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SIB2021

**Congresso Nazionale Biomateriali  
Lecce, 11-14 Luglio 2021**



DEAR COLLEAGUES,

This year, the annual conference on biomaterials of the Società Italiana dei Biomateriali will take place in Lecce at the Sede del Rettorato of University of Salento.

The conference is jointly organized by the Department of Engineering for Innovation of the University of Salento and the Institute of Polymers, Composites and Biomaterials of the National Research Council (IPCB-CNR).

The overall aim of the annual conference is to provide a forum for scientists, clinicians, industry members to meet, promote and share ideas on cutting-edge research, and novel innovation strategy on biomaterials and their applications. In this context, young scientists are strongly invited to participate. They will have the opportunity to share their research results to a wide public of experts in an unique interdisciplinary/transdisciplinary environment: from chemistry, engineering, biology, material science, translation to clinical practice and industrialization. Indeed, networking, dissemination and the open curiosity are the driving force of the conference as represented by the SIB2021 logo: Six degrees of separation theory.



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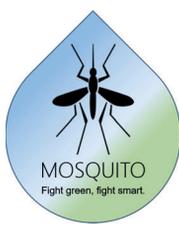


## Topics

- Drug delivery, therapeutic biomaterials & nanocarrier systems
- Biomaterials for hard and soft tissue repair/regeneration
- Cell-material interactions
- Scaffold-based tissue engineering
- Tissue and organ models
- Additive manufacturing and 3D bioprinting / Nanodevice
- Innovative biomaterials for antibiotic-resistance
- Wound healing
- Clinical applications



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OUR INNOVATION STARTS FROM NATURE



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A wireframe illustration of a human figure, composed of numerous thin, light-colored lines that form the head, neck, shoulders, and arms. The figure is positioned in the upper right quadrant of the page, appearing to look towards the left. The background is a solid dark color, and a large, white, rounded shape is centered on the page, containing the text and logo.

# INVITED LECTURES



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# Innovatory routes for tissue engineering and precision medicine approaches

Rui L. Reis <sup>1</sup>

<sup>1</sup>3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal; 2ICVS/3B's-PT Government Associate Laboratory, Braga/Guimarães, Portugal

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## Introduction

We believe that the use of natural origin polymers, including a wide range of marine origin materials, is the best option for many different approaches that allow for the regeneration of different tissues. In addition to the selection of appropriate material systems it is of outmost importance the development of processing methodologies that allow for the production of adequate scaffolds/matrices, in many cases incorporating bioactive/differentiation agents in their structures.

Furthermore, an adequate cell source should be selected. In many cases efficient cell isolation, expansion and differentiation, and in many cases the selection of a specific sub-population, methodologies should be developed and optimized. The development of dynamic ways to culture the cells and of distinct ways to stimulate their differentiation in 3D environments, as well as the use of nano-based systems to induce their differentiation and internalization into cells, is also a key part of some of the strategies that are being developed in our research group.

The potential of each combination materials/cells, to be used to develop novel useful regenerative and precision medicine therapies will be discussed. The use of different cells and their interactions with different natural origin degradable scaffolds and smart hydrogels will be described. Several examples of strategies to regenerate different types of tissues will be presented. Several precision medicine strategies will also be described.

## Experimental methods

### Biosketch

Rui L. Reis, PhD, DSc, Hon. Causa MD, Hon Causa PhD, FBSE, FTERM, member of NAE, FAIMBE, FEAMBES, is the founder and Director of 3B's Research Group, I3Bs – Institute for Biomaterials, Biodegradables and Biomimetics & Director of the ICVS/3B's LA of UMinho. He is the CEO of the European Institute of Excellence on TERM, the Past-World President of the Tissue Engineering and Regenerative Medicine International Soc. (TERMIS) and Editor-in-chief of the Journal TERM (Wiley). He is a recognized World expert in the TERM and biomaterials fields, he has 1460 publications listed in ISI WoK, around 125 filled/awarded



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patents, 18 books, around 285 book chapters in books with international circulation and on major encyclopedias, and more than 2200 communications in conferences, as well as 275 plenary or invited talks. His work has been cited around 46000 times in ISI WoK (around 33 citations per article, around 68500 citations in Google Scholar, and 50500 citations in Scopus), and he has an ISI h-index of 98 (123 according to Google Scholar, and 102 for Scopus).



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# Balancing bioactivity and mechanical properties of calcium phosphate bone graft substitutes

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Regeneration of large and complex bone defects remains a clinical challenge, despite decades of research into bone grafts and bone graft substitutes. Moreover, a result of ageing and a more active life style is an increasing need for successful bone regenerative therapies. Synthetic bone graft substitutes with an intrinsic osteoinductive potential, i.e. the ability to induce de novo bone formation, have shown the potential to become effective and affordable substitutes for natural bone grafts. A majority of current synthetic osteoinductive biomaterials are based on (porous) calcium phosphate ceramics, which have the advantage of being bioactive, and, depending on the composition, biodegradable. Nevertheless, porous calcium phosphates are intrinsically brittle, strongly impairing their clinical applicability from a mechanical and handling perspective. In this lecture, our recent efforts to improve mechanical and handling properties of calcium phosphate bone graft substitutes, without compromising their bioactivity, will be discussed.

## Biosketch

Pamela Habibovic holds a PhD degree from the University of Twente, the Netherlands. Following post-doctoral research at Children's Hospital Boston and McGill University, in 2008, she started her research group at the University of Twente. In 2014, she moved to Maastricht University, the Netherlands, where she became Full Professor of Inorganic Biomaterials and where she cofounded MERLN Institute for Technology-Inspired Regenerative Medicine. Currently, she is the Scientific Director of the Institute and chair of MERLN's Department of Instructive Biomaterials Engineering. The main focus of her research group is on synthetic bone graft substitutes, bioinorganics, nanomaterials for theranostics in regenerative medicine and high-throughput approaches in biomaterials research. For her research she received prestigious Veni, Vidi, Aspasia and Gravitation grants of the Netherlands Organisation for Scientific Research among other external research funds. Since 2013, she serves as a council member of the European Society for Biomaterials (ESB), and since 2017 she holds the role of the ESB President. Habibovic is an Associate Editor of the RSC journal Biomaterials Science and an editorial board member of several other journals in the field. She has published over 100 peer-review articles. In 2013, she received the Jean Lera Award of the ESB.



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# The microbiota: a new organ essential for our well-being

**M. Rescigno**<sup>1</sup>

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The human gut is home to trillions of microbial cells with over 1,000 diverse microbial species that contribute to the primary functions of the gastrointestinal tract, including nutrition, mucosal immunity and pathogen defence. The gastrointestinal tract mucosa constitutes the major interface that separates the luminal environment from the internal milieu and it is also the body's primary site for interaction with the microbial world living within the gut lumen. The surface of the gastrointestinal mucosa is estimated at up to 4,000 square feet when laid out flat and, most importantly, contains adapted structures allowing bi-directional host-microbe communication. The gut barrier has to guarantee nutrient and metabolite exchange with the microbiota but at the same time also protection against the microbial world. The gut barrier consists of three major components including a mucus layer, an intact epithelial monolayer and a lamina propria with mucosal immune cells. All three layers contribute to well-functioning of the gut barrier. The epithelial monolayer is not a static structure and the tight junctions that seal spaces between epithelial cells are regulated by the gut microbiota and dietary components. Below the mucosal barrier there is an additional barrier, the gut vascular barrier that controls what enters in the systemic circulation, and avoids bacterial translocation at systemic sites. When the microbiota composition changes due to inflammatory conditions, dietary misbehaviour or antibiotic treatment, these defence mechanisms may be altered. Here we show how the barrier is preserved and what happens when it is modified.

## **Biosketch**

Maria Rescigno is full professor, vice-rector and delegate of research at Humanitas University and group leader at Humanitas Research hospital, Milan. She graduated in Biology at the University of Milan, received her PhD in Pharmacology and toxicology and was a post-doc at the University of Cambridge, UK. From 2001 to 2017 she has been the director of the Dendritic cell biology and immunotherapy Unit at the European Institute of oncology. She was the first to show that dendritic cells actively participate to bacterial uptake in the gut and the existence of a gut vascular barrier that resembles the blood brain barrier. She authored more than 165 publications. She was nominated EMBO young investigator in 2007. In 2008-2013 she was visiting professor at the University of Oslo. In 2011 Maria Rescigno was elected EMBO member and from 2019 a member of the EMBO council. She has been the recipient of three ERC grants (starting, proof-of-concept and consolidator). She is in the scientific board of several charities (AIRC, L'Oreal) and different



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companies (MilBo, SiFi). In 2016 Maria Rescigno founded Postbiotica s.r.l. that exploits microbiota-derived metabolites as new pharmaceutical agents. Postbiotica has won two competitions: Bioupper and MyStart BCN. H-index: 65



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# Artificial vs god-made scaffold for wound covering

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Preventing fibrosis or hypertrophic scar formation following tissue damage is still a big challenge notwithstanding the numerous approaches clinicians currently use. Hitherto, no written account was available of a successful case of scarless skin healing after a severe burn injury. Here, we report 400 cases of porcine collagen-based artificial scaffold and a case of natural CTLA4Ig-gene transferred pig skin graft for re-construction of deep wounds.

Artificial scaffold grafting substituted the lost cutaneous tissue, induced the newly formed tissue and significantly improved the cosmetic appearance. However, the difference between the normal skin and the artificial scaffold healed wound is apparent, including deformity and contracture of the healed wound. Interestingly, a case of the perfect regenerative healing of a severe burn wound with no hypertrophic scar formation in which a postage stamp skin autograft was covered with the natural scaffold, ie., human cytotoxic-T-lymphocyte associated antigen4-immunoglobulin (hCTLA4Ig) gene-transferred pig skin. We also discuss the mechanisms involved in scarless healing of human burn wounds.

## Biosketch

Director, Department of Burn and Plastic Surgery, The First Affiliated Hospital of Shenzhen University

Past President, Chinese Burn Association

President, Chinese Wound Repair Society

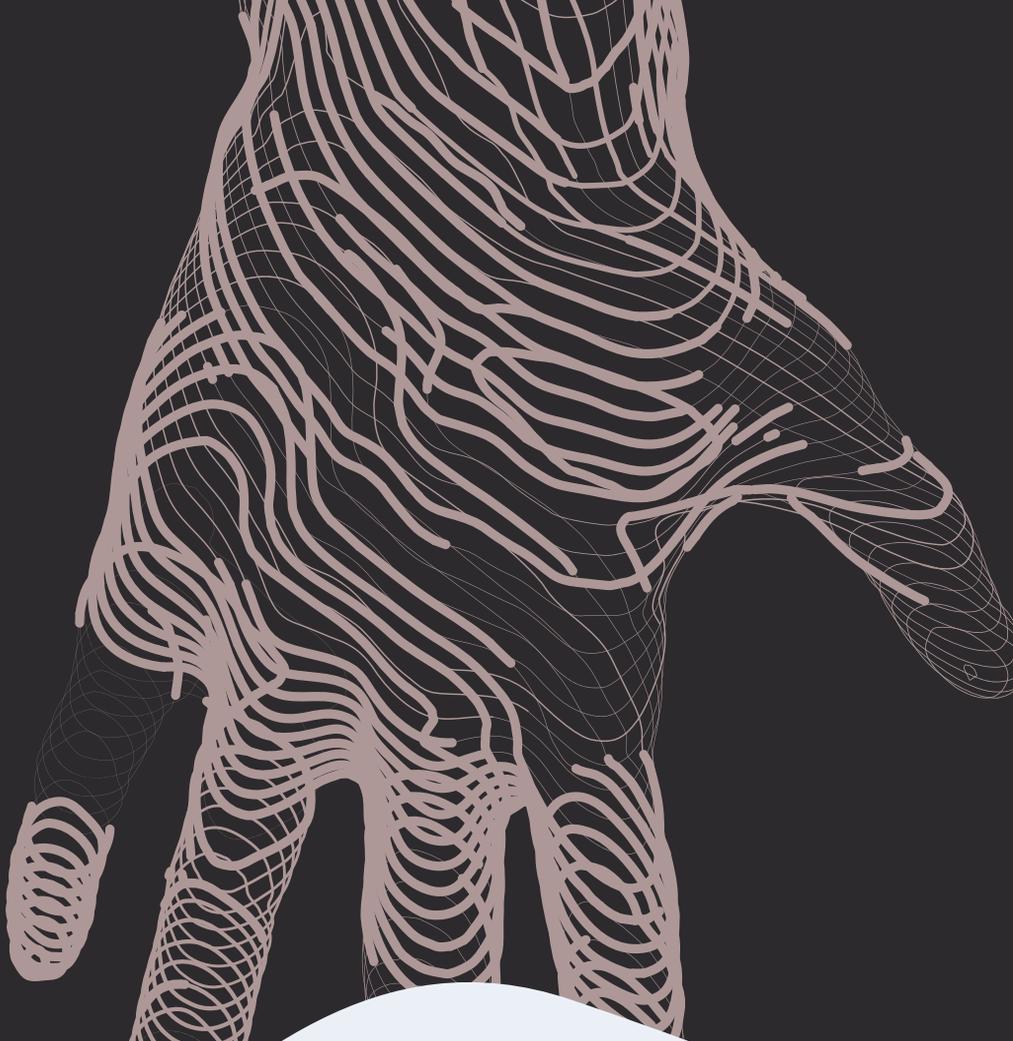
Past President, Chinese Burn Rehabilitation

Executive member of International Society of Burn Injury

Editor-in Chief, Burn & Trauma



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## KEYNOTE LECTURES



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# Can an engineered biohybrid cardiac patch improve ischemic ventricular wall remodeling?

A. D'Amore <sup>1</sup>

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As an intervention to abrogate ischemic cardiomyopathy, the concept of applying a temporary, local patch to the surface of the recently infarcted ventricle has been explored from a number of design perspectives. Two important features considered for such a cardiac patch include the provision of appropriate mechanical support and the capacity to influence the remodeling pathway by providing cellular or biomolecule delivery. The objective of this report was to focus on these two features by first evaluating the incorporation of a cardiac extracellular matrix (ECM) component, and second by evaluating the impact of patch anisotropy on the pathological remodeling process initiated by myocardial infarction. The functional outcomes of microfibrous, elastomeric, biodegradable cardiac patches have been evaluated in a rat chronic infarction model. Ten weeks after infarction and 8 wk after patch epicardial placement, echocardiographic function, tissue-level structural remodeling (e.g., biaxial mechanical response and microstructural analysis), and cellular level remodeling were assessed. The results showed that the incorporation of a cardiac ECM altered the progression of several keys aspects of maladaptive remodeling following myocardial infarction. This included decreasing LV global mechanical compliance, inhibiting echocardiographically-measured functional deterioration, mitigating scar formation and LV wall thinning, and promoting angiogenesis. In evaluating the impact of patch anisotropy, no effects from the altered patch mechanics were detected after 8 wk, possibly due to patch fibrous encapsulation. Overall, this study demonstrates the benefit of a cardiac patch design that combines both ventricle mechanical support, through a biodegradable, fibrillary elastomeric component, and the incorporation of ECM-based hydrogel components.

## Biosketch

Dr. Antonio D'Amore is a Research Assistant Professor in the Department of Surgery and Bioengineering at the University of Pittsburgh. He also currently serves as Group Leader and Head of the Tissue Engineering Program at Ri.MED Foundation. He is the author of more than 150 publications including peer-reviewed journal articles, book chapters, international conference proceedings and abstracts, biomedical devices patents applications, and software to model biological systems. Dr. D'Amore's research seeks to couple a mechanistic understanding of the relationship between scaffolds micro-structure, mechanics, and



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endogenous tissue growth with the development of novel biomaterials for tissue engineering strategies. The research interests are generally in the area of cardiovascular engineering, including: quantitative histology and biomaterials microstructure image-based analysis, structural modeling strategies to guide tissue engineering scaffold fabrication, mechanical and topological conditioning for tissue elaboration, development of cardiac restraint devices, vascular grafts and engineered heart valves. Dr. D'Amore's project funding comes from the National Institutes of Health, Ri.MED and University of Pittsburgh. In 2020 he was awarded with the European Research Council Consolidator Grant for his project BIOMITRAL on advanced strategies for mitral valve regeneration.



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# Nanoinnovation on titanium surfaces to counteract bacterial adhesion

S. Ferraris <sup>1</sup>

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Bacterial adhesion is a critical issue for implantable biomaterials and can lead to multiple surgery, prolonged antibiotic therapies with the consequent increase of hospitalization time and costs. The problem is worsened by the increasing antibiotic resistance. In this context the development of implantable biomaterials able to reduce bacterial adhesion without the use of antibiotics appears an extremely promising strategy.

The talk will present an overview of different possible surface modifications developed, by the author research group with national and international collaborations, to counteract bacterial adhesion on titanium surfaces without the use of drugs. The proposed solutions are mainly based on nanotextures and on the application of a reduced amount of active substances. These strategies are aimed at the reduction of bacterial adhesion and biofilm formation avoiding the limitations connected to the release of active substances (limited action, cytotoxicity concerns, certification and cost issues).

A nanotextured titanium oxide layer obtained by means of a chemical treatment, nanosteps produced on titanium by means of electron beam structuring and zirconia based nanometric coatings will be discussed and compared. The grafting of natural molecules is finally cited. Morphology, roughness, mechanical and chemical stability, wettability, surface charge, chemical composition and eventual ion release will be presented together with bacterial and cellular adhesion. Particular attention will be dedicated to the possibility to apply these technologies to real implants in terms of durability, technology upscaling, possibility to be sterilized, required storage conditions, eventual certification issues and supposed costs.

## Biosketch

Sara Ferraris received a PhD in Biomedical Engineering in 2010 from Politecnico di Torino and she is actually assistant professor at the Department of Applied Science and Technology, in Politecnico di Torino.

She is active in the field of surface modification and characterization of biomaterials. Her research activities deal with metallic materials, surface functionalization of biomaterials with natural molecules (of animal or vegetal origin), often obtained from industrial byproducts, natural coatings, surface nanotexturing and chemical treatments or surface activation procedures (e.g. plasma treatments). Moreover, she is active in the field of surface characterization by means of several complementary techniques (FESEM-EDS, FTIR,



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XPS, UV, contact angle, zeta potential, fluorescence microscopy, confocal microscopy, AFM-KPFM, optical microscopy, tape and scratch adhesion tests, contact profilometry) as well as in the design of customized ageing test, durability, stability and release tests. She is guest editor of two special issue of Coatings (MDPI), co-guest editor of a special issue of Materials (MDPI), review editor for Frontiers in Materials, Frontiers in Bioengineering and Biotechnology and co-organizer of two symposia for the Materials Science & Technology international conference. She is author of more than 100 publications on international journals and 6 patents (h-index 22, Scopus).



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# X-ray scattering scanning microscopies of pathologic collagen-based tissues

C. Giannini <sup>1</sup>

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X-ray Small and Wide-Angle Scattering (SAXS/WAXS) scanning microscopies have been employed to inspect morphological and structural properties of human tissues at the atomic and nano scale. Different specific pathologies have been diagnosed by means of these techniques, by inspecting changes in the type 1 collagen (supra-molecular and molecular) structure in keratoconus, diabetes mellitus, coxarthrosis, aneurysms and breast cancer affected tissues. In aneurysms and breast cancer tissues we have been able to detect also micro-calcifications and to identify their chemical and crystallographic nature.

SAXS/WAXS microscopy is a valuable tool to non-invasively

1. (co)localize soft tissues (collagen, myofilament, elastin) and hard tissues (metals, microcalcifications)
2. determine collagen fiber direction, periodicity, and electron density
3. differentiate normal from scarred collagen, the latter being denser and more aligned
4. identify the crystallographic origin of the microcalcifications (examples: Ca/P structures, cholesterol).

## **Biosketch**

PhD in Physics at the University of Bari. From 2001 in the National Research Council, where now she leads the Institute of Crystallography. Expert in the structural characterization of materials, with X-ray based scattering techniques. Her research interests span the structural analysis of inorganic (nano)materials, biomaterials, natural and bio-engineered tissues. A recent area of interest is x-ray micro and nanoimaging techniques, also with coherent x-rays.

She has published more than 280 papers in international peer-reviewed journals, h index: 41 (scopus); 46 (google scholar).



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# Innovative strategies and biomaterials for antibiotic resistance

**L. Gritsch**<sup>1</sup>

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Resistance to antimicrobial agents, especially antibiotics, recently gained worldwide attention as a major problem for the future of healthcare. Authorities recognized an increase in the failure rate of known drugs due to excessive use or misuse: an estimated 50% of antibiotic usage is reported to be unnecessary. The risk of regression to pre-antibiotic death-by-infection rates is thus considered a close reality. This threat demands a strong multidisciplinary effort by the scientific community to develop new antimicrobial medical technologies, preferably avoiding the use of conventional antibiotics. Metallic therapeutic ions, chemical functionalization (e.g. quaternary ammonium), nanotechnology-based antimicrobials and plant-derived compounds such as polyphenols or essential oils are just a few of the several approaches that have been investigated with encouraging results. As it is often the case, a critical point to transfer novel strategies remains the difficulty of harmonizing the needs of industry with the interests and drives of academia. The development of new technologies, however, is just one way to tackle the problem. Reducing and optimizing the usage of existing antibacterial treatments, a concept that goes by the name of antimicrobial stewardship, can go a long way towards minimizing resistance development. The problem is complex and multifaceted, but critical for the well-being of our society. And if we want to win this fight it is mandatory that we learn to continuously adapt and evolve our scientific community at the same pace bacteria learn to protect themselves from us.

## **Biosketch**

Lukas Gritsch is a biomaterials scientist specialized in antimicrobial technologies and 3D manufacturing for tissue engineering. Working at the crossroad between biology and engineering, his investigations explored, among others, the electrospinning of chitosan for therapeutic ion delivery and the fused deposition modeling of hybrid materials for bone grafting. As a Marie Curie fellow, his research was focused on tackling the problem of antibiotic resistance using metallic ions as antimicrobial agents. His achievements granted him the Julia Polak European doctoral award by the European Society of Biomaterials (ESB). He holds an MSc and a BSc honors in Biomedical Engineering obtained at Politecnico di Milano. He gained an industrial PhD in Materials Science pursued under the supervision of Prof. Aldo Boccaccini at the Institute of Biomaterials of Erlangen. His career also includes a



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two years post-doc at Université Clermont Auvergne and visiting research positions at Lucideon Ltd. in Stoke-on-Trent, UK and at Polytechnique de Montréal. To date, he is the author of 9 scientific papers, 3 book chapters and presented at >10 international conferences.



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# Aquaporin water channel as novel target for biomaterial design and engineering: implications in health and disease

**G.P. Nicchia** <sup>1</sup>

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The design of advanced bio-functional materials represents a great challenge in nanomedicine, tissue engineering and regenerative medicine. The dynamic interaction between cell and biomaterial is orchestrated by plasma membrane proteins, as the water channel protein Aquaporin-4 (AQP4), able to tune cell fate, therefore representing an essential focus in this context. AQP4 mediated water fluxes, as well as AQP4 supramolecular structure, are the key to control brain cell behaviour. In particular, by finely controlling cell volume changes, they can switch cells from migration to adhesion/differentiation (healthy brain) or from migration to apoptosis (diseased brain).

The possibility to use biomaterials to control AQP4-dependent behaviour of brain cells, called astrocytes, was recently investigated by our group by the use of nanostructured biocompatible interfaces, Hydrotalcite-like compounds (HTLc), able to induce astrocyte differentiation not associated with inflammatory (gliotic) reaction. We demonstrated that HTLc induced an AQP4 upregulation-dependent increase in cell volume change efficiency associated with major differences in AQP4 and actin organization at nanoscale, assessed by stimulated emission depletion (STED) super-resolution microscopy. HTLc-dependent effect on AQP4 expression and function in turn caused an improved capability of astrocyte sensing and effector mechanisms mediated by two other plasma membrane protein (TRPV4 and VRAC) which showed an AQP4-driven gain of function.

Based on these results we propose AQP4 as a novel target for the design and engineering of innovative biomaterials. In particular, we advise the use of tunable biomaterials to modulate AQP4 function and/or supramolecular structure and, in turn, cell behavior in healthy and diseased brain.

## **Biosketch**

Grazia Paola Nicchia is Professor of Physiology and Vice-Director at the Department of Bioscience, Biotechnology and Biopharmaceutics, University of Bari Aldo Moro, Visiting Professor at the Dominick P. Purpura Neuroscience Department, A. Einstein College of Medicine, New York, USA, and Associated to the CNR - Institute for Organic Synthesis and Photoreactivity, Bologna, Italy. Her research career has focused on the cellular and



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molecular physiology and biophysics of aquaporin (AQP) water channel proteins, in health and disease. The main interest is the brain AQP4 and its peculiar plasma membrane organization into well-ordered structures called Orthogonal Arrays of Particles (OAPs). In particular, she has discovered that OAPs are the target of autoantibodies in Neuromyelitis Optica, a severe form of Multiple Sclerosis. Research in her laboratory is focused on the molecular mechanisms by which AQPs promote cell volume regulation, changes in cell morphology and calcium signalling, working in concert with TRP and Connexin ion channels. Her expertise ranges from biophysical techniques for functional studies, (TIRF-M, stopped flow light scattering, calcein quenching microscopy) to live cell imaging with confocal microscopy and super resolution microscopy (gSTED) along with gene expression, biochemistry and molecular biology. Currently funded by AFOSR, NIH, Marie Skłodowska-Curie Action - Innovative Training Networks, ITN.



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# Magnetic nanoparticles and clusters and clusters as platforms for combing magnetic hyperthermia with other therapeutic treatments to tackle cancer

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The use of heat to cure cancer is very ancient. Among the techniques that enable to precisely deposit the heat in very specific body regions thus providing more selective heat treatment with less side effects, “magnetic hyperthermia” (MHT) exploits magnetic nanoparticles as heat agents that can be excited under alternating magnetic fields. MHT can be applied at magnetic field conditions (field intensity and frequency) that are clinically safe for patients with no tissue-depth attenuation for nanoparticle actuation. In addition, magnetic nanoparticles that show superparamagnetic behaviour at body temperature are also ideal carries for drugs or other therapeutic elements.

This talk aims at providing an overview of our last five years research efforts to combine magnetic hyperthermia with other clinical therapeutic modalities (i.e. chemotherapy, induced cytotoxicity, radiotherapy). In the first part of the presentation, I will focus on our progress on non-hydrolytic methods for the preparation of magnetic nanoparticles with optimal heat performance in MHT by specifically control size, size distribution, shape, composition and their magnetic properties. In a second part, I will report our in vitro studies on tumor cell models to determine the MHT effects, with or without the association of chemotherapeutic drugs, on different subpopulations of cancer cells. Finally, in a third part, I will discuss our preclinical results to evaluate the magnetic hyperthermia efficacy of some of our magnetic materials on xenograft murine tumor model and the bio-distribution studies of some of our best performing materials.

## Biosketch

Teresa Pellegrino, received her master in Chemistry in 2000 and her Ph.D. in Chemical Synthesis and Applied Enzymatic Chemistry in 2005 from the University of Bari, Italy. During her PhD she was visiting student for 18 months in the group of Prof. P. Alivisatos at University of Berkeley, California and later for additional 18 months, in the group of Prof. W. J. Parak at the Center for Nanoscience in Munich-Germany, where she started working in nanoscience. After being Post Doc at National Nanotechnology laboratory in Lecce, then permanent staff scientist at the Nanotech Center of CNR-Lecce, since 2014 she is tenured team leader of the Nanomaterials for Biomedical Applications group at the Italian Institute of Technology, Genoa, Italy. Her current research interests focus on the development of



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inorganic nanostructures for drug delivery, magnetic hyperthermia, photo-thermal treatment and radiotherapy exploiting cation exchange radio-protocols. She has been the recipient of the ERC-Starting grant ICARO (GA 678109) and the ERC-proof of principle Hypercube (GA 899661). At IIT, she has generated 9 families of patents and 23 applications. She has co-authored over 130 peer-reviewed papers in international journals in the field of nanoscience, chemistry, nanomedicine and drug delivery.



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# Antibacterial and antiadhesive biomaterials: strategies and applications from nanomaterials to medical devices

L. Russo <sup>1</sup>

<sup>1</sup>Università degli Studi di Milano-Bicocca

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Infection of implanted medical devices still represents an unresolved issue with enormous economic and healthcare costs. The development of new antibacterial and antiadhesive methods able to maintain intact the properties of implanted devices without inducing antimicrobial resistance is becoming urgent in order to reduce chronic infections and consequently medical device failure. The generation of new chemical methodologies employable in the design of new antibacterial devices represents an open challenge in the biomedical field. Here in this lecture, the current chemical methods to induce antibacterial and antiadhesive properties will be presented, taking into consideration the open challenges and the current limitations.

## Biosketch

Laura Russo is Assistant Professor at University of Milano-Bicocca, Adjunct Lecturer at National University of Ireland, Galway and Visiting Researcher at Cùram – Center for Excellence of Biomaterials and Regenerative Medicine - Ireland. Since 2021, LR is in the Scientific Advisory Board of Biocompatibility Innovation (BCI), company operating in the development of medical devices. LR is also President of AlumniBicocca, the association of Alumni from University of Milano-Bicocca. In 2010 LR joined the research group of Prof. Julian R. Jones, at Imperial College of London, as Visiting Researcher, working on hybrid biomaterials for osteochondral tissue regeneration. In October 2016 Dr. Russo awarded a SFI Starting Investigator Research Grant (SIRG) at Cùram, National University of Ireland - Galway – where she started her research as Principal Investigator on Glycofunctionalized hydrogels and biomaterials for tissue engineering applications.

In March 2017 she was appointed as Assistant Professor in Organic Chemistry at the University of Milano – Bicocca. LR has awarded the prestigious Junior Research Award for Organic Chemistry in Life Science of the Italian Chemical Society for her scientific contribute on organic chemistry applied to biomedical and life science field. LR has been invited as lecturer in international conferences and serves as evaluator of international projects. Her research interests are focused on biomaterials for medical devices, tissue engineering and 3D bioprinting applications.



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# MSC based intervertebral disc regeneration: from bench to bedside

G. Vadalà <sup>1</sup>

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Low back pain caused by intervertebral disc disease (IDD) presents a large, unmet medical need which results in a disabling loss of mechanical function. Today, no efficient therapy is available. Chronic cases often receive surgery, which may lead to biomechanical problems and accelerated degeneration of adjacent segments.

Bone marrow mesenchymal stem cell (MSC) based regenerative therapies showed encouraging results in in vitro, in animal model and in phase 1 and 2 clinical trials.

Our group devoted 15 years of research moving from in vitro cells studies and organ culture studies to preclinical studies and randomized clinical trials.

The lecture aims to present the state of the art of bone marrow MSC based Intervertebral disc regeneration focusing on our contribution in this field of research.

## Biosketch

Gianluca Vadalà, MD, PhD, is Assistant Professor of Orthopaedic Surgery at the Campus Bio-Medico University (UCBM) of Rome, Italy, in the Dept. of Orthopaedic and Trauma Surgery where he actively performs regenerative and reconstructive orthopaedics and spine surgical cases. He is the Director of the Laboratory for Regenerative Orthopaedics at UCBM focused on developing and translating new biological therapies included stem cell-based therapies in Orthopaedic and Spine Surgery.

