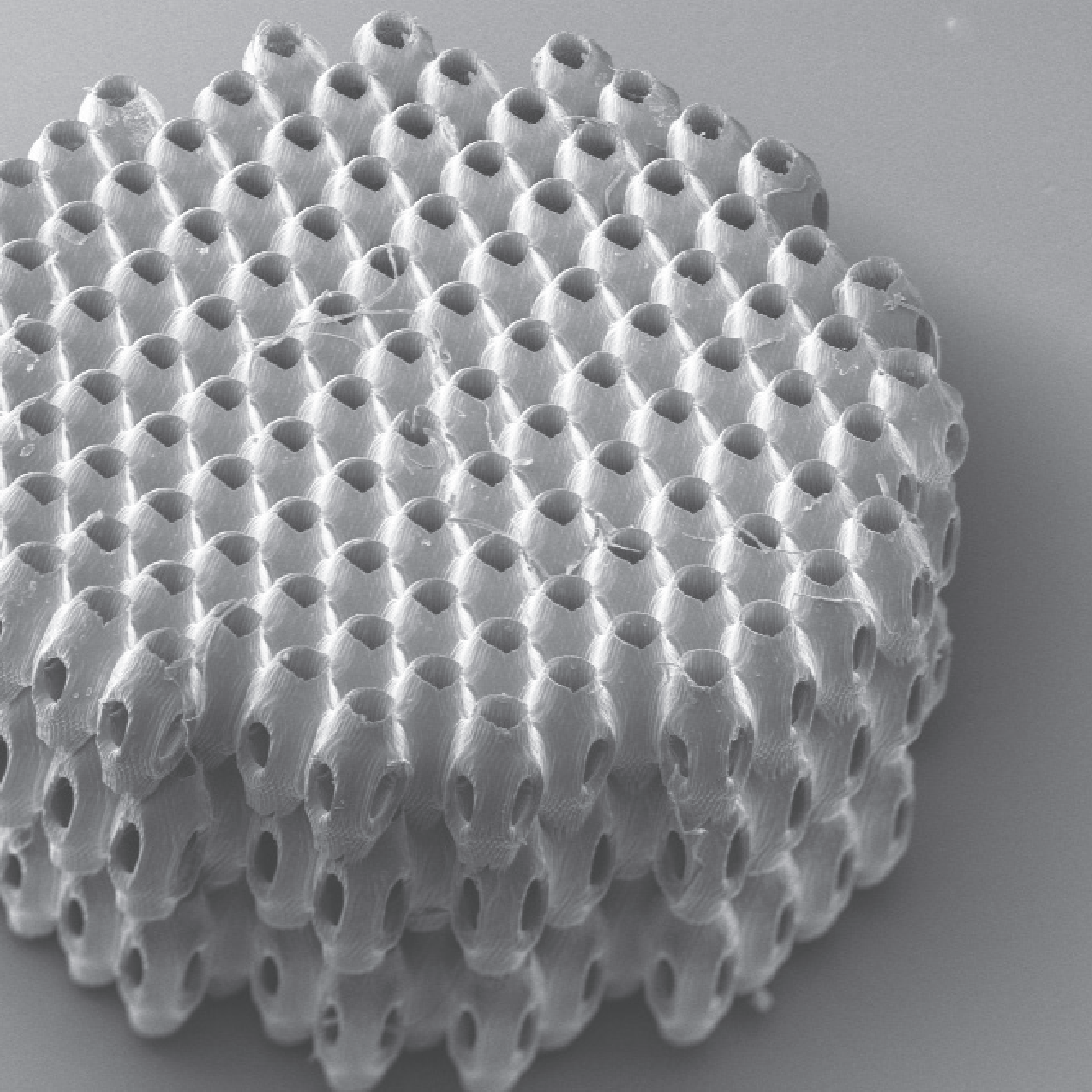

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DR NICOLE HAUPTMANN

Nicole Hauptmann studied chemistry at the University of Technology in Dresden and received her Diploma in 2009. Between 2010 and 2013 she worked on her PhD-thesis at Leibniz-Institute of polymer research in Dresden in the department bioactive and responsive polymers. Since 2014 she is working at Institute of Bioprocess- and Analytical Measurement techniques Heiligenstadt e. V. in the group of Prof. Dr. Liefeith.

DIPL CHEM LEANDER POOCZA

Leander Poocza studied chemistry at the Albert-Ludwigs University in Freiburg, Germany, with a focus on macromolecular chemistry. This was followed by a period in which he worked as a researcher at the iba Heiligenstadt (Germany) in the biomaterials group on applied biomaterials. In July 2015 Leander Poocza started his PhD on the use of recombinant proteins for tissue engineering at Valladolid University, Spain, in the frame of a Marie Curie innovative training network.

DR GERHARD HILDEBRAND LEADER

Gerhard Hildebrand studied materials sciences at the University of Technology in Ilmenau and received his Diploma in 1989. Between 1989-1991 he worked as Scientist for Material Sciences at the Department of Biotechnology and Engineering of the Academy of Science. Since 1992 he is working at Institute of Bioprocess- and Analytical Measurement Techniques Heiligenstadt e. V. in the biomaterials group of Prof. Dr. Liefeith. Between 2002 and 2005 he worked on his PhD-thesis at University of Saarbrücken in the faculty of chemistry, pharmacy, and biomaterial sciences. Since 1992 Gerhard Hildebrand is one of the manager of the Thuringian Society for Biomaterials e.V.

PROF KLAUS LIEFEITH

Klaus Liefeith studied “Applied Mechanics and Material Sciences” at the Friedrich-Schiller-University of Jena and received his Diploma in 1982. In the following years (1982-1988) he completed successfully his PhD with “Summa cum laude”. Between 1987-1991 he worked as Project Leader for Material Sciences at the Department of Biotechnology and Engineering of the Academy of Science and changed 1992 to the Institute for Bioprocessing and Analytical Measurement Techniques (iba) in Heiligenstadt as Head of the Department for Biomaterials with a focus on interfacial interactions between biological systems and technical material surfaces. In 2009 followed the appointment as Professor at the Technical University of Ilmenau for “Biofunctional Surfaces and Structures”. Klaus Liefeith is President of the Thuringian Society for Biomaterials and Leader of the Technical Committee „Surfaces and Coatings in Biotechnology and Medicine“ of the European Society of Thin Films (EFDS). Further he is Reviewer for the German Federation of Industrial Research Associations (AiF), the European Commission (EC), the German-Israelic Science Foundation (GIF), the Austrian Research Promotion Agency (FFG) and numerous scientific journals.

ABOUT

INDUSTRIAL PRODUCTION OF HIGH RESOLUTION SCAFFOLDS UTILIZING 2-PHOTON-POLYMERIZATION

TETRA

TETRA Gesellschaft für Sensorik, Robotik und Automation GmbH

During the InnovaBone project TETRA developed an industrial approach to utilize the 2-photon-polymerization process.

Two-photon polymerization (2PP) is based on the two-photon adsorption which is defined as the simultaneous absorption of two photons by a molecule. The adsorption leads to an energetically excited state of a photoinitiator resulting in cross-linking of the exposed polymer. The advantage of this effect is a very small polymerized area. In combination with a kinematic structure to move the so called voxel, 2PP is capable to write 3D structures with resolutions below one µm. Thus the 2PP process dramatically expands the possibilities of typical lithography processes used in semiconductor technology.

Within the last years 2PP-processes targeting applications like tissue engineering, photonic applications, microfluidics and MEMS have been presented. Today, 2PP process units produce structures like scaffolds, photonic crystals and single optical interconnects. Until recently, a major drawback of 2PP-processes was that only structures in small spatial dimensions and at low speed could be manufactured. For this reason, no industrial application for 2PP processes was available. Tetra, with its strong expertise in drive technology and mechatronics developed an industrial system performing 2PP processes. For the first time, a possibility for the industrialization of the 2PP process is now offered with higher speed of production resulting in higher outer dimensions of the structures.

In 2014 TETRA presented the world largest monolithic structure written by 2PP. By using biopolymers developed by the partners of the InnovaBone project TETRA supported the InnovaBone project by writing of more than 1000 scaffolds for research purposes. ■

TETRA

TETRA Gesellschaft für Sensorik, Robotik und Automation GmbH

Ilmenau, Germany

Tetra was founded in 1991, it is a manufacturing and service provider and specialises in standard and customised solutions in the following business segments: PRECISION MECHATRONICS Machines and components for precision automation; precision handling in the sub-µm range (resolution better than 10 nm) by using precision drives and positioning systems, special sensors and image processing; special measuring and test units for applications in laser machining, coordinate measurement, wafer and solar cell inspection, etc.; SENSORS / SCIENTIFIC DEVICES: Force and position measurement systems based on fibre optic and interferometric sensors in the nano range; scientific devices for analysis of material and surface properties, like friction, abrasion, adhesion, viscosity; ELECTRONIC CONTROL SYSTEMS: Development and production of control and communication devices based on newest micro controller systems (device controls, current supply, drive controls, remote controls, networks); analogue and digital circuit.

The products and technologies of TETRA are changing the relationship between humans and technology. The assistance robots of TETRA work hand in hand with humans. Based on our precision technologies fundamental structures of human organs can be generated

ABOUT

IN VITRO DEGRADATION & CYTOCOMPATIBILITY OF PHOTOPOLYMERISED POLY (LACTIDE-CO-CAPROLACTONE) SCAFFOLDS

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INTRODUCTION

The InnovaBone project aims to develop a biomimetic product that consists of a bespoke scaffold and a bioactive self-setting gel, which will provide a microenvironment that contains active elements such as growth factors and CaP nanoparticles to promote bone repair. Scaffolds composed of different lactic acid (LA) and ε -caprolactone (CL) ratios were produced using a two photon polymerisation (2PP). In this study, *in vitro* degradation and compressive properties were conducted for the produced scaffolds in phosphate buffered saline (PBS) at 37°C. Cytocompatibility of the scaffolds was assessed using human mesenchymal stem cells (MSCs)[1].