He received several awards including the AOSpine Europe Young Research Award in 2013 and 2018, one of the most prestigious European award for research in the Spine Surgery field, for his enthusiastic research on intervertebral disc regeneration. He received several national and international research grants in the field of regenerative orthopaedics as PI.

He authored over 100 scientific articles in peer-reviewed journals in orthopedic surgery with an H-index of 27 (Scopus).

He serves as President of the European Orthopaedic Research Society (EORS).



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# ANTIMICROBIAL NANOCOATINGS ON BIOCOMPATIBLE AND ECO-FRIENDLY MATERIALS

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## Introduction

Antimicrobial surfaces represent a primary approach to prevent the occurrence and diffusion of clinical infections, foodborne diseases, and propagation of pandemics in everyday life. Addition of antimicrobial agents in the form of coatings onto a surface results in a very effective strategy to confer such desired feature, inducing the inhibition of microorganism spread and biofilm formation, preventing cross-contamination phenomena, contrasting antimicrobial resistance (AMR) and, also, suitably modifying other materials properties at the same time. In this framework, this presentation reports about some antibacterial inorganic nanocoatings developed in our laboratories and made of a combination of different active materials together. Nanostructured thin films and nanoparticles are applied by physical vapour deposition methods on different kinds of biocompatible substrates, like bioplastic sheets and soft electrospun polymer micro/nano fibers. Preferential action of the different coating materials is evidenced against different bacterial species, so that the materials combination can act against the different species simultaneously, allowing a complete bacterial suppression even in a few minutes. The influence of the coatings on the optical properties and surface wettability is also assessed. At the same time, considerations about particle release and cytotoxic effects on human cells are drawn in order to evaluate the possible usages of the coated systems for biomedical applications, food packaging, filtration, textiles, etc.

## Biosketch

Daniele Valerini, Ph.D., is a researcher at ENEA – Italian National Agency for New Technologies, Energy and Sustainable Economic Development, in Brindisi (Italy). He graduated in Physics in 2004 (full marks cum laude) and earned his Ph.D. in Physics in 2008. Since 2010 he is researcher with permanent position at ENEA, where his main R&D activities are related to development and characterization of coatings and nanostructured materials for antimicrobial applications, mechanical machining, transportation, energy and environment. His expertise is mainly focused on deposition of materials by PVD techniques and study of their morphological, structural, compositional, optical, electrical, mechanical, tribological and antimicrobial properties. Involved in several national and international projects, coordinator of the European network of infrastructure “EXTREME” and of the corresponding project funded by the KIC “EIT RawMaterials”, and WG member of COST



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Action CA15102 “CRM-EXTREME”. Organizer, committee member, chairman and speaker in several international events and symposia. Lecturer and trainer in R&D projects, professional training and University courses, and co-supervisor of bachelor students. Coauthor of currently more than 60 international publications like peer-reviewed papers, reviews, editorials, conference proceedings, books and book chapters. Co-editor and guest editor of volume and special issue in international journals, and reviewer for more than 20 international journals.



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# CARDIAC AND NERVOUS REGENERATION



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# Injectable thermosensitive hydrogel for cardiac delivery of biotherapeutics

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**Introduction** Many genes and pathways have been indicated to play essential roles during heart regeneration and manipulation of these pathways using mRNAs or small molecules have been shown to be promising therapeutic strategies [1-3]. Successful and efficient induction of cardiac regeneration relies on simultaneously activating/suppressing several pathways, which is technically challenging. In this study, a dual delivery system composed of mRNA polyplexes and micelle-containing thermosensitive hydrogel, previously complexed with CHIR99201 (a small hydrophobic GSK3 inhibitor and Wnt agonist), was designed allowing local sustained release of mRNA as well as small molecular drugs.

**Experimental methods** In this study, the synthesis of a methoxypoly(ethylene glycol) poly[2-(dimethylamino)ethyl methacrylate] (mPEG-pDMAEMA) diblock copolymer was optimized via RAFT polymerization and this polymer was used as polymeric carrier for mRNA condensation. Particle size and polydispersity index (PDI) of mRNA polyplexes were determined by dynamic light scattering while  $\zeta$ -potential was measured by laser Doppler electrophoresis at 37 °C in HEPES buffer (20 mM, pH 7.4). Subsequently, the loading of mRNA polyplexes into a thermosensitive pNIPAM-PEG-pNIPAM (NPN) hydrogel was evaluated to facilitate local and sustained mRNA release. The NPN triblock copolymer synthesized by ATRP polymerization, was used as a loading carrier for CHIR99021 (CHIR) by forming flower-like micelles that encapsulate the hydrophobic drug via heat-shock procedure [4]. The CHIR-NPN gel was then formulated by increasing the CHIR-NPN polymer content, reaching the final concentration of 20% w/w. CHIR release studies were conducted at 37 °C upon addition of PBS (pH 7.4) supplemented with 4.5% w/w bovine serum albumin on top of the gels. Placebo NPN and CHIR-NPN gels were characterized by rheological analyses and



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swelling/degradation studies to investigate the mechanical properties and the stability of the formulations. Gelation and injectability of the final system, made of mRNA polyplexes loaded into CHIR-NPN gel, were tested.

**Results and discussion** A well-defined mPEG-pDMAEMA (PD) diblock copolymer of 35.2 kDa was used as cation carrier for mRNA. Condensed mRNA polyplexes showed an average size of  $146 \pm 11$  nm at N/P charge ratio of 10, with a narrow distribution (PDI  $\sim 0.30$ ) and a positive zeta potential of  $10.1 \pm 0.3$  mV in HEPES buffer. Agarose gel electrophoresis exhibited good mRNA binding at N/P ratio of 2 and above. To investigate the role of CHIR in the system, swelling and degradation studies were performed comparing NPN placebo hydrogel with CHIR-loaded system. Surprisingly, after 15 days NPN placebo hydrogels were fully dissolved while drug-loaded hydrogels exhibited much longer degradation times (up to 54 days). This proves that the presence of CHIR affects the stability of the hydrogel, presumably due to its interaction with the dehydrated moieties of pNIPAM. Experimental studies revealed a sustained release of the drug over 54 days, demonstrating release kinetics mainly governed by hydrogel erosion. To highlight the influence of CHIR on hydrogel mechanical properties, rheological measurements were also performed. Placebo and CHIR-loaded gels showed temperature-sensitive behavior with a gel point below  $37^\circ\text{C}$ , proving their injectability and in situ gelation upon administration, as previously demonstrated [4]. The drug conferred to the system higher storage and loss moduli, regardless of the temperature or frequency applied. Interestingly, both NPN and CHIR-NPN hydrogels showed viscoelastic behaviour: at frequencies higher than 10 rad/sec, the systems displayed a gel-like properties, while at lower ones, a liquid-like behaviour. The final dual delivery system, composed of mRNA polyplexes and CHIR-loaded NPN hydrogel, showed the same mechanical properties of the CHIR-NPN gel and demonstrated injectable features at room temperature.

**Conclusion** PD copolymer can stably entrap mRNA. Moreover, to achieve a local and prolonged release of biotherapeutics, the NPN thermosensitive hydrogel proved to be a potent candidate for cardiac delivery thanks to its erosion-dependent releasing characteristics and injectability. For therapeutic purposes, the synergistic release of mRNA polyplexes and CHIR-NPN micelles offers a promising therapy for myocardial regeneration. The next steps in our research will focus on the use of this dual delivery system as a tool for local treatment for myocardial infarction.

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# Injectable hydrogels for the delivery of miRNA-loaded lipoplexes in cardiac regeneration

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**Introduction** Myocardial infarction leads to the gradual formation of a non-contractile fibrotic scar. Alginate-based injectable hydrogels are promising for cardiac regeneration and currently investigated in clinical trials<sup>1</sup>. However, alginate presents poor degradability and no cell adhesion *in vivo*. Herein, degradable alginate dialdehyde (ADA) hydrogels were prepared, able to encapsulate and release therapeutic miRNAs-loaded lipoplexes triggering transdifferentiation of cardiac fibroblasts into cardiomyocytes.

**Experimental methods** ADA was synthesized by oxidation of sodium alginate using sodium metaperiodate and characterized by ATR-FTIR and <sup>13</sup>C MAS NMR analyses. Novel miRNA-loaded lipoplexes (6.7-13.4 µg/mL) were physically encapsulated in 2-4% w/v ADA hydrogels formed by calcium ions crosslinking and rheological properties were analyzed<sup>2</sup>. Cy5-labelled lipoplexes release was monitored under a fluorescence microscope and miRNA release was studied by Qubit fluorimetric assays.

**Results and discussion** Under optimal conditions, ADA was prepared with an oxidation degree of 23% at an average yield of 68%. ATR-FTIR and <sup>13</sup>C MAS NMR analyses confirmed the successful formation of aldehyde groups. Injectable hydrogels with different viscoelastic properties were obtained by using different amounts of calcium ions. Lipoplex distribution and progressive release was followed under a fluorescence microscope. Release studies showed a prolonged miRNA release over time, reaching 100% release after 9 days.

**Conclusion** ADA injectable hydrogels releasing miRNAs-loaded lipoplexes were designed for myocardial regeneration. In the future ADA will be functionalized with bioactive molecules able to improve transdifferentiation of cardiac fibroblasts into cardiomyocytes.

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**Acknowledgments** This project is supported by ERC BIORECAR- EU H2020 GA N° 772168.



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# Production and characterisation of a bioartificial and functionalised cardiac patch prototype

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**Introduction** The main purpose of cardiac tissue engineering is to develop new biomaterials able to induce myocardium regeneration after infarction. A micro-structured PLGA/gelatine patch showed to have suitable physico-chemical and mechanical properties as well as to promote adhesion and an early myocardial commitment of human mesenchymal stem cells (Patent WO2014108814A1). In this study, the first steps towards a technological transfer of the cardiac patch have been taken.

**Experimental methods** The effects of sterilisation on the PDMS moulds (ThunderNIL srl) were evaluated. Maintenance of the micropatterning was assessed by optical microscopy and SEM. The effect on mechanical properties was studied using tensile tests. Rheological studies were performed to optimise the preparation of the bioartificial blend. The patches obtained at several production steps were characterised by means of mechanical tests, SEM, HPLC, GPC, FT-IR Chemical Imaging and mass loss. Drug release from patches was quantified by HPLC. Biological tests were performed using H9C2 cardiomyoblasts.

**Results and discussion** Sterilisation of the moulds was effective in removing bacteria without damaging the geometry. Production of the matrix was optimised, with a reduction of production time and a better reproducibility of the matrices. Mechanical anisotropy, typical of cardiac ECM, was confirmed in the prototype samples. Biological studies proved the cytocompatibility of the patches and their capability to favour cell adhesion and elongation. The kinetics of patch degradation products was evaluated. The micropatterning improved drug release due to an increased surface area in contact with the medium.

**Conclusion** This study represents an important starting point of a cardiac patch translational path that will require further scientific studies and fruitful interaction with biomedical companies.

**Acknowledgments** This work was supported by PoC Instrument-CSP 2020 and by transnational EU project INCIPIT (4158) M-ERA.NET 2 call 2016, MIUR FIRST.



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**Introduction** Sutures are the most utilized implants in surgical and clinical practice, they play an important role in wound management and tendons and ligaments repair. However, suture materials are often affected by mechanical mismatch, excessive fibrosis, and inflammation.

**Experimental methods** In this work, a mandrel-less electrodeposition method, able to fabricate continuous microfiber wires, with controlled microarchitecture and mechanics, was introduced. Poly(ester urethane) urea (PEUU) microfiber wire morphology and mechanical properties have been characterized by scanning electron microscopy and uniaxial tensile test respectively. The macrophage response to PEUU degradation products and scaffold macro and microarchitecture was evaluated *in vitro* by immunoblotting and immunolabeling. The host response to microfiber wires was tested *in vivo*: twenty rats, randomized in 5 groups, received a 2cm infra-scapular incision and the skin was closed using PEUU microfiber and cast wires. Common suture materials, including polyglycolic acid, polydioxanone, and polypropylene, were used as controls. After one month, mechanical and histological evaluation of explants and suture wires was performed.

**Results and discussion** *In vitro* results have shown an anti-inflammatory macrophage response associated with PEUU microfiber scaffold and wires. *In vivo*, PEUU microfiber wires showed better mechanical performance compared to the other groups, a favorable collagen remodeling comparable to the healthy group, and a mild host response reaction.

**Conclusion** These results suggest that microfiber wires reduce macrophage pro-inflammatory response and improve collagen deposition, which makes it an ideal candidate for soft tissue suture applications.

**Acknowledgments** I would like to acknowledge the other authors of this research work: Joseph G. Bartolacci, Marco G. Traina, William R. Wagner, Antonio D'Amore



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# SPERMIDINE AS NEUROPROTECTIVE AGENT AND CROSS-LINKER FOR GELLAN GUM NANOFIBERS IN THE TREATMENT OF NERVOUS TISSUE INJURIES

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**Introduction** Nervous tissue injuries affect more than one billion people worldwide and dramatically impact on the patient's quality of life. Aim of the work was to develop a novel neural scaffold to be implanted at the injury site: nanofibers, consisting of gellan gum (GG), an anionic polysaccharide, and spermidine (SP), a bioamine, were produced by electrospinning. SP was selected for its neuroprotective activity: it preserves neurons from oxidative damage and modulates the over-expression of pro-inflammatory cytokines at the injury site. SP cationic nature makes it an ideal GG cross-linking agent.

**Experimental methods** Six GG/SP mixtures (GSP1-GSP7), containing 1.5% w/w GG and increasing concentrations (0-0.125% w/w) of SP were prepared in distilled water. Mixture rheological properties and compression resistance were measured. A rheological study was performed to identify the GG/SP mixtures which were iso-viscous with 1.5% w/w GG solution. Such GG/SP mixtures were blended with two grades of poly(ethylene oxide) (PEOs) and poloxamer (P407) and, then, electrospun. Fiber morphology and mechanical resistance to deformation were investigated.

**Results and discussion** More and more structured GG/SP mixtures were obtained by increasing SP concentration, to demonstration of its cross-linking potential. GSP3 (containing 0.05% w/w SP) diluted 3:1 w/w showed a shear stress profile similar to that of 1.5% w/w GG solution and was blended with PEOs and P407 (S1). The electrospinning of S1 allowed to obtain homogenous nanofibers (NFs), which, however, freely dissolved in water: therefore, GG concentration was increased, fixing GG:SP ratio, PEOs and P407 amounts (S2). S2 NFs were homogenous and, after hydration, formed a thin gel layer. S2 NFs underwent a plastic deformation without structure break, suggesting a good mechanical resistance.

**Conclusion** S2 NFs represents a potential neural scaffold to be applied at the injury site: S2 NFs should support and guide axonal outgrowth, releasing SP in a controlled manner. Further studies are ongoing to assess S2 NFs capability to be colonized by Schwann cells.



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# Electroconductive and injectable hydrogels based on gelatin and PEDOT:PSS for nervous tissue regeneration

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## Introduction

Hydrogels are hydrated networks able to mimic the natural Extra Cellular Matrix. Nervous tissue is an excitable system, with poor self-healing ability. Recently, electroconductive hydrogels emerged to support recovery of the degenerated nervous tissues, by enhancing cell growth and formation of functional neuronal networks.

## Experimental methods

Hydrogels were fabricated by using i) gelatin, ii) genipin, a crosslinking agent and iii) PEDOT/PSS, an electroconductive polymer. Gelation time and mechanical properties were investigated by rheology. Electroconductive properties of resulting hydrogels were assessed by Electrochemical Impedance Spectroscopy.

Furthermore, swelling and degradation were considered. Hydrogels biocompatibility was investigated by using different cell types, including primary rat astrocytes.

## Results and discussion

Genipin was able to promote gelation of the resulting networks.

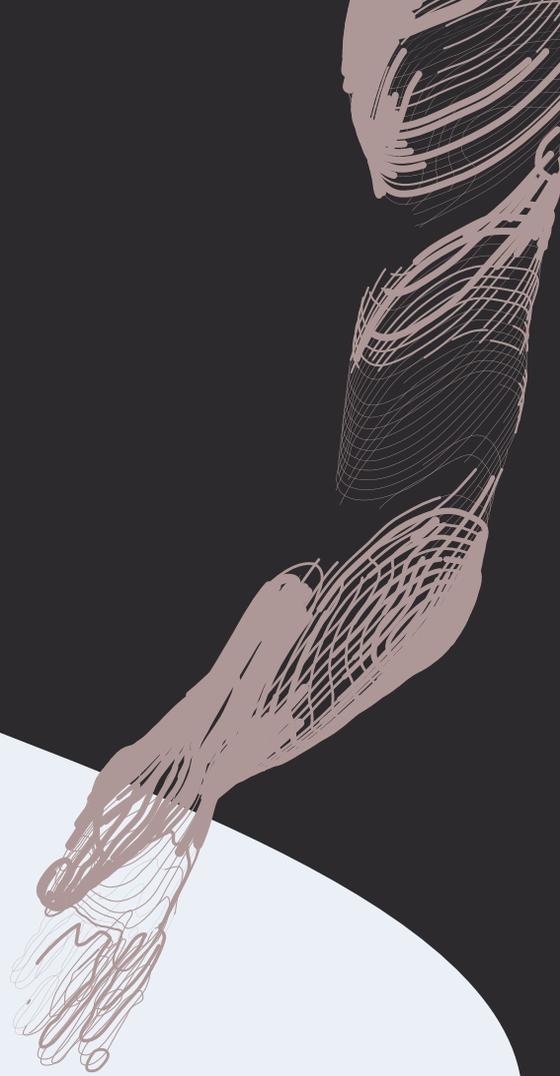
Gelation time in the presence of PEDOT/PSS was reduced down to 30 minutes. Hydrogels fabricated in the presence of PEDOT/PSS displayed an increase of the shear and elastic modulus but resulted to be less resistant to deformation. This behavior was attributed to the ability of conductive polymer to partially prevent networks bending and stretching. All hydrogels displayed an excellent stability and biocompatibility. Additionally, the presence of PEDOT/PSS enhanced the electroconductivity of resulting hydrogels.

## Conclusion

The presence and the amount of PEDOT/PSS resulted to finely tune gelation time, mechanical performance and electroconductive properties of resulting hydrogels. These hydrogels can be proposed as interfaces with human tissues and as injectable



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# BIOACTIVE SURFACE TREATMENTS



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# Development and characterization of doped TiO<sub>2</sub> coatings for orthopedic implants

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**Introduction** Periprosthetic Joint Infections (PJIs) represent one of the most serious and frequent complications related to prosthetic devices [1]. A key moment during the pathogenesis of PJIs is the ability of microbial populations to produce "biofilm" [2]. Sol-gel process is being increasingly used to modify surface because it allows to produce homogeneous materials with the desirable properties [3]. In this work, sol-gel method is employed to realize titanium dioxide (TiO<sub>2</sub>) coatings doped with copper ions (Cu<sup>2+</sup>) and zinc ions (Zn<sup>2+</sup>) both capable of exerting antibacterial properties [4].

**Experimental methods** Coatings are obtained in three steps: (i) synthesis of TiO<sub>2</sub> sol added of antibacterials salts of copper and zinc; (ii) deposition of coating by dipping the substrates (small discs of CoCr alloy) in the sol dispersion; (iii) thermal treatment (120 °C). Several physical-chemical techniques have been carried out to characterize the samples: XRD; SEM-EDS; GD-OES; ICP-OES and static contact angle measurements. Antibacterial activity of treated samples have been evaluated towards proliferations of *Staphylococcus Aureus*.

**Results and discussion** XRD investigation reveals anatase crystalline phase of TiO<sub>2</sub>. SEM images have shown uniform coatings well distributed over the entire surfaces of substrates. In addition, thickness of coating near 100 nm has been estimated through GD-OES measures. Both EDS and ICP-OES analyses have ensure the incorporation of Cu<sup>2+</sup> and Zn<sup>2+</sup> within the coating. Moreover, treated samples exhibit hydrophobic behavior respect to the untreated. Then, preliminary antimicrobial tests show a very promising bactericidal effect of TiO<sub>2</sub> doped coatings, that are able of eliminating more than 85% of bacterial strains.

**Conclusion** The sol-gel strategy here performed could represent a simple route for surface treatments of orthopedic implants. Undoubtedly, further assessments will be required to understand also cytocompatibility of obtained coatings.

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# Innovative ZrO<sub>2</sub>-Ag nanometric layer on titanium alloy

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**Introduction** Orthopedic implants are mainly divided into permanent and temporary devices. The last class include materials such as plates and screw<sup>1</sup> externally or internally implanted to fix small fractures. When the bone healing is obtained, the device is removed by following a secondary surgery. The main issues associated with these implants are bacterial colonization and bone tissue overgrowth, which can increase hospitalization time and cost and also health risks for the patient. Differently from permanent implants, the surfaces of temporary fixation devices are still poorly investigated to improve their performances. The aim of the present study is the biological characterization of ZrO<sub>2</sub> based coatings doped with Ag nanoclusters to evaluate their suitability for temporary fixation devices.

**Experimental methods** Ti6Al4V was used as substrate as the principal material for the production of temporary fixation devices. ZrO<sub>2</sub> coatings doped with different amounts (low and high) of Ag nanoclusters were obtained by co-sputtering of ZrO<sub>2</sub> and Ag in Ar atmosphere. Antiadhesive and antibacterial properties have been tested against a multi-drug resistant Staphylococcus aureus strain by applying the ISO 22196 standard. Cytocompatibility was evaluated in direct contact with Bone-marrow derived mesenchymal stem cells. The strength of adhesion of the cells to the material has been estimated by using (i) static conditions, (ii) dynamic conditions and (iii) fluidic conditions. Further analysis on proteomics and transcriptomic have been implemented to better understand the adhesion mechanism.

**Results and discussion** Homogeneous and well adhered coatings were obtained for both Ag contents. Bacterial adhesion test showed an increase in bacterial concentration in the supernatant, suggesting materials' antiadhesive properties. Considering the adherent bacteria, the Ag doped materials showed an antibacterial activity in comparison to the untreated ones, suggesting a promising application for the reduction of bacterial contamination on temporary fixation devices. In vitro cytocompatibility showed a significative reduction in cells' metabolic activity for the Ag-doped specimens. ZrO<sub>2</sub> coatings with a low amount of Ag nanocluster evidenced a biocompatible behavior.



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Proteomic analysis confirmed difference in cytoskeletal proteins expression between base material and ZrO<sub>2</sub> based coatings with low amount of Ag nanoclusters.

**Conclusion** The present research demonstrate that ZrO<sub>2</sub> based coatings with low amount of Ag nanoclusters are promising surfaces for temporary fixation devices.

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**Acknowledgments** Easy-Fix project, MANUNET call 2017 is acknowledged for founding (European Commission Through Local Agency Finpiemonte).



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# Development of an antibacterial borate-based Plasma Electrolytic Oxidation coating on titanium for bone-contact applications

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**Introduction** Plasma Electrolytic Oxidation (PEO) has recently raised great interest as a surface modification technique to produce porous and biocompatible oxide coatings on light metals, while also allowing the incorporation of specific ions or particles [1]. However, conventional Ca-P PEO electrolytes typically have a low power efficiency or require strict thermal control to form optimal coatings. In our work, we focused on the development of an alternative PEO treatment to produce tuned coatings embedding copper and zinc as antibacterial and pro-osteogenic agents for bone-contact applications.

**Experimental methods** Grade 2 cp-Ti samples were treated in a novel alkaline electrolyte [2], in pulsed DC conditions, by applying various duty cycles (10 ÷ 90%) and treatment frequencies (50 ÷ 1000 Hz), up to 300V, for 7 minutes. Pore diameters and porosity of samples were evaluated over SEM micrographs, while their chemical-physical features were studied by XRD, GDOES, and wettability testing.

**Results and discussion** The electrolyte showed high thermal stability between 10 and 65°C. The treated samples' average pore size, porosity, and coating thickness increased with the duty cycle and decreased with the frequency, and highly homogeneous, 4 µm thick coatings were produced at frequencies lower than 200 Hz and 50% or higher duties. All the films presented a bilayer structure, with a compact inner layer (related to borate presence) and a highly porous outer layer, mainly due to silicate; moreover, the GDOES spectra indicated the presence of Cu and Zn in the outermost coating region. The XRD analysis showed that, while anatase is predominant for duty cycles lower than 50%, the rutile content grows with higher duties. Furthermore, all the coated samples were almost superhydrophilic, showing a contact angle dramatically lower (3°) than that of cp-Ti (89°).

**Conclusion** The alternative PEO treatment and process parameters here reported allowed the production of compact and homogeneous coatings on titanium, showing promising morphological and physical-chemical properties for bone-contact applications.

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# Development of Nanostructured Antibacterial Coatings to Control Bacterial Growth on Hospital Surfaces

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**Introduction** Recently worldwide, a major challenge is represented by the prevention of Health Care Associated Infections (HCAI). The risk of infections in health facilities is an extremely important issue also considering current epidemiological emergency. Pathogenic microorganisms can persist for a long time in hospital setting, contaminating items and equipment in patients' rooms and causing serious infections and epidemic outbreaks<sup>1</sup>. To fight the infectious risk for patients and healthcare workers, the main strategies adopted include collective protection measures such as disinfectant procedures on High Touch Surfaces (HTS)<sup>2</sup> and personal protection equipment (PPE) such as gloves, gowns and masks. However, nowadays there is an increased interest in the identification of new approaches to control surface microbial contamination using nanostructured Antibacterial Coatings (AC)<sup>3</sup>. Several active compounds and new technologies are presented on the market, but only few studies demonstrated their performance in hospital setting<sup>4</sup>. On this basis, the aim of this study was i) to develop and characterize a new nanostructured antibacterial coatings to apply on high touch surfaces and ii) to evaluate its efficacy in vitro studies using bacterial strains well-characterized responsible for HCAI.

**Experimental methods** Nanostructured antibacterial coating was obtained by dissolving polycaprolactone granules in suitable solvent and subsequently adding in the polymer solutions chlorhexidine-based nanoparticles as active agent. This dispersion was used to coat polypropylene (PP) substrates. Surface properties have been investigated by contact angle measurements. As concerns the in vitro assays, a technical standard (ISO 22196: 2011) and a new protocol were performed. Known concentrations of two reference bacterial strains (*Escherichia coli* ATCC® 8739 and *Staphylococcus aureus* ATCC® 6538P) were deposited on AC samples and controls.

Antibacterial activity was assessed in relation to the contact time (t0-t1-t2-t24) needed to obtain a reduction (R) in the number of colonies in conditions of temperature and humidity similar to indoor environments. Antibacterial efficacy has been attributed to AC samples capable of determining a reduction in the number of bacterial colonies  $\geq 2 \text{ Log CFU/cm}^2$  within a contact time of 24 hours.



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**Results and discussion** Contact angle measurements show an improvement of hydrophobicity of the developed AMCs materials. Both methods show that AC samples have antibacterial activity against *E. coli*, but the R value obtained with the technical standard ( $R=5,78 \text{ Log UFC/cm}^2$ ) is higher than that detected with our method ( $R=2,30 \text{ Log UFC/cm}^2$ ). As for the effectiveness against *S. aureus*, also in this case the data obtained with the two methods are discordant because the value of reduction was 2 Log UFC/cm<sup>2</sup> only when the protocol ISO 22196:2011 was applied. They exhibit antibacterial activity against *E. coli*, but the R value obtained with the technical standard ( $R = 5.78 \text{ Log CFU / cm}^2$ ) is higher than that detected with new method ( $R = 2.30 \text{ Log CFU / cm}^2$ ). These differences are mainly due to the different incubation conditions of the two methods as the microclimatic parameters ( $UR>90\%$ ;  $T= 35\pm 1^\circ\text{C}$ ) in ISO 22196:2011 are very different from those commonly found in indoor environments. This would explain why some coatings found to be active in vitro may be less effective when tested in hospital setting.

**Conclusion** Results show that the developed AC samples are effective in reducing bacterial growth, even in common indoor environment conditions. Furthermore, in vitro results show that a rapid bactericidal action is a very important aspect for the correct identification of the AC to be used being as a collective protection measure from infectious agents carried by objects or hands of patients and healthcare personnel. The new method proposed in this study provides information on the real antibacterial efficacy in vitro of new materials under temperature and relative humidity conditions comparable to those in the target environments.

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**Acknowledgments** The project NANOBIO SAN, funded by INAIL BRIC ID49, is kindly acknowledged.



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# Tissue on-chip for modeling in vitro the cancer microenvironment

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**Introduction** Cancer is the leading cause of death around the world. The lack of prognosis, the genetic complexity and the tumor heterogeneity make the discovery of new therapeutic options extremely difficult. For this reason, the establishment of a scalable in vitro model able to recapitulate the tumoral microenvironment is urgently needed (1). Here, we developed two microfluidic in vitro models of the acino-ductal unit and the alveolar barrier which serve as powerful tools to deeper understand the early evolution steps of pancreatic ductal adenocarcinoma (PDAC) and lung cancer.

**Experimental methods** The microfluidic devices were fabricated by replica molding using poly-dimethylsiloxane (PDMS) (2). For PDAC, the microfluidic device was designed to contain an upper layer and a lower layer divided by an electrospun polycaprolactone/gelatin (PCL/GL) membrane. The bottom layer recreates the stroma by collagen gel loaded with the pancreatic stellate cells (PSCs) while the top layer was designed to incorporate PDAC cells. The chip mimicking the alveolar barrier was composed by two layers separated by an extra cellular matrix (ECM) like electrospun membrane of PCL/GL serving as substrate for the coculture of epithelial and endothelial alveolar cells.

**Results and discussion** A biomimetic lung-on-a-chip device was obtained to better mimic the architecture of the basement membrane of the alveolar wall and a PDAC-on-a-chip was designed allowing to better study the influence of stroma on the tumor's evolution.

**Conclusion** These microfluidic devices reproduce in vitro the tumor microenvironmental interactions that occur between adjacent cell types in vivo.

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**Acknowledgments** This work is partially supported by Deflect Project (Regione Piemonte).



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# Surface Treatments in Chitosan Coating of Titanium and Alumina Substrates for Biomedical Application

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**Introduction** Surface modification plays an important role in improving service life and performance of components, enabling features such as antibacterial effect. In this framework, implant coating is a way to place a barrier between substrate and body environment, effectively tuning contact stiffness. Herein, different medical implant substrates have been coated with chitosan, analyzing mechanical properties and adhesion.

**Experimental methods** Substrates were made of Ti6Al4V and  $\alpha$ -Al<sub>2</sub>O<sub>3</sub>. Coating was deposited by dip-coating, leaving samples drying at room temperature. Mechanical characterization has been performed through nanoindentation and scratch tests.

**Results and discussion** Ambient drying was found necessary as faster drying procedure would imply, under conditions of poor chemical affinity between coating and substrate, a detachment of the coating. Concerning nanoindentation and scratch, a good repeatability has been found in terms of mechanical properties of coating and scratch failure, although its reduced thickness. For both types of substrates, several failure zones of interest have been recognized: (i)plowing; (ii)cracking; (iii)substrate exposure; (iv)substrate damage. During tip penetration the coating gradually gets thinner and penetration depth stabilizes at the onset of substrate damage. Ti6Al4V substrate performed better, in terms of cohesive failure, which may be related to local variations in coating properties and thickness and adhesive failure, likely due to different surface topography of substrates. Indeed, it has been confirmed that irregularities induced on the substrate surface provide mechanical interlocking effect between coating and substrate, improving its performance.