MATERIALS AND METHODS

Scaffold production

Scaffolds were provided by IBA and TETRA and manufactured by a 2PP polymerisation technology[2] in which the 3D structure was based on a Schwarz Primitive minimal surface derived unit cells. Three different LA:CL ratios (16:4, 18:2 and 9:1) were investigated and samples were coded LC16:4, 18:2 and 9:1.

In vitro degradation and mechanical testing

Degradation study of the scaffolds was performed according to the standard BS EN ISO 10993-13:2010 at 37°C using PBS buffer (pH =7.4 ± 0.2) and accelerated testing was also applied at 50 and 65°C. Compression testing was conducted using Hounsfield tester according to the standard ASTM 1621-10: 2010 at 25±1°C.

Cell culture

Human MSCs[1] were seeded at a concentration of $1x10^6$ cells per scaffold and cultured in DMEM supplemented with 10% foetal calf serum, 1% L-Glutamine, 1% non-essential amino acids, 1% penicillin/streptomycin [standard medium], supplemented with 0.1 μM dexamethasone, 50 μM ascorbic acid phosphate, and 10 mM β-glycerophosphate [Osteogenic (OS) medium)] at 37°C and 5% CO_2.

Cell viability, metabolic activity, and markers of osteogenic differentiation were measured using Neutral Red, PrestoBlue, SigmaFAST & Alizarin Red assays respectively.

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1 Rashidi, H., et al., Cells Tissues Organs, 2012. 195(6): 484-94.

2 Davis, K.A.et al., Biomaterials, 2003. 24(14): 2485-95.

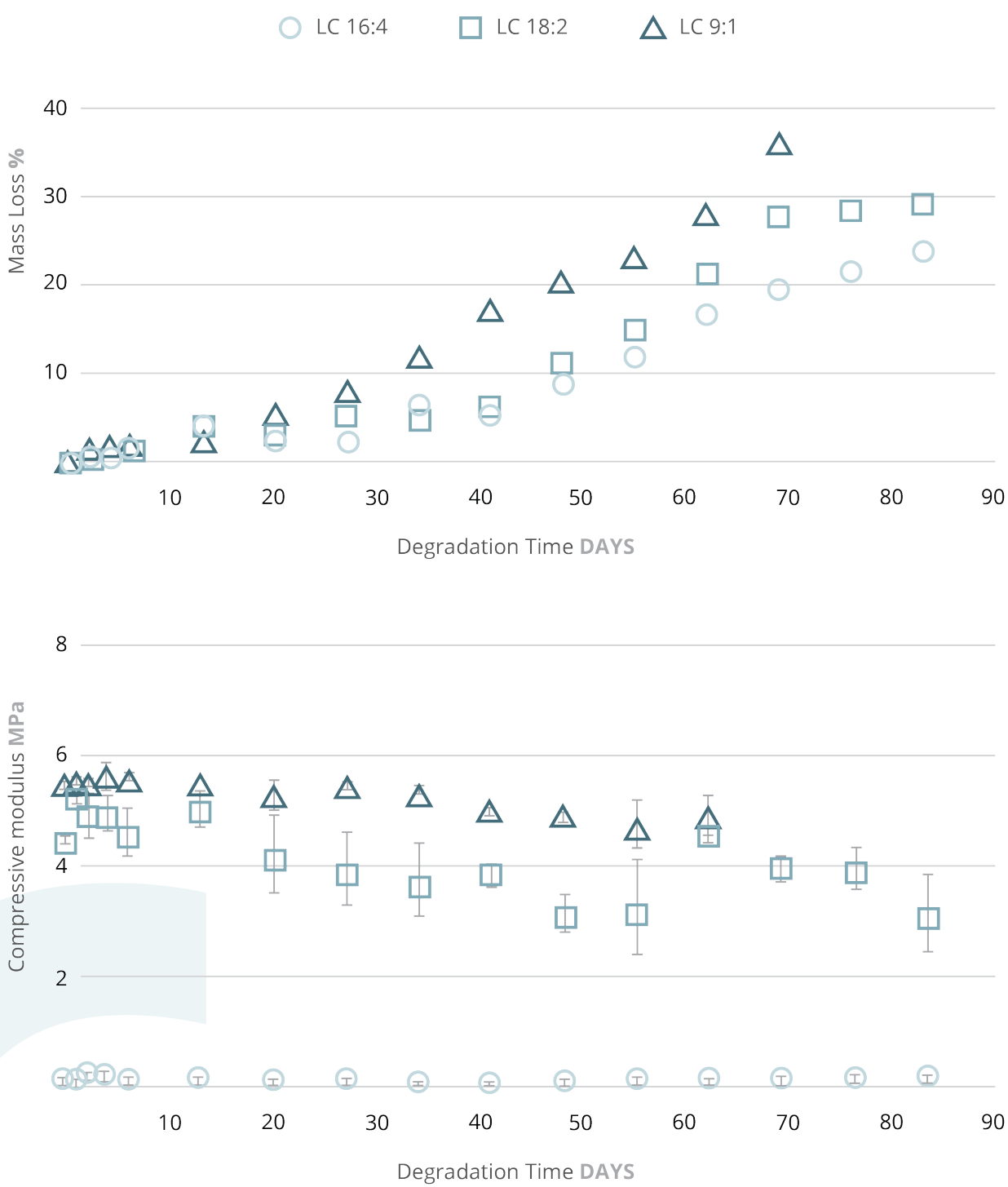


Figure 1 *Change in mass loss and compressive modulus of LC scaffolds against degradation time at 37oC.*

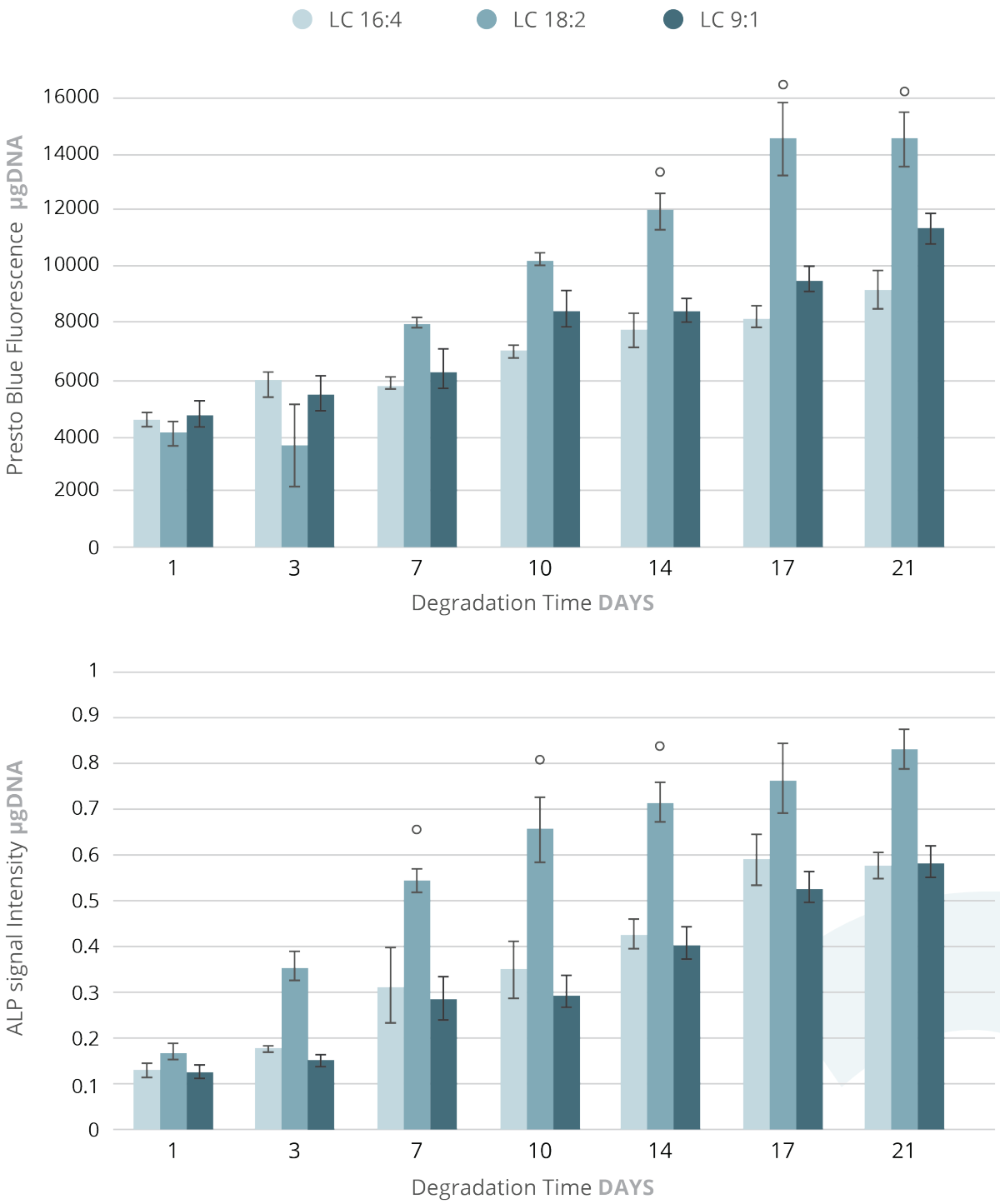


Figure 2 *Metabolic activity and ALP for LCM scaffolds over 21 days of MSCs culturing.*

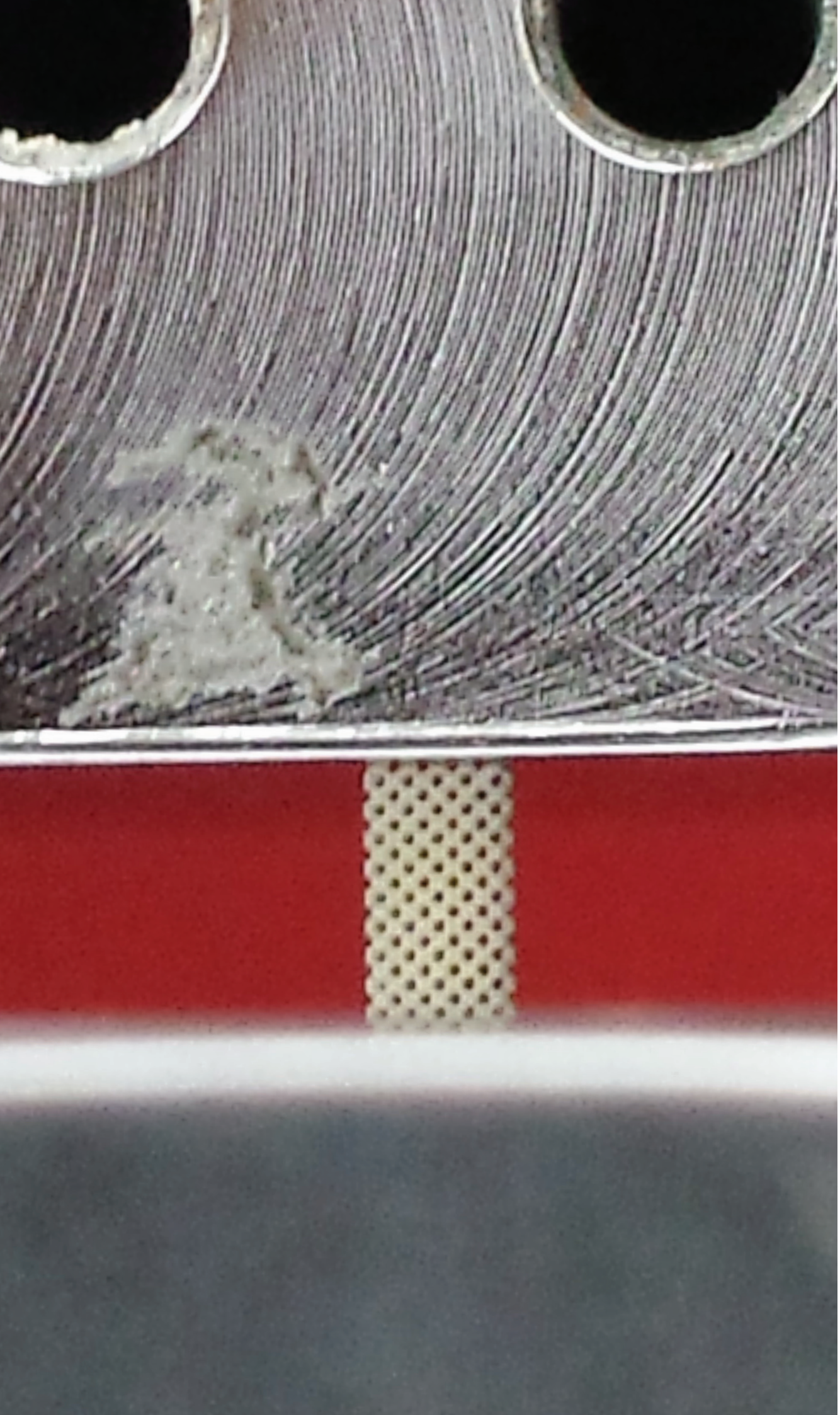
RESULTS & DISCUSSION

Percentage of mass loss for LC16:4, 18:2 and 9:1 scaffolds showed a gradual increase versus degradation time as can be seen from *Figure 1*.

LC16:4 showed lower mass loss (ca. 20%) in comparison with LC18:2 and 9:1. This could be ascribed to the variation in ε-caprolactone to D,L lactide ratio (CL/LA) ratio between the scaffold materials. Compressive properties for LC18:2 and 9:1 scaffolds were also significantly higher ($P<0.001$) than LC16:4. Mechanical properties of these scaffolds are mainly dependent on their materials composition (i.e. CL/LA ratio) as all scaffolds have similar architecture. Accelerated degradation results show prediction of degradation rates, and the activation energies from half mass loss results were found to be 87.9, 82.7 and 94.9 kJ mol^{-1} for LC16:4, LC18:2 and LC9:1 respectively.

Metabolic activity and ALP activity of cells within the scaffolds are shown in Figure 2. LC18:2 promoted higher cell metabolic activity but earlier time points showed no significant difference ($p>0.05$) between the three compositions. LC18:2 had also the highest ALP activity in comparison with LC16:4 and 9:1 scaffolds.

LC18:2 scaffolds showed significantly higher cellular activity (ca. 20%) and mineralisation (ca. 30%) in comparison with LC16:4 and 9:1.



CONCLUSION

Variation of degradation and mechanical properties of LC scaffolds are related to their chemical composition and accelerated degradation was predictable. All three types of scaffold were capable of supporting cell proliferation and osteogenic differentiation of human MSCs over 21 days and LC16:4 showed the best bone cytocompatibility response. These scaffolds have a potential for use in bone repair applications. ■

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DR MIQUEL GIMENO-FABRA
Research Fellow for the Centre of Innovative Manufacturing in Medical Devices (MeDe). Originally a chemist, he obtained a PhD in Materials and Chemical Engineering at the UNOTT, working on the development of continuous synthesis methods for Hydroxyapatite. He is now working on a wide range of projects, from thin-film ceramic coatings for electrical engineering applications to formulation of novel nano-Hydroxyapatite morphologies and photphate glass coatings for medical implants. He worked on the study of the mechanical properties and degradation behaviour of the InnovaBone scaffolds.

DR AMY PROSSER
PhD at the UNOTT in the School of Engineering and the School of Medicine. She also studied for her undergraduate degree in Human Genetics at the University of Nottingham and is currently working at Sygnature Discovery, a drug discovery CRO based in Nottingham.

DR COLIN SCOTCHFORD
Associate Professor in the Advanced Materials Research Group in the Faculty of Engineering of the UNOTT. He studied Zoology with Marine Zoology at Bangor University, before completing a PhD at the Institute of Orthopaedics, University College London. He carried out post-doctoral research at the Interdisciplinary Research Centre in Biomedical Materials and then University of Nottingham where he was subsequently appointed as lecturer. His research interests are centred around cell-material interactions, particularly for orthopaedic biomaterial interactions.

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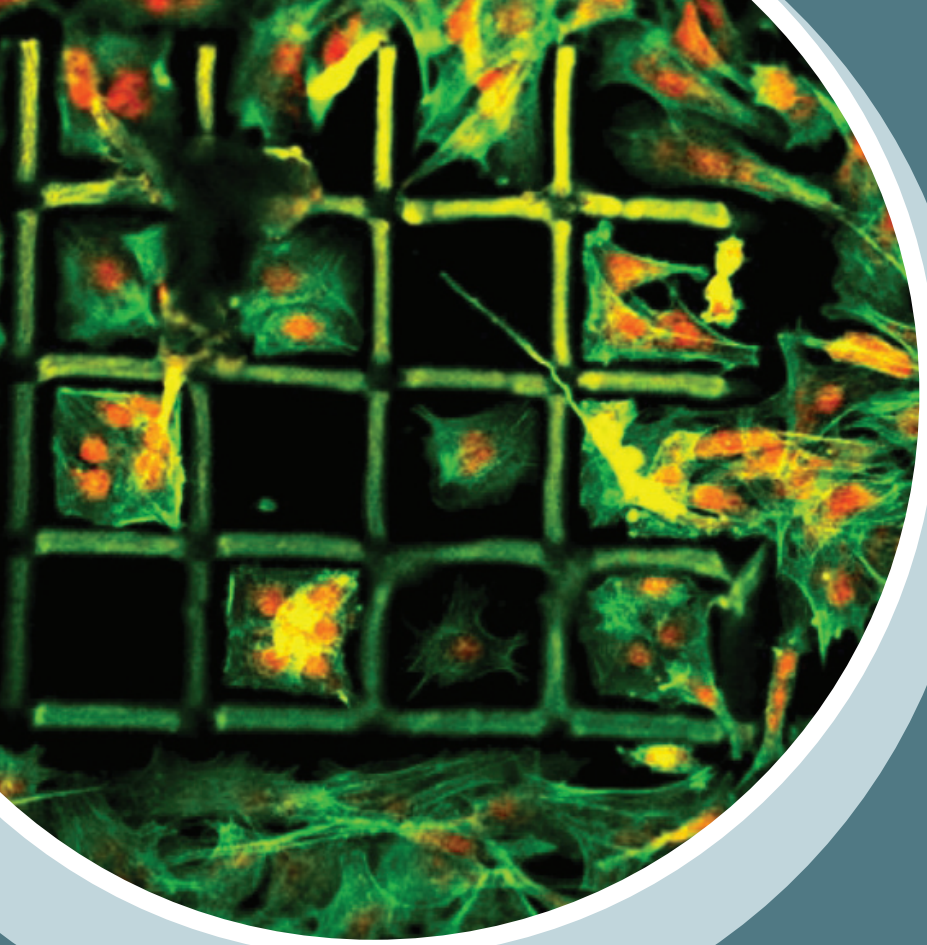
Associate Professor at the Wolfson Centre for Stem Cells, Tissue Engineering & Modelling (STEM) at the School of Medicine - UNOTT. She studied cellular and molecular biology at the University of Nice (France), carried out her PhD research at Novartis (Switzerland) before joining the Roslin Institute (UK). She then joined the UNOTT where she started the adult stem cell group in 2007, now leading an active research group focused on the regulation and translational applications of stem cells for regenerative medicine.