**Conclusion** Concluding, it can be stated that: (i)the preparation of chitosan coating requires attention to avoid delamination phenomena due to thermal gradients; (ii)surface roughness and topography become important elements in case of poor chemical affinity; (iii)failure mechanism substantially remains the same for both investigated cases. Further research will lead to improve performance of biomedical devices varying coating thickness.



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**BIOMATERIALS WITH  
ANTIBACTERIAL PROPERTIES**



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# Antibacterial injectable bionanocomposite based on Hyaluronic acid containing green synthesized silver nanoparticles for bone regeneration

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**Introduction** In bone tissue engineering, biomimetic scaffolds comprising calcium phosphate bioceramics, e.g. beta-tricalcium phosphate (beta-TCP), are aimed to serve as an artificial temporary extracellular matrix (ECM) in order to support cell adhesion and guide new bone tissue formation. The combination of bioceramics with organic materials, such as hydrogels containing hyaluronic acid (HA), naturally occurring polysaccharide that serves as a major component of extracellular matrix in mammalian connective tissues, is highly advantageous to overcome the brittleness of their intrinsic nature. Among the different types of hydrogels, thermosensitive hydrogels based on amphiphilic copolymers, such as Pluronics (polyethylene oxide-polypropylene oxide copolymers, PPO-PEO-PPO), thanks to their ability to undergo thermal gelation as well as low cytotoxicity, have attracted significant attention in biomedical applications and pharmaceutical industries [1,2]. However, infections during or post scaffold transplantation are still challenging which reduce the efficacy of bone healing. After the transplantation, infections may also be distributed to the scaffold from other sources of inflammation through bloodstream. Silver nanoparticles have shown a strong capability to inhibit or decline infections and have been also utilized for bone regeneration applications. Among different methods, biosynthesis of silver NPs, by using plant extracts, has received considerable attention due to the growing need to develop environmentally and non-toxic technologies [3].

On considering the properties of beta-TCP, HA, Pluronic, and Ag NPs, our work was aimed to fabricate thermosensitive hydrogel biocomposites for bone tissue repair, which can be injected easily and possess antimicrobial properties to prevent infection.

**Experimental methods** Ag NPs were synthesized within corn silk extract (CSE) by microwave assisted method. The hydrogels were prepared by dissolving different amounts of HA, beta-TCP, Pluronics F127 and F68 in CSE with and without AgNPs. The hydrogels composition was optimised by rheological analysis.

**Results and discussion** The results of DLS analysis of silver NPs show a monodispersed



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population with a narrow size distribution and a mean diameter of about 49 nm. TEM experiments show well-dispersed silver nanoparticles which spherical shape and an average size of about 27 nm. Rheological experiments demonstrated that the thermosensitive hydrogels have good mechanical properties with gelification temperature (T<sub>gel</sub>) close to body temperature. The systems showed antibacterial activity toward gram-positive (*Bacillus Subtilis*, *Staphylococcus Aureus*) and gram-negative (*Pseudomonas Aeruginosa*, *Escherichia Coli*) bacteria. L929 cells exhibited a noncytotoxic and typical mouse fibroblast-like cellular morphology after 24 h of the incubation with the samples. Thermosensitive hydrogels, which possess good retention at the application site, have appealed a great deal in biomedical and clinical fields. At room temperatures, the systems are liquid and could be injectable whereas, at the body temperature, they became a gel. This fast sol-to-gel phase transition behavior is beneficial for cell entrapment to give a uniform distribution of cells within the gelled matrix. HA offers many advantages as a tissue scaffold which including biodegradability, biocompatibility, and bioresorbability which have been shown in many studies. Hyaluronic acid could activate cell surface receptors, influencing intracellular signaling cascades for cell growth, migration, proliferation, and differentiation [4]. One of the main reasons of scaffold failures is due to the implant-associated bacterial infections. Hence, using antibacterial scaffolds helps more success for bone tissue formation which are of clinical importance. In this study, we used spherical particles of Ag to impart antibacterial activity to the thermosensitive hydrogels to avoid bacterial contamination [5].

**Conclusion** In conclusion, silver NPs were biosynthesized in an aqueous medium of corn silk extract without using toxic chemical reagents. The new thermosensitive HA-based nanocomposite hydrogels demonstrated good mechanical properties with T<sub>gel</sub> close to the body temperature. The system revealed desired antibacterial activity against several gram-positive and gram-negative bacterial strains which can prevents bacterial infection. In addition, from the biological point of view, the nanocomposites revealed appropriate biocompatibility in comparison with the control samples. In addition, from the biological point of view, the nanocomposites revealed appropriate biocompatibility in comparison with the control samples.

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# In-situ synthesis of silver nanoparticles and production of Ag-hyaluronic acid based nanocomposite bioink with potential antibacterial activity

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**Introduction** The increasing popularity of research of AgNPs in the last decades is closely associated to their potential applications for several purposes such as anticancer agents, electronic devices, water treatment and broad-spectrum activity against Gram-positive and Gram-negative bacteria, fungi, protozoa and viruses [1]. Antibiotic loaded hydrogels composed of natural polysaccharides, proteins and synthetic polymers have been used to have sustained drug release minimizing the cytotoxic effects of the systemic antibiotic therapies [2]. In this study we proposed a new diethylenetriamine derivative of hyaluronic acid (HA-DETA) as a capping agent to synthesize silver nanoparticles and further formation of a Schiff base hydrogel by reaction with oxidized hyaluronic acid, for potential antibacterial activity, with a 3D extrusion printer.

**Experimental methods** Diethylenetriamine derivative of hyaluronic acid (HA-DETA) was synthesized, characterized and used as a capping agent for silver nanoparticles obtained by UV-photo reduction. Hydrogels were obtained via Schiff base linkages between HA-DETA and HA-Ald (i.e. dialdehyde derivative of HA obtained by periodate oxidation). <sup>1</sup>H-NMR and IR spectroscopy confirmed the functionalization. Cytocompatibility of both HA-DETA and HA-Ald derivatives was confirmed with MTS and Live/Dead assays. UV spectroscopy and TSEM analysis confirmed the presence of silver nanoparticles.

**Results and discussion** HA-DETA/HA-Ald Schiff base hydrogels were produced in order to obtain scaffolds sensitive to acidic conditions.

Four hydrogels with different molar ratio CHO/NH<sub>2</sub> were obtained and characterized in terms of swelling, degradation, gelation time, cross-linking efficiency, rheological and self-healing properties. Rheological properties showed a rapid recovery for all hydrogels with different molar ratio and an increase in the storage modulus is evident as the molar ratio increases. Macroscopic tests showed an increment of the self-healing capacity with a decrease of the molar ratio.

3D printing tests were performed to obtain different surface/volume ratios to control AgNPs release.



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**Conclusion** Here we propose a dynamic system hydrogel to take advantage of the pH sensitive linkage to increase the AgNPs release in the presence of a bacterial acid environment. 3D printing is used to further modulate the release. Studies of silver release, antifouling and antibacterial activity are in progress.

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# Photothermal antimicrobial nanocomposite hydrogel based on amine functionalized Gellan Gum and polydopamine

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**Introduction** The topical delivery of antimicrobial drugs to the infected wound aims to enhance the local drug concentration and limits their systemic exposure. Unfortunately this approach cannot overcome the antibiotic resistance phenomenon and there is an urgent clinical need to develop new therapeutic approaches to treat infections of multi-resistant bacteria strains [1,2]. Photothermal therapy exploits the localized raise in temperature, given by the conversion of near infrared (NIR) irradiation into heat, to exert a direct non-specific bactericidal effect and to potentiate the effect of the antibiotic by increasing the permeability of the microbial wall or membrane [3]. Here we produced a nanocomposite hydrogel based on amine functionalized gellan gum (GG-EDA) [4] crosslinked with 4-arm polyethylene glycol in the presence of polydopamine (PDA) nanoparticles and ciprofloxacin to obtain an advanced antimicrobial wound dressing with on demand activatable photothermal effect.

**Experimental methods** Nanocomposite hydrogel was produced by mixing amine functionalized gellan gum (GG-EDA) [4] dispersion with a dispersion of the crosslinker 4-arm polyethylene glycol vinylsulfone terminated in the presence of polydopamine (PDA) nanoparticles and ciprofloxacin.

The viscoelastic properties were studied through rheological analyzes while the photothermal effect was studied by irradiating the samples with 810 nm laser and measuring the temperature with a photothermal camera.

Drug release experiments were conducted both irradiating or not the samples and the bactericidal effect was studied in vitro effect against staphylococcus aureus and pseudomonas aeruginosa.

**Results and discussion** The crosslinking process occurs spontaneously and quickly in aqueous environment without activating agents thanks to the chemical versatility of GG-EDA. PDA nanoparticles confer marked photothermal and photostability characteristics and contribute to potentiate the hydrogel physicochemical properties. By irradiating the PDA containing samples it was possible to reach temperatures from 30 to 55°C.



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PDA containing hydrogels can be irradiated several times without losing their photothermal effect.

Beside to trigger a localized heating process, NIR irradiation at 810 nm influences the release of the loaded antibiotic bursting its release and potentiating its activity toward both staphylococcus aureus and pseudomonas aeruginosa.

## Conclusion

The chemical versatility and the physicochemical properties of GG-EDA allowed to obtain, through a facile and reproducible crosslink process, hydrogels with with superior viscoelastic features.

The hydrogel photostability allowed to perform several consecutive cycles of irradiation with unchanged photothermal effect.

A synergistic effect of ciprofloxacin release and photothermal therapy caused a better bactericidal effect against both Gram-positive and Gram-negative bacteria.

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# Assessment of antifouling and cytotoxicity properties of superhydrophobic Ag-Cu-Zr metallic glass coating

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**Introduction** Healthcare-associated infections (HCAI), also referred to as nosocomial infections, are major cause of health-care complications leading to prolonged hospitalization, long-term disabilities, and unnecessary deaths (1). HCAI prevalence in high-income countries is estimated to be 7.5% (2). The fiber-based textile materials are widely used in health-care sector (e.g. surgical gowns, nurse aprons, bedding, mask) and are often contaminated by pathogenic microorganisms. Therefore, it is crucial to develop medical textile surfaces that inhibit the adhesion and as a consequence the proliferation and spread of pathogenic germs to minimize the spread of multidrug-resistant germs in hospital and high-risk environments.

Aim: in this study, antifouling and cytocompatibility properties of three metallic glasses co-sputtered with Ag, Cu and Zr were investigated.

**Experimental methods** We have developed a Ag-Cu-Zr metallic glass coating with antifouling properties on Polybutylene terephthalate (PBT) textile material. Then, their physical-chemical characterizations were assessed using SEM-EDS instrument and static contact angle measurement. Since the presence of contact-killing antibacterial agents such as Cu and Ag inside our Zr-based metallic glass system, in the next step the generated coating is investigated for its antifouling properties using *Staphylococcus aureus*, and its cytocompatibility is evaluated by Human bone marrow-derived mesenchymal stem cells (hBMSC). Finally, antibacterial and cytocompatibility properties of ions released were performed through indirect experiments.

**Results and discussion** According to the physical-chemical results, water-contact angle of each sputtered sample ranging from 130° to more than 150°, thus demonstrating a superhydrophobic behavior and EDS analysis was confirmed the successful co-sputtering process. Direct experiments showed that sputtered samples declined considerably



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attachment of *S. aureus* and also no cytotoxicity effects were detected. ICP result revealed after 5 days, the maximum ion released was Cu at concentration 120 ppb but indirect experiments' results revealed this concentration was significantly lower than antibacterial ones. However, after 5 days, metabolic activity of hBMSC reduced slightly that was for cells confluent beyond 90% and lack nutrients after 5 days. The May-Grumwald-Giemsa histology demonstrated that ions were internalized by cells that demonstrated to be viable by the live and dead assay.

**Conclusion** In conclusion, metallic glasses have superior wear resistance compared to traditional metallic systems and therefore, creation of changes in their surface properties is extremely optimistic for reduction of HCAI and emerge of drug resistance microorganisms.

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# Apatitic bone cements with effective drug release: from antibacterial and antibiofilm ability to anticancer therapy

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**Introduction** Self-hardening pastes and cements based on calcium phosphates are widely used biomaterials for several clinical applications, including spinal fusion, vertebroplasty and periodontal surgery. Their preparation involves the mixing of solid powder and liquid components, which then self-harden in vivo and transform into nanostructured, nanocrystalline apatitic phases. However, the occurrence of bone infections, i.e. osteomyelitis or bone tuberculosis, still represents a major responsible of therapy failure in orthopaedics. If bone infections occur, the clinical protocols usually include the long-term oral administration of antibiotics, which however is characterized by limited bioavailability of the drug at the healing site and, often, provoking the occurrence of systemic adverse side effects. A further drawback is represented by the bacterial resistance to antibiotics, which is widely considered as a major threat in medicine and particularly in orthopaedics in the incoming years. In this scenario, the development of biomaterials with regenerative ability and effective antibacterial and antibiofilm properties is a goal of ever-increasing relevance. Key aspects are the ability of biomaterials to prevent bacterial adhesion and proliferation, thanks to bioactive composition and surface texture, as well as the ability to act as carrier able to release drugs in situ in a sustained and therapeutically effective manner.

**Experimental methods** The present work describes a novel approach to prepare osteointegrative Sr-doped apatitic bone cements featuring nanostructured architecture and capable of controlled release of tetracycline (TC), intended as broad-spectrum antibiotic, but also chemically close to anthracyclines (e.g. doxorubicin (DOX)), another wide group of drugs commonly used as first-line chemotherapeutic treatment of osteosarcoma and other solid tumors as breast cancer. Firstly, the synthesis of nanocrystalline biomimetic apatites was performed by wet chemistry, followed by surface adsorption of TC. Then, drug-loaded bone cements were obtained by mixing proper amounts of TC-loaded nanoparticles (TC-NPs) or DOX-loaded nanoparticles (DOX-NPs) to Sr-doped  $\alpha$ -tricalcium phosphate powders and aqueous solutions enriched with sodium alginate. The injectability, setting times and drug release profile of these final pastes were optimized according to the clinical need. In



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order to achieve an antitumoral effect, specific concentrations of DOX should be delivered. Clinical pharmacokinetic profiles of the drug administered systemically and locally were used to identify the correct concentration to be delivered in order to optimise the therapeutic effect and avoid toxicity. Chemotherapeutic concentrations were set at 0.6-1.2 µg/ml with a delivery duration within three to four days.

**Results and discussion** An extensive physico-chemical characterization of the cements was performed. The presence of TC-NPs or DOX-NPs allowed to modulate the drug release profile in physiological conditions, if compared with the formulation without NPs. The antibacterial and antibiofilm effectiveness of native and TC-loaded cements was evaluated by microbiological tests assessing the viability of *Staphylococcus aureus* and *Escherichia coli*, among the most diffuse bacterial strains in nosocomial infections, proving substantial bacteriostatic and bactericidal properties in the native cement and complete eradication of bacterial cultures in the TC-loaded device. The biological performance of the DOX-NPs loaded cements on triple negative breast cancer (TNBC) cell line MDA-MB-231 was also assessed by MTT assay, showing a significant decrease in the TNBC cell line proliferation rate when compared to unloaded control.

**Conclusion** This work proved the ability of apatitic cements to contrast bacterial infections as well as provide anticancer therapy, by modulating the drug release profiles with loaded nanoparticles. These results are relevant in the view of design and development of new devices for bone regeneration, more effective to improve the patient safety and clinical outcomes, to contrast the bacterial resistance to antibiotics and to favour the local treatment of cancer.

**Acknowledgments** This work was supported by the Italian Ministry of Health (Bando Ricerca Finalizzata 2016, no. GR-2016-02364704)



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# DISEASES MODELING AND HEALING



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# A cytokine-induced *in vitro* model of osteoarthritis pathogenesis

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**Introduction** Osteoarthritis (OA) is the main chronic form of joint disease, whose long-term surgical treatments are still unsuccessful, mainly due to the lack of awareness on its pathogenesis<sup>1</sup>. In this work, we designed and manufactured an Articular Cartilage *in vitro* spheroid-based model in Healthy (H) and OA conditions, emulated by combining a cocktail of pro-inflammatory cytokines, aiming to study the disease evolution at cellular level.

**Experimental methods** Spheroids of human articular chondrocytes (HC) and mesenchymal cells differentiated in chondrocytes (Y201-Cs) were cultured in three conditions: H (DMEM/F12), Low concentration OA (LC-OA) (DMEM/F12 with: interleukin (IL)-1 $\beta$ :1ng/mL, tumor necrosis factor (TNF)- $\alpha$ :1ng/mL, IL-6:10ng/mL) and High Concentration OA (HC-OA) (DMEM/F12 with: IL-1 $\beta$ :5ng/mL, TNF- $\alpha$ :5ng/mL, IL-6:50ng/mL). Spheroid's growth kinetics and metabolic activity were evaluated over 10days. Then, spheroids were assembled and cultured up to day21 on a gelatin-coated PLGA membranes (10 spheroids/cm<sup>2</sup>), using a protocol in line with the clinically approved Chondrosphere® (CO.DON AG) technique<sup>4</sup>. Expression of anabolic (SOX9,COL2A1,ACAN) and catabolic (MMP13 and ADAMTS-5) genes, histology (H&E, AlcianBlue, PicrosiriusRed) and immunohistochemistry (IHC) (Collagen II and Aggrecan) were performed.

**Results and discussion** HC and Y201-C spheroids showed a diameter decrease over culture in H compared to OA conditions, whereas it maintained a stable dimension. Cell metabolic activity decreased over culture for HC and increased for Y201-Cs in all conditions, but both cells showed a higher metabolic activity in HC-OA condition at day 10, possibly due to the biosynthesis of OA-related inflammatory and degradative enzymes<sup>3</sup>. At day 21, in OA conditions, both cells showed a significant decreased expression of anabolic markers and an upregulation of catabolic markers, as well as a significant lower amount of collagen (PicrosiriusRed) and glycosaminoglycans (AlcianBlue). IHC confirmed this tendency.

**Conclusion** Our approach allowed the obtainment of reliable *in vitro* models of OA, where even LC-OA environment was sufficient to favouring cellular catabolism over the anabolism.

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# Functional in vitro engineered osteochondral tissue for modeling osteoarthritis

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**Introduction** Osteoarthritis (OA) is a joint degenerative pathology characterised by a complex interplay of inflammatory processes involving synovium, articular cartilage (AC) degradation, and subchondral bone (SB) remodeling (1,2). With the increase of life expectancy, OA ranks the fourth among the cause of global disability and needs significant national spending (3). The OA pathophysiology is complicated to be understood due to a not recognised early phase of the disease, and the lack of reliable and consistent in vivo and in vitro models able to reproduce the entire pathology.

This work aims to develop an engineered in vitro model of osteochondral human tissues reproducing the main features of OA as a tool for investigating the disease physiology, biology, and progression.

**Experimental methods** For the SB layer, a polylactic acid (PLA) porous trabecular-like construct was 3D printed via Fused Deposition Modelling (Rokit INVIVO Bioprinter) and functionalised with gelatin (Gel) and hydroxyapatite (nHA). For the AC deep zone, a photocurable Gellan Gum (GG 3%w/v) multi-channels hydrogel structure was obtained via soft-lithography, adherent to the PLA scaffold. Chondrocytes (differentiated from Y2O1 bone marrow mesenchymal stromal cells) (4) were encapsulated in each channel of the GG layer within a chondroitin sulfate-based hydrogel, while Y2O1 cells were seeded on the PLA scaffold. The OA milieu was induced by the addition of IL-6, IL-1 $\beta$ , and TNF- $\alpha$ . Cell behavior was assessed, for both phases of the construct, via Live/Dead, and analysis of metabolic activity and cytoskeleton. qRT-PCR, Alcian blue, and Alizarin Red were used to assess cell chondrogenic potential and the effect of cytokines.

**Results and discussion** GG hydrogel showed high water uptake capability and porosity (pore range size of 100-150  $\mu$ m 30%) and suitable AC mechanical properties (48 $\pm$ 5 kPa as compressive Young's modulus). Cytokines decreased the mechanical properties at day 14, altered the expression of chondrogenic markers (COL2A1, ACAN, SOX9), the



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mucopolysaccharide production ( $0.22\pm 0.02\mu\text{g}$  in OA vs  $0.36\pm 0.01\mu\text{g}$  in Healthy at day 21), increased OA-related molecule expression (COLL1, MMP9-13, ADAMTS5) and Alizarin Red values ( $680\pm 20\mu\text{M}$  in OA vs  $550\pm 10\mu\text{M}$  in Healthy model).

**Conclusion** A high reproducible 3D in vitro OA model, characterised by quickly manufacturing and easy manipulation was developed. This construct could be applied to test new treatments and to study cell crosstalk.

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**Acknowledgments** Giorgia Cerqueni acknowledges support from UNIVPM for her period abroad within her Ph.D. project; Annachiara Scalzone acknowledges support from EPSRC for her Ph.D. studentship (EPSRC Grant EP/R51309X/1).



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# Investigating protein adsorption on surfaces for biomedical applications: a comparative study on bioactive materials

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**Introduction** Adsorption of proteins on biomaterials and biomedical devices is a major field of interest. Proteins are thought to cover medical device surfaces almost immediately after they are in contact with body fluids and form the interphase for cellular interactions. Understanding how surface properties affect the adsorbed proteins will provide guidelines for novel and better biomaterial design. This work aims to study how proteins interact with titanium based materials designed for bone-bonding and to define a set of techniques that can be applied to characterize protein-surface interaction on different materials.

**Experimental methods** Ti and Ti6Al4V alloys with three different chemical treatments for osteointegration were soaked in a solution of bovine albumin (20 mg/ml) at physiological pH 7.4. Surfaces were characterized by confocal microscopy, zeta potential titration, XPS, Kelvin probe force microscopy (KPFM), ATR-FTIR, and contact angle measurements with different liquids. The adsorbed proteins were quantified by BCA assay and analyzed by using conventional (XPS, fluorescence microscopy, FTIR) and novel (zeta potential titration and KPFM) techniques to determine their conformation and the layer structure.

**Results and discussion** The investigated surfaces differ for chemistry (density and chemical reactivity of the surface OH groups) and thickness of the surface oxide layer, surface zeta potential, wettability, and roughness. BSA is adsorbed and forms continuous layer on all surfaces. Comparing the results of different techniques, BSA shows different interaction mechanisms with the different surfaces: its conformation and chemistry upon adsorption may vary on different substrates.

**Conclusion** Protein adsorption is deeply affected by surface properties, such as surface charge, surface energy, and surface functional groups. They dictated amount and conformation of the adsorbed proteins and the interactions with the substrates, which may vary even on materials with similar chemical composition. Our results suggest a protocol of characterization useful to obtain information on the adsorption process and protein structure on bulk materials that are intended and designed for biomedical applications.



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# MULTIFUNCTIONAL BIOINSPIRED MELANIN-CeO<sub>2</sub> NANOSTRUCTURES FOR WOUND HEALING APPLICATIONS

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**Introduction** Nanosized CeO<sub>2</sub> holds huge promise for the design of cutting-edge devices for skin regeneration due to its dual surface oxidation state (Ce<sup>3+</sup>/Ce<sup>4+</sup>) that makes it a red-ox active material. Similar properties are shown by melanin, a class of hydrophobic natural pigments. In this study, CeO<sub>2</sub> and melanin are combined at the molecular scale into a core-shell architecture. Antioxidant and antimicrobial assays were carried out to prove the efficacy of the proposed strategy in boosting intrinsic biocide and antioxidant properties of organic and inorganic components.

**Experimental methods** CeO<sub>2</sub> nanoparticles were produced following a one-step hydrothermal synthesis. Melanin coatings were produced by dropwise addition of DHICA-in-ethanol solutions to a CeO<sub>2</sub> water suspension. Physico-chemical characterization was carried out through FTIR, UV-vis, EPR. The antioxidant properties of all the samples were tested through DPPH assay. Finally, the antimicrobial power of the nanosystems was assessed towards Escherichia Coli and Stafilococcus Aureus strains.

**Results and discussion** FTIR confirmed the adsorption of DHICA onto nanoceria was achieved thanks to a Ce(IV)-catechol complex in the form of a bidentate bridge. Melanin formation was assessed by EPR and UV-vis. Melanin-coated nanosystems exhibited DPPH radical scavenging activity higher than bare CeO<sub>2</sub> thanks to melanin poly hydroxy indole planar structures with extended resonance. Finally, melanin-coated CeO<sub>2</sub> nanosystems experienced a remarkable boost in the biocide power against Gram (-) strains with respect to bare nanoceria.

**Conclusion** Melanin coating onto CeO<sub>2</sub> nanoparticles was successfully obtained via Ligand-to-metal charge transfer mechanism. Obtained multifunctional nanomaterials hold huge promise in regenerative medicine.



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# Photocrosslinked hyaluronic acid hydrogels for adipose tissue in vitro model.

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**Introduction** Hyaluronic acid (HA) is a linear polysaccharide, mainly found in extracellular matrix (ECM) of connective tissues, giving them a mechanical support [1]. HA could be directly modified by methacrylation reaction (HAMA) [2] [3] [4]. Methacryloyl groups allowed covalent bonds formation via photocrosslinking leading to HAMA hydrogels formation, widely investigated in 3D modelling or bioprinting [1]. Anyway, optimizing all the involved photocrosslinking parameters remains still challenging. Furthermore, although UV-rays led to an efficient photocrosslinking process, cell encapsulation in HA-based hydrogels remains limited, due to high time exposure [1]. For this purpose, a novel photocrosslinking approach was investigated in this work. Since visible-light photocrosslinking has been already reported for GelMA [5], giving promising results, complex photoinitiator (i.e., ruthenium, Ru) and photocrosslinker (i.e., sodium persulfate, SPS) were investigated in this work for adipose tissue (AT) mimicking.

**Experimental methods** HA methacrylation was performed by following a protocol described elsewhere [2]. Briefly, high molecular weight ( $1.0 \times 10^6$  Da) HA was dissolved in distilled water (2 % w/v). Methacrylic anhydride was then added keeping the solution at 4 °C. Overall, pH was carefully monitored and eventually adjusted in the range 7.5-8.5 by NaOH addition. Solution was dialyzed and subsequently lyophilized. Degree of functionalization (DoF) was assessed by <sup>1</sup>H NMR spectroscopy. Photocrosslinked hydrogels were obtained by dissolving HAMA in DPBS (1 % w/v) and then pouring the solution in PDMS moulds for light source exposure (20 min). Briefly, different Ru/SPS ratios (0.05/0.5, 0.1/1, 0.2/2 mM/mM) were selected for visible light exposure, and Irgacure 2959 (0.05 % v/v) for UV-rays crosslinking, as control. Hydrogels were characterized for their physical (i.e., weight variation, gel fraction), mechanical (i.e., compressive tests) and biological (i.e., in vitro tests using 3T3-L1 preadipocytes) properties.

**Results and discussion** Degree of Functionalization was equal to 52.5 % by <sup>1</sup>H NMR spectroscopy. HAMA hydrogels appeared morphologically well-defined, with different colour



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(FIG. 1.A) depending on the photocrosslinking method (UV rays or visible light). Although hydrogels with three different Ru/SPS concentrations were obtained, the lowest concentration (0.05/0.5 mM/mM) was selected for further investigation. Weight variations trend was similar comparing UV and visible light hydrogels (FIG. 1.B), reaching absorption plateau at 24 h, and complete degradation for both HAMA formulations after 14 days. Gel fraction parameter revealed a higher photocrosslinking efficiency for UV hydrogels ( $p < 0.05$ , FIG. 1.C), which is overall reflected in mechanical properties (FIG.1.D). Higher stiffness and maximum stress were exhibited by UV hydrogels ( $p < 0.05$ ), while residual deformation resulted lower in visible light samples ( $p < 0.05$ ). In vitro indirect cytotoxicity was performed to evaluate potential toxic effects of the investigated samples (FIG. 2.A). Cell viability resulted higher than 70 % threshold for both HAMA hydrogel formulations, after 3T3-L1 preadipocytes culture in eluates obtained at different time points (i.e., day 1, 3, 7). Encapsulated 3T3-L1 preadipocytes (cell density =  $1.0 \times 10^6$  cells  $\text{mL}^{-1}$ ) maintained a rounded morphology after 1 day of culture (FIG. 2.B).

**Conclusion** HA methacrylation resulted an optimal functionalization approach to enhance HA ability in forming stable hydrogels. Moreover, visible light photocrosslinking revealed a challenging alternative to most common photocrosslinking approaches (i.e., UV-rays), which often involved high time exposure that could damage cell viability [1][4].

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# Nanogel-mediated hydroxytyrosol release as a therapeutic strategy for nonalcoholic fatty liver disease

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**Introduction** Nonalcoholic fatty liver disease (NAFLD) represents the most common chronic liver disease, still demanding for effective therapies. NAFLD is a multifactorial disease, which may progress to cirrhosis and hepatocellular carcinoma, due to the enhanced fat infiltration as triglycerides inside hepatocytes (1,2). Hydroxytyrosol (HT), a naturally occurring polyphenol peculiar of olives, has been shown to contrast the development of hepatic steatosis through its lipid-lowering, antioxidant and anti-inflammatory activity. However, the efficient delivery of HT to the target site remains a crucial aspect, which could be addressed through smart nanoencapsulation techniques. Here, we propose the design of formulated nanogels (NGs) as nanocarriers to enhance the intracellular HT uptake.

**Experimental methods** NGs were synthesized through interfacial chemical crosslinking between imidazole-activated polyethylene glycol (PEG) and linear polyethyleneimine (PEI), in a o/w emulsion and solvent evaporation technique (3). Rhodamine was grafted to PEI via click chemistry (copper-catalyzed azide-alkyne cycloaddition) to ensure the permanent traceability of NGs in in vitro study. The formulated nanoscaffolds are characterized by a hydrodynamic diameter around 250 nm and showed high biocompatibility, stability in aqueous environment, and an optimized swelling behavior, which can be exploited to encapsulate bioactive molecules. Indeed, the transition from dry to swollen state, preserving the NG architecture, was used to entrap HT within the polymeric matrix, without any chemical modification of the drug that could affect its therapeutic potential. Two different encapsulation conditions, namely 0.1 mg\*mL<sup>-1</sup> (NG0.1) and 0.5 mg\*mL<sup>-1</sup> (NG0.5) were tested in an in vitro model of hepatic steatosis (induced by an overload of free fatty acids, FFAs) by high-content analysis tools (4).



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**Results and discussion** NGs showed different drug release profiles, depending on the chosen concentration: NG0.5 presented a sustained release until 24 h, whereas in NG0.1 the drug release was almost completed after 7 h. In vitro results showed a significant decrease in the intracellular triglyceride accumulation only when HT was delivered by the nanovectors, especially at the higher concentration (NG0.5). This lipid lowering effect was paralleled by an effective restoration of cell viability levels to those of healthy controls, without any toxic side-effect, outperforming HT administration in its non-encapsulated form.

**Conclusion** The formulated nanogels represent a promising strategy for delivering HT, thereby protecting liver cells from NAFLD manifestations, outperforming the administration of the non-encapsulated phytopharmaceutical.

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**AcKnowledgments** This work is supported by Regione Puglia (CUP B84I18000540002) and FISR MIUR-CNR (CUP B83B17000010001).



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# Hyaluronic acid thermosensitive hydrogels containing green corn silk extract antibacterial nanosilver for wound healing application

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**Introduction** Hyaluronic Acid (HA) based hydrogels have attracted research attention for wound healing applications since hydrogel dressings are an excellent source for providing moisture to a dry lesion, monitoring fluid exchange from within the wound surface, helping to cool down a wound, as well as provide temporary pain relief [1]. HA a naturally occurring glycosaminoglycan, presents in mammalian connective tissues, promotes dermal regeneration [2]. Thermosensitive amphiphilic block copolymers, polyethylene oxide-polypropylene oxide copolymers (Ploxamers or Pluronics, PPO-PEO-PPO), thanks to their ability to undergo thermal gelation, to their good tolerability, and low irritancy/toxicity, have been used in the biomedical field [3]. Infection is a crucial and generally unsolved issue in wound healing. Therefore, materials containing antimicrobial compounds, such as Ag nanoparticles (Ag NPs) have shown the capability to inhibit or decline infections. Corn silk extract (CSE), a waste material of the crop, has been used for AgNPs biosynthesis as both a reducing and stabilizing/capping agent [4]. In this work we propose novel and green thermosensitive nanocomposites hydrogels based on HA, Pluronics and AgNPs for wound healing applications.

**Experimental methods** The corn silk were heat extracted. AgNPs were synthesized within CSE by microwave. The hydrogels were prepared by dissolving different amounts of Pluronics CSE with and without AgNPs. Subsequently HA was added. The hydrogels composition was optimised by rheological analysis. The formation of AgNPs was confirmed by UV-Vis surface plasmon band while their morphology was evaluated by DLS. Bacterial cell suspensions were prepared, for each tested Gram-positive and Gram-negative, and analysed by optical density measurements at a wavelength of 620 nm. In vitro biocompatibility was performed by Alamar Blue assay on L929 fibroblast cells culture. The wound healing potential of the realized formulations was assessed by wound healing scratch assay on Human Dermal Fibroblast (HDF) cells after 24 and 48.

**Results and discussion** Separate Pluronic F127 and F68 solutions do not show an appropriate Tgel but by formulating Pluronic F127/F68 blends at specific concentrations, it



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was possible to obtain a medium with a Tgel close to Tb. The addition of HA slightly affects Tgel, but very interestingly, improved significantly the final gel viscoelastic properties. AgNPs were spherical with average diameter of 8 nm and the presence of AgNPs did not alter the hydrogels rheological properties. The AgNPs hydrogels showed an excellent bactericidal activity against the tested Gram-positive and Gram-negative bacterial strains. It has been demonstrated the biocompatibility of the hydrogel on L929 cells. The in vitro wound healing assay of the realized thermosensitive HA CSE AgNPs hydrogels was performed on HDF cells; the results demonstrated that the wound surface area decreases with the increasing exposure times (24 to 48h) of the tested hydrogels compared to the controls.

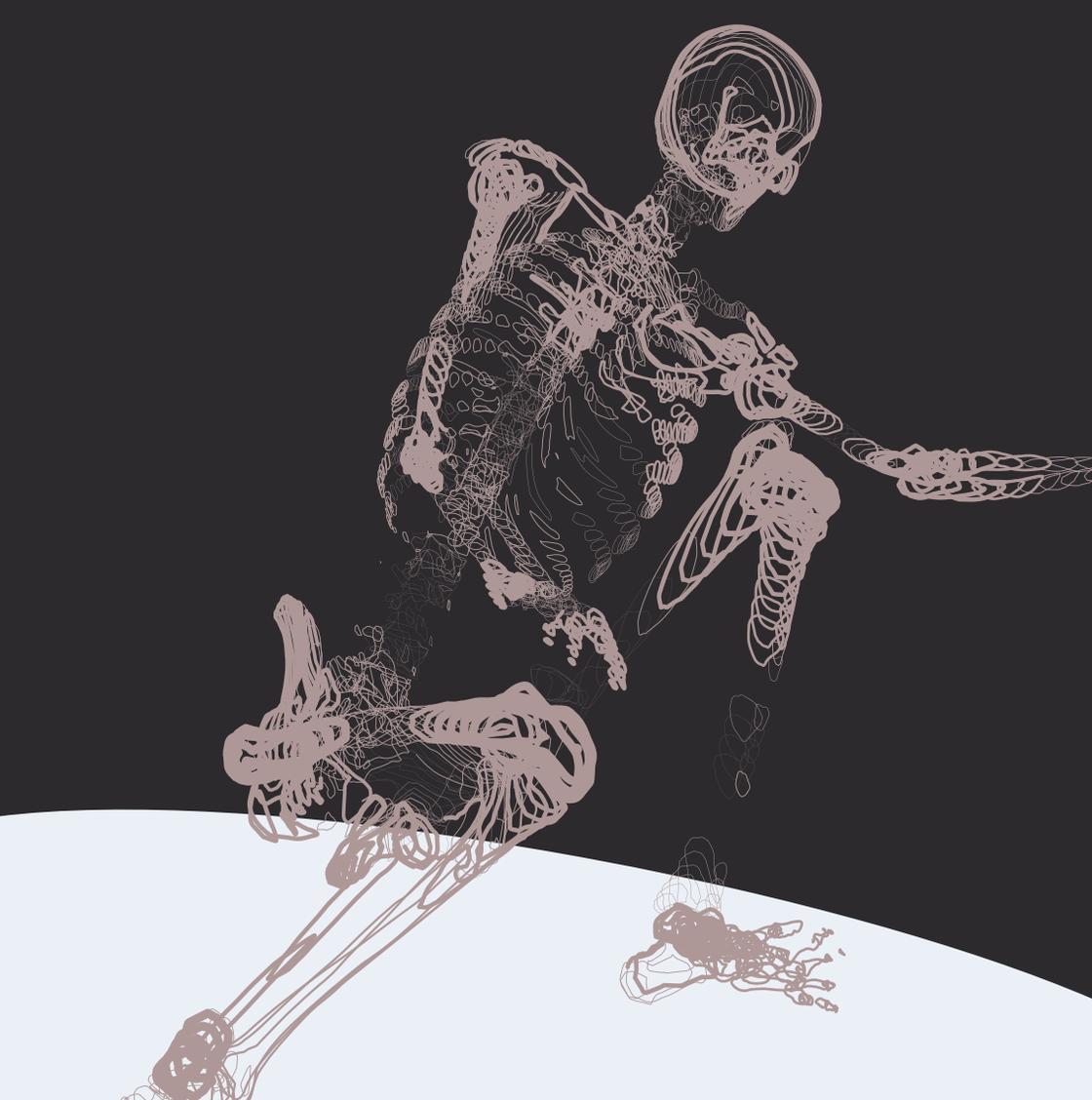
**Conclusion** We developed a Novel and green thermosensitive injectable hydrogels based on HA, CSE and Ag NPs. In-vitro model of wound healing revealed that the nanocomposites allow faster wound closure compared to the control. The obtained results highlight the potential application of these novel injectable hydrogels as wound dressing.

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# FIBROUS MATERIALS



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# A versatile platform for Biomaterials characterization : a tool to assess mechanical properties

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**Introduction** Biomaterials come in a variety of different shapes and with a variety of purposes and requirements. Mechanical properties such as Modulus, damping capabilities, friction coefficient and others are as well important as bio-compatibility.

A versatile platform that can assess several of these properties under various testing conditions is essential in order to characterize such materials.

Here we present a review of two case studies related to different materials and purposes: CS-PEG hydrogels for regenerative medicine and Proteoglycan as a lubricating medium for contact lenses.

**Experimental methods** Chondroitin Sulfate - Polyethylene Glycol (CS-PEG) are a family of hydrogels recently developed at Johns Hopkins School of Medicine for self-healing purposes. CS-PEG (with optional addition of Hyaluronic Acid) is intended to be used as an adhesive tissue regenerating integrator, especially for cartilage. Therefore its adhesive properties and the mechanical modulus and damping properties must be tested.

The ESG3200 platform by TA Instruments is used as a high force DMA (up to 225 N) to assess the material under several production conditions: pH and Relative Humidity during synthesis, different amount of HA added that changes the CS:PEG ratio.

The samples are tested under compression with a variable strain up to 10%. Triangular waves are used to study the visco-elastic properties ( $E'$ ,  $E''$ ,  $\tan\delta$ ) of the different specimens, while regular tack tests are used to assess the adhesive strength.

The same instrument, with a torsion configuration and a custom-made saline bath is used to measure the lubricating properties of solutions at different concentrations of Proteoglycan 4 (PRG 4) with and without HA.

The lens is put in contact with a resected cornea and the torsional motor is used to apply different rotational speeds (from 0.3 to 30 mm/s) under a constant 0.2 N load in order to measure the resistance offered to rotation, thus the friction coefficient.



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**Results and discussion** CS-PEG shows a clear dependence on preparation conditions: decreasing pH increases the damping capabilities (ie.  $\tan\delta$ ) as well as Relative Humidity. A significantly greater adhesivity with respect to competitive materials such as Fibrin is observed.

The amount of HA shows relatively little effect over Storage Modulus values, but a more evident one on  $\tan\delta$  (thus on  $E''$ ).

PRG 4 shows a good lubricant effect especially when combined with HA. Regular commercially available solutions contain HA only, but their lubricating effect is quite similar to plain saline solution. The addition of PRG 4 has a synergistic effect.

**Conclusion** Different biomaterials require different characterization setups, depending on the final purpose and the actual properties we want to assess. When it comes to mechanical properties, whether they are visco-elastic properties, or adhesive strength or friction coefficient, a multi-purpose, versatile mechanical platform is essential for accurate measurements.

In this work we have shown just a few examples of what the high force DMA model ESG3200 from TA Instruments is capable of.

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# Electrospun polyurethane scaffolds loaded with Cerium oxide and Chondroitin sulphate for tendon tissue engineering

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**Introduction** Tendon pathologies are medical conditions that include ruptures and overuse injuries, accompanied by inflammatory and degenerative alterations, such as tendinopathies. The aim of this work was the design and development of tubular scaffolds, by means of electrospinning technique, based on thermoplastic polyurethane (TPU) and combined with chondroitin sulphate (CS) and cerium oxide nanoparticles (CeO<sub>2</sub>).

**Experimental methods** TPU was solubilized in water/acetic acid at 12% w/w, in a second moment CS (1% w/w) and CeO<sub>2</sub> (1 mg/ml) were added, alone and combined, and the blends were electrospun using an electrospinning apparatus equipped with a rotating drum to obtain scaffolds with a tubular shape. The systems morphology was assessed using SEM and TEM analysis, and the wettability of the scaffolds was measured. The scaffolds mechanical properties were thereafter evaluated.

**Results and discussion** The morphological analysis of the systems showed that TPU 12% w/w was the optimal concentration to obtain a continuous jet and uniform fibers. The presence of CS and CeO<sub>2</sub> did not affect the electrospinning process and the obtained scaffolds were insoluble in water. All systems were characterized by nanometric dimensions, moreover the fibers collected in tubular shape presented an aligned structure, mimicking the collagen nanofibrils typical of native tendon tissue. The scaffolds had hydrophobic surface and the mechanical properties of the scaffolds confirmed that the presence of CeO<sub>2</sub> increased the force at break (F<sub>max</sub>) and the Young modulus of the systems. Moreover, the scaffolds reached F<sub>max</sub> values of 13 MPa, which is comparable to that of the native tendon (12 MPa).

**Conclusion** In conclusion, electrospinning was successfully used to prepare scaffolds based on TPU in association with CS and CeO<sub>2</sub>. The scaffolds possessed mechanical properties comparable to that of the tendon, representing a promising structure to stand the mechanical loads during the tissue regeneration. Further analyses are ongoing to test the cell adhesion and proliferation onto the scaffolds and their ability to produce ECM.



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# Maltodextrin-amino acids scaffold in wound healing reparation

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**Introduction** In chronic wounds, as venous leg ulcers, arterial ulcers, diabetic ulcers, and pressure ulcers, the healing process does not lead to anatomical and functional recovery<sup>1</sup>. The aim of this study was the design and the development of nanofibrous scaffolds, based on maltodextrin and amino/polyamino acids, crosslinked via Maillard reaction, as dermal substitute for skin reparation and regeneration.

**Experimental methods** Maltodextrin (47% w/w aqueous solution) was associated with arginine (5.8 %w/w)/lysine (11.8 %w/w)/polylysine (2.5 %w/w) and electrospun using an electrospinning apparatus. A thermal treatment was applied to obtain insoluble scaffolds in aqueous environment, taking the advantage of amino acids-polysaccharide conjugates formed via Maillard reaction. The nanofiber morphology was assessed by means of SEM, and the mechanical properties of scaffolds were measured using a TA-XT plus Texture Analyzer. The biocompatibility and TNF- $\alpha$  were assayed on monocyte-derived macrophages. Finally, the scaffolds were subjected to in vivo characterization on a murin model, and the histology of biopsies were performed.

**Results and discussion** The morphological analysis showed that the scaffolds had cylindrical and uniform nanofibers with smooth surfaces independently of amino acids/polyamino acids type, crosslinking and hydration and that the crosslinking by heating did not significantly change the nanofibers' dimensions. Furthermore, the scaffolds retained the nanofibrous structure after hydration, showing a swelling of the individual fibers. The tensile test showed that scaffolds were resistant to break and highly elastic with a maximum deformation lower than 14.5%. The scaffold cytocompatibility shows a full biocompatibility and, when looking at cytokine secretion, scaffolds did not show any significant pro-inflammatory activity. The in vivo experiments show the healing of skin injury: after 18 days of treatment with each formulation, fast wound closure with completely regenerated epithelium was assessable and collagen fibers were remodelled in orderly pattern.

**Conclusion** The electrospinning was successfully used to obtain nanofibrous scaffolds based on maltodextrin in association with amino acids/polyamino acids. Nanofibers showed



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regular shape and smooth surface and the resistance of scaffolds toward solubilization in aqueous fluids seems attributable to Maillard reaction occurring at crosslinking heat treatment. The scaffolds showed distinctive properties and optimal features for promoting cell proliferation leading tissue reparation towards a complete skin restore.

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**AcKnowledgments** This work was funded by Horizon 2020 (Grant Agreement No 81460).



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# Sustainable fibrous 18 $\beta$ -glycyrrhetic acid/poly(lactic acid) templates for oral pathologies treatments

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**Introduction** In the field of regenerative medicine, combined strategies, aimed at involving supports ('scaffolds') for cell adhesion, proliferation and growth, cells and bioactive factors (e.g., drugs, growth factors, antibiotics, anti-inflammatory, antimicrobial ...) are attracting a lot of interest [1]. In the present work poly(lactic acid) (PLA) fibrous mats, loaded with 18 $\beta$ -Glycyrrhetic Acid (18 $\beta$ ), were successfully fabricated by electrospinning technique. PLA was selected as polymeric matrix due to its renewable resources derived nature, biocompatibility, transparency, stiffness and thermoformability [2]. 18 $\beta$ -Glycyrrhetic Acid was selected being a natural derivate from liquorice supplied with outstanding features, such as antiviral, anti-inflammatory, antihepatotoxic, antiestrogenic, antileukemogenic, antineoplastic properties [3].

**Experimental methods** PLA based fibrous membranes, loaded with different concentration (1.0-10 %) of 18 $\beta$ -Glycyrrhetic Acid, were fabricated by electrospinning technique. 18 $\beta$ /PLA solutions were prepared in a CHCl<sub>3</sub>:DMF solvent mixture (67:33, in volume ratio) [4], poured in a glass syringe, and electrospun in air at room temperature. The microstructure, thermal and mechanical properties were investigated by scanning electron microscopy (SEM), differential scanning calorimetry (DSC) and uniaxial tensile tests. Preliminary cytotoxicity tests were carried out, using as cell line PE/CA-PJ15 (carcinoma of the human oral cavity) and as a reference the 3T3-Swiss cells (murine fibroblasts). The cell growth was also monitored by means of ECIS® (Electric Cell-substrate Impedance Sensing) measurements, a real-time, label-free method, based on the monitoring of the electrical impedance presented by the electrodes on which the cells are seeded and subjected to stimuli of different nature (physical, chemical, biological).

**Results and discussion** Uniform and homogeneous fibrous mats were successfully produced. They resulted composed of randomly oriented and defect-free submicrometric fibers (average fiber diameter between (591 $\pm$ 123) and (660 $\pm$ 104) nm). The 18  $\beta$  loading caused a shift of the glass transition temperature toward lower values, associated to an increment of the polymer crystallinity degree, suggesting its plasticizing action. Concerning



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the release kinetics, in the case of PBS, a release up to 20% was recorded, whereas in the case of ethanol, almost complete release (95%) was detected in the first hours of incubation. MTT results evidenced no cytotoxicity in all conditions and they were confirmed by the ECIS data.

**Conclusion** The obtained 18 $\beta$ /PLA fibers could be potentially used for several biomedical applications, and have been thought for the treatment of the squamous carcinoma of the oral cavity (CSO), a malignant tumor that originates from the lining epithelium of the oral cavity and can affect all sites of the oral cavity, i.e. lips, tongue, gums and floor of the mouth. Indeed, they could properly deliver the encapsulated natural corticosteroid agent to the action site, and could be proposed as a promising alternative to the traditional topical approaches. These latter ones, based on the use of drugs/pastes/ointments, are often ineffective, due to poor compound retention in contact with saliva, consequent dilution and further swallowing, and to a poor compliance of the patient caused by bad taste or by the viscous consistency of the same.

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**Acknowledgments** The Authors acknowledge the project POR FESR 2014–2020 ‘Gruppi di Ricerca’, titled ‘Sistemi Innovativi sensorizzati mediante Manifattura Additiva per la Cranioplastica” (SISMAC, A0375-2020-36603), financed by Lazio Region.



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# Hyaluronan hydrogels for medical purposes: new insights in the correlation between biopolymer chemical modification parameters and hydrogel biophysical features and their implications in view of application

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**Introduction** Hyaluronan (HA) hydrogels via crosslinking with 1,4-Butanediol diglycidyl ether (BDDE) are widely used for medical purposes, especially as dermal fillers, for skin rejuvenation. Following crosslinking, HA turns into a water-insoluble polymeric network that swells in aqueous medium, and exhibits elastic/shear thinning behavior and improved resistance to hyaluronidases. These features are responsible for in vivo gel volumizing and hydration ability, injectability and longer permanence in the tissue, compared to unmodified HA. The extent of HA crosslinking is expected to affect hydrogel biophysical properties and, in turn, clinical performance. Lately, in vitro characterization studies aiming at assessing gel biophysical properties have been strongly intensifying. However, the correlation between these properties and HA chemical modification parameters, which is crucial to design gels with specific performance, has not been studied so far. To fill this gap, clinically available HA-BDDE hydrogels were investigated here for the type and extent of HA modification, rheological properties and sensitivity to enzymatic degradation. Collected data were used to investigate the above correlation.

**Experimental methods** <sup>1</sup>H-NMR and <sup>13</sup>C-NMR analyses were performed to derive the total HA modification degree (BDDE/HA molar percentage) and to quantify the amount of HA effectively crosslinked by BDDE (HA-BDDE bridging) and the one bearing pendant (one-side anchored)-BDDE. Rheological analyses were carried out on samples as commercialized and, for the first time, on the same after increasing dilution in physiological medium. An oscillatory rheometer, equipped with a parallel plate geometry was used to record sample dynamic moduli in the linear viscoelastic range and their variation with frequency. Sensitivity to hyaluronidases was evaluated by monitoring the extent of hydrogel solubilisation in the presence of bovine testicular hyaluronidase.

**Results and discussion** <sup>1</sup>H-NMR analyses indicated that the BDDE bonded to HA varied from very low amount (2) up to around 31 (molar percentage). <sup>13</sup>C-NMR data highlighted that only less than 30 % of the BDDE incorporated into the product effectively bridges two HA disaccharides units while the majority of the crosslinker is only one-side anchored to the



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polymer. Unexpectedly, quantitative chemical data did not directly correlate with gel biophysical properties. Expected correlation between HA crosslinking degree and gel rheology was not found for the gels, as commercialized but only for the gels equilibrated in PBS. This latter finding has important implications in view of application. Further, the extent of modification was found to significantly affect sensitivity to enzymatic hydrolysis only from a certain value on.

**Conclusion** Quantitative data on HA chemical modification extent and type as well as rheological and stability parameters were obtained for four HA hydrogels, clinically available as dermal fillers. Data on the correlation between chemical modification parameters and gel biophysical properties were then provided for the first time. Overall, the findings are of interest for the design, the in vitro characterization and the prediction of performance of this type of hydrogels. Results highlighted unexpected aspects of these hydrogels, especially in respect to the prediction of in vivo performance in relation to gel rigidity, as measured in vitro.



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# ADDITIVE MANUFACTURING



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# CENTRIFUGAL SPINNING: AN ALTERNATIVE TECHNOLOGY FOR THE PRODUCTION OF POLYDIOXANONE MICROFIBERS

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**Introduction** PDO is a bioabsorbable polyester derived from p-dioxanone monomers which recently gained particular interest in the biomedical field thanks to its peculiarity such as optimum biocompatibility, modular degradation rate, and excellent mechanical properties. This study aimed to achieve polydioxanone (PDO) fibers by centrifugal spinning and avoid the use of toxic solvents typical of other spinning techniques as electrospinning.

**Experimental methods** A commercial candy floss machine was properly improved to apply the same theoretical principles of the patented Forcespinning®. To improve the capability of PDO to be spun in melt form, two different deep eutectic solvents (DESs) were employed as polymer plasticizers. For this study choline chloride/citric acid (ChCl/CA) and betaine/citric acid (Bet/CA) DESs were respectively employed. Physical mixtures were prepared by blending different polymer/DES weight ratios and maintaining the DES (ChCl/CA or Bet/CA) molar ratio equal to 1:1. The physical mixtures were then poured into the spinneret and melted at 140°C for 5 min. After complete melting, the blends were spun for 1-2 min at 700 rpm.

**Results and discussion** Except for PDO alone, all PDO/DES mixtures spun using the handmade centrifugal spinning apparatus gained fibers and the suitability of DES as a plasticizer agent was demonstrated. Scanning electron microscopy analysis (SEM) showed smooth and continuous fibers with dimensions ranging from 10 to 20 μm and the hydration did not affect the fiber structure. Microfibers were assembled in aligned and random macro structures and the mechanical behaviors were assessed. Both macro conformations exhibited excellent elastic nature and high tensile strengths (600 MPa). PDO/DES microfibers resulted biocompatible on normal human fibroblasts ensuring cellular proliferation within 18 days of culture.

**Conclusion** PDO was successfully spun by centrifugal spinning technique. Thanks to the excellent mechanical behaviors and the good biocompatibility, the resultant microfibers can be envisioned as optimal biomedical devices to treat, depending on their conformation, skin (random, cotton-like structure) or tendon (aligned, high tough woven band) lesions.



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# From human tissue to regenerative approaches: development of 3D printed composite scaffolds mimicking the TGF- beta1 features within the bone

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**Introduction** Bone remodelling is a lifelong process where the balance between bone formation and resorption is maintained by cell mutual signaling. During resorption, collagenous fibers disassembly and biomolecules stored in the extracellular matrix (ECM) as Transforming Growth Factor beta 1 (TGF-β1) are released and activated, with crucial effects on bone remodelling. (1) Considering the pivotal role of growth factors in bone biology, their controlled release is one of the crucial aspects of bone regenerative approaches. To this aim, several drug delivery systems have been developed, and among polymers poly(lactic-co-glycolic) acid (PLGA) is one of the most used (2).

The purpose of our research was to investigate morphological human bone tissue features and design a biomimetic 3D printed scaffold able to reproduce the natural bone architecture and biology.

**Experimental methods** Localization and quantification of TGF-β1 in human bone samples were examined by immunohistochemistry and ELISA assay. To develop the 3D printed scaffold mimicking the bone ECM TGF-β1 distribution, TGF-β1 was encapsulated into PLGA nanoparticles (PLGA\_TGF-β1) before being incorporated in a type I collagen matrix. 3D structures were printed using a commercial bioprinter (Bio-X) and cross-linked with genipin. The release kinetics of TGF-β1 and its localization inside the printed meshes were assessed.

**Results and discussion** Immunostaining revealed that TGF-β1 was mainly stored as dots, covering  $4.47 \pm 3.03\%$  of the total bone ECM area. A TGF-β1 concentration of about  $1.75 \times 10^{-10} \pm 6.37 \times 10^{-11}$  pg/mg of bone was detected.

Spherical PLGA\_TGF-β1 nanoparticles ( $257 \pm 7.3$  nm in diameter) showed high encapsulation efficiency ( $64.9 \pm 7.2\%$ ) and a release of about 38% of the incorporated TGF-β1 after 24 hours, reaching 97.6% on the 28th day. PLGA\_TGF-β1 nanoparticles were then



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combined with Type I Collagen and mesh-like scaffolds were printed. The TGF-  $\beta$ 1 release from the scaffolds was comparable to that from PLGA nanocarriers, confirming the correct embedding of PLGA nanoparticles inside the collagenous matrix. Finally, the histological and immunohistochemistry analyses revealed that the incorporated PLGA\_TGF- $\beta$ 1 nanocarriers covered  $3.39 \pm 1.15\%$  of the 3D printed scaffold area, showing no differences with human bone tissue.

**Conclusion** Our 3D printed scaffolds are capable to reproduce TGF- $\beta$ 1 amount and localization of bone ECM and manage the release of the growth factor, suggesting its promising use for devices in bone regeneration.

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**Acknowledgments** This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme, grant agreement No. 681798-BOOST ([www.ercprojectboost.eu](http://www.ercprojectboost.eu)) and by the Italian Minister of Education, Universities and Research (MIUR), Progetto FARE Ricerca in Italia (GRACE).



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# PEG-coated Mesoporous Silicas to Release Large Biomolecule in Acidic Environment and Their Use in 3D Printed Collagen Scaffolds

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**Introduction** During the resorption phase of bone remodelling, active osteoclasts (OCs) generate a local acidic microenvironment that, in combination with the secreted enzymes, causes the bone matrix degradation and the release of the encased growth factors (GFs) [1]. In the field of biomolecule/drug release, mesoporous silica particles (MSs) are well known due to their excellent biocompatibility and high loading capability [2] due to the presence of nanopores in the range of 2-8 nm [3]. In order to host large biomolecules such as GFs, the nanopores need to be enlarged. The present study aimed at developing smart MSs with large-pores (LP-MSs) able to upload GFs, coated with a pH-responsive polymer, poly(ethylene glycol) (PEG), to release the cargo in response to a pH decrease thus mimicking the GF release during bone resorption. The smart carrier system was then incorporated in a type I collagen solution to 3D print bone-like scaffolds.

**Experimental methods** LP-MSs were obtained combining the traditional sol-gel method with a hydrothermal treatment, assessing the influence of temperature and time over the final mesostructure. Horseradish peroxidase (HRP) was used as model protein to evaluate the ability of LP-MSs to adsorb and release large biomolecules. These carriers were then coated with a silane functionalized PEG and the release kinetics were investigated up to 24 h at different pHs. Finally, PEG-coated LP-MS particles were incorporated into a type I collagen solution to enable 3D printing of bone-like scaffolds that were enzymatically crosslinked with transglutaminase and analysed.

**Results and discussion** The synthesised LP-MSs presented micrometric dimensions and a cage-like mesoporous structure with accessible pores of diameter up to 23 nm. LP-MSs produced at 140 °C for 24 h showed the best compromise in terms of specific surface area (428 m<sup>2</sup>/g), pores size (17-23 nm) and volume (1.09 cm<sup>3</sup>/g) and hence, were selected for further experiments. HRP was successfully adsorbed into LP-MS mesopores with an adsorption efficiency of about 81.1%. PEG-coated carriers tested at acidic pH enabled a faster release compared to those observed under physiological conditions after 24 h, due to the protonation of PEG at low pH that catalyses polymer hydrolysis reaction. In the 3D printed scaffolds, PEG-coated LP-MSs were homogeneously distributed and embedded in the



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collagenous matrix.

**Conclusion** Our findings indicate that LP-MSs can host large molecular weight molecules as GFs and that PEG can be an effective pH-responsive coating. The obtained collagen-based hybrid formulation is a suitable biomaterial ink and was used to design 3D printed scaffolds able to release GFs for bone tissue engineering applications.

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**Acknowledgments** This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme, grant agreement No. 681798-BOOST ([www.ercprojectboost.eu](http://www.ercprojectboost.eu)) and by the Italian Minister of Education, Universities and Research (MIUR), Progetto FARE Ricerca in Italia (GRACE).



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# Topological, Mechanical and Biological Performances of Ti6Al4V Scaffolds for Bone Tissue Regeneration Manufactured with Reused Powders via EBM

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**Introduction** Cellularized scaffold is emerging as the preferred solution for bone tissue regeneration. The focal point of scaffolds is unit cell topology, which influences mechanical and biological performances [1]. Scaffold design must be balanced to allow nutrients and oxygen supply, metabolic waste removal, cell penetration and reduction of stress shielding to prevent mechanical failure [2].

Finite element analysis (FEA) [3] and numerical analysis by computational fluid dynamics (CFD) [4] showed that porous structures with rhombic dodecahedron (RD, midline symmetrical) or diamond (DO, diagonal symmetrical) unit cell geometry are valid alternatives for bone tissue regeneration.

Accurate control of the topology of scaffold's elementary unit cell can be efficiently obtained by additive manufacturing (AM) technology. Typically, AM processes for metal alloys consist of melting powders through an energy source in a layer by layer process, according to a CAD file. Electron beam melting (EBM) is commonly employed in patient's customized bone implants [5]. Combination of EBM technology and Ti6Al4V alloy has been investigated by Popov et al. [6] for clinical cases of patient-specific implants with reduction of operation time and patient recovery. Titanium alloys have elastic modulus in the range 50-118 GPa, closer to natural cortical bone (10-30 GPa) than any other widely investigated alloy for permanent implant. Despite the key advantages of combining EBM technology with Ti-based alloys, only a small percentage of tissue engineering research in this field achieves clinical application, due to the high cost of preclinical studies. To overcome this gap, Martinez-Marquez et al. [7] proposed a material feedstock recycling as the first step to ensure the cost-effectiveness of customized scaffolds, affecting material feedstock in EBM processes up to 31% of the cost of built parts [8]. Although, powder reuse means repeated



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preheating steps and prolonged thermal treatments in vacuum during the EBM process that can induce changes in the reused powder, in terms of chemical composition, morphology and physical properties [9].

Another engineering aspect to be improved for implant success is surface functionalization, which must fulfill scaffold-bone interface requirements. The most common functionalization treatment is coating the scaffold's surface with a layer of biocompatible material, to avoid the release of toxic ions and improve implant osteointegration [10]. Currently, the researchers' interest is focused on synthetic bioresorbable and biocompatible polymers. Among them, PCL is the most promising due to slow degradation time [11]. However, to overcome the PCL drawbacks concerning elevated hydrophobicity, poor bioactivity and low cell adhesion, PCL is usually combined with ceramic materials such as hydroxyapatite (HA), which shows excellent biocompatibility, bioactivity and osteoconductive properties, allowing enhanced bone formation and implant-site recovery [12].

The aim of the work is to fabricate suitable Ti-based alloy scaffolds for in vivo perspectives starting from industrial EBM process based on cost and waste reduction in order to overcome issues connected to the high costs of preclinical studies.