PROF DAVID GRANT — LEADER
Professor of Materials Science at the UNOTT. He heads the Advanced Materials Research Group and has wide ranging research interests in Biomaterials such as surface modification, coatings, characterisation, nano-composite structures and scaffolds, degradation behaviour, cell surface interactions. Other research interests include hydrogen storage materials such as intermetallic and complex light metal hydrides and multicomponent systems.

ABOUT



40 TESTING I

11 PLATFORMS

INVESTIGATION OF THE INNOVABONE PRODUCT WITH PRIMARY HUMAN CELLS

David Shepherd
Serena Best

UCAM - University of Cambridge, CCMM, Department of Materials Science and Metallurgy, Cambridge, UK

INTRODUCTION

Biomaterials can show promise as implants both theoretically and in terms of materials characteristics, however, before a material can go into clinical trials it will have to have proved successful in *in vitro* and *in vivo* tests.

The aim of the work in Cambridge was to use primary human osteoblast and monocyte cells to investigate the osteogenic and inflammatory responses to the materials (both scaffolds and ELRs) produced by the other partners in the project. By measuring cell response to the materials it is possible to make predictions about the outcome following implantation of the InnovaBone product in the body.

MATERIALS AND METHODS

The materials to be characterised were provided by other partners in the project (scaffolds and discs from IBA and ELRs by UVa). These were sterilised using gamma irradiation.

The scaffolds or discs were pre-wetted in 70% ethanol, washed in sterile deionised water and placed in culture medium for 24 hours before being used in experiments. The ELRs were made up as a 1% solution in the culture medium used in the experiment.

The osteoblast response was measured using assays to measure cell proliferation and activity. Cells were also stained to measure alkaline phosphatase (ALP) activity and mineralisation using alizarin red. An assay to measure the increased calcium produced by the cells at day 14 was also used as an additional measure of mineralisation. The medium in all cases except the negative control had osteogenic supplements added. Positive controls included the addition of BMP 2 and BMP 7; other controls were culture medium with and without osteogenic supplements.

The inflammatory response of the materials was measured by seeding them with monocytes. The cytotoxic response was determined by measuring the amount of lactate dehydrogenase (LDH) released from the cells. The inflammatory response was detected by measuring the release of the cytokines IL–6 and TNF α from the monocytes. Positive controls had zymosan or lipopolysaccharide added to the cells.

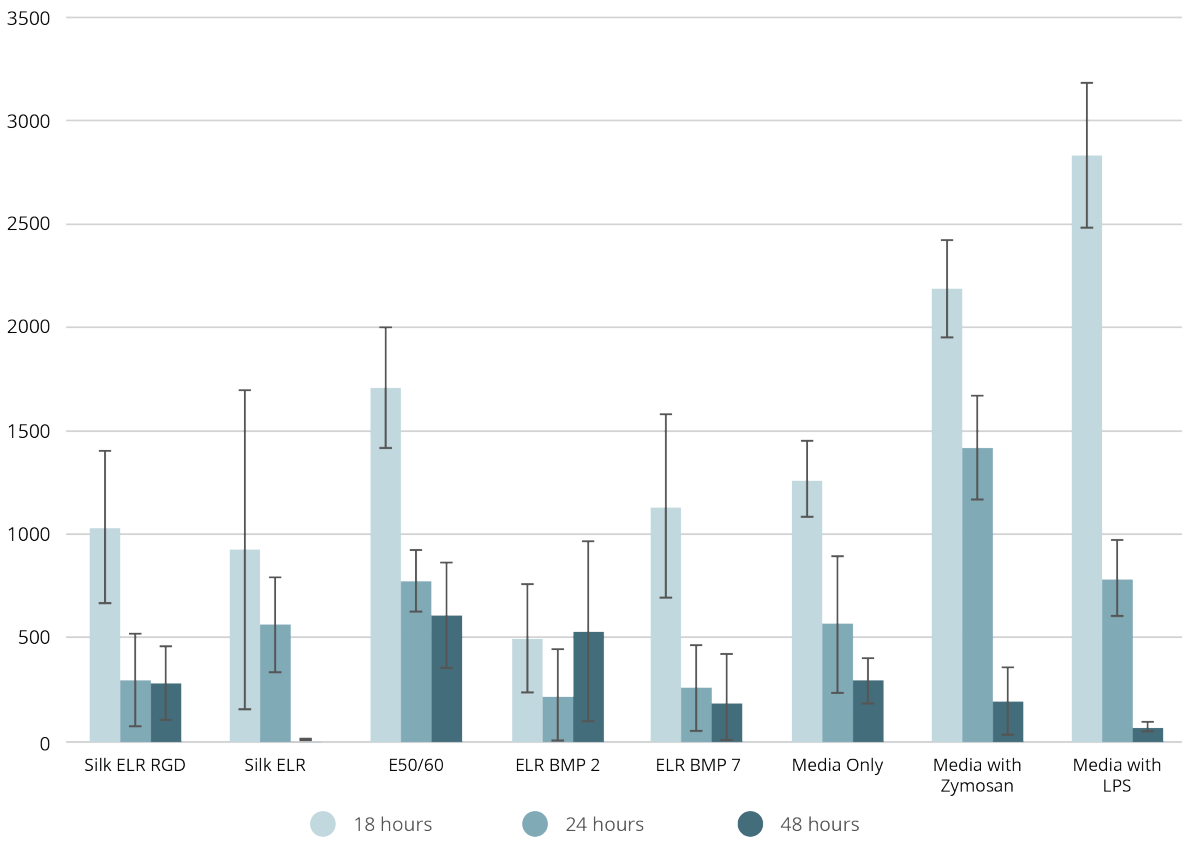


Figure 1 *Inflammatory response (TNF α release) of human monocytes cultured in the presence of various ELRs and controls*

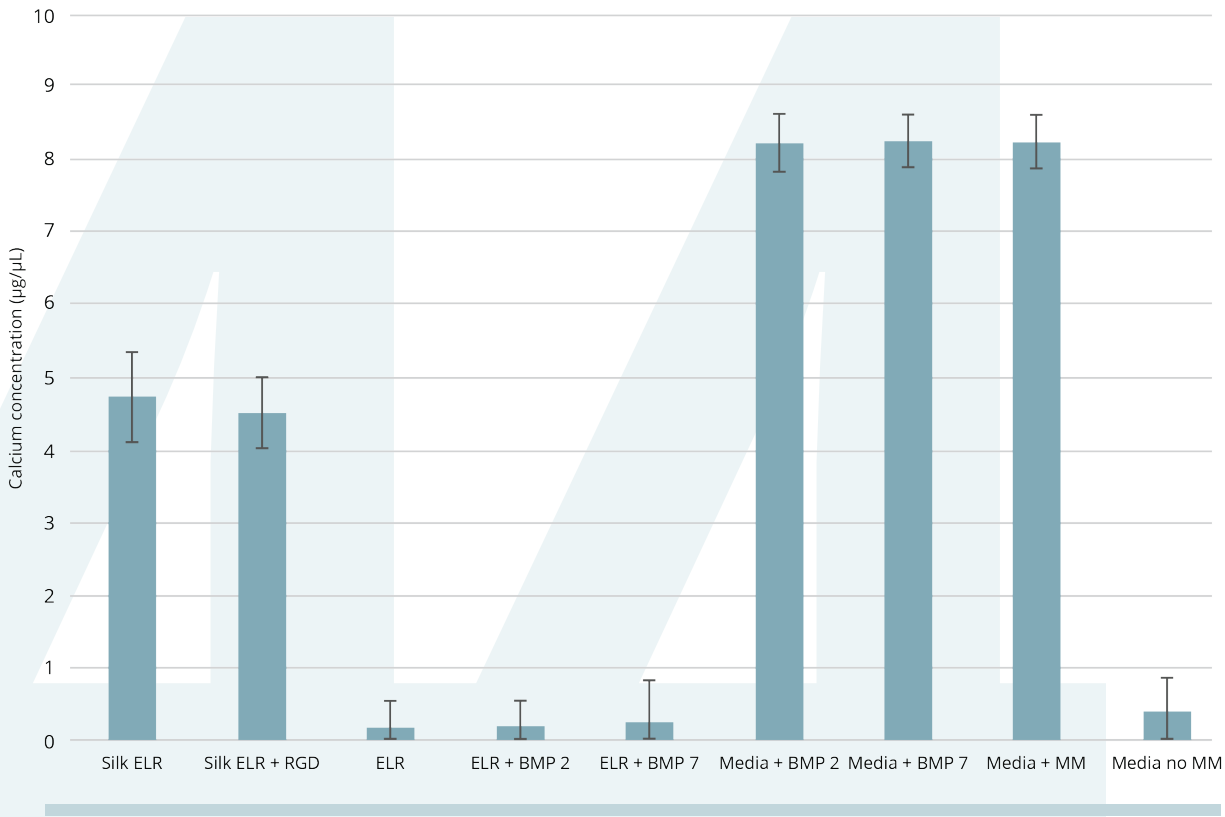


Figure 2 *Calcium presence (mineralisation) for cells cultured for 14 days with various biogels and controls*

RESULTS

Discs and subsequently scaffolds were found not to be toxic with low LDH release. Scaffolds of all materials were found to support osteoblast differentiation with cells showing good ALP activity. The release of inflammatory markers from cells seeded on the scaffolds was also found to be low.

Some of the ELRs were found to show an inflammatory response at a concentration of 1% but they were not toxic (*Figure 1*).