**Experimental methods** Starting from Ti6Al4V reused powder blended with Ti6Al4V virgin new powder as the raw material of the EBM process, scaffolds with DO and RD elementary unit cell geometry were produced. After production, scaffolds were submitted to topological and mechanical characterization to compare their performances with the experimental and simulated results reported in literature for identical structures (same alloy composition, production technology and elementary unit cell geometries). Afterwards, scaffolds were coated with a single layer of PCL or PCL/HA for surface functionalization and then submitted to biological tests. Human mesenchymal stem cell cultures for 24 h and 4 days were used as a fast check of the scaffold's biological response. The role of unremoved residual powder in the scaffold core on mechanical and biological behavior was also considered.

**Results and discussion** EBM technology is suitable for the production of porous structures with controlled topology and well-defined unit cell geometry showing appropriate biological performance and surface characteristics for in vivo perspectives. Low surface roughness typical of the EBM process promotes hMSC proliferation, while the partially sintered residual powder in the scaffold's core is responsible for the porosity mismatch between experimental and CAD design values.

The mechanical performance of scaffold is mainly influenced by elementary unit cell geometry. The presence of residual powder becomes evident for compression values outside the range of in vivo use of the device and tends to cancel out the differences between geometries. In general, the compression behavior of the scaffolds is quite similar to natural bone tissue and follows trends reported in literature, although strut size and the absence of structural defects inside the struts are responsible for the higher compression strength of DO against RD.

Short-term cell viability and metabolic protein quantification identifies DO as a better biological environment than RD, in agreement with literature-reported numerical



simulations. After 4 days incubation, hMSCs start to penetrate inside the scaffold, favored by adherent, continuous and protective layers of PCL and PCL/HA.

**Conclusion** From the above results it is evident that powder recycling represents a convenient industrial practice applicable to the manufacture of biomedical devices to heavily reduce preclinical costs without altering biomechanical performance.

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# 3D bioprinting of a cell-laden thermogel: an effective tool to assess drug-induced hepatotoxic response

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**Introduction** The formulation of bioinks with cytocompatible gelation mechanisms and tailored chemo-mechanical behavior represents one of the recent trends in bioprinting [1]. Here, a thermoresponsive Pluronic/alginate semi-synthetic hydrogel was developed for the fabrication of 3D hepatic constructs, with the aim to investigate liver-specific metabolic activity compared to conventional 2D cultures [2].

**Experimental methods** Pluronic/alginate cell-laden constructs were fabricated in combination with HepG2/C3A cells by pressure-assisted deposition, exploiting the multi-step gelation mechanism of the bioink. The bioprinting method was validated in terms of cell viability and metabolic activity. Hepatotoxicity testing was also performed using acetaminophen (paracetamol, APAP) as a model drug.

**Results and discussion** A novel method for bioprinting hepatic cells was developed, via a robust and reproducible manufacturing process, characterized by high-shape fidelity, mild depositing conditions and easily manageable gelation mechanism. The dissolution of the sacrificial Pluronic templating agent significantly ameliorated the diffusive properties of the printed hydrogel. High cell viability and liver-specific metabolic activity were reported for the 3D constructs. A markedly increased sensitivity to a well-known hepatotoxic drug (acetaminophen) was observed for cells in 3D constructs compared to 2D cultures.

**Conclusion** The developed 3D model may represent an innovative in vitro platform for investigating drug-induced hepatotoxicity.

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# Polymethylmetacrilate 3D printed implants: set-up of the fused deposition process and biomedical applications

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**Introduction** The advent of additive manufacturing has allowed the creation of objects of complex geometries, without waste of material, commonly revealed in the case of traditional processing techniques. Among the most widely used materials for this type of technology, polymers certainly play a predominant role, from polylactic acid (PLA), through less conventional polymers such as polymethyl methacrylate (PMMA), up to technopolymers (e.g., polyetheretherketone, polyetherimide) [1]. Particularly, PMMA lends itself to various applications in the biomedical field, especially in the cranioplasty and maxillofacial sectors for the creation of custom-made prostheses [2-4]. Therefore, in this work the main printing parameters for PMMA by fused deposition modelling (FDM) have been selected in order to obtain a good quality of the printed piece, in terms of microstructure and mechanical properties, and some case studies related to cranio-mxillofacial implants are presented.

**Experimental methods** In a preliminary step the process parameters (i.e. extrusion temperature, plate temperature, printing speed) were set up, printing discs (10-20 mm diameter, 0.5-1.0 mm thickness) by means of a fused deposition modelling (FDM) 3D printer (Creality Ender 3 Pro) using PMMA filament (HIRMA, TreedFilament). Different orientations and deposition patterns (i.e. Line pattern, Grid, Gyroid, Concentric, Octet, Triangle, Zig-Zag) were tested. The reproducibility was investigated, measuring weight, dimensions and thickness of the produced specimens. The microstructure, the thermal properties, the mechanical properties were studied by observation at optical and scanning electron microscopies, differential scanning calorimeter (DSC), and tensile, compression and bending tests, respectively.

**Results and discussion** The optimal process parameters were identified, allowing to obtain final samples with good surface quality, and negligible weight and dimension variation with respect to the designed model. From the comparison between the DSC thermograms of PMMA filament and printed disc a decrease of 10 °C of the glass transition temperature was revealed, evidencing an influence of the 3D printing process on the material thermal behaviour. The mechanical tests results pointed out a remarkable variation of the mechanical resistance in function of the texture chosen for the deposition of the material. Among the geometries proposed in the most common Slicing softwares (e.g., Cura, slic3r, idea-maker...), the Line Pattern was able to ensure higher mechanical



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properties, with elastic modulus E of 1.7 GPa and yield stress of 11 MPa.

**Conclusion** The optimal FDM parameters for printing PMMA were identified by a systematic study, considering different conditions, orientations and patterns. After having selected the optimal process parameters, some biomedical devices, such as cranial implants and maxillofacial fixation systems were successfully produced.

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**Acknowledgments** The Authors acknowledge the project POR FESR 2014–2020 ‘Gruppi di Ricerca’, titled ‘Sistemi Innovativi sensorizzati mediante Manifattura Additiva per la Cranioplastica” (SISMAC, Project No: A0375-2020-36603), financed by Lazio Region, and the project Erasmus+ Programme-Strategic Partnership, titled ‘Brain Revealed: Innovative Technologies in Neurosurgery Study (BRAIN IT)’ (Project No: 2018-1-RO01-KA203-049317), financed by the European Committee.



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# DRUG DELIVERY



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# Polysaccharide-based hydrogels for local berberine delivery: an innovative strategy for wound healing

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**Introduction** The cutting-edge innovations in dermatology entail the combination of antiinflammatory, antioxidant, antimicrobial, biocompatible and moisturizing features in composite, green systems. Nevertheless, it is uncommon to get such performances from environmentally sustainable, biodegradable materials [1]. Skin moisturization is an essential requirement for wound care materials, as well as for skin drug delivery systems, patches and beauty masks. In this respect, hydrogel-based formulations provide the right water content for skin issues management, while ensuring tunable loading and release properties [2]. In this work, it is described the development and characterization of an eco-friendly composite hydrogel, based on a mixture of polysaccharides and clays. The hydrogel has been loaded with berberine, a polyphenolic extract with antioxidant, antiinflammatory and antimicrobial properties, already used to treat several skin conditions. The physico-chemical properties of the hydrogel have been extensively studied, as well as its cytocompatibility and berberine skin permeation kinetics.

**Experimental methods** CMC-based hydrogels have been developed from our patented technology with modifications [3]. Berberine loading has been achieved through direct dissolution in water employed for hydrogel preparation or through prior intercalation in bentonite, one of the clays included in the composite. The hydrogel chemical composition and thermal properties have been assessed by FT-IR/ATR, XPS, XRD and TGA. Furthermore, hydrogels water uptake, gel fraction and berberine in vitro skin permeation have also been investigated. In vitro assessment has been performed on HaCaT and NhDF cells, evaluating viability and changes in cytoskeletal organization.

**Results and discussion** The optimization of the hydrogel composition and crosslinking procedure enabled to tune the gel stiffness, as well as its water uptake. In particular, gallium ions were used as source of ionic crosslinking of the polysaccharide-based hydrogel composite, for the first time. The obtained hydrogels, after 24 hours, displayed a water



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uptake above 100 g/g. Transdermal permeations studies, performed by Franz diffusion cell, highlighted that both berberine containing hydrogels provided a gradual berberine release, reaching a plateau after 24h. Our results showed that at 48 h cells indirectly exposed to the hydrogels, with or without berberine, modify their behavior. In HaCaT, migratory features with the presence of lamellipodia and filopodia, as well as cell-cell separation with total or partial loss of intercellular junctions were observed.

**Conclusion** The developed hydrogels, after a careful optimization of their composition, have been able to tune berberine release and water uptake, while being nontoxic for human keratinocytes. Overall, these systems represent a promising tool for future applications to treat both intact and damaged skin.

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# Inhalable colistin-loaded calcium phosphate nanoparticles for the treatment of infections related to cystic fibrosis.

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**Introduction** Cystic fibrosis (CF) is a recessive genetic disease that leads to a hypersecretion of thick mucus difficult to clear. The over-production of mucus results in the airway obstruction that makes the lungs susceptible to recurrent and persistent bacterial infections [1]. Colistin is a cyclic polypeptide widely used for its antibacterial properties having a wide spectrum of action against the CF pathogens. The aim of this work is to synthesize a new therapeutic formulation based on inhalable and biodegradable calcium phosphate (CaP) NPs functionalized with colistin, and test it in vitro and in murine models of infection relevant for CF.

**Experimental methods** CaP NPs were prepared using a one-pot synthesis reported by Rodriguez Ruiz et al. [2]. Colistin was loaded to CaP NPs by a post synthesis surface functionalization. Physical, chemical, morphological, and structural composition of loaded and unloaded CaP NPs were evaluated. Microbiological experiments were performed on RP73, a *P. aeruginosa* clinical strain isolated from a CF patient. In vitro RP73 biofilm treated with CaP NPs-colistin were investigated by colony forming unit valuation assay and by SEM. Furthermore, CaP NPs-colistin were also investigated in vivo by using a murine model of chronic infection with agar beads containing RP73 treated through inhalation route.

**Results and discussion** Loading of CaP NPs with colistin by surface functionalization was successfully achieved. CaP NPs were able to provide a sustained colistin release in simulated lung fluid and did not induce mucus thickening. In vitro tests revealed good microbicidal and antibiofilm activity of the colistin loaded CaP NPs. In vivo efficacy is under investigation.



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**Conclusion** A promising new therapeutic formulation based on biodegradable CaP NPs functionalized with colistin was achieved in order to potentially impair biofilm formation and microorganism vitality as well as to lower the drug resistance phenomenon in the treatment of CF related infections.

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**Acknowledgments** The present study was supported by a grant from Fondazione per la Ricerca sulla Fibrosi Cistica-Onlus (Verona, Italy; Project FFC#20/2018)



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# Biomedical application of hyaluronic acid based non-spherical nanoparticle by microfluidics: Potential usefulness in chemotherapeutics delivery for cancer therapy

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**Introduction** Hyaluronic acid (HA), a naturally anionic polysaccharide has attracted research attention for tumour-targeted delivery since it is biocompatible and non-immunogenic, and can even specifically bind CD44-protein, which is overexpressed in a variety of cancer cells [1]. Recently, the shape of nanoparticles (NPs) is emerged as an important design parameter able to tune the fate of NPs in biological systems [2]. In literature, a manufacturing technique to generate non-spherical (NS) NPs, from the spherical (S) NPs, such as film-stretching method (FSM), has been described [3]. The FSM, based on the immobilizing spherical polymeric NPs in a thin plastic film, does not lead to gain reproducibility in physicochemical properties of NPs, as size distribution, and consequently in vitro and in vivo drug delivery tests. Recently, newly continuous-flow nanoprecipitation assisted by microfluidic systems, in contrast to conventional NPs synthetic methods, are widely investigated for their unique properties such as precise control, rapid mass transfer, mixing efficacy, large reaction interfaces[4]. In the microfluidic apparatus, the nucleation, growth and agglomeration steps for the NPs formation can be separated as a function of distance from the position where solution mixing occurs to obtain an absolute control of the particle size and morphology [5]. In this work, blank and irinotecan-loaded (IRI) NS and equivalent (E) S NPs composed of poly(lactic-co-glycolic acid) (PLGA) have been formulated and coated with HA by means of microfluidics for tumor targeting toward CD44-overexpressing cell lines. The stability of the formulated NS and ES blank and IRI-loaded NPs was evaluated by measuring their size and in vitro, the IRI release. z-potential analysis and ELISA tests were further employed to investigate polymer assembly in NP formulations. Finally, in vitro cell uptake biological test on L-929 cell line was performed.

**Experimental methods** NS and ES blank and IRI-loaded NPs were prepared by a nanoprecipitation-assisted by microfluidic method. Briefly, for the NS and ES NPs organic phase (OP) PLGA (RG504H) 50:50 and poloxamers (PP) (PF68/PF127) (1:0.3:0.3) powder were solubilized at 2.0% (w/w). Afterwards, the dispersion was placed into a 15 mL tube connected to the micromixer chip having 12 mixing phases. In particular, PLGA/PP solution was introduced in the internal channel pumped at 250  $\mu\text{L}\cdot\text{min}^{-1}$ . A solution of HA (803 kDa) at 0.08% (w/w), was used in the external channels with fluxes of 1250  $\mu\text{L}\cdot\text{min}^{-1}$  to set a flow ratio (i.e., internal flow/external flow) equal to 0.2. In the case of drug-loaded NPs, IRIN



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(1 mg) was solubilized in the OP. The sizes and morphologies of all NP formulations were evaluated by DLS, z-potential, TEM techniques. Drug entrapment efficiency was evaluated by UV-vis and in vitro release kinetic of IRI by spectrophotometric assay. In vitro cell uptake assay of IRI-loaded NPs was performed on L929 cell line (IC50=51.7mM).

**Results and discussion** TEM micrographs of NS and ES HA/PLGA/PP NPs fabricated by microfluidics are have shown a regular and reproducible shape of an oblate ellipsoid ( $a=b>c$ ) with dimensions 240.31x101.2 nm, while their ES shown a diameter  $d=260$  nm. Single peaks and a polydispersion index lower than 5 % have been found in all size distributions and for all formulations, and a NP size are in agreement with TEM micrographs. In NS NPs HA addition resulted into a significant reduction of z potential from  $\sim-41.7$  mV (ES HA/PLGA/PP) to  $\sim-51.7$  mV. In the case of NS HA/PLGA/PP NPs, the drug entrapment efficiency (EE) was found to be 92.3%, while for their ES was 87.3% that is higher than EE obtained for the spherical HA/PLGA/PP synthesized by a traditional nanoprecipitation (62.7%). Moreover, it was observed a decreasing for NP HA yield from 97.8%, for ES HA NPs, to 92.3%; in any cases, the yield values of all NP formulation resulted higher than the traditional nanoprecipitation fabrication NPs method (48%). ELISA data revealed a loss of 6.4% w/w of HA in the supernatant. In vitro assay release profiles of IRIN for ES and NS HA/PLGA/PP showed that IRIN is completely released in less than two weeks, with >95% of the loaded drug eluted within 7 days. Cell uptake studies of ES and NS HA/PLGA/PP were performed on an L-929 cell line up to 48h of incubation and in both cases show that the internalization increases with exposure time, reaching a plateau after approximately 24 hours. Furthermore, from the analysis of biological assays it is clear that the NS HA/PLGA/PP NPs show a higher cellular uptake than their ES NPs, especially evident in the longer times of exposure. This trend of biological assay and a reduction of z potential was observed due to the greater surface area exposed by NS NPs [6].

**Conclusion** In conclusion we developed a novel non-spherical and equivalent spherical blank and IRI-loaded HA/PLGA/PP NPs by means of microfluidics. In vitro assays of HA/PLGA/PP NPs revealed that the non-spherical NPs allow better internalization than their ES NPs on the L-929 cells. The obtained results highlight the potential application of novel non-spherical HA/PLGA/PP NPs for cancer therapy.

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**Acknowledgments** The authors acknowledge the research project "ADVISE DRUGS AND ANTI-TUMORAL VACCINES FROM THE SEA" - POR CAMPANIA FESR 2014-2020 e con l'asse 1 OO.SS. 1.2.2 / 1.1 CUP B43D18000240007"



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# Hyaluronic acid-coated nanoparticles for active tumour targeting: Influence of polysaccharide molecular weight on cell uptake

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**Introduction** Hyaluronic acid (HA) is an anionic naturally occurring polysaccharide, main constituent of the extracellular matrix, able to bind specifically CD44 receptor, which is widely overexpressed in cancer cells. HA, due to its outstanding characteristics, aroused a noteworthy research interest for the engineering of nanoplatforms endowed with active targeting features. HA has been chemically anchored onto many drug loaded nanodevices, and their enhanced tumor targeting ability assessed. Nevertheless, the need for a chemical reaction poses serious regulatory challenges for FDA approval. Moreover, HA arrangement play a cornerstone role in dictating the specific interaction and binding proneness to CD44 receptor, which in turn strongly depends on HA size and molecular weight (MW). Here we aimed to correlate different MW of HA, 200, 800 and 1437 kDa, used to decorate poly(lactico-glycolic acid) (PLGA)-based nanoparticles (NPs), to their cell uptakes, considering that HA decoration on NPs surface was obtained by exploiting polymer selfaggregation driven by a lipophilicity gradient between oil and water phases.

**Experimental methods** NPs were produced by modified nanoprecipitation technique, forcing a PLGA/F68/F127 solution in acetone through a syringe at 333 microliter min flow rate by a Syringe Pump [1]. The solution was precipitated in an aqueous phase, containing poloxamers as surfactants and different amounts of HA, depending on the specific MW at 200, 800 and 1435 kDa, and the NPs formulations were named HA2, HA8 and HA14 respectively. HA NPs were characterised for their technological and thermal properties. by TEM, DLS and DSC. NP internalization kinetics were quantified in CD44-overexpressing breast carcinoma cells (HS578T), using healthy fibroblast (L929) cells as a reference by spectrofluorimetric assay on the cell lysate to quantify NP associated fluorescence at scheduled time points. Experimental results were compared with the numerical simulations obtained with a kinetic internalization model based on a cell membrane adsorption-desorption pseudo-stoichiometric balance [2].

**Results and discussion** Spherical NPs with a minor 200 nm mean size were obtained and ZP was about -50 mV in all cases, thereby indicating HA arrangement on NP surface. DSC results suggested the spontaneous interaction among PLGA, poloxamers and HA. The



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lowest melting heat evolved in the case of HA8 NPs was associated to its favoured interaction with the hydrophilic segment of poloxamers. In the case of HA2 and HA14 formulations the melting heats were comparable. This has been correlated to a promoted NP internalization and active cell targeting. Cell uptake experiments and model simulations indicated a significant tropism of HA decorated NPs towards tumour cells and, more interestingly NPs uptake were higher for HA8, compared HA2 and HA14. HA8 NPs, seemingly possess an optimized interaction with CD44 receptor. As for HA2, the HA segment protruding outwards is long enough to allow a significant, although suboptimal, tumor targeting. Anyhow, most of the targeting ability is lost in the case of HA14 formulation, and this puzzling finding can be reasonably ascribed to an excessive chain length, which favours HA macromolecule self-interactions and, consequently, the formation of random coils on NP surface, which reduces the possibility to interact with CD44 receptors on the surface of tumor cells.

**Conclusion** Overall, results obtained in this work point at how HA molecular weight, is pivotal project parameter in NP formulation to promote active targeting in the CD44 overexpressing cancer cells.

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**Acknowledgments** The authors acknowledge the research project ADVISE DRUGS AND ANTI-TUMORAL VACCINES FROM THE SEA - POR CAMPANIA FESR 2014-2020 AND WITH THE AXIS 1 OO.SS. 1.2.2 / 1.1 CUP B43D18000240007



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# Thermoresponsive copolymers as nanovectors to improve the bioavailability of retrograde inhibitors in the treatment of Leishmania infections.

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**Introduction** Leishmania species are parasites which are the causative agents of different types of leishmaniases. Visceral leishmaniases (VL) can be lethal and require curative treatment. A few studies have reported on the antileishmanial efficacy of a small, organic, retrograde pathway inhibitor (Retro-2, R2) and some analogs (DHQZ 36 and DHQZ 36.1). However, the use of these hydrophobic drugs is limited by their low solubility in water. Encapsulation of water insoluble drugs within nanoparticles represents a promising method to significantly improve their solubility and bioavailability, while also ensuring protection against degradation and reduced toxicity of the compound of interest.

Thermoresponsive polymers are particularly interesting as encapsulation vectors for hydrophobic drugs. The solubility in water of these amphiphilic copolymers dramatically depends on the temperature. In particular, below a critical temperature (low critical solution temperature, LCST) they form water-soluble micelles, but when the temperature exceeds the LCST, they self-assemble forming intermicellar aggregates, which can be employed as intracellular drug delivery vectors.

Herein, copolymers (PFG) of oligoethyleneglycol methacrylate (OEGMA) and pentafluorostyrene (PFS) were synthesized at variable compositions by radical polymerization, and their potential application for the delivery of R2 and its analogs to Leishmania-infected cells was investigated.

**Experimental methods** PFG copolymers were prepared via radical polymerization using different amounts of OEGMA and PFS. Copolymers were characterized by <sup>1</sup>H-NMR and GPC analyses, and the self-assembly process was studied by Dynamic Light Scattering (DLS). Coumarin 6 (C6), was used to evaluate the uptake of a hydrophobic molecule into PFG30 aggregates by fluorescence analyses.



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RAW264.7 murine macrophages were infected with Promastigotes of *L. amazonensis* and *L. donovani* strains, and then treated 24 h-post infection with R2 and analogs or PFG30-encapsulated R2 to assess the effect of encapsulation on the antileishmanial efficacy of the drugs. Cell lysates were analysed through mass spectrometry.

**Results and discussion** The study of PFG self-assembly process highlighted that the relative composition of copolymer strongly affect both LCST and aggregate size. It was observed that when the PFS molar fraction in the feed was 30% (PFG30) the coil to globule transition occurred at around 30°C leading to the formation of nanoparticles of 100 nm. C6 was used to investigate PFG30 efficiency of encapsulating hydrophobic compounds, demonstrating that copolymers significantly enhance its solubility in water. R2, DHQZ 36 or DHQZ 36.1 were therefore encapsulated into the PFG aggregates and their cell uptake was studied. Mass spectrometry results showed considerably greater delivery of the DHQZ analogs into infected cells. Moreover, encapsulation in PFG enhanced the efficacy of Retro-2 and its analogs to clear both *L. amazonensis* and *L. donovani* infections, as evidenced by the decrease of EC50 and the more rapid shrinkage of *L. amazonensis* communal vacuoles.

**Conclusion** The thermo-responsive PFG copolymers self-assemble at temperature higher than LCST. The resulting nanoaggregates are able to encapsulate hydrophobic drugs, and they are efficiently taken up by RAW264.7 macrophages. Therefore, the encapsulation of R2 and its analogs is a viable approach to dramatically increase their bioavailability and efficacy to clear *L. donovani* and *L. amazonensis* infections of macrophages in vitro.



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# Methylcellulose based biocomposites for bone tissue regeneration

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**Introduction** Methylcellulose (MC) hydrogels have been described as noteworthy materials in the field of tissue engineering. Due to their thermo-responsive behavior, MC gels undergo sol-gel transition upon temperature increase (e.g., for  $T \geq 37$  °C), thus representing ideal candidates for the development of injectable, in situ gelling hydrogels [1]. The aim of this work is to prepare organic-inorganic biocomposites based on MC and CaP or graphene oxide-modified CaP (CaPGO), capable to recapitulate the biological environment of bone tissue [2] and provide additional antimetastatic properties (i.e., for CaPGO) [3].

**Experimental methods** MC hydrogels (2 % w/v MC in 150 mM Na<sup>2</sup>SO<sub>4</sub>) were prepared as previously reported [4]. Biocomposites were prepared by embedding the CaP/CaPGO, synthesized at the IPCB (CNR; Naples), into the so obtained MC dispersion. The swelling behavior of the hydrogels was assessed in PBS up to 28 days. The presence of CaP/CaPGO and their potential chemical interactions with MC were evaluated via FT-IR spectroscopy. The injectability of the hydrogels was calculated using a MTS machine in strain-controlled mode. Lastly, gelation temperature and gelation time of the obtained hydrogels were assessed by rheological characterization.

**Results and discussion** All the tested hydrogels displayed a maximum swelling rate of ~ 150% 6 hours after immersion in PBS, and resulted stable up to 28 days. FT-IR data revealed the presence of phosphate and carbonate groups of CaP in the biocomposites, confirming the presence of the inorganic phase into the obtained hydrogels. Injection tests revealed, for all the tested hydrogels, an injection force < 30 N, as required for manual injection [2]. Rheological tests confirmed that all the tested hydrogels undergo gelation at  $T < 37$  °C and within 2-3 min after injection.

**Conclusion** The prepared hydrogels, due to their thermo-responsive character and suitability as injectable scaffolds represent ideal candidates for in situ gelling biocomposites for bone tissue regeneration.

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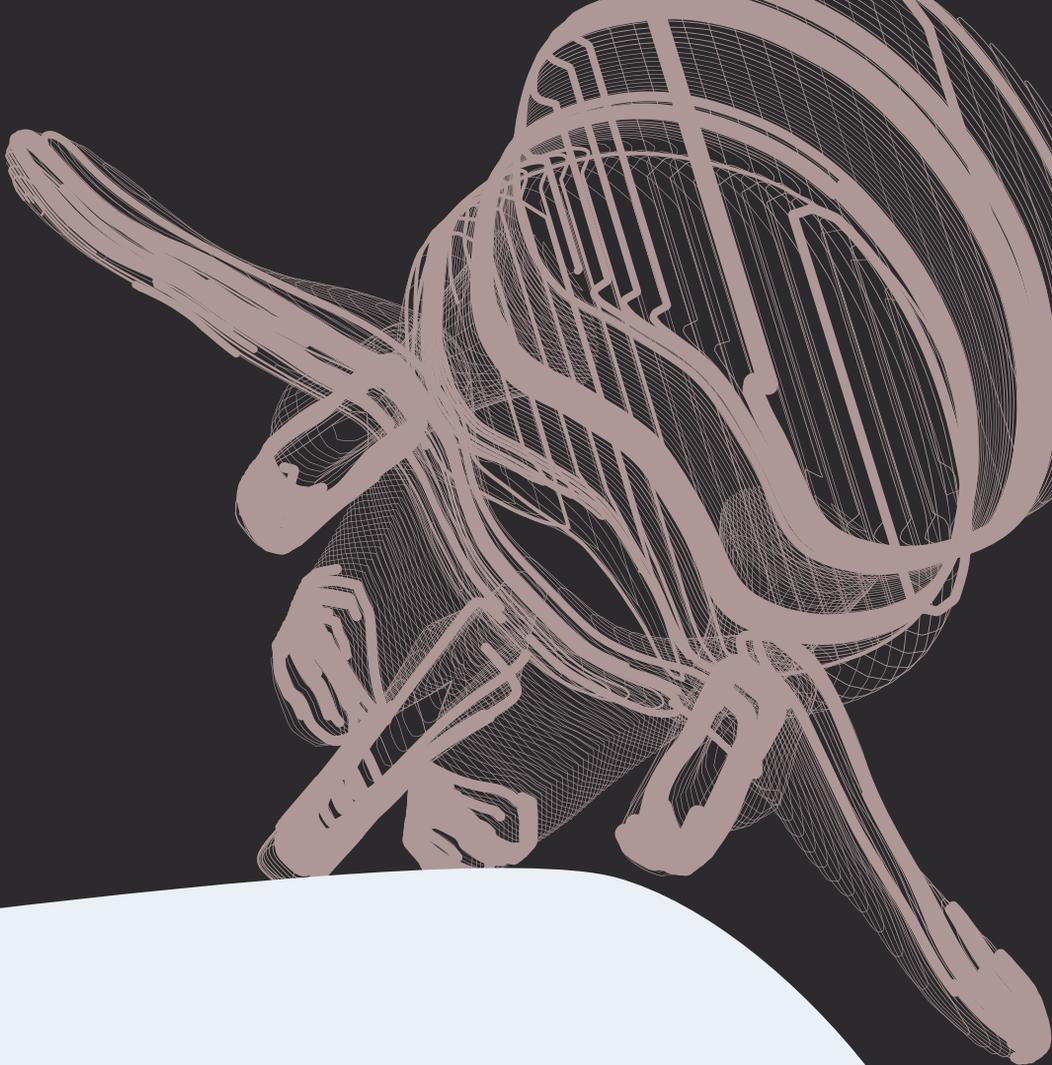
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**Acknowledgments** Authors thank PRIN2017 ACTION program for founding



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# BIOCERAMICS



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# Tellurium: a new active element for multifunctional bioactive glasses

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**Introduction** Multifunctional biomaterials are aimed to promote target tissue repair and to influence at the same time surrounding tissues cross-talk in order to favor a complete self-healing. Moreover, they are also able to prevent bacterial infections as they represent nowadays the main reason for implant failure in orthopaedic implants [1]. Bioactive glasses are attractive materials for bone replacement due to their tailorable chemical composition that is able to promote bone healing and repair. Moreover, bioactive glasses can be doped with different therapeutic element (e.g. Ag, Cu, Zn...) modification of a surface by a combination of metal (such as Tellurium) to confer antibacterial and antioxidant properties during the bone healing process [2].

**Experimental methods** Three glass compositions (STe0, STe1 and STe5) were developed by partially substituting SiO<sub>2</sub> with TeO<sub>2</sub> and the glasses were synthesized by melt and quenching process. Specimens cytocompatibility was evaluated by direct contact with Human bone marrow- derived stem cells (hMSCs) by means of metabolic evaluation with Alamar blue. Antioxidant activity of Te was evaluated towards hMSCs by means of ROS/RNS scavenge activity. Furthermore, hMSCs osteogenesis differentiation was performed with inflammation and evaluated by means of ALP activity and alizarin red staining. Antibacterial activity by infecting specimens with the orthopaedic-related pathogens *S aureus* and *S epidermis*. Antibacterial activity was evaluated by direct contact with bacteria by means of metabolic evaluation with Alamar blue. Furthermore, co-culture of cells and pathogenic bacteria and evaluated by cell count (hMSCs) and CFU (Bacteria).

**Results and discussion** Te-doped bioactive glass did not show any toxicity towards the hMSCs as the cell viability was comparable for all specimens and controls. Under oxidative stress Te insertion was effective in protecting cells from apoptosis induced by the H<sub>2</sub>O<sub>2</sub> treatment, whereas in controls cells viability was significantly reduced. During osteogenesis, the specimens did not show any significant differences in terms of ALP activity by comparing controls (STe0) and Te-doped specimens. Furthermore, ALP activity was further evaluated in the presence of H<sub>2</sub>O<sub>2</sub> induced oxidative stress: no significant differences were noticed between control (STe0) and Te-doped specimens and the ALP values are comparable. Alizarin results for STe1 & STe5 were comparable between treated and control



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groups. On the opposite, antibacterial evaluation assays demonstrated the strong ability of Te-doped glasses to inhibit biofilm formation. Furthermore, STe5 specimens were effective in significantly reduce bacteria number and preserve cells viability when they were cultivated in the same environment.

**Conclusion** In the present in-vitro study Te doping onto bioactive glass preserved the cytocompatibility. Moreover, tellurium insertion was effective in protecting cells metabolic activity from apoptosis induced by  $H_2O_2$  treatment. Furthermore, antibacterial activity showed a clear antibacterial effect of Te containing glasses and a strong ability to inhibit biofilm formation. Osteogenic expression confirmed the ability of Te-containing glasses to preserve the ALP and Alizarin red expression in presence of oxidative stress. Finally, cells and bacteria co-cultures confirmed that Te presence significantly preserves cells from the infection, in particular for STe5 glass.

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# Assessment with an indirect co-culture system of human osteoblasts and osteoclasts of nanostructured collagen-based materials for bone tissue engineering approach

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**Introduction** Osteoporosis is a worldwide disease resulting in increased bone fragility and enhanced fracture risk. Since the conventional treatments such as antiresorptive agents can cause rare but very serious adverse effects, such as mandible necrosis and atypical femur fracture[1], in the last decades bone tissue engineering is considered a potential approach for osteoporotic fractures. The development of bone substitutes combining bioactive materials and cells is today an attractive alternative therapy. Recently, cell co-culture systems have been demonstrated to be a useful tool for the pre-screening of innovative biomaterials for bone regeneration due to the simulation of the natural cell crosstalk[2]. In this scenario, two bioactive constructs were developed using a hybrid formulation composed of a natural polymer, type I collagen (Blafar), and inorganic bioactive components such as mesoporous bioactive glasses containing strontium ions (Sr-MBG) and rod-like hydroxyapatite nanoparticles (n-HA) respectively. The ability of the composite constructs to interact with bone cells promoting cell adhesion, proliferation and differentiation was assessed with an indirect co-culture system of human bone-derived cells, i.e. osteoblasts and osteoclasts, showing the potential positive effect of the two hybrid materials on the promotion of bone regeneration.

**Experimental methods** Osteoporosis is a worldwide disease resulting in increased bone fragility and enhanced fracture risk. Since the conventional treatments such as antiresorptive agents can cause rare but very serious adverse effects, such as mandible necrosis and atypical femur fracture[1], in the last decades bone tissue engineering is considered a potential approach for osteoporotic fractures. The development of bone substitutes combining bioactive materials and cells is today an attractive alternative therapy. Recently, cell co-culture systems have been demonstrated to be a useful tool for the pre-screening of innovative biomaterials for bone regeneration due to the simulation of the natural cell crosstalk[2]. In this scenario, two bioactive constructs were developed using a hybrid formulation composed of a natural polymer, type I collagen (Blafar), and inorganic bioactive components such as mesoporous bioactive glasses containing strontium ions (Sr-



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MBG) and rod-like hydroxyapatite nanoparticles (n-HA) respectively. The ability of the composite constructs to interact with bone cells promoting cell adhesion, proliferation and differentiation was assessed with an indirect co-culture system of human bone-derived cells, i.e. osteoblasts and osteoclasts, showing the potential positive effect of the two hybrid materials on the promotion of bone regeneration.

**Results and discussion** The hybrid formulations combining type I collagen respectively with Sr-MBGs and n-HA were successfully crosslinked, and the material stiffness and stability were improved thanks to the optimized genipin chemical crosslinking. Using the indirect osteoblast-osteoclast co-culture the ability of the two systems to promote cell adhesion and proliferation up to 21 days, as well as the differentiation of cells towards mature phenotype without the addition of external factors in the culture medium, was demonstrated. Moreover, a different osteoblast morphology was observed in relation to the inorganic phase added to the collagen matrix.

**Conclusion** Bulk materials were successfully obtained starting from a bioactive formulation composed of type I collagen added with respectively Sr-MBGs and n-HA, with the aim to reproduce the microenvironment and structure of native bone. The biological assessment with human osteoblasts and osteoclasts co-culture system proved the ability of the designed scaffold to support cell adhesion, proliferation, and differentiation by paracrine activity in basal conditions.

### **Acknowledgments**

This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 681798-BOOST).

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# Nano-functionalized Mg-hydroxyapatite scaffolds improve antimicrobial property and bone regeneration

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**Introduction** Hydroxyapatite (HA) is the main inorganic mineral that constitutes bone matrix and represents the most used biomaterial for bone regeneration. Although HA is biocompatible, minimally inflammatory, osteoconductive, osteo-inductive and biodegradable, its low antibacterial properties and its fragile nature restricts its usage in bone graft applications [1]. Recently, several studies focused on HA scaffolds functionalization with nanoparticles (NPs), including metal ones, to improve the matrix antimicrobial activity, mechanical strength, and capability to stimulate osteogenic and angiogenic properties [2].

**Experimental methods** In this study, Mg-HA scaffolds were functionalized with several NPs including gold nanorods (Au-NRs), silver NPs (Ag-NPs), and maghemite NPs ( $\gamma$ -Fe<sub>2</sub>O<sub>3</sub>-NPs). Their antibacterial effect and ability to induce the proliferation and differentiation of human adipose-tissue derived mesenchymal stem cells (hADSCs) were assessed.

**Results and discussion** Our results clearly highlight that  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub> nanofunctionalization substantially improves cell proliferation and differentiation compared to the control, whereas Au-NRs and Ag-NPs inhibit them. On the contrary, the scaffolds functionalization with  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub>-NPs shows low antibacterial activity, whereas Au-NRs and Ag-NPs improve this property with a bacterial reduction of almost 100%. These results suggest that Au-NRs and Ag-NPs are not able to stimulate cell proliferation and differentiation, probably due to their cytotoxicity. The latter can be ascribed to their physico-chemical properties, such as size, shape, concentration, and interaction with a biological system, and to the oxidative stress induced by ROSs [3]. The cell proliferation and differentiation improvement observed in the  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub>-NPs functionalized Mg-HA scaffolds can be likely attributed to the magnetic properties of the nanomaterial [4]. In term of bactericidal mechanism, the cytotoxic activity of Au-NRs and Ag-NPs can also depend on their size, surface functionalization, and aggregation state, possibly preventing endocytic processes. Once inside the cells, the bactericidal effect blocks some genes expression, inhibits ATP synthesis, dissipates the membrane potential, and leads to ROSs production [5].

**Conclusion** The results presented in this study pave the way for the development of innovative nanostructured scaffolds for the design of effective biomaterials for bone



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regeneration. Further investigations exploring the effects of Au, Ag and  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub> NPs having different size, shape, magnetic properties as well as concentration will be performed.

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# Calcium phosphate bone cement reinforced with self-assembling fibers

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**Introduction** Calcium phosphate bone cements are biocompatible and osteogenic systems but their mechanical properties are far from those of bone and a mechanical reinforcement is often required. The incorporation of fibers into the cement matrix has been used for this purpose, but, on the best of our knowledge, they have always been introduced inside the pasty material after their synthesis, lacking a good cohesion between fibers and cement paste.

In this work we demonstrate the feasibility of forming self-assembling fibers by the introduction of a low-molecular-weight gelator (DOPA(OBn)<sub>2</sub>-OH) able to form supramolecular structures stabilized by weak interactions.

**Experimental methods** The cement powders are composed of a gelatin/ $\alpha$ -TCP mix and CaHPO<sub>4</sub>·2H<sub>2</sub>O. The liquid phase is made by an aqueous solution of the gelator at two different concentrations: 1 and 2.6% w/w. Mechanical properties in compression and in bending were evaluated, as well as porosity studies, rheological measurements and morphological investigations. *In vitro* biocompatibility was performed to ensure the biocompatibility of the materials.

**Results and discussion** SEM images showed a high number of fibers in all the FRCPC formulation. XRD patterns and the FT-IR spectra indicated that the cements were totally converted after 7 days of soaking. The mechanical properties of the FRCPC were significantly improved by the presence of the gel. MicroCT analyses showed no significant variation on the porosity of the composite materials. Moreover, biological assays ensured the biocompatibility of the materials loaded with 1% w/w and their ability to express the main gene markers that are necessary for bone formation.

**Conclusion** The approach used in this work could represent a new, simple and effective method to obtain cements reinforced with self-assembled fibers in just a single step. The formation of fibers during the hardening reaction provided structural and mechanical support to the material without interfering with their porosity and the hardening reaction. The obtained fiber-reinforced CPC with 1% w/w of fibers is injectable, biocompatible and able to promote the deposition of extracellular matrix.



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# Zirconia toughened alumina femoral components developed with ceramic injection molding for Total Knee Replacement

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## Introduction

Total Knee Replacement (TKR) have been used in the human joint for over 40 years. So far metals such as cobalt-chromium and titanium alloys have been used. Metal replacement may suffer of allergic reactions, aseptic loosening, and of tiny particles (debris) and metal cations release into the body as a result of friction. These particles can sometimes cause reactions in the human body. Aseptic loosening is the failure of joint prostheses without the presence of mechanical cause or infection. It is often associated with osteolysis and inflammatory cellular response within the joint.

In this study Ceramic femoral components for (TKR) has been developed and characterized with microstructural, wear, and mechanical tests. The components are made of Zirconia Toughened Alumina (ZTA) formed by ceramic injection moulding; selected composition was Al<sub>2</sub>O<sub>3</sub> (83%vol) and ZrO<sub>2</sub> (17%vol). ZTA have been proposed because of its minimum wear, excellent biocompatibility, mechanical resistance, wear and scratch resistance and improved wettability. Ceramic injection molding is an innovative and convenient approach to the production of inert bioceramics.

## Experimental methods

ZTA femoral replacementent and test samples were manufactured by ceramic injecton molding by Salentec s.r.l. starting from a proprietary feedstock. A detailed mechanical characterization has been performed with three point bending resistance, Single-edge V-notch beam tests and Vickers indentation. An XRD and SEM microsctructural characterization anticipated and followed the mechanical tests.

Wear resistance of ZTA has been investigated with pin on disk tribometer and with 6-degrees-of-freedom knee simulator. Tetragonal to monoclinic phase ratio was evalutated by XRD before and after wear test to evaluate the stability of tetragonal ZrO<sub>2</sub> phase.

## Results and discussion

The study showed that both friction and wear rate are very low compared to similar studies on metal components and surface and bulk microstructure modification under severe wear



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is negligible as revealed by SEM and XRD and other tests. Minimal conversion of tetragonal to monoclinic phase was found in the bulk and the surface of ZTA after tribological tests. As a consequence, the strength and toughness of the components was not altered under simulated wear conditions.

### **Conclusion**

Fully densificated, tough and reliable femoral Knee components were obtained by ceramic injecton molding. ZTA knee femoral component under lubricated conditions possesses low and constant friction coefficient, minimizing friction and wear.

The use of ceramic materials for knee replacements can assess several issues such as aseptic loosening and other concerns related to the actual use of metal parts. The risk of brittle mechanical failure is minimized with tough and resistant ZTA.

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### **AcKnowledgments**

Financial support of Italian Ministry of Innovation and Economic Development Project, "Horizon 2020 - PON 2014/2020" project F/050427/01-02/X32 is acknowledged.



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# Ti-doped hydroxyapatite based eco-sustainable physical filter for novel sunscreen formulation

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**Introduction** The sunlight is essential for our well-being, because it is responsible for regulating the metabolism, immune systems, and for the production of vitamin D essential for healthy bones; however, excessive sun exposure can be dangerous for human health, particularly for skin. In this regard, the use of sunscreen to protect ourselves from solar radiation, especially harmful UVA and UVB rays, is becoming an increasingly important issue, to avoid skin photo-aging and the onset of malignant tumours. Furthermore, the more attention for the preservation of the marine environment has brought out the need to develop eco-sustainable materials. With these evidences, this research would propose innovative and eco-sustainable hybrid UV-physical filters composed of ions doped-hydroxyapatite and biopolymers obtained by a nature-inspired biomineralization process.

**Experimental methods** Through the biomineralization process, titanium-doped-hydroxyapatite (TiHA) crystals were nucleated on different organic matrices to develop biomimetic physical filters with eco-friendly properties and without photocatalytic effect. The hybrid composites were investigated through chemical-physical (XRD, FTIR, TGA, ICP-OES) and morphological (SEM) analysis. Moreover, the interaction with the UV-VIS radiations (adsorption and reflection spectra) and the photodegradation potential were evaluated.

**Results and discussion** The major phase (more than 85 %) is a nanostructured TiHA featured by a micrometric size due to the presence of organic phase on which the apatitic crystal are grown which avoids the penetration into epidermis. Furthermore, the low TiHA crystallinity very close to human bones and obtained through the biomineralization process, allow to create a fully biodegradable material releasing only harmless ions for health and aquatic environment. The results obtained by UV-VIS spectroscopy have shown excellent reflectance and absorption properties of the composites thanks to the presence of Ti ions. Moreover, since titanium is not present in the form of titanium dioxide (TiO<sub>2</sub>), as in commercial UV-physical filters, but as Ti(IV) ions, any photocatalytic effect was not revealed avoiding the photo-degradation of the other ingredients in sunscreen formulations and the generation of radicals and/or reactive species under irradiation, harmful for skin and corals.

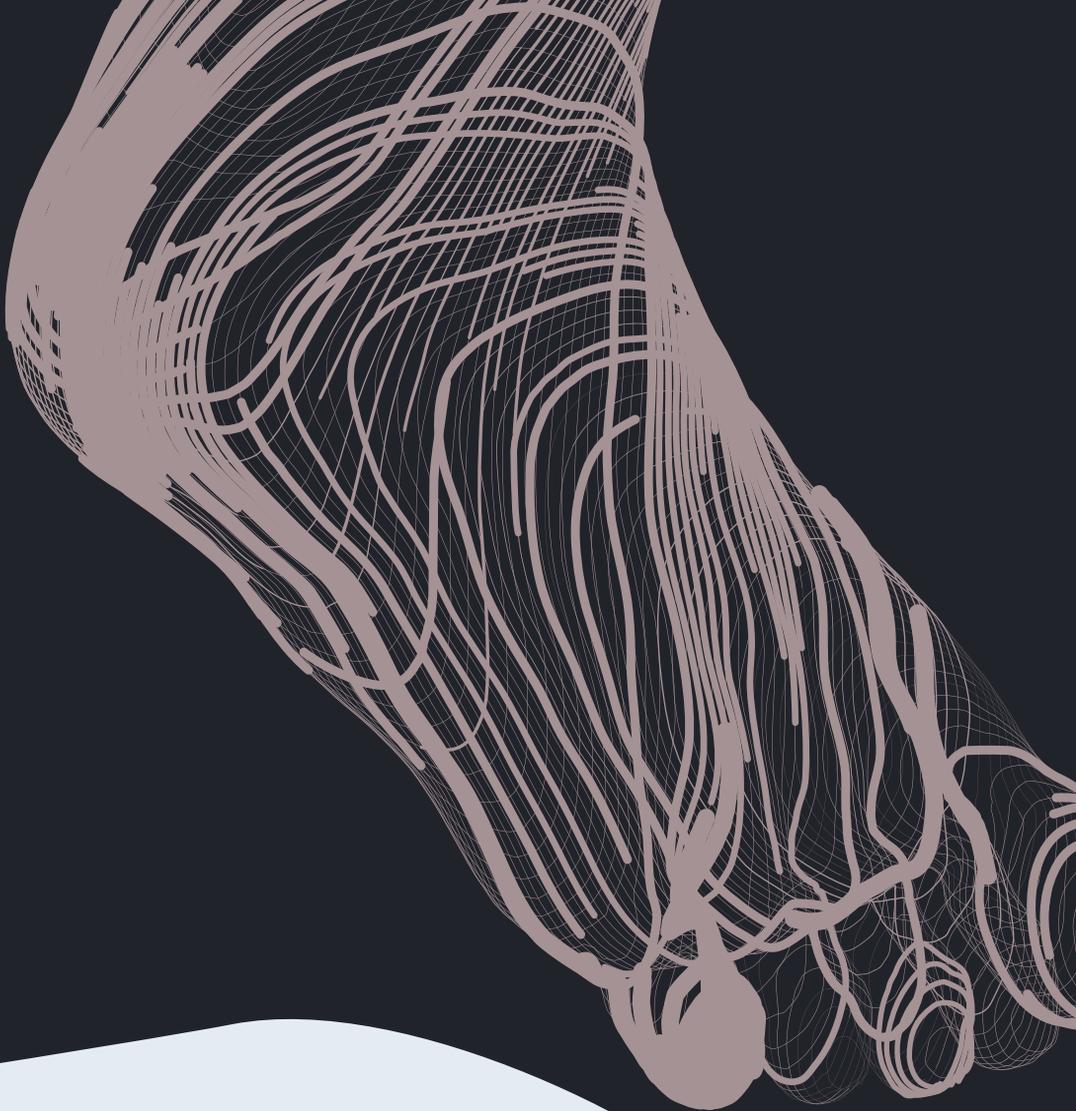


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**Conclusion** Effective and eco-friendly Ti-HA based sunscreens have been developed as new safe physical filters compared to commercial ZnO and TiO<sub>2</sub>. Thanks to the introduction of Ti ions within the biomineralization process it was possible to obtain the partial substitution of both Ca (Ti<sup>4+</sup>) and P (TiO<sub>4</sub><sup>4-</sup>) ions into the hydroxyapatite latex, realizing innovative solar filters featured by high biocompatibility, bands of UV radiations absorption/reflection in the desired range and zero photocatalytic effect.



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## POSTERS



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# Study, Characterization and Comparison of Commercially Available Collagen-Based Membranes GTR-GBR

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**Introduction** Polymeric barrier membranes are widely used in reconstructive periodontal surgery to enhance periodontal-bone regeneration. They are placed between the mucogingival flap and the bone to prevent epithelial ingrowth into the defect thus allowing for the slower-growing periodontal-bone tissues to regenerate into the void volume. Both non-resorbable and resorbable membranes are available. Collagen-based devices are the most widely used among the latter: they provide excellent biocompatibility while showing the same efficacy as non-resorbable products. A huge number of collagen-based devices differing for collagen origin and processing-stability are currently on the market. Several reports, investigating specific properties of some membranes, are available in the literature. However, a whole comparison of diverse types of collagen devices, considering all the biophysical and biochemical features potentially affecting in vivo performance, is still lacking. Availability of these data could support clinicians to optimize the use of these products also providing valuable information for the development of new highly performing similar devices. To this aim, here, we present a rather complete in vitro characterization of four type of collagen devices, highlighting similarities and differences in chemical structure, superficial morphology, porosity, swelling, stability, mechanical features, cell occlusivity and cytocompatibility.

**Experimental methods** 1) Collagen from Achille tendon (Bioactiva); 2) Collagen from cortical lamina (Bioactiva); 3) Collagen from pericardium equine (Bioactiva); 4) bilayer collagen membrane (Bio-Gide) were used. Samples swelling in PBS (pH 7.4, 37°C) was analyzed by gravimetric measurements. Resistance to collagenase was evaluated by monitoring mass loss and collagen solubilization (spectrophotometric measurements) during incubation with 4U/mL collagenase. Porosity was determined by a fluid replacement method. Fourier transform infrared spectroscopy (FT-IR) and scanning electron microscope (SEM) were to study the chemical structure and the surface morphology. Mechanical analyses are carried out using a dynamometer. primary human fibroblasts and osteoblasts are used to study cytocompatibility. An innovative test aiming at evaluating cell occlusivity, by using cell culture inserts, is being setting.

**Results and discussion** FTIR spectra confirmed the presence of the classic collagen



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peaks. The spectra of the Bioactiva membranes are comparable, while the Bio-Gide membrane shows additional peaks rationally related to chemical processing. All membranes rapidly absorbed water and reached equilibrium in PBS within 6h. The highest swelling degree was recorded for the pericardium membrane (10fold weight increase) while the cortical lamina showed the lowest water absorption. All membranes degraded in the presence of collagenase confirming resorbability. Samples 1 and 4 degraded faster than samples 2 and 3 with the cortical lamina showing highest stability. Sample 3 showed the highest porosity, also confirmed by SEM. SEM analyses highlighted great differences in surface morphology for sample 2. Cytocompatibility tests and mechanical tests are in progress.

**Conclusion** Data collected up to now highlighted significant differences among the membranes mainly in terms of swelling, stability and porosity. Based on the biophysical analyses, the product 4 is expected as the one most occlusive to cells. Data from mechanical and biological analyses will interestingly complete the panel of features characterizing the tested products.



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# A sol-gel derived organic/inorganic nanohybrid for bone regeneration

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## Introduction

As an organic/inorganic composite, bone has a complicated hierarchical architectural structure. Inspired by nature, there is a class of biomaterials known as organic/inorganic hybrids holding a promising potential in bone tissue regeneration. These materials are endowed with the best of ceramics and polymers simultaneously—appropriate compressive and tensile strengths besides osteogenic and biocompatible properties [1].

Bioactive glass (BG)-based sol-gel derived hybrid materials are of particular interest in bone tissue regeneration. These materials are endowed with recapitulating the advantages of polymers and ceramics into a package. As calcium is vital for BG's bioactivity and osteoblasts activities, its incorporation into the BG hybrids is still problematic. Due to lack of thermal treatment for organic/inorganic BGs, calcium ions cannot diffuse into glass structure. Moreover, it is critical to bear in mind that the burst release of calcium ions in the cells medium has potential to cause a significant decrease in the cell viability and so these issues are still a real challenge [2]. Considering the mentioned issues, we successfully gave birth to an innovative hybrid biocomposite composed of pluronic-F127/BG.

**Experimental methods** The hybrid, which is composed of F127/BG, is synthesized through evaporation-induced self-assembly sol-gel technique followed by being in the exposure of a microwave irradiation (MWI, Biotage Initiator). Different calcium precursors (nitrate and chloride) and MWI are applied to assess their effects on the physicochemical and biological properties of hybrid biocomposite.

## Results and discussion

The obtained results indicated that the F127 had a key role in homogenous distribution and controlled release of  $\text{Ca}^{2+}$  from the hybrid. The samples w/o F127 induced toxicity when exposed to L929 due to burst release of  $\text{Ca}^{2+}$ . The samples treated with MWI turned out as glass-ceramic. The MWI had a stronger effect on the chemical stability and the samples treated with it showed a significant increase in the cell viability.

## Conclusion

Two issues in the field are successfully addressed as follows: homogenous distribution of



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Ca<sup>2+</sup> in the sol-gel derived hybrid BG structure and the ions controlled release from the hybrid. The obtained hybrid biocomposite can be potential biomaterials with wide variety of applications in biomedical engineering.

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### Acknowledgments

This study was supported through funds provided by Progetto MIUR PRIN2017–ACTION, Grant N. 2017SZ5WZB.



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# Targeting her-2 positive breast cancer cells with photothermal-responsive gold nanoparticles

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**Introduction** In 2018, breast cancer was the most common type of cancer in women worldwide, causing about 6.4% of women's cancer deaths. Hence, there is a need to develop a more efficient therapeutic approach to improve breast cancer prognosis and enhance its survival rates. Among gold nanoparticles, gold nanostars (GNSs) are emerging as promising tools for cancer photothermal therapy (PTT).<sup>1,2</sup> The study aimed to synthesize and characterize a novel Herceptin (H) conjugated polyethylene glycol-gold nanostars (H-PEG-GNSs), to be used as a selective PTT platform against HER2 (human epidermal growth factor receptor-2) expressing breast cancers.

**Experimental methods** GNSs were synthesized, pegylated, and characterized using Transmission electron microscopy (TEM), Dynamic Light Scattering (DLS), UV-Vis spectrophotometry, and Thermogravimetric Analysis. The conjugation between PEG-GNSs and Herceptin was carried out using the previously reported EDC-NHS protocol.<sup>3</sup> The conjugated GNSs were characterized using UV-Vis spectroscopy absorption studies, DLS, BCA analysis, and dot-blot assay. Viability (MTT) and uptake studies were carried out to verify the interaction of Herceptin conjugated PEG-GNSs with the target breast cancer cells.

**Results and discussion** PEG-GNSs showed an intense LSPR (Localized Surface Plasmon Resonance) absorption with the maximum wavelength in the bio-transparent window (750-950 nm), making it a suitable tool that can be used for deep tissues PTT. Next, H-PEG-GNSs were prepared and characterized using DLS and UV-Vis spectroscopy: Herceptin conjugation resulted in particle size increase, less negative zeta potential, and UV-Vis absorption spectrum redshift of PEG-GNSs. The presence of Herceptin on the surface of GNSs also revealed by BCA and dot blot assays. The targeting properties of the nanosystems were observed by in vitro studies against HER-2 overexpressing cells, SKBR-3, and compared to normal fibroblast cell line, NIH-3T3.



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**Conclusion** Preliminary data showed the successful formation of Herceptin-PEG-GNSs conjugate. Currently, experiments are ongoing to explain other aspects related to the efficacy and the uptake mechanism of the nanoconjugate systems in the target tumor cells. 3D cancer models and vivo studies are required to assess the toxicity and efficacy of this PTT nanoplatform against HER2-positive breast cancers.

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**AcKnOwledgments** This research was supported by a grant from the Italian Ministry of Education, University and Research (MIUR) to the Department of Molecular Medicine of the University of Pavia under the initiative “Dipartimenti di Eccellenza (2018-2022)” and Pavia University’s Crowdfunding on breast tumor studies (2015 and 2021 <https://universitiamo.eu/en/campaigns/tumore-seno-diagnosi-cura-nanoparticelle-doro> <https://universitiamo.eu/campaigns/tumore-al-seno-sconfiggerlo-con-nanosfere-doro-intelligenti-nuove-sfide/>



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# Chemically crosslinked methylcellulose substrates for cell sheet engineering

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**Introduction** Methylcellulose (MC) hydrogels have been described as noteworthy materials in the field of cell sheet engineering (CSE) since they allow to control cell attachment/detachment on their surface by means of a temperature trigger [1]. However, their reduced water-stability and mechanical properties may limit the breadth of their possible applications [2].

This work aims at exploiting the possibility to use citric acid (CA) crosslinked MC-based hydrogels [3] to obtain cell sheets (CSs). The crosslinked MC substrates were characterized from a mechanical point of view, *in vitro* tested, and the obtained CSs were studied to assess their regenerative potential.

**Experimental methods** MC hydrogels (8 % w/v MC in 50 mM Na<sub>2</sub>SO<sub>4</sub> [3,4]) were crosslinked by adding CA (1, 3, 5 % w<sub>CA</sub>/w<sub>MC</sub>) to the hydrogel solutions, followed by oven-drying and thermal treatment [4]. Three crosslinked MC hydrogels, MC-L, MC-M, MC-H (low, medium, and high crosslinked, respectively), were obtained. Non-crosslinked MC hydrogels were used as control. All the specimens were tested, investigating their tensile mechanical and *in vitro* biological characteristics. Indirect cytotoxicity was assessed culturing L929 murine fibroblasts in contact with 24, 48, and 120 h extracts for 24 h. CSs were harvested 48 or 120 h after L929 cells seeding on the MC substrates, simply by lowering the temperature to 4 °C. The detached CSs were characterized by immunofluorescence and image analysis (ImageJ, NIH). Lastly, the CSs regenerative potential was assessed by evaluating their adhesion and proliferation capability after their transfer to a new tissue-culture substrate.

**Results and discussion** CA crosslinking effectively modulated the mechanical properties (i.e., Young's Modulus, E) of MC hydrogels. The E values were found in the range 5 kPa - 3.5 MPa, increasing the crosslinking degree.

Cell viability around 100 % was observed for each specimen (MC, MC-L, MC-M, MC-H), suggesting that the CA amounts used to achieve crosslinking did not cause any *in vitro* cytotoxic effect on L929 cells.

CSs were then harvested simply by lowering the temperature at 4 °C. Intact CSs were detached from MC, MC-L, and MC-M hydrogels. Conversely, CSs harvesting was not feasible from MC-H hydrogels, confirming previous results which disclosed that a high crosslinking



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degree leads to the loss of MC hydrogels thermo-responsiveness [4].

Fluorescence images of the detached CSs revealed the presence of widespread actin filaments in the cytoskeleton, contributing to cell-cell junctions in the obtained CSs. For all the detached CSs, cell count was found to increase ( $p < 0.05$ ) between 48 and 120 h of cell culture. No significant differences ( $p > 0.05$ ) in terms of cell count were observed among the CSs obtained for the differently crosslinked samples (MC, MC-L, and MC-M).

Adhesion and proliferation assay on a new tissue-culture multiwell plate revealed that the CSs started to adhere to the new substrate shortly ( $\sim 20$  min) after their transfer. Optical microscopy observation 72 h after CSs transfer revealed that L929 cells migrated from the CS to the bottom of the new well, indicating the potentiality of the obtained CSs for tissue regeneration.

**Conclusion** This work reports for the first time the use of crosslinked MC substrates in CSE. CSs were successfully detached from the surface of mild crosslinked MC hydrogels (MC-L and MC-M). Conversely, high crosslinked MC hydrogels (MC-H) did not exhibit the expected thermo-responsive character, preventing CSs harvesting. The obtained CSs displayed no differences in terms of cell viability, compared to CSs obtained from control (pristine MC hydrogel).

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# BRAIN IT-Innovative technologies in Neurosurgery Study: an interdisciplinary approach

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**Introduction** Neurosurgery is the branch of surgery that requires the highest accuracy and needs complementary and interdisciplinary skills. Scientific technology in neurosurgery is progressing much faster than the evolution in a medical department, and it would be very important to try to align the scientific and clinical realities. In this context, the project “BRAIN IT- Innovative technologies in Neurosurgery Study”, financed by the Europe Committee in the context of the Erasmus+ Key Action 2 - “KA203 - Strategic Partnerships for Higher Education” call, aimed at promoting international strategic partnerships with teaching and research activities, involves three different countries, i.e. Romania (University Lucian Blaga-Sibiu), Spain (University of Las Palmas de Gran Canaria) and Italy (University of Rome “Niccolò Cusano”). The priorities followed in the project have been to develop skills for biomedical engineering and medicine students and curricula relevant to the labor market and societal needs, to open innovative education practices in a digital era, to contribute to innovation, supporting the transfer of latest research outputs back into education, with the final aim to make a closer connection between European neurosurgery, biomaterials and technologies research centres.

**Experimental methods** In order to achieve BRAIN IT purpose, different activities were planned. Three summerschools were organised at the University of Sibiu-Romania in 2019-2021, about the main biomaterials, technologies and clinical topics correlated to neurosurgery. In this regard, 14 days ‘traineeships’ in a working hospital were planned for every summerschool, and 4 ECTS were ascribed after passing a test. Employing the strategic use of ITC technologies in teaching/training activities was considered, as well as the production of interactive teaching and training aids for students using 3D technology – 3 D reconstruction of the skull in various pathologies (tumours, traumas..), and the development of a reader-friendly online platform which can facilitate the access to all students, residents and practitioners to new technologies and new discoveries in neurosurgery.

**Results and discussion** An innovative e-learning platform for Telemedicine was



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developed as a support to provide an open on-line course in all the topics addressed by the project in the field of neurosurgery and biotechnology. It contains streaming functionalities for the telemedicine applications showing the surgical procedures, a role concept allowing the visualisation of different types of content depending on the user role, web 2.0. features (discussion forums for the students; chatting functionalities, Facebook and twitter feeds), e-learning functionalities, a network feature allowing users to work together on different tasks and topics. Three summerschools have been organised: the first one “Trauma in Neurosurgery” in July 2019 and the second and the third ones “Neuro-oncology” and “Neurovascular surgery” in August 2021. The course participants had the possibility to be involved in a real life working experience, accompanying the whole diagnostic process. Specific training materials, such as printed skulls, were prepared for the organization of the practical activities and workshops during the summerschools. The handbook conceived to comprise all the important information delivered during the three years project is available both in printed and online version on the project e-platform.