When the response of osteoblasts to biogels was studied it was found that the silk containing ELRs supported osteoblast proliferation and subsequent mineralisation. The presence of the ELRs prevented any staining through alizarin red and so it was only possible to see mineralisation shown using the calcium assay (*Figure 2*).

CONCLUSION

The silk ELRs were found to support the development of osteoblasts and their subsequent mineralisation. They evoked a small inflammatory response but were not found to be toxic. The scaffolds allowed osteoblasts to differentiate and showed good ALP activity. The scaffolds were found to be non-toxic and promoted minimal inflammatory responses. ■

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DR DAVID SHEPHERD

David Shepherd is currently a Research Associate at the Cambridge Centre for Medical Materials. He is a member of the Institute of Physics and has several publications and book chapters in the field of biomaterials with a particular emphasis on hydroxyapatites and substituted hydroxyapatites. He is also a reviewer for multiple Biomaterials Journals.

PROF SERENA BEST — LEADER

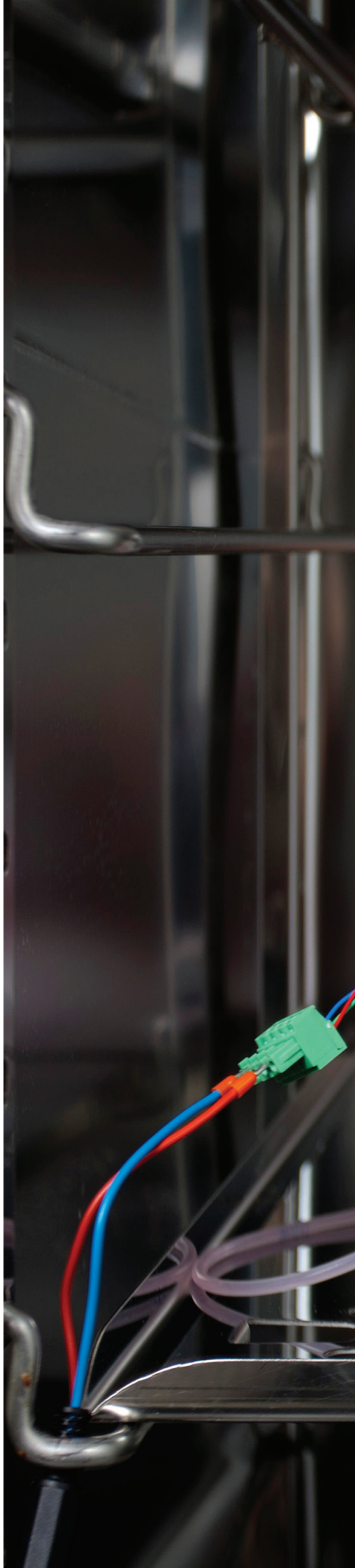
Professor of Materials Science and Fellow of St. John's College, Cambridge. She co-directs the Cambridge Centre for Medical Materials. She has published around 250 journal papers, books and book chapters and holds 9 patents in the fields of biomaterials and skeletal repair. She is a Fellow of the Royal Academy of Engineering and also the Institute of Materials, Minerals and Mining. She is an Editor of the Journal of Materials Science: Materials in Medicine and has been invited to act as a specialist on both national and international assessment panels.

ABOUT

A BIOREACTOR TO MODEL 3D BONE REPAIR

Marta Giazzon
Réal Ischer
Mélanie Favre
Rita Smajda
Martha Liley

CSEM - Centre Suisse d'Electronique et Microtechnique SA - Neuchâtel - Switzerland



Culture of a bone scaffold in the IB1 bioreactor

The development of predictive *in vitro* tests remains a major challenge for toxicology, pharmacology and also for tissue engineering.

We have developed a simple bioreactor to host bone scaffolds and bone substitutes. It allows scaffolds filled with bone cells to be tested for a period of several weeks.

During this period the bioreactor is placed inside a standard cell culture incubator, which controls both the temperature and the partial pressure of CO_2. A fluidic system guarantees the supply of nutrients and the removal of toxins from the culture. A key feature of the bioreactor is the possibility to apply a mechanical stimulus to the scaffold during culture. A stepper motor, spring and piston together apply a compressive force which mimics *in vitro* the effect of daily movement on bones and bone substitutes *in vivo*. A force sensor allows accurate control of the forces applied as well as determination of the mechanical properties of the scaffold.

The bioreactor allows scaffolds to be cultured in quasi-physiological conditions. However, new tools are needed to analyse and monitor the behaviour of cells inside the 3D scaffolds.

While many tools are available for 2D cell culture, very few of these can be applied to 3D scaffolds.

We are currently developing optical sensors that can be used gather information about the conditions in the centre of the scaffold. Oxygen and pH sensors using reactive dyes incorporated into porous sol-gel matrices. The combination of these matrices with optical fibres is now being explored. ■

CSEM

Centre Suisse d'Electronique et Microtechnique SA

Neuchâtel, Switzerland

Swiss Centre for Electronics and Microtechnology, founded in 1984 is an independent, private, non-profit research and development centre specialised in microtechnology, nanotechnology, microelectronics, system engineering and communications technologies. It offers its customers and industry beneficiaries tailor-made innovative solutions based on its technological expertise. The division Nanotechnology and Life Sciences deals in particular with micro-optical components and nanoscale photonics for applications in lasers and spectrometers. Nanoscale structuring of surfaces and functional materials for improved optical and chemical surface properties, nanoporous membranes and security features. Micro- and nanoscale components for biological applications, such as cell studies, toxicology testing, improved implants and nanoscale tools that allow new approaches in biological research. Also innovative applications of biosensing for integrated and wearable sensing, in textiles, in food quality, diagnostics and health.

A B O U T

IN VITRO AND IN VIVO PLATFORMS FOR TESTING SMART SCAFFOLD MATERIALS, BIOACTIVE ELR-GELS AND HYDROXYAPATITE NANOPARTICLES ON BONE REGENERATION

Carina Kampleitner Oskar Hoffmann
Christian Richard

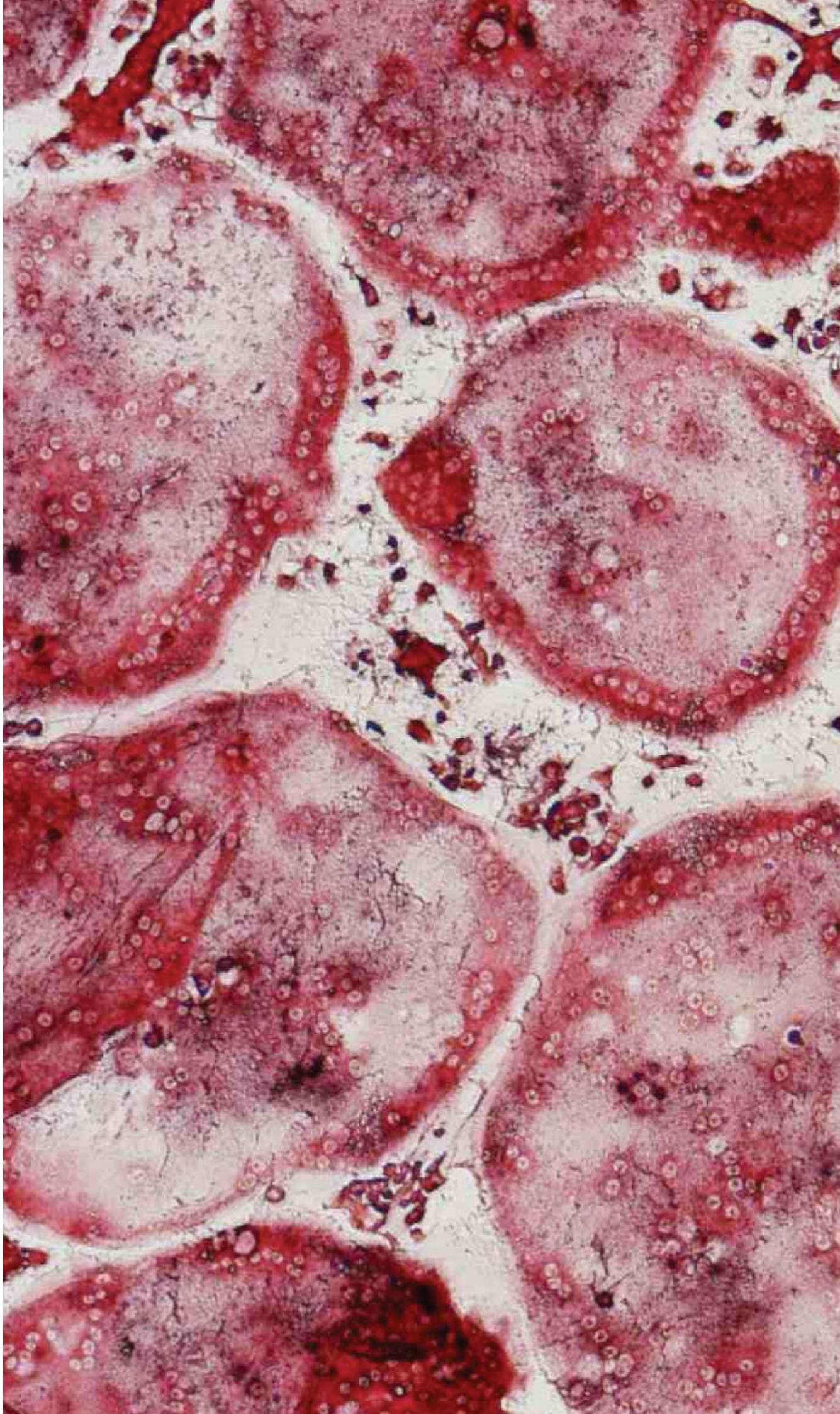
UNIVIE - University of Vienna, Vienna, Austria

The UNIVIE Bone Biology Laboratory established *in vivo* and *in vitro* platforms to evaluate biomimetic biomaterials for bone regeneration.