**Conclusion** BRAIN IT project has gained the proposed aims, highlighting the importance of combining several and complimentary expertises and cultures. Students from Italy, Spain and Romania and with different backgrounds (engineering and medicine) joined the organised summerschools, acquiring new interdisciplinary competences and knowledge. BRAIN IT allowed to really create an efficient and fructose strategic partnership between the involved research groups who have started to strictly collaborate and submit new proposals.

**Acknowledgments** The Authors acknowledge the project “Brain Revealed: Innovative Technologies in Neurosurgery Study (BRAIN IT)” (Project No: 2018 - 1 - RO01 - KA203 - 049317, <http://grants.ulbsibiu.ro/brainit>), financed by European Committee, under the Erasmus+ Key Action 2 - “KA203 - Strategic Partnerships for Higher Education” call.



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# Development of a novel alginate/hyaluronic acid dressing for light-activated therapies in wound healing

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**Introduction** The application of the photodynamic therapy in chronic wound treatment, which consists of the localized administration of a non-toxic light-sensitive drug/compound (photosensitizer) followed by exposure to appropriate light to produce singlet oxygen or other reactive oxygen species, could be a promising approach to ameliorate this global health problem. The aim of this research is the development of a novel alginate/hyaluronic acid (ALG/HA) advanced wound dressing loaded with a light-activated molecule based on the incorporation of microemulsions (MEs) into a biocompatible hydrogel to fight bacterial infections and enhance the wound healing rate.

**Experimental methods** An aqueous titration method was used for the preparation of MEs loaded with the natural photosensitizer curcumin. Labrafac lipophile WL 1349 (oil), and Kolliphor HS 15 (surfactant) were selected as the main component of the MEs based on their ability to form stabile MEs and encapsulate the photosensitizers. Stability, dynamic light scattering, and microscopy studies were carried out to characterize the MEs before their introduction into the ALG/HA dressing. To obtain a uniform structure, ALG/HA hydrogels were prepared by internal gelation using glucono- $\delta$ -lactone and CaCO<sub>3</sub>. The light activated wound dressings were tested for a series of functional characteristics including mechanical strength, porous microstructure, stability, swelling, and in vitro mucoadhesion. The release profile of curcumin was evaluated using a model with an air-liquid interface to mimic the real conditions on the wound area

**Results and discussion** A thermodynamically stable MEs was adopted as the vehicle to encapsulate curcumin with the help of the pseudo-ternary phase diagram. Dynamic light scattering analysis and encapsulation efficiency results showed that the developed microemulsion had a  $37.9 \pm 2.0$  nm size and a near-neutral zeta potential. Hydrogels with different shapes, sizes, and MEs loading were prepared and later freeze-dried to obtain a dry dressing with a macroporous uniform structure. The prepared curcumin-loaded ALG/HA dressings were soft, elegant in appearance, non-brittle in nature, and able to maintain their physical integrity once applied to a simulated wound environment. The curcumin release from the dressings showed continuous release for over 7 days, a period suitable for a long-lasting antimicrobial effect.



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great potential as load-bearing materials for biomedical applications.



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# Membranes for endotoxin-retentive filters for the online preparation of ultrapure dialysis fluid and substitution fluid: state of the art

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**Introduction** Poor water treatment and the concentrate powders and solutions used for the preparation of dialysis fluids favor bacterial growth and the production of pyrogens, such as endotoxins, that may cross hemodialysis membranes and get in the blood circulation. When this happens, hemodialysis patients are at risk of acute bacteremia and pyrogenic reactions and long-term complications that put their lives at risk and worsen their quality of life. For this reason, it is recommended that ultrapure dialysis fluid be used for routine hemodialysis. There is also increasing interest for the online production of sterile non-pyrogenic substitution fluid from ultrapure dialysis fluid to render hemodiafiltration treatments effective but also safe, sustainable and accessible to a large cohort of patients. Ultrapure dialysis and substitution fluid are often produced by filtering online standard dialysis fluid through a cascade of bacteria- and endotoxin-retentive filters (ETRFs) upstream from the hemodialyzer. Commercial ETRFs vary for the membranes used, operating principles and mode, performance, and disinfection protocols. In this paper a critical analysis is reported on the most relevant characteristics of the membranes used in commercial ETRFs and how their separation properties are exploited in current module design.

**Experimental methods** Available commercial and scientific literature was analyzed in non-systematic fashion, and some ETRFs were subjectively selected among those commercially offered for the preparation of ultrapure dialysis fluid and non-pyrogenic substitution fluid. The properties of the membranes therein used and their effectiveness in removing bacteria and endotoxins were analyzed in comparison to commercial hemodialysis membranes and based on the ETRFs performance and prospected use.

**Results and discussion** The selected ETRFs are equipped with hollow fiber membranes arranged in similar configuration to hemodialysis modules. The configuration of some ETRFs permit their use in dead-end and/or cross-flow operation. Membranes are generally made of similar polymers and geometry to the membranes used by each manufacturer in its own hemodialyzers, likely to minimize the costs for obtaining permission from regulatory bodies.



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Membrane polymer and wall structure generally enable endotoxin removal by combining sieving and adsorption mechanism, although to varying extent. Only in one case membrane geometry is varied to enhance endotoxin adsorption at the operating conditions imposed by the rate at which ultrapure dialysate and substitution fluid have to be produced in routine hemodialysis and hemodiafiltration, respectively. Membrane surface area in commercial ETRFs varied by one order of magnitude as a function of the specific membrane endotoxin removal effectiveness and the possibility of cleaning and disinfecting the membranes for repeated use.

**Conclusion** The study evidenced the difficulty of easily gathering information on the characteristics of commercial ETRFs. In most cases, the selected commercial ETRFs seem to feature similar membrane material, configuration and membrane surface area to commercial hemodialyzers. Only in a few cases, membrane material and geometry appears to be modified to enhance their endotoxin rejection and adsorption capacity.



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# Complex scaffold geometry by vat photopolymerization for bone regeneration

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**Introduction** The surface properties and the architecture parameters of bone substitutes affect cell adhesion and response, as well as angiogenesis and bone extracellular matrix production (1). Vat photopolymerization technology allows a unique control over the micro- and macro-architecture of bone scaffolds that cannot be achieved with traditional fabrication techniques or other additive manufacturing (AM) technologies (2). Its application in combination with mathematical processing models can boost this technology to its maximum potential and makes available the upgrading of architectures with a high biomimetic structural degree. DS3000 bioresin has been successfully used for the development of surgical planning guides (3), dental impression models (4) and the in vitro modelling of breast cancer bone metastasis (5). Up to now, DS3000 has never been tested for creating a 3D bone regenerative environment.

This work aims to provide a preliminary feasibility study on the combination of an innovative biocompatible resin and vat photopolymerization technology to obtain a bio-mimic scaffold for bone tissue regeneration.

**Experimental methods** Vat Photopolymerized DS3000 bulks were preliminarily produced to compare structural and mechanical behavior with bone tissue performances. Bulk samples were created by developing a grid with porosities of 20  $\mu\text{m}$  and infill of 30  $\mu\text{m}$ . Surface morphology, chemical composition and crystallinity were studied by scanning electron microscope (SEM), energy dispersive spectroscopy (EDS) and X-ray diffraction (XRD), respectively, while bulk density and mechanical test were performed by X-ray computed tomography (XCT). Short-term cytotoxicity and viability tests, with osteoblast-like cells (MG63), were carried out as first check of material-technology biological response. Cell behavior was assessed, via the analysis of metabolic activity, cytoskeleton and ultrastructural studies.

**Results and discussion** Bulk characterization revealed a fully dense sample, with a surface regular pattern, free of harmful elements and semicrystalline. No cytotoxic activity was detected up to 72 hours. Cells adhered quickly to the material showing a good viability



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at 24h, and spread following the surface structure, probably attracted by the capillary effect that porosity created.

**Conclusion** Biological results clearly suggest that the bulk surface pattern promotes cells adhesion, encouraging an increase of the complexity degree from 2D to 3D structure biomimicking bone tissue.

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**Acknowledgments** This study was conducted under the collaboration with Prosilas - Rapid Prototyping, Via Terracini 14 - zona Industriale A, 60212 Civitanova Marche (MC), Italia and PUNTOZERO team, Seconda Strada, 20096 San Felice (MI), Italia.



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# 3D in vitro model of the human gut microbiota and bone tissue crosstalk

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**Introduction** The gut microbiota shares a mutually beneficial relationship with its host, by producing various metabolites that maintain the homeostasis of the gut and contribute to the fitness of the host (1). Recent reports revealed a complex association between the gut and bone health: during intrauterine and early postnatal life, the exposure or restriction to the environmental factors regulates the growth retardation, bone mineralization, and gut microbial composition (2). The intrinsic difficulty to frequently access the human gut for monitoring microbial composition hampered clinical or animal model studies and, therefore, in vitro models represent a valid alternative (3). Attempts have been made in culturing single bacterial strains on electrospun synthetic scaffolds (4), but challenging is the culturing of complex microbial populations as those residing in the gut.

The present work aimed at developing a 3D in vitro model of the human gut microbiota, culturing the microorganisms on an electrospun gelatine structure, for shedding light on the interplay between the composition of the human gut microbiota and bone cells.

**Experimental methods** A gelatin solution crosslinked with (3-Glycidioxypropyl)-trimethoxysilane (GPTMS) was used to fabricate the electrospun structures. Faecal samples were collected following the European guidelines for faecal microbiota transplantation. Human intestinal epithelial cell line Caco-2 and human osteoblast-like cells were used for biological experimentation. Scaffold mechanical and physical properties were assessed. The microbial behaviour within the electrospun gelatine structures was quantitatively and qualitatively analysed by Scanning Electron Microscopy (SEM), real-time qPCR, and Next Generation Sequencing (NGS) techniques. Caco-2 monolayers were treated up to 72 hours with the sterile gut microbiota supernatant; then the supernatant obtained from Caco2 was tested on osteoblasts. Viability and morphofunctional analyses were performed in both cell populations.



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**Results and discussion** Our results revealed the three-dimensionality of the structure-adhered microbial consortia that maintain the bacterial biodiversity of the original sample. Initial data on the effect of the gut microbiota supernatants on Caco2 shows dose-related differences in cell viability.

**Conclusion** Our preliminary data demonstrate the validity of our system for in vitro culturing the human gut microbiota and evaluate its crosstalk bone tissue

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# Smart biopolymers endowed with antimicrobial properties

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**Introduction** Human Elastin-like Polypeptides (HELPS) represent a very interesting stimuli-responsive drug delivery platform. They are artificial bio-inspired polypeptides derived from major extracellular matrix components, and biodegradable without releasing toxic degradative compounds, properties that make them have higher biocompatibility compared to synthetic biomaterials. At the same time, their smart properties, i.e. their response to different environmental stimuli, constitute an advantage during purification, self-assembling and drug release. Within the “Antimicrobial Integrated Methodologies for orthopaedic applications” project (AIMed), taking advantage of the HELP properties, we will develop a range of smart biomaterials with antimicrobial properties that are suitable to be used on the surfaces of orthopaedic implants.

**Experimental methods** Multifunctional HELPS will be developed and assayed for the realization of prototypic devices and coatings capable of smart release of the bioactive domain upon proteolytic stimuli. By the means of DNA recombinant technologies, HELPS will be fused with known antimicrobial domains, expressed in *Escherichia coli* and purified taking advantage of the reverse phase transition properties of the HELPS. They will be characterized by biochemical and biophysical methods and the antimicrobial activity as well as the cytotoxicity of the fusion polypeptides will be assessed. Those fusion products that will show the desired biochemical and antimicrobial properties towards the model microorganisms tested, will be used to develop prototype hydrogel-like coatings.

**Results and discussion** As a result, we expect to realize smart bio-interfaces endowed with antimicrobial activity, that will allow the controlled release of the bioactive domains upon cellular stimuli, like those released by the tissue inflammation process.

**Conclusion** Herein, we develop novel biomaterials with antimicrobial properties suitable for future implant interventions without resulting in new bacterial resistance mechanisms.

**Acknowledgments** This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement AIMed No 861138



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# Tuneable gellan-gum based bioink formulations for 3D printed bone constructs

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## Introduction

Over the past years, gellan-gum (GG) is emerging as a promising noncytotoxic biopolymer for bone tissue engineering (BTE)<sup>1</sup>. GG consists of repeating units of glucose, rhamnose, and glucuronic acid. The secondary structure changes upon the temperature from a random coiled form, at high temperature, to a double-helix form, at room temperature. However, the mechanical performances as well as the stability are the main issues limiting its application as bioink for 3D printing<sup>2</sup>. To this aim, GG was chemically modified in order to add functional moieties and making possible a further crosslinking of the hydrogel by UV light using a photo-initiator. Furthermore, the material was enriched by incorporation of hydroxyapatite nanoparticles (HAp), through sol-gel method, as osteoinductive signal.

## Experimental methods

GG (Gelzan™, Sigma Aldrich) was chemically modified by reaction with methacrylic anhydride to produce a photocrosslinkable derivative (GGMA) with a shear thinning behaviour and tuneable mechanical properties. The materials were fully investigated in terms of physico-chemical properties to validate the success of the functionalization. The bioprinting was performed using a 3D printer "In vivo Rokit" (Rokit Healthcare Inc.). Different formulations of biocomposites based on GGMA (2 and 4 %wt/v) and HAp (10 and 30 %wt referred to GGMA amount), were developed. The morphology of the 3D structures was investigated by SEM, while mechanical properties were assessed by DMA tests. Preliminary cytocompatibility was assessed by using murine fibroblast cell line according to ISO guidelines.

## Results and discussion

The methacrylation of GG was confirmed by the presence of carbon double bond peak at 1640 cm<sup>-1</sup>, as shown by ATR-FTIR spectra. GGMA and GGMA/HAp constructs were successfully 3D printed with a good shape fidelity without collapse in the z-direction. A homogenous porosity with lateral pores clearly visible was confirmed by SEM. Mechanical properties of GGMA/HAp structures showed values of the storage modulus generally higher than GGMA. Furthermore, HAp improved the nanoroughness and bioactivity, showing a



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different cell adhesion and proliferation.

## **Conclusion**

3D well-organized constructs were produced by 3D bioprinting proving the potential use of these materials as bioinks for BTE. Future studies will be focused on the optimization of GGMA-based hydrogels as 3D models for studying bone tissue diseases.

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## **Acknowledgments**

The authors would like to thank PRIN 2017, "SAPIENT - A system approach for identifying connective tissue degeneration in diabetic analogues", Grant N° 2017CBHCWF and Progetto Premiale di AREA SCIENCE PARK "OPEN LAB 2 - A System of Open Research Facilities" for financial support.



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# PEGylated cationic nanoassemblies based on triblock copolymers to combine siRNA therapeutics with anticancer drugs

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**Introduction** Small interfering RNAs (siRNAs) are emerging as innovative nucleic acid medicines for the treatment of incurable diseases such as cancers. In this context, more than 20 RNAi are in clinical trials. Nevertheless, the clinical administration of siRNA therapeutics is still challenging due to the need of safe and efficient delivery carriers. Herein, we propose novel biodegradable NPs based on poly(ethylene glycol) (PEG)- poly(2-dimethyl(aminoethyl) methacrylate) (pDMAEMA)- polycaprolactone (PCL) triblock copolymers with different lengths of the blocks and hydrophilic/lipophilic balance to deliver siRNA alone or in association with a conventional anticancer drug for a potential synergic anticancer therapy. Copolymers were synthesized by a combination of chemical methodologies and characterized by NMR analysis, Fourier Transform Infrared (FTIR) spectroscopy, Gel Permeation Chromatography (GPC) and Differential Scanning Calorimetry (DSC). Copolymers were then employed to prepare NPs through nanoprecipitation. NPs were complexed with a therapeutic siRNA against bIII-tubulin, involved in Multi Drug Resistance mechanisms of different anticancer drugs (TUB-siRNA), and loaded with Docetaxel (DTX). Colloidal properties at pH 7.4 (cytoplasmic pH) and 5.5 (endosomal pH) and buffer capacity of NPS were assessed. Release studies of DTX and siRNA were performed. The transfection efficiency, cytotoxicity and efficiency in silencing TUB gene expression of NPs was evaluated in human melanoma cells (A375).

**Experimental methods** Poly(ethylene glycol) methyl ether (mPEG2k and mPEG5k),  $\epsilon$ -caprolactone ( $\epsilon$ -CL), 2-(dimethylamino)ethyl methacrylate (DMAEMA) and all reagents were purchased from Sigma-Aldrich. DTX was purchased from Alfa Aesar (Germany). A Silencer™ Negative Control and TUB-siRNA were provided by Eurogentec (UK). Release of DTX was assessed at pH 7.4 (PBS) and 37°C in a dialysis bag. TUB-siRNA release studies were performed on NPs dispersed in PBS at pH 7.4 and 37°C. A375 cell line (ATCC, Italy) was cultured in Dulbecco's Modified Eagle's Medium. Cytotoxicity effect was assessed through MTT assay. Total RNA was extracted from A375 cells and quantified on a ND-8000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). qPCR analysis was carried out using SensiFAST SYBER® No-ROX kit (Bioline, London, UK).



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**Results and discussion** PEG-pDMAEMA-PCL triblock copolymers were prepared through a multistep technique: a) conversion of mPEG-OH to  $\omega$ -bromoisobutyrate macroinitiator (mPEG-Br); b) atom transfer radical polymerization (ATRP) of DMAEMA from mPEG-Br; c) end-group conversion from bromine to azide ( $-N_3$ ) of mPEG-pDMAEMA-Br diblock copolymer; d) ring opening polymerization (ROP) of  $\epsilon$ -CL initiated by  $\alpha$ -butynyl-1-ol to form alkyne-terminated PCL (butynyl-PCL); e) conjugation of mPEG-pDMAEMA-N<sub>3</sub> to butynyl-PCL through azide-alkyne Cu(I) catalysed click reaction. Different combination of blocks molecular weights were studied and NPs obtained by copolymers with a long PCL block (4000 Da) were selected on the basis of their greater stability and PEG surface exposure. In particular, copolymers with short length of PEG and PDMAEMA blocks and long PCL block length (SSL) gave NPs characterized by higher buffer capacity and stronger interaction with siRNA. SSL-NPs showed a sustained release of DTX as well as of siRNA within 48 h and an efficiency in transfection similar to Lipofectamine. The *in vitro* cytotoxicity of NPs loaded with DTX, with or without TUB-siRNA, was evaluated in A375 cell line. Results showed that the co-delivery of TUB-siRNA and DTX appears to potentiate the anti-proliferative activity of DTX, especially at DTX lower doses. Finally, the NPs delivering TUB-siRNA and DTX are highly efficient in silencing TUB gene expression.

**Conclusion** Herein, we have reported the development of novel biodegradable NPs based on mPEG-pDMAEMA-PCL triblock copolymers and demonstrated how, through the proper modulation of the copolymer block length and its hydrophilic/hydrophobic balance, we can have core-shell nanostructures able to condense with siRNA with high efficiency and eventually deliver a second anticancer drug for a dual therapy.



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# OSTEOGENIC DIFFERENTIATION CAN BE INDUCED BY CONCENTRATED GROWTH FACTORS (CGF) IN HUMAN BONE MARROW STEM CELLS

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**Introduction** In the field of regenerative medicine and particularly in the bone regeneration area there is a growing interest in identifying new effective strategies for inducing osteogenesis, in order to speed up the process as well as to avoid potential risks for the patient. A new autologous biological matrix, the Concentrated Growth Factor (CGF) is a blood derived product, obtained by centrifugation of venous blood using alternating speed rate. CGF releases several growth factors involved in proliferation and extracellular matrix mineralization.

In this study the ability of CGF to induce osteogenic differentiation of human Bone Marrow Stromal Cells (hBMSC) *in vitro* has been investigated

**Experimental methods** Blood samples were taken via venipuncture from donors in good general health and then processed by a device (Medifuge MF200; Silfradent srl, Forlì, Italy), following the manufacturer's instructions. Informed consents were obtained from the donors. In all the experiments CGF, as it is, was placed directly into the cell dishes.

The hBMSC were cultured with MSC Basal Medium (BM), or Osteogenic Medium (OM), or BM+CGF (CGF) for 14 days for the analysis of Alkaline phosphatase (ALP) activity or for 21 days for all the other analyses. ALP activity was measured by using an ALP assay kit (MyBioSource) according to the manufacturer's instructions. Alizarin red S stain (Sigma) was performed as described in [1]. For the Real-Time PCR analysis, total RNA was extracted from cells grown in culture dishes and then the reverse transcriptase reaction was carried out. For the Western-blot analysis, proteins were determined using the Bio-Rad protein assay kit. The subcellular localization of RUNX2 was evaluated by immunofluorescence assay.

**Results and discussion** The osteogenic differentiation efficiency induced by CGF was analyzed measuring the ALP activity after 14 days of treatment, being ALP an early



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osteogenic marker. In hBMSC cultured in OM, ALP enzymatic activity enhanced by about 50% with respect to BM, whereas CGF treatment induced an increment higher than 70% with respect to CTR.

The matrix mineralization of hBMSC was revealed by Alizarin Red Staining (ARS). After 21 days the staining was not evident in BM-treated hBMSC, whereas it was significantly observable in OM-treated cells. The treatment with CGF induced cell morphology modifications in hBMSC, although a very low red staining was detected. In hBMSC the matrix mineralization requires the addition of substrates, such as  $\beta$ -glycerophosphate (BGP) and ascorbic acid 2-phosphate (AA); in fact, CGF+BGP+AA strongly increased ARS.

In stem cells the decrease of surface markers such as CD90 and CD105 has been reported as a differentiation signal. To clarify if CGF could determine hBMSC differentiation, quantification of CD90 and CD105 protein contents was carried out by Western blotting. The results showed that CGF abolished the expression of both CD90 and CD105 proteins, while OM treatment only reduced CD90 and CD105 by about 40% and 50%, respectively.

To evaluate the effects of CGF on hBMSC osteogenic differentiation, the mRNA abundance of RUNX2, the transcription factor key regulator of osteogenesis, of COL1a1 and of OCN, extracellular matrix proteins used as osteogenic differentiation markers, was quantified. The treatment with CGF and CGF+BGP+AA markedly augmented the mRNA abundance of RUNX2 and OCN with respect to CTR. RUNX2 and OCN mRNA levels significantly increased in cells incubated in OM as well, with respect to CTR. Instead, all the treatments induced a statistically significant reduction of COL1a1 mRNA abundance, when compared to CTR.

The effect of CGF on the proteins involved in osteogenic differentiation was also evaluated by the quantification of the protein content of RUNX2 and COL1a1. In hBMSC, CGF determined a very strong increase of both RUNX2 and mature COL1a1 (mCOL1a1) protein levels when compared to the untreated CTR cells. OM increased both RUNX2 and mCOL1a1, as well, when compared to CTR. The increment induced by the CGF treatment was remarkably higher than that one caused by the OM on the same protein.

The subcellular localization of RUNX2 was assessed by immunofluorescence. RUNX2 was expressed in the cytosol of hBMSC grown both in BM and in presence of CGF, whereas in the cells cultured in OM it was localized in the nucleus.

**Conclusion** The treatment with CGF stimulated ALP activity and promoted matrix mineralization compared to control and seems to be more effective than the osteogenic medium alone. Also, after CGF treatment, hBMSC lost mesenchymal markers and showed other osteogenic features. Our study showed for the first time that CGF alone is able to induce osteogenic differentiation in hBMSC. The application of CGF on hBMSC osteoinduction might offer new clinical and biotechnological strategies in the tissue regeneration field.

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# A systematic study of the dependence of biochemical and biophysical properties of hyaluronan solutions on molecular size and concentration in a wide range of clinical interest

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**Introduction** In nutraceutical, cosmetical and biomedical applications, hyaluronic acid (HA) is extensively used in relation to its adhesive properties, flowing behavior injectability and resistance to mechanical stress. It has been shown that molecular weight (Mw) and concentration of the biopolymer affect formulation's features such as dynamic viscosity and sensitivity to degradation, that are key to clinical performances. Studies aiming at establishing the correlation between HA hydrodynamic parameters and concentration and the biophysical aspects of the formulations are, therefore, of great scientific, industrial and clinical interest.

**Experimental methods** We involved 9 molecular weight distributions of pharma grade HA with Mw ranging from the lowest (about 50kDa) to the highest (about 2500kDa) and 13 different concentration values (from 0.1mg/mL to 32mg/mL).

A complete hydrodynamic characterization was provided for each HA employed, using a Size Exclusion Chromatography-Triple Detector Array (SEC-TDA) system, and all rheological measurements were performed using a rotational rheometer. Stability to enzymatic degradation was investigated by incubating HA with Bovine testicular hyaluronidase (BTH).

**Results and discussion** Zero shear viscosity was correlated to concentration highlighting a different behavior and trend (equation parameters and interpolated curves) depending on both Mw and concentration. Switching points between diverse regimes were clarified, finding a lower concentration threshold for higher Mw families. In addition, degradation due to enzymatic mechanisms were explored showing a lower degradative ability for bovine testicular hyaluronidase on samples with Mw below 100 kDa.

**Conclusion** The comprehensive approach reported permitted to derive functional equations to predict either viscosity or stability in relation to defined Mw of pharma grade hyaluronan.

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# EXFOLIATED BLACK PHOSPHORUS AS THERAPEUTIC NANOMATERIAL ON 3D IN VITRO OSTEOSARCOMA MODEL

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**Introduction** Bone cancers derive from primitive mesenchymal cells including osteosarcoma (OS) and occur frequently in children, adolescents, and young adults aging 15 to 29 years. OS is one of the most frequent morphological subtypes of bone cancers presenting a worldwide distribution of about 20 to 40% of all bone tumors. Despite improvements in treatment, children and adolescents with bone cancers still present a high mortality [1]. Consequently, the development of novel effective strategies for OS treatment represents a crucial challenge. In this context, anticancer nanomaterials such as exfoliated black phosphorus (2D BP) nanosheets injected in tumor site may be a potentially strong tool to achieve this aim. Here, we have developed a 3D OS in vitro model to evaluate the efficacy of injectable 2D BP.

**Experimental methods** 2D BP nanosheets were obtained using liquid exfoliation process of BP microcrystals and dissolved in H<sub>2</sub>O/(DMSO) solution. To test 2D BP purity and morphological features, Inductively coupled plasma mass spectrometry (ICP-MS) analysis and scanning electron microscopy (SEM) were carried out. In addition, biological investigations were performed by injecting 2D BP solution at different concentration (5-75 µg/mL) on in vitro 3D OS tumor model, obtained by culturing Saos-2 cells on Matrigel® as support for 6 days. To investigate antiproliferative effects of 2D BP, cell proliferation was evaluated through Alamar blue assay and Ki-67 expression by confocal analysis after 3 days of 2D BP exposure. Furthermore, cytological analysis was carried out by hematoxylin-eosin staining.

**Results and discussion** In vitro 3D model demonstrated that 2D BP solution is able to inhibit in higher percentage the tumor cell proliferation (Saos-2) already at 25 µg/mL. This data were confirmed by the inhibition of Ki-67 expression in 3D structures treated by 2D BP 25 µg/mL. Finally, results obtained using hematoxylin-eosin staining showed that 2D BP treatment for 3 days destroyed 3D in vitro tumor structure.

**Conclusion** Nanostructured 2D BP is able to inhibit OS cancer cell survival in in vitro



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tumor model better mimicking in vivo microenvironment, thus suggesting the opportunity to develop innovative therapeutic protocols based on 2D BP injection in tumor site of patients affected by OS.

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**Acknowledgments** This study was supported through funds provided by Progetto MIUR PRIN2017 - ACTION, Grant N. 2017SZ5WZB. The authors also thank Mrs. Cristina Del Barone of LAMEST laboratory for SEM investigations, Mariarosaria Bonetti for lab technical support & data elaboration and Dr. Roberta Marzella for support to project management.



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# Addition of bioinsecticide *Beauveria bassiana* to biomimetic luring substrates for tiger mosquito control

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**Introduction** Tiger mosquito (*Aedes albopictus*) is a very difficult to control vector of extremely serious pathologies for humans. Lethal ovitraps and *Beauveria bassiana* (a bioinsecticide fungus) could be an effective low environmental impact control method but actually are limited in use due to low cost/effectiveness. The work proposes to control tiger mosquitoes imitating an ideal oviposition microenvironment on a biocompatible biopolymer-based substrate, to integrate it with *Beauveria bassiana*, and to use it in lethal ovitraps. The substrate could improve ovitraps cost/effectiveness and promote employment of bioinsecticide by acting as matrix for survival, growth and delivery of the fungus.

**Experimental methods** A macromolecular hydrogels was tested for tiger mosquito in oviposition assay (in lab and field conditions) and then was evaluated as matrix for the survival, growth (also before and after freeze-drying) and delivery of *Beauveria bassiana* (Bb). Furthermore, the effectiveness of Bb-Gel system was tested on *Aedes albopictus* eggs through hatching tests.

**Results and discussion** Trials proved that a cellulose based physical hydrogel shows a superior efficacy in terms of reached eggs and lasting (up to 30 days vs few days) compared to common ovitraps and standard substrates Masonite and absorbing paper (also on field). Tests verified the biocompatibility between hydrogel and *Beauveria bassiana*. Furthermore, the lethality of the matrix (due to fungal and material action on the eggs and larvae) was verified.

**Conclusion** It is possible to produce a cellulose based physical hydrogel acting both as oviposition substrate (based on biomimetic lure) and as biocompatible matrix *Beauveria bassiana*.



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# Superabsorbent hydrogel reverses high fat diet induced intestinal damage and slows progression of hepatic steatosis in DIO mice

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**Introduction** Obesity, insulin resistance and disrupted gut homeostasis are major contributors to the pathophysiology of NAFLD and its progression to NASH. Therapies exploiting the gut liver axis may offer a unique treatment option for metabolic liver disorders. A novel orally administered hydrogel platform using crosslinked modified cellulose, was developed by Gelesis to restore gut barrier function and has been shown to protect against the deleterious effects of a high fat diet (HFD) in a mouse model of NAFLD. Here, we examined the potential therapeutic effects of hydrogel administration in DIO mice with established hepatic steatosis.

**Experimental methods** C57BL/6J wild type mice were fed HFD (45% lard) for 12 weeks. From week 12 to 24, mice were treated with either HFD alone (n20), HFD plus hydrogel 2% (n18) or 4% (n18). In addition, a control group (n21) remained on chow alone. At 4 and 12 weeks of treatment, changes in body weight and EAT size were recorded. Intestinal barrier integrity was evaluated using a FITC dextran permeability assay and expression of zonula occludens1 (ZO-1). Liver triglyceride (TG) accumulation was graded using a semi-quantitative scoring system on Oil red O stained samples.