IN VITRO

To characterize biomaterials *in vitro*, we used mouse and human bone cells:

1. For mouse cell assays, we cultured mouse calvaria-derived osteoblasts (OBs) with the biomaterial test samples and analyzed:
   - cell viability,
   - alkaline phosphatase activity,
   - differentiation into mature matrix mineralizing OBs, by Alizarin S staining for Calcium.
2. To evaluate osteoclast (OC) development and bone resorption, we used a mouse co-culture of OC precursors from bone marrow and OBs derived from calvarial bones and quantified multinucleated OC formation using a TRAP (tartrate-resistant acid phosphatase) assay and evaluated the Cathepsin K secretion, respectively.
3. For human cell assays, we generated human OCs from peripheral blood mononucleated cell precursors, then incubated them with the test samples, and used the TRAP assay.



IN VIVO

To study bone regeneration *in vivo*, we used a mouse calvarial defect model, in which a critical size defect (Ø = 4 mm) was created and then filled with the test biomaterials compared with an empty defect (negative control) and a commercially available bone repair material (Vitoss™; positive control). The mice were then evaluated up to 12 weeks after surgery using µComputed Tomography (CT) and histological sections of the implant area.

The materials tested included:

1. Polymer scaffolds composed of various combinations of lactide (LA), caprolactone (CL), and methacrylate (MA) produced by two-photon photopolymerisation;
2. Thermosensitive Elastin-Like-Recombinamer (ELR) biogels containing BMP2 and BMP7;
3. Hydroxyapatite nanoparticles.

RESULTS

Our results indicate that scaffolds support OB differentiation and elicit similar responses in mouse and human OC assays. Furthermore, scaffolds, biogels and nanoparticles in combination enhanced new bone formation *in vivo*. Taken together, our data suggest that mouse models may be predictive for human bone cell responses and that our biomimetic biomaterial approach for bone regeneration may have important clinical implications. ■

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CARINA KAMPLEITNER

Carina Kampleitner is a Ph.D student in the Bone Biology Laboratory. She graduated in pharmacy from the University of Vienna, Austria. Mag. Kampleitner entered the working group during her diploma studies and has a competent knowledge of bone biology and practical experience in *in vitro* bone cell culture work as well as animal defect models to study bone regeneration.

DR CHRISTIAN RICHARD

Christian Richard Ph.D. has graduated in biochemistry from Laval University in Quebec, Canada. Dr. Richard completed post-doctoral fellowships in USA, France and Canada. Dr. Richard holds a solid expertise in bone biology with multidisciplinary skills and practical experience on animal models of bone diseases and in cell and molecular biology.

PROF OSKAR HOFFMANN — LEADER

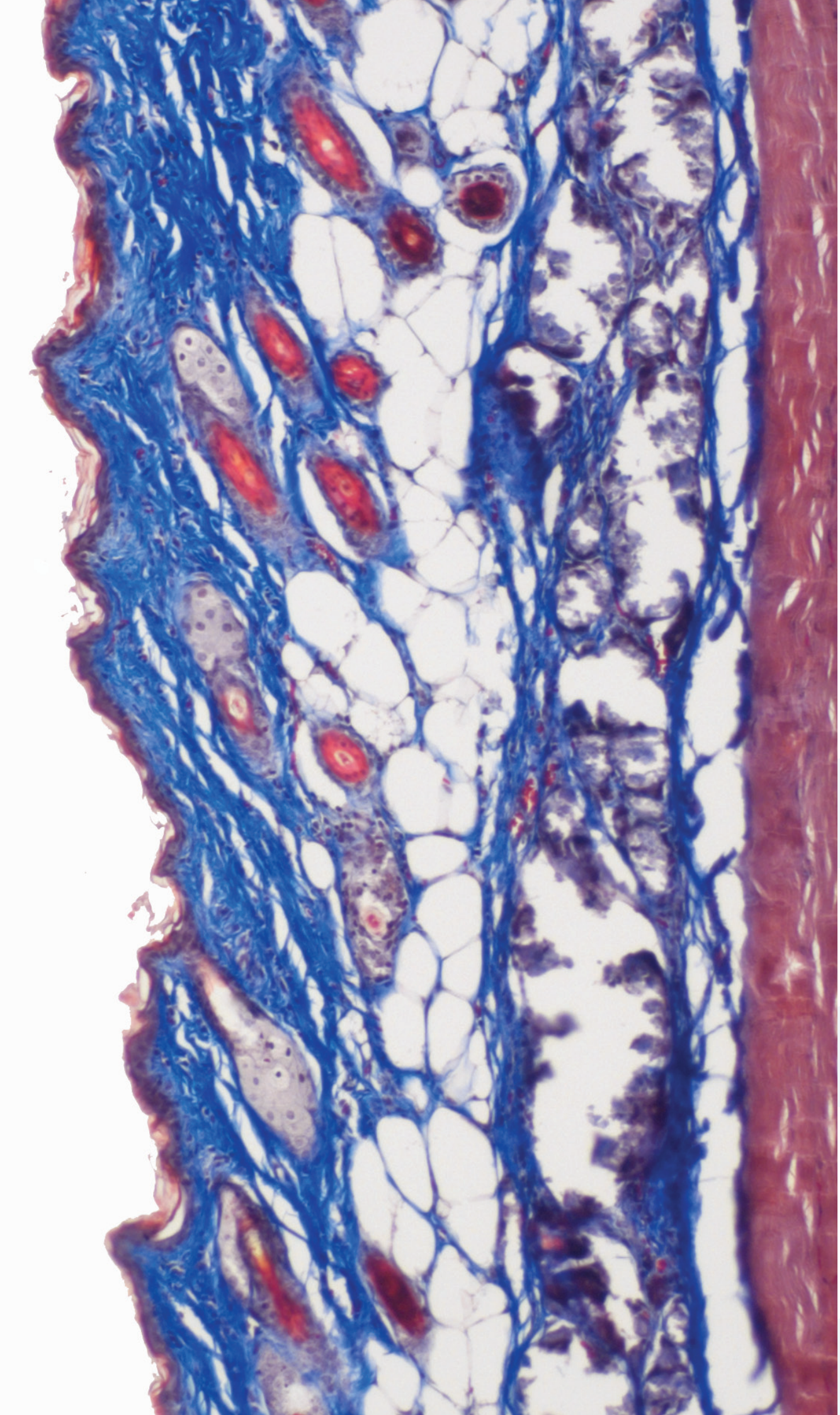
Professor Oskar Hoffmann, is a Pharmacist and Pharmacologist who graduated from the University of Vienna and went on to Yale University where he did post doctoral studies with Roland Baron. Upon his return to Vienna, he established the Bone Biology laboratory research group that combines his interests in pharmacology and cell biology. Soon after, Dr. Hoffmann did a sabbatical at the Harold Varmus Laboratory at the NIH, thereby increasing his armamentarium of technology. The current focus of the Bone Biology Laboratory is on elucidating the mechanisms underlying bone disease and healing and evaluating the use of bone biomaterials for bone regeneration utilizing state-of-the-art *in vivo* mouse models and *in vitro* mice and human bone models.

ABOUT

EVALUATING FOREIGN BODY REACTIONS TO BIOMATERIALS

Katayoon Changi
Berislav Bosnjak
Michelle Epstein

MUW - Medical University of Vienna, Vienna, Austria



MUW focused on *in vitro* **evaluation** of ELR biogels and scaffold materials using spleen cell cultures from BALB/c and C57Bl/6 mice. We analysed *in vitro* cytokine responses to the scaffold disks and ELR biogels with cytokines measurement and cell proliferation assays. *In vitro* immune cell studies revealed that LCM3, LCM4, LCM6.1, ELR-RGD, ELR-BMP2 and ELR-BMP7 were inert. These results illustrate that these biomaterials did not induce an immune response and were not toxic to the cells. Intraperitoneal (i.p.) models in BALB/c and B6 mice were used to test the biomaterials *in vivo*. Mice were implanted with scaffold, ELR biogels or a currently available biomaterial for bone repair: Vitoss Foam (OVF, Orthovita, USA). The intraperitoneal model is a 7-day protocol that provides a quick read out for early signs of foreign body reactions (FBRs). We evaluated biocompatibility by enumerating inflammatory cells and measuring cytokine levels in peritoneal lavage fluid. OVF-implanted group had a significant increase in the total number of cells with significant increases in macrophages, eosinophils and neutrophils compared to naive cells. There was no significant increase in total cell infiltration into the peritoneum in response to the scaffolds and ELR biogels demonstrating that there is no early acute inflammatory response to our biomaterial *in vivo*.The

subcutaneous (s.c.) model was used to address longer term biocompatibility and to correlate the results from short term i.p. and longer term s.c. experiments. We sought to determine whether there were late immune responses including a foreign body response and fibrosis. We established a subcutaneous (s.c.) implantation mouse model and examined the late response by H&E and Masson's trichrome-stained tissue sections and qPCR of the implant site at 3 and 8 weeks after s.c. implantation. OVF induced chronic inflammation with giant cells and collagen deposition compared with mild inflammation and few collagen fibers induced by scaffolds (LCM3, LCM4 and LCM6.1) and ELR biogels (ELR-RGD, ELR-BMP2, ELR-BMP7). QPCR assays revealed high upregulation of inflammation, fibrosis- and wound healing-related genes following OVF implantation compared to ELRs (ELR-RGD, ELR-BMP2, ELR-BMP7). Although the ELRs which have NP (nano-particles) induced more inflammation, collagen deposition and gene expression compared to ELR without NP and normal wound healing, but it was still less than OVF. Serum antigen-specific antibody titration showed OVF and ELRs generated antigen-specific IgG1 and IgE titres. Our data demonstrate that InnovaBone biomaterials induce minimal immune responses compared with OVF and suggest that they are safe and could be modified to increase the immune/inflammatory response in bone, if necessary by altering the ELR biogel bioactive molecues. Currently, we are profiling gene expression of calvaria defect implantation sites with the best candidate of scaffold and ELR biogels for inflammation, fibrosis or bone regeneration. ■

MUW

Medical University of Vienna

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Katayoon Changi is a PhD student in the Experimental Allergy Laboratory, Department of Dermatology, Medical University of Vienna, Austria. She studied medicine at Zahedan University of Medical Science, Zahedan, Iran.

BERISLAV BOSNJAK

PhD student in the Experimental Allergy Laboratory, Department of Dermatology, Medical University of Vienna, Austria. He graduated with a BSc. in molecular biology at Faculty of Science, University of Zagreb, Croatia, worked in the Research and Development Division on novel anti-inflammatory substances and accomplished a MSc in the Physiology and Immunobiology at Faculty of Science, University of Zagreb, Croatia. He joined the Medical University of Vienna in 2009.

DR MICHELLE EPSTEIN — LEADER

Dr. Michelle Epstein is a medical doctor who has specialized in Internal medicine and Allergy and Clinical Immunology recognized in both Canada and in the USA. After post doc fellowships in basic immunology at the Howard Hughes Medical Institute at Yale University and the National Institute of Allergy, Immunology and Infectious Diseases at the National Institutes of Health, she established a research group in Vienna combining her interests in clinical medicine and basic research focusing on allergic models in mice. Her research addresses three areas related to immunologic and allergic disease. Her laboratory group has established several models to study acute and chronic immune and allergic animal models. Dr. Epstein is the WP leader for WP4 and was supervising the experiments investigating the foreign body reaction to the biomaterials.

ABOUT

IMAGING OF BONE REGENERATION AND INFLAMMATION OF MICE WITH CALVARIAL IMPLANTS

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Andrea Markus
Frauke Alves

UMG - University Medical Center of Göttingen, Göttingen, Germany

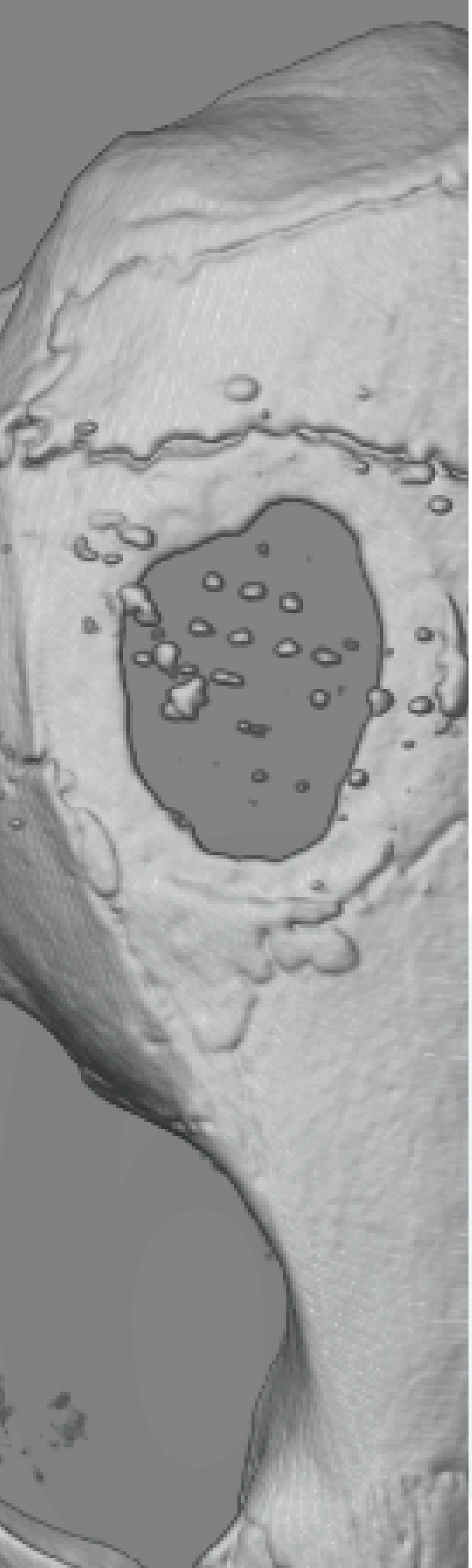
ESTABLISHING BONE MINERAL DENSITY

Initial work by UMG-GOE included the establishment of bone mineral density (BMD) measurement with x-ray based imaging techniques, the setup of x-ray based 3D virtual histology of mouse soft-tissue stained *ex-vivo* with a heavy ion containing contrast agent (PTA), as well as the development of a dedicated analysis scheme to analyze bone morphologic parameters in mice in 3D utilizing the software Scry (Kuchel & Sautter GbR).

To define bone regeneration and/or osteolysis, CT studies (Quantum FX, Perkin Elmer) were performed either at low dose longitudinal *in vivo* or at higher resolution post-mortem in order to better characterize the only weakly mineralized new formed bone in the scaffold area. For 3D data analysis the software Scry was used. New bone formation was calculated by comparing the area of defect 12 weeks post surgery with the defect area immediately after surgery. 12 weeks after implantation of discs, scaffolds or scaffolds plus biogel and/or nanoparticles into calvarial defects of different sizes by UNIVIE, UMG-GOE received heads of mice fixed in 70% ethanol from UNIVIE. Live mice with different implants were received 1 week after surgery. CT results indicated that the scaffolds can be visualized by CT but have poor contrast against the surrounding soft-tissue. All discs and LCM6 scaffolds appeared osteolytic as shown by an increase of the area of defect 12 weeks after surgery when compared to the size of the original defect. The consortium decided that LCM3 was the most suitable scaffold, as it has the following advantages: 1) did not cause any bone resorption, 2) was more bone conductive than the other LCMs, 3) showed more regrowth of bone from the edges of the defect, 4) has some bone growth in the pores of the scaffold, and 5) was not resorbed at 12 weeks after surgery. UMG-GOE quantified the mineralisation of the regrown bone by measuring the bone mineral density (BMD) using CT in combination with a custom made density phantom. We found that the mineralisation of new bone in the mice calvaria was similar between all LCM scaffolds, but lower than normal bone.

For the evaluation of bone growth or resorption over time, longitudinal studies in live mice were performed by weekly CT as well as optical imaging in the near infrared range (NIR). For this purpose, NIR fluorescent bisphosphonate imaging agents (Osteosense) were intravenously injected at diagnostic concentrations every 4 weeks. The obtained results demonstrated a steady increase of fluorescent bisphosphonate levels over the 10 weeks of measurements for all implants. *In vivo* CT scans showed that the combination of LCM3 plus BMP-containing biogel plus nanoparticles is the most osteoconductive material within the scaffold. Optimization of this implant combination, *in vivo* scanning thereof and calculation of bone growth is ongoing.

Two steps were taken to improve the contrast of the scaffold to be able to visualise and calculate the degradation of



the scaffold over time. Firstly, UMG-GOE scanned the implants using in-line free propagation phase contrast CT at the SYRMEP beamline of the synchrotron light source 'Elettra' (Trieste, Italy), a method which results in much higher soft-tissue contrast than classical CT, which was still not sufficient for quantitative analysis of scaffold resorption or new bone formation. Secondly, IBA incorporated 5nm or 100nm gold nanoparticles into LCM cylinders, which were scanned by UMG-GOE and showed an increased x-ray attenuation, thereby raising the contrast-to-noise ratio by a factor of 20 for the larger gold nanoparticles.

ASSESSING INFLAMMATION

To assess inflammation *in vivo* we used optical imaging in combination with near infrared fluorescent (NIRF) activatable probes (ProSense) which assess the activity of proteolytic enzymes released at the site of inflammation. ProSense was biweekly injected intravenously in mice with different implants starting from week 2 after surgery until week 12 and measured by *in vivo* optical imaging. The fluorescence intensities measured over the defect conclusively showed that maximum inflammation occurred at the closest time point after implantation and steadily declined over the following weeks. All implant combinations so far tested showed a considerable decrease of inflammation to near background levels by week 12 after surgery, suggesting that neither scaffold nor biogel or nanoparticles cause prolonged inflammation in the environment of the implants. ■

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DR CHRISTIAN DULLIN

Christian Dullin studied physics at the Friedrich Schiller Universität Jena. Following his degree, he first worked as software designer at the 3di GmbH Jena, which produces patient-customized implants. In 2004 he moved on to the Department of Diagnostic and Interventional Radiology, Göttingen. Christian is currently finishing his PhD and is responsible for the technical needs of the imaging unit, the development of adapted software, as well as data analysis..

DR ANDREA MARKUS

Andrea Markus received her degree in biology from the Johannes Gutenberg University, Mainz. Following her PhD in 1998 at the Helmholtz Center in Munich, she moved to Australia, where she worked for many years in molecular biology of cancer at the University of Sydney. In 2010 she returned to Germany and took up a position at the Department of Hematology and Medical Oncology at the University Medicine Göttingen. She is mainly involved in optical imaging and CT imaging of different animal models.

PROF FRAUKE ALVES — LEADER

In addition to being a clinician, Prof. Frauke Alves has a long standing track record in basic research and preclinical evaluation of tumour therapies by applying molecular imaging techniques in oncology. She heads a research group in the Dept. of Hematology and Medical Oncology and the Dept. of Diagnostic and Interventional Radiology at the University Medicine Center, Goettingen. Since 2008 she is leading a second “Translational Molecular Imaging” research group at the Max-Planck-Institute for Experimental Medicine in Goettingen, in the Dept. of Molecular Biology of Neuronal Signals, with the aim to translate basic knowledge into clinical practice. The focus of her interdisciplinary tumour imaging group is the investigation of mechanisms of tumour progression, angiogenesis, and the evaluation and optimization of novel anti-tumour therapies in orthotopic and transgenic tumour mouse models by applying noninvasive optical imaging techniques in combination with novel fluorescent probes in the near infrared range as well as computed tomography.

ABOUT

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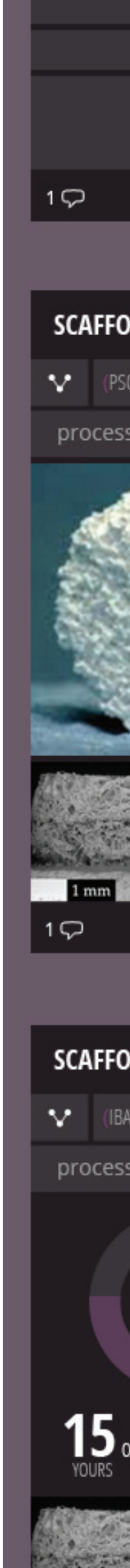
SME INV

VOLVEMENT

NETSCIENCE: A CASE STUDY OF HOW EUROPEAN FUNDING ALLOWS TO CREATE TOOLS FOR A SMOOTH RUNNING OF SCIENTIFIC EXPERIMENTS

Promoscience

Promoscience srl, Trieste, Italy



Digitalisation of R&D processes is acquiring a key role in scientific production, as it allows to share experimental procedures and data, assuring their replicability and consistency; this, in the long term, can boost achievement of reliable results.

Nevertheless, in most European research laboratories, information and datamanagementonlyreliesonfileservers and conventional laboratory notebooks, which considerably limit the possibility to organize and share information about experimental processes.

SampleLife was designed to respond to the needs of the EU-funded InnovaBone project, and specifically:

- **To trace samples and their datasheets** in order to track any material and data and reenact the relation between the single parts of joint experiments;
- **To manage the logistics of samples**, such as information on material flows from production to testing phase: availability, location, order and shipping status;
- **To harmonize datasheets** through a shared and systematic framework of data structures to be customized by its users for their research needs;
- **To allow replication of experimental processes and integrated data comparison**.

When InnovaBone started, on the market there was no software which was flexible enough to meet such complex

needs; this lead us to develop a new online tool to plan and monitor scientific experiments by managing information on materials, on their functionalization and improvement techniques, and on their *in-vitro* and *in-vivo* tests.

NetScience is also integrated with an Electronic Laboratory Notebook, which allows researchers to link sample and process data with free annotations, pictures, files and tables, as well as to comment and alert colleagues on relevant events.

NetScience also includes an online platform to manage contents, documents and project commitments towards the EC and among project partners. The tools has been developed in response to the coordination needs of InnovaBone, and is currently used by its partners to monitor the progress made in the different project tasks, as well as to upload and download project documents in an intranet where different access rights have been previously set in line with the definition of the governing bodies of the project.

Given the success obtained with the two prototypes so far, we intend to verify in LEAN mode whether it can be expanded beyond the field of biomaterials in both single laboratories and big research networks. ■

PROMOSCIENCE

Promoscience SRL

Trieste, Italia

Promoscience is a spin-off of SISSA (Scuola Internazionale Superiore di Studi Avanzati – International School of Advanced Studies) in Trieste, founded in May 2004 by Mr. Riccardo Brancaleon, a young research engineer who specialised in the design of advanced solutions for Knowledge Management. Promoscience was born with the purpose of exploiting market with the expertise and the results of the research and development activity performed by the founder, Mr. Riccardo Brancaleon.
Following a direction it had already taken working for SISSA - institution with which it still has constant and successful collaborations - Promoscience wants to integrate innovative methodologies of Knowledge Management with efficient and flexible information systems, joining advanced technology competences and effective communication strategies.
The working group is composed of highly qualified professionals with a documented experience. The Promoscience Company Curriculum reported here, describes their great experience in designing and producing web tools supporting knowledge management and scientific communication.

ABOUT

CLUSTERING, TRAINING AND NETWORKING: PARTICIPATIVE METHODOLOGIES TO SUPPORT INNOVATIVE DISCUSSIONS AMONG RESEARCHERS IN EUROPE

Moverim

Moverim sprl, Brussels, Belgium

InnovaBone implemented an inter-disciplinary approach to foster collaborative discussions and exchanges among researches mainly from EU funded project. Moverim boosted internal training (MOOCs) and clustering actions with other projects to build up a common vision, exchange information and good practices on bone regeneration and biomaterials. Multidisciplinary fora enable to create solid new relationships and push for further collaborations. Moverim is a SME located in Brussels working on funds for R&D for many clients like European universities, Research Centre's, SMEs, non-profit organisations, industries. The SME supports the project management, organises training activities and contributes to dissemination strategy and the results exploitation. This project enhanced Moverim competences in raising researchers skills, build up participative networking methodologies to keep research group active beyond the EU project financing. ■

MOVERIM

Moverim sprl

Brussels, Belgium

Moverim premises are in Brussels, it has been created in 2001 thanks to the professionality and experience accumulated by its founders since early '90s in raising and managing funds for R&D of many clients like European universities, Research Centre's, SMEs, non-profit organisations, industries. Moverim is willing to fully participate in EU projects assuring assistance to coordination, clustering and training activities to foster knowledge potential, cooperation and cross sectoral fertilisation among all beneficiaries. Moverim as an SME wants to improve the capacity to: develop tailored training plans, contribute to dissemination strategy and to results exploitation, build capacities required to get the best value from European projects, investigate commercial and philanthropic opportunities to keep the group research activity alive beyond the EU project financing.

ABOUT

BENEFITS OF MEDICAL DEVICE REGULATIONS IN APPLIED RESEARCH

Qserve

Qserve® Consultancy BV, Amsterdam, the Netherlands

The orthopedic medical device industry challenges the advanced materials science to investigate new biomaterial designs for medical devices with enhanced performance and controlled safety profiles. When the medical device regulations are not recognized during research, the industry cannot judge the feasibility of the design and does not take the risk of transferring it to the market. It will benefit the valorization process if research institutes take the medical device regulations into account early phase their research projects. For this purpose, we have introduced a systematic risk based approach to establish a 'frozen' design and to identify suitable design controls from a regulatory compliance point of view. This should ease the industry decision-making for accepting biomaterial designs into medical devices. ■

QSERVE

Qserve® Consultancy BV

Amsterdam, the Netherlands

Qserve is an international independent ISO 9001 registered medical device compliance consulting firm in QA/RA/CA with offices in The Netherlands, China and USA. Qserve provides expert advice and support, including certified training and auditing, for the medical device industry since 1998 covering a wide range of medical devices. Qserve's experts provide services on medical device regulations (CE Mark, 510K and more), quality system requirements (ISO 13485, CANCAS and QSR), application of design controls, risk management (ISO 14971), product validation and preclinical testing (electrical, software, packaging, sterilization, biological, animal, etc.), clinical evaluation (ISO 14155, MEDDEV 2.7.1) and technical documentation for regulatory submissions/ registrations world-wide.

ABOUT

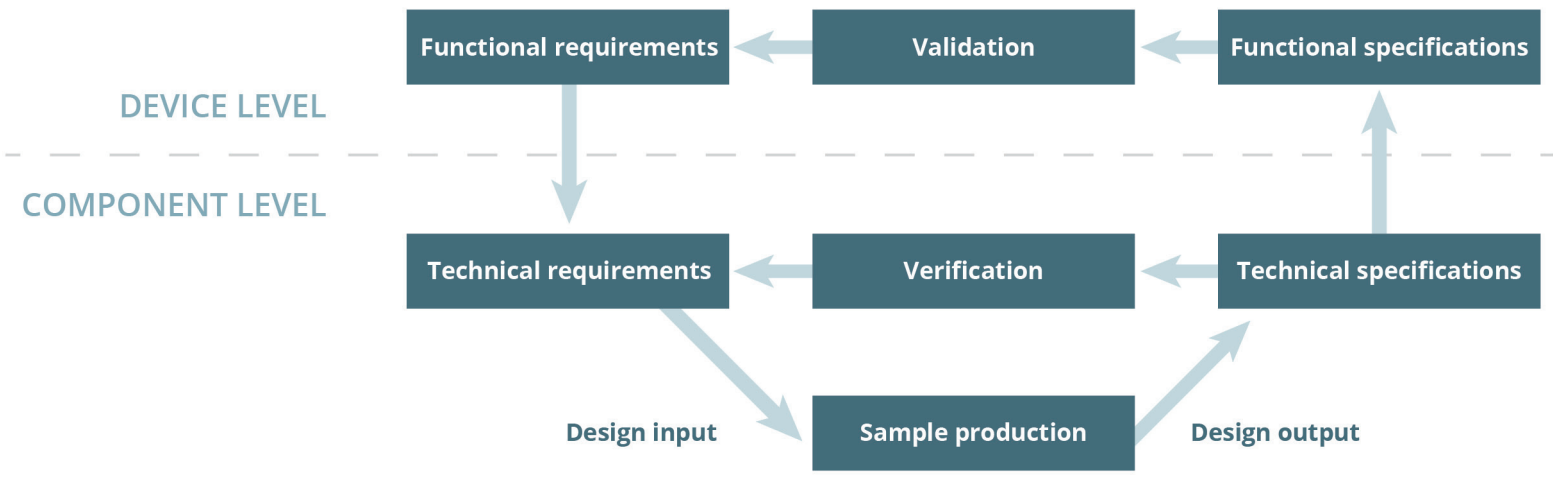


Figure 1 *Design control cycle (simplified scheme)*

SOURCE

The abstract book of the InnovaBone conference is available at **conference.innovabone.eu**

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