**Results and discussion** High-fat feeding for 12 weeks led to significantly greater body weight ( $p=0.03$ ), larger adipocytes ( $p=0.0001$ ), intestinal atrophy ( $p=0.007$ ), fatty liver, increased intestinal permeability and reduced ZO-1 expression ( $p=0.0084$ ) compared to controls. After 12 weeks of hydrogel 2 or 4% treatment, body weight and adipocyte size were significantly reduced compared to mice continuously fed HFD (body weight  $p0.02$  for 2% hydrogel and  $p<0.0001$  for 4% hydrogel; adipocyte size  $p=0.0001$  for both 2% and 4% Hydrogel). Hydrogel treatment prevented intestinal atrophy induced by high fat diet, driven by changes in small intestine length (Hydrogel 2%  $p=0.0017$ ; 4%  $p<0.0001$  by 12 weeks). A reduction in serum FITC dextran was found in Hydrogel groups compared to HFD at 12



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weeks (Hydrogel 2%  $p=0.0025$ ; 4%  $p<0.0001$ ), and was associated with an upregulation of intestinal ZO-1 expression in both Hydrogel groups at 4 weeks (2%  $p=0.0052$ ; 4%  $p=0.0003$ ), though not significant at 12 weeks (2%  $p=0.1385$ ; 4%  $p=0.0803$ ). Hepatic TGs accumulation was hampered by hydrogel 4%, as 5/10 HFD mice had greater than grade 3 accumulation, compared to 2/10 in hydrogel 4%.

**Conclusion** We showed that in mice with established fatty liver and intestinal damage, due to prolonged high fat feeding, both doses of superabsorbent hydrogel were able to restore intestinal barrier function and slow accumulation of lipids in the liver.



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# Beta-glucan and protein mixtures extracted from barley and filled with ZnO to develop bioactive wound dressings

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**Introduction** Barley is one of the most produced and cultivated cereal crops worldwide. Its grains are mainly composed by proteins and polysaccharides, in particular  $\beta$ -glucans that are linear polysaccharides showing hypocholesterolemic and hypoglycemic activity and having interesting antimicrobial and wound healing properties. However, the process of extraction and purification for isolating  $\beta$ -glucans from the other components is complex, expensive and may cause polymer chain degradations. In light of this, the aim of this work was the development of low-cost simultaneous extractions of proteins and polysaccharides from barley by-products, to prevent polymer degradation. It is well known that ZnO is widely used to prevent or treat topical and systemic diseases, due to its antimicrobial activity. Therefore, films based on different polysaccharide and protein extracted mixtures were prepared with and without ZnO to get cost-effective bioactive wound dressings.

**Experimental methods** Extraction of polysaccharide and protein mixtures from barley grains was performed at different pH, in particular in mild (MA) and high alkaline (HA) conditions. The water soluble obtained extracts were recovered by centrifuging and films without and with different amount of ZnO (0.5, 1.0, and 2.0 % w/v) were prepared by casting at room temperature. Films were characterized by means of FT-IR spectroscopy, Scanning Electron Microscopy (SEM), thermogravimetric analysis (TGA), Differential Scanning Calorimetry (DSC), and tensile properties in order to evaluate the effect of the extraction conditions and of the presence of zinc particles on film properties. The cytotoxicity and the antibacterial activity against *Staphylococcus epidermidis* and *Escherichia coli*. of films were investigated. A qualitative test was carried out to evaluate whether the films are safe for direct contact with human skin.

**Results and discussion** The pH conditions of extraction strictly influenced the composition of the extracted mixture and the chain-to-chain physical interactions among proteins and polysaccharides, as evidenced by FT-IR, DSC and TGA measurements. As a matter of fact, a higher Tg, a higher elastic modulus, a lower deformability were found for HA films, suggesting that a more compact polymer network was obtained when the



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extraction was carried out in high alkaline media. The presence of ZnO differently affected the mechanical properties. In MA samples, the elastic modulus and tensile strength decreased and the elongation at break slightly increased with ZnO amount, probably due to the plasticizing effect of water molecules bound to zinc particles. Conversely, films obtained in high alkaline conditions showed an elastic modulus decrease and a strength and elongation at break increase up to a ZnO concentration of 1 wt%. Finally, all the films show lack of cytotoxicity and have intrinsic antimicrobial properties that were enhanced by the addition of an antimicrobial agent such as ZnO.

**Conclusion** Bioactive films were obtained by casting from protein and polysaccharide mixtures obtained by a low-cost extraction process. The mechanical properties together with permeability, the lack of cytotoxicity and the intrinsic antimicrobial activity make the investigated films promising candidates for wound healing applications.



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# In vitro antioxidant effect of an arbutin-loaded coating on bone progenitor cells

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**Introduction** Bone prosthesis implantation can lead to excessive production of Reactive Oxygen Species (ROS) that, causing oxidative stress, has consequences on the osseointegration and implant success (1). Arbutin is a plant-derived glycosylated hydroquinone with antimicrobial and antioxidant features, with potential usage in several medical applications. (2)

In vitro, Arbutin is capable to reduce oxidative injury induced by hydrogen peroxide on cultured retinal ganglion cells (3), implicated in proliferation and differentiation of osteoblasts (4), and inhibits osteoclast differentiation, by suppressing RANK-L-mediated superoxide, one of the main sources of ROS in osteoporotic tissues (5).

This study aimed to explore the in vitro protective role of Arbutin in periosteum-derived progenitor cells (PDPCs) seeded on a polyacrylate-based system designed to in situ release an effective concentration of Arbutin.

**Experimental methods** An innovative poly(ethylene-glycol diacrylate)-co-acrylic acid (PEGDA-AA) copolymer coating containing Arbutin was electrosynthesized on titanium sheets, investigating physicochemical and morphological features to achieve an optimized system (6). PDPCs were cultured on the PEGDA-AA-Arbutin system and exposed to oxidative stress induced by hydrogen peroxide. Cell viability and morphology were evaluated by MTT assay and immunofluorescence, respectively. In PDPCs cultured in a differentiative medium, osteogenic markers were analyzed by RT PCR and Western Blotting.

**Results and discussion** Morphological evaluations highlighted the good compatibility and the reparative effect of the Arbutin-loaded coating. A restoration of viability in cells cultured in oxidative conditions was observed, confirming the antioxidant role of Arbutin in bone cells.

We also showed that the PEGDA-AA-Arbutin system stimulated the expression of differentiation markers in PDPCs, even under oxidative stress. Our results indicated that cells exposed to oxidative stress were protected by Arbutin, which preserved both cell



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viability and differentiation capability.

**Conclusion** Taken together, these results open interesting prospects for the further development of natural bioactive coatings for orthopedic titanium implants, capable of restore non-oxidative conditions after prosthesis implantation.

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# Design of hyaluronic-based scaffolds with anisotropic pore architecture

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**Introduction** The aim of this work was the development and characterization of biomimetic scaffolds based on hyaluronic acid with axially oriented pore channels for nerve regeneration. Matrices with elongated microstructure, suitably oriented porosity and controlled degradation rate have the potential to improve the regeneration of peripheral nerves and spinal cord by physically supporting and guiding the growth of neural structures across the site of injury [1]. Highly porous and functionalized tubular scaffolds were successfully produced using methacrylate and photo-crosslinked hyaluronic acid. In addition, to create axially oriented pore channels, scaffold manufacturing technique based on unidirectional freezing and subsequent freeze-drying was optimized.

**Experimental methods** . Hyaluronic acid (HA) was methacrylated by means of a glycidyl methacrylate (GDM) grafting reaction, with an excess of GDM equal to 10 with respect to the disaccharide unit [2]. Next, a few photocrosslinked hydrogels were synthesized by exposing 26 mg/ml methacrylate HA (HAmE) to UV light in the presence of increasing concentrations of the photoinitiator Irgacure 2959 (1%, 2% and 3% wt), in order to obtain different photo-crosslinked HAmE hydrogels. To create axially oriented pore channels, the optimized HAmE solution resulting by rheological tests (HAmE 26mg/ml + 3 % wt PhI) was freeze-dried using three different strategies. They distinguish for the methods of preparation of support plates, before inserting the gel into the tube, in: Full ice (the cavities between guide tubes were completely filled with water and frozen), ice bottom (cavities filled only for 1/3 of their height) and no ice (water added after filling of tubes with gel). In addition, three HAmE concentrations (26, 35, 50 mg/ml) and three different sequences of photo-crosslinking and freeze phase were studied: (i) photo-crosslinked of HAmE, filled tube, cavities filled with water, frozen and freeze-drying; (ii) filled tube, cavities filled with water, frozen and freeze-drying, UV exposure of scaffold; (iii) filled tube, cavities filled with water, frozen, UV exposure and freeze-drying. All scaffolds produced were observed by SEM and the evaluation of the weight loss % in PBS at 37°C up to 28 days of soaking was carried out.

**Results and discussion** The metacrylated and photo-crosslinked hyaluronic acid hydrogel was optimized and characterized. By assessing the variation of photo initiator concentration, the best rheological properties were achieved by hydrogel produced with PhI at 3% by weight. Starting from these results, the optimized solution based on HAmE 26 mg/ml + PhI 3% was used for the production of tubular nerve scaffold by freeze-drying



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technique. From different strategies of freeze-drying tested, scaffolds with elongated and suitable porosity have been obtained using strategy called NO ICE, in which water at room temperature is placed around the material. The use of material in which photo-crosslinking was interposed between two successive stages of freezing and the control of temperature gradients during freezing, allows the columnar growth of crystals thus obtaining a structure totally oriented and aligned according to the longitudinal axis of the scaffold. The scaffolds produced using method described above and with three different HAme concentrations were tested up to 28 days of soaking in PBS. The scaffolds HAme 26 mg/ml and HAme 35 mg/ml showed loss of structure and swelling after 1 day compared to scaffold HAme 50 mg/ml, which presented good stability.

**Conclusion** Highly porous and functionalized scaffolds were successfully produced using methacrylate and photo-crosslinked hyaluronic acid, using tubular guides and water at room temperature as a means of controlling the columnar growth of ice crystals within the hydrogels, which subjected to the freeze-drying process left room for an engineered scaffold. In order to mediate between the high concentration of methacrylate hyaluronic acid, necessary for stabilizing the support in a physiological environment, and the growth of columnar crystals, an alternative method of production has been developed and optimized.

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# Collagen-Hydroxyapatite composite doped with magnesium and silicon for hard tissue repair

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**Introduction** The bioceramic scaffold composed of pure hydroxyapatite (HA) and doped HA with osteoinductive ions like magnesium (Mg) or silicon (Si) are widely used for bone tissue regeneration. Many works have reported the co-doping with both ions in the crystal structure of HA [1], whereas in this work we doped  $MgO^{2+}$  and  $SiO_4^{-4}$  ions separately during powder synthesis and made composite scaffolds by mixing the doped HA powders. Subsequently, to increase the bioactivity, these scaffolds were impregnated with a collagen matrix and freeze-dried, using a new approach that led to the formation of open pores on composite scaffolds surface. The bioceramic scaffolds and composite scaffolds impregnated with Collagen were evaluated for physical, mechanical, biodegradation and biocompatible properties.

**Experimental methods** Pure and substituted (Mg and Si) HA were synthesized by aqueous precipitation reaction. Scaffolds were prepared by polyurethane sponge replica method using a slurries with HA-Mg/HA-Si calcinated powder (weight ratio kept 50/50). The ceramic scaffolds obtained were impregnated with collagen type I slurry (0.5 % w/v). The innovative freeze-drying approach (scaffolds were placed on ice plates, immersed in cold distilled water  $\sim 0^\circ C$ , cooled to  $-20^\circ C$  and then lyophilized) and DHT crosslinking ( $121^\circ C$  for 24h under vacuum) were carried out. The ceramic powders and scaffolds were analyzed by TEM and XRD. For all were evaluated the morphology (SEM) mechanical properties, biostability in Tris-HCl, pH 7.4 up 28 days and citocompatibility using Human Bone Marrow-Derived Mesenchymal Stem Cells (BMSC).

**Results and discussion** The XRD analysis on powders and scaffolds after calcination and sintering processes revealed the presence a secondary  $\beta$ -TCP in HA-Mg and HA-MgSi, absent in the other ones. All ceramic scaffolds presented Mg and Si ions homogeneously distributed (XRF analysis), porosity  $>90\%$  and pore size ranging between 200- 850  $\mu m$ . TEM analysis and microstructural analysis (SEM) of powders and scaffolds HA-Si showed littler grain size compared to the others ones. This decrease seems positively influence the mechanical properties of ceramic scaffolds. Indeed, HA-Si and HA-MgSi scaffolds revealed better compression trends compared to HA and HA-Mg. This trend was observed also after 28 days of soaking in TRIS, despite the overall decrease observed for all scaffolds. To increase the



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bioactivity, the bioceramic scaffolds are impregnated with collagen using a new freeze-drying approach studied firstly in this work. SEM analysis showed scaffolds with more open surface porosity compared to tradition freeze-drying. This new approach avoids the formation of collagen skin layer, thanks the formation of ice crystals on the scaffolds surface that lead to have open pores on the outer layer of the scaffold. The mechanical tests on bioactive scaffolds shown the same trend observed for ceramic ones but with a considerable decrease of  $\sigma_{max}$ , probably due to the poor compressive strength of type I collagen fibrils. The HA-Si\_Coll and HA-MgSi\_Coll scaffolds showed better mechanical resistance before and after immersion in physiological solution. In particular, for both scaffolds after a week of soaking, the loss of the collagen component from the scaffolds made the ceramic component prevail, thus leading to an increase in  $\sigma_{max}$  with values similar to those observed for the corresponding ceramic ones. Cells proliferation experiment showed that all scaffolds where biocompatible and composite scaffold (HA-MgSi\_Coll) is more effective for cells adhesion and proliferation compared to all other scaffolds.

**Conclusion** In this study, bioactive scaffolds with a composite structure made with a new ceramic mix (HA-MgSi) and collagen were fabricated successfully using a new freeze-drying approach. Firstly, bioceramic scaffolds produced with the new blend of HA doped were fabricated. These scaffolds showed improved mechanical properties also up 28 days of soaking in physiological conditions and good biocompatibility compared to HA, HA-Mg and HA-Si scaffolds In addition, to improve the bioactivity, the optimized HA-MgSi scaffolds were impregnated with collagen using a new freeze-drying approach that allow having open pores on surface. The presence of collagen and open pores have in fact promotes better cells proliferation than corresponded bioceramic scaffolds.

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# Carbon dots as a promising biomaterial for biological applications

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## Introduction

In the last decades nanotechnology became decisively in the biomedical research and carbon-based nanomaterials are among those most promising in a plethora of bioapplications [1]. In particular, Carbon Dots (CDs) that are the youngest member of this family are quasi-spherical carbonaceous nanoparticles less than 20 to 60 nm with an irregular surface rich of polar functional groups that confers intriguing chemical and mechanical properties [2]. Aqueous disperdibility, brilliant photoluminescence and good biocompatibility together with easy synthetic procedures make these nanomaterials appealing systems for multiple biological applications [3] and biocompatible platform.

## Experimental methods

In this work CDs, prepared according to Sawalha et al method [3] from olive solid waste (recovered from an oil mill in Puglia, Italy), have been tested for their biocompatibility on different cell lines and for antibacterial activity on a Gram-positive and a Gram-negative bacterial strain.

## Results and discussion

Inspired from the natural source, we choose to assess the biological behaviour of sustainable Carbon dots, prepared recycling the discard of the olive oil production. We found excellent biocompatibility in many human cellular lines and antibacterial activity that set the base for the realization of new CDs-based nanostructured biomaterials.

## Conclusion

*In vitro* results of this study, obtained with different human cellular lines and bacterial strains, demonstrate that Carbon dots, prepared from olive solid waste, could be promising candidates for the development of new nanostructured materials for biological applications. Further experiments will be performed for the realization of new CDs-based biocompatible scaffolds against antibiotic resistance promising also for tissue engineering.

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# Identification and quantification of potential degradation products from polymeric medical devices according to EN ISO 10993-13:2010

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**Introduction** The present study is addressed to a chemical-physical comparison of newly designed Gelesis Medical Devices with previously developed devices, which have documented history of biocompatibility testing and/or clinical use in humans with accepted risk profile, for the toxicological risk assessment described in Annex B of EN ISO 10993-1:2018. As recommended by the standard, the presence and nature of degradation products has been characterized in accordance with EN ISO 10993-13:2010. Gelesis medical devices under examination are Sodium Carboxymethylcellulose (CMC-Na) based hydrogels, thermal x-linked by citric acid, with slightly different physical properties and addressed to different intended uses e.g., weight management, glycemic control, NAFLD, constipation.

**Experimental methods** The new medical devices under examination, MD3 and MD4, were compared to MD1 and MD2 with a well-known risk profile, by following three analytical steps.

Chemical characterization of raw materials was the first step for the comparison of hydrogels. In particular chemical composition of CMC-Na was verified by Fourier-Transform Infrared Spectroscopy (FTIR) according to USP <197A> compared to a standard. Molecular Weight Distribution was investigated by Size Exclusion Chromatography (SEC) according to pullulans calibration curve, viscosity determination by using Brookfield apparatus and Differential Scanning Calorimetry (DSC). The second step was the comparison of the final properties of the hydrogels, including absorption capacity (MUR) in simulated gastric fluid and G' determination by rheological analysis. The third analytical step was the analysis of the degradation residues under accelerated condition (6.25 mg/ml @70°C for 48h at pH 2.1). The degradation products were examined by FTIR analysis to prove the chemical equivalence after degradation and SEC to calculate the MWD of the residuals.

**Results and discussion** The most relevant results for the first step of characterization were the FTIR analyses on the CMC-Na of MD1, MD2, MD3 and MD4 which revealed a good



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chemical equivalence, being the comparative indices respectively 0.993, 0.992, 0.986, 0.986 relative to the standard. The second analytical step showed some differences in the properties of the hydrogels i.e., MD1 recorded the lower  $G'$  (1043 Pa) and the higher MUR (84 g/g). Whereas MD2 ( $G'$ =2132 Pa; MUR=71 g/g), MD3 ( $G'$ =2265 Pa; MUR=66 g/g), MD4 ( $G'$ =2248 Pa; MUR=62 g/g) showed no significant differences.

The third step examined the degradation residues under accelerated conditions, revealing an important degradation of CMC-Na. The MWD of CMC-Na residuals obtained by SEC, showed a loss of  $M_w$  compared to the initial condition of the 64% for MD1, 60% for MD2, 49% for MD3 and 54% for MD4.

**Conclusion** MD3 and MD4 were comparable to the references MD1 and MD2 for both raw materials and residuals. The FTIR spectra were completely overlapped, SEC analysis showed a decrease of  $M_w$  after acidic degradation due to polymer chains cleavage, for all the devices. When slight differences were observed, they remained always in the range between MD1 and MD2. This activity provided chemical data suitable for the toxicological assessment of the devices MD3 and MD4.

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# Tailored synthesis and functionalization of nanoparticles for biomedical approaches.

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**Introduction** Colloidal-particle-based materials combine a large surface-to-volume ratio with excellent control over building block size, shape and biochemical functionalization. The interactions of colloidal particles with their local environment are dominated by the chemical organisation on their interface. This provides unique opportunities to develop particle-based sensors.

For the use of nanomaterials in biomedical approaches, the protocol optimized to obtain highly monodispersed, stable and well-defined nanoparticles is expected. Providing precisely engineered and highly characterised fluorescent Silica (SiO<sub>2</sub>), gold (AuNP) and gold core with silica shell (Au@Si) nanoparticles. That means the study of the number and distribution of active sites deduced from super-resolution images to modify chemical conditions such as pH, ionic strength and buffer composition.

**Experimental methods** For the synthesis of fluorescent silica (SiO<sub>2</sub>) nanoparticles with a narrow size-distribution (<5-10%), the reverse microemulsion technique was performed to obtain three different particle sizes: 25, 50 and 100 n.m. In the case of Gold (AuNP) nanoparticles, a reflux system was used. Once the techniques have been optimized, the synthesis of core-shell (Au@Si) nanoparticles will be carried out.

It is intended to attach functional groups (amines, carboxyls, etc) on the nanosystems surface to study the active sites by dye-labelling and stochastic super-resolution imaging (STORM). And later, functionalise the particles with single-stranded DNA to quantify the organisation of strands using DNA-PAINT technique.

**Results and discussion** Making use of super-resolution characterization techniques it is possible to analyze the surface chemistry by modifying the parameters in the synthesis and functionalization of the nanosystems. It is expected to obtain well-defined fluorescent Silica (SiO<sub>2</sub>), gold (AuNP) and gold core with silica shell (Au@Si) nanoparticles for subsequent live study and future biomedical applications such as bioimaging, biosensors, drug delivery, etc.

**Conclusion** The use of biomolecules to functionalize the surface of colloidal nanoparticles



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is a widely known methodology for their use in biomedical applications as drug delivery, biomarkers, pathogens binding and now, increasing the possibilities as potential diagnostic and therapeutic systems. Quantifying and controlling the number and distribution of active sites while minimizing particle-to-particle differences is of fundamental importance for the development of responsive and super-selective nanosystems.

**Acknowledgments** Project 860914 financed through Maria Skłodowska Curie, Innovative Training Networks. Call: H2020-MSCA-ITN-2019



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# Exfoliated black phosphorus guides in vitro healthy and cancer bone cell behaviors

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**Introduction** Currently, osteosarcoma is the most common bone cancer, which mainly affects young people. Surgical resection of tumor followed by chemotherapy for micro-metastasis inhibition constitutes the current standard procedure. In recent years, several studies have focused the attention on Photodynamic Therapy (PDT) or Photothermal treatment (PTT) as minimally invasive therapeutic procedure that can apply a selective cytotoxic activity toward cancer cells [1]. In this context, we propose the use of 2D photothermal transducing agent based on few-layer black phosphorous (2D BP) as an alternative tool for osteosarcoma treatment and report how 2D BP can inhibit cancer cell proliferation and simultaneously to stimulate newly forming bone tissue generation after osteosarcoma resection without PTT.

**Experimental methods** 2D BP was obtained by liquid exfoliation process of BP microcrystals. The purity was ascertained by Inductively coupled plasma mass spectrometry (ICP-MS) analysis, the morphological characterization was carried out by transmission electron microscopy (TEM). In vitro 2D model was used to investigate the selective effect of 2D BP on human healthy (HOb) and cancer cells (Saos-2), with and w/o PTT, by analyzing cell proliferation, oxidative stress (Reactive oxygen species- ROS, nitrites levels) and osteogenic differentiation (ALP activity) at day 3 of cell culture. Cell morphology was evaluated by SEM and confocal microscopy (CLSM). The effect of 2D BP on inflammatory response through pro and anti-inflammatory cytokine investigations on co-culture model (Saos-2 and HOb) was also investigated.

**Results and discussion** 2D BP was obtained by liquid exfoliation process of BP microcrystals. The purity was ascertained by Inductively coupled plasma mass spectrometry (ICP-MS) analysis, the morphological characterization was carried out by transmission electron microscopy (TEM). In vitro 2D model was used to investigate the selective effect of 2D BP on human healthy (HOb) and cancer cells (Saos-2), with and w/o PTT, by analyzing cell proliferation, oxidative stress (Reactive oxygen species- ROS, nitrites levels) and osteogenic differentiation (ALP activity) at day 3 of cell culture. Cell morphology was



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evaluated by SEM and confocal microscopy (CLSM). The effect of 2D BP on inflammatory response through pro and anti-inflammatory cytokine investigations on co-culture model (Saos-2 and HOb) was also investigated.

**Conclusion** These selective effects prompt the application of 2D BP as a highly promising candidate for bone biomedical applications.

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**AcKnowledgegments** This study was supported through funds provided by Progetto MIUR PRIN2017 - ACTION, Grant N. 2017SZ5WZB. The authors also thank Mrs. Cristina Del Barone of LAMEST laboratory for SEM investigations, Mariarosaria Bonetti for lab technical support & data elaboration and Dr. Roberta Marzella for support to project management.



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## Promising bioglasses for medical applications

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**Introduction** Infectious diseases are one of the most important causes of death in the World. The constant use of antibiotics to treat infections has led to the development of resistant bacterial strains. Therefore, noticeable efforts from different scientific fields have been devoted to solve this problem. In materials science, several metal ions have been studied for their antibacterial properties. Copper (Cu) is one of the most used due to its low toxicity to mammalian cells<sup>1</sup>. The aim of this work is to evaluate the antibacterial effects and the biocompatibility of un-doped calcium phosphate glasses (CPG) and Cu<sup>2+</sup>-doped (CPG\_Cu) ones, which have shown in previous studies interesting degradative, mechanical and optical properties to be used in biophotonic applications<sup>2</sup>.

**Experimental methods** CPG and CPG\_Cu were analyzed with inductively coupled plasma–optical emission spectroscopy (ICP-OES) to evaluate their content of Ca, P, Na, Mg, B, Si and Cu. The antimicrobial effect of CPGs against four of the most common causing-infections bacteria, namely *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* PAO1 and *Klebsiella pneumoniae* were investigated; the biocompatibility was performed using NIH-3T3 cells. These viability assays in both bacteria (after 24h) and eukaryotic cells (after 24 and 48h) were evaluated through the quantitative 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) test, which was performed in both direct and indirect contact and measures dehydrogenase activity as an indicator of the metabolic state. Moreover, we investigated the viability of adherent cells with MTT and confirmed it with scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM).

**Results and discussion** The obtained results demonstrated the promising antibacterial properties of CPG\_Cu with respect to CPG, in particular against Gram-negative bacteria. The particular efficacy against *E. coli*, *P. aeruginosa* and *K. pneumoniae* is due to the different



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composition of bacterial surface. The preliminary quantitative and qualitative (SEM and CLSM) studies performed using 3T3 cells showed that CPG and CPG\_Cu were not toxic.

**Conclusion** The results suggested that the tested bioresorbable bioglasses showed biocompatible and antibacterial properties useful for medical applications.

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**AcKnOwledgments** D.P. and D.J. acknowledge the Interdepartmental Center “PhotoNext” of Politecnico di Torino for the partial support of this research effort. The study was supported by a grant of the Italian Ministry of Education, University and Research (MIUR) to the Department of Molecular Medicine of the University of Pavia under the initiative “Dipartimenti di Eccellenza (2018-2022)”. L.V. and E.R. acknowledge the following members of University of Pavia: Roberta Migliavacca for providing bacterial strains and Patrizia Vaghi for CLSM analyses.



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# Electrochemical sensors based on nanostructured electrodes as devices for the measurement of OP of PM

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**Introduction** The electrochemical sensors are useful in many fields connected with the human life, such as the environmental analysis and the monitoring of air quality. In this work an innovative electrochemical sensor for thiols detection is described. This sensor can be considered a device for the measurement of OP (Oxidative Potential) of PM (Particulate Matter). The term “particulate” refers to the solid and liquid particles dispersed in the atmosphere and it’s responsible for the generation of the biological oxidative stress. Long and short-term exposure to atmospheric particulate matter (PM) has detrimental effects on human health being associated with morbidity and mortality (Lionetto et al., 2021). Over the years, multiple cellular and acellular assays have been developed to quantify the OP in order to predict the potential toxicity of PM. Among them, the acellular dithiothreitol (DTT) assay (Cho et al., 2005) is one of the most frequently used methods. The sensor described in this work can be considered an alternative to the classic spectrophotometric methods. In parallel, the cytotoxicity and the induction of oxidative stress by the particulate have been evaluated respectively by MTT assay and use of the fluorescent probe sensitive to reactive oxygen species, CM-CM-HD2CFDA.

**Experimental methods** The operating sensor’s principle is the determination of the PO, through the measurement of the loss in dithiothreitol content. The DTT-based chemical reactivity is indeed a quantitative probe for assessment of the capacity of a particulate matter (PM) sample to catalyse Reactive oxygen species (ROS) generation which will result in induction of oxidative stress (Koehler et al., 2014). All the experiment have been carried out in Batch Injection Analysis-BIA and Flow Injection Analysis-FIA.

The Glassy Carbon electrodes (traditional and SPE) have been modified by electrochemical deposition with Gold nanoparticles (GC/AuNPs). The modified electrodes were prepared by direct drop casting of the AuCl<sub>3</sub> solution (15μL) 0,137 mM on the Glassy Carbon ones. The Au electrodeposition had been performed by chronoamperometry at the applied potential of -0.9V vs Ag/AgCl for t=200s. Cyclic Voltammetry between -1.0V and 0V and then between 0V and 1.3V was performed until the steady state (20 cycles).



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**Results and discussion** The MTT assay shows an increase in cell mortality after 24h of exposure to the PM aqueous extracts. The CM-CM-H2DCFDA shows the intracellular oxidative stress induction by PM which, in turn, results in an increased cytotoxicity. A dose-dependence of the fluorescence of A549 cells charged with the probe is observed, with the increasing concentration of PM. The higher the PM concentration, the greater the endogenous stress induction on A549 cells. Correlation analysis between the MTT test results (expressed as mortality) and the results were obtained on the same samples with the CM-H2DCFDA fluorescence. The higher the intracellular oxidative stress, the higher the mortality observed.

The sensor is reactive towards the DTT and shows low LOD, an high sensitivity and selectivity. The aqueous extracts of PM don't interfere with its detection.

**Conclusion** The electrochemical properties of the sensor are considerably influenced by the Gold nanoparticles, which were optimized to yield a stable and reproducible response. In the trend of sensitive detection, the electrochemical sensor for PM oxidative potential promotes the simplicity and the highly sensitivity, with a minimal equipment.

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# Modified hyaluronic acid-PEGDA double network as promising bioink for 3D scaffolds with enhanced mechanical properties

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## Introduction

3D bioprinting is a rapidly developing technology based on additive manufacturing of biological materials and living cells to fabricate highly organized 3D structures. Currently, hydrogels are used as bioinks with extrusion 3D bioprinting technique.

However, due to the soft mechanical properties and inability to be self-supporting for layer-by-layer fabrication, the printing fidelity is very limited and it is always difficult to produce large-scale tissue constructs. Double network (DN) hydrogels are a kind of interpenetrated hydrogel prepared by a two-step gel formation<sup>1,2</sup>. A combination of DN hydrogels with 3D bioprinting technique may offer the possibility to produce customized 3D scaffolds with enhanced mechanical properties. In this study, a chemically modified hyaluronic acid sodium salt (HAS) was processed by 3D bioprinting and interpenetrated with a poly(ethyleneglycol) diacrylate (PEGDA) in order to obtain a DN hydrogel porous scaffold.

## Experimental methods

Hyaluronic acid sodium salt (HAS,  $M \approx 340$  kDa) was Methacrylated (MeHA) and Maleated (MaHA) in order to have a photocrosslinkable hydrogel with tunable properties. Separately a PEGDA/dH<sub>2</sub>O solution (200mg/mL) has been prepared using 2-Hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (Irgacure 2959) as photoinitiator. DN hydrogels scaffolds were synthesized by two-step reaction procedure as reported below. First step: MEHA and MAHA were dissolved in deionized water (dH<sub>2</sub>O) containing 0.1 wt% of Irgacure 2959. Porous rectangular MaHA and MeHA scaffolds, measuring 15x15x5 mm<sup>3</sup>, were printed with a porosity of 40% using a Rokit Invivo 3D Bioprinter (Rokit Healthcare). Second step: the porous scaffolds (1st network) were dipped in the PEGDA (2<sup>nd</sup> network) solution for 4 days. The extracted samples were exposed to UV light for 300 s, washed in dH<sub>2</sub>O and stored at 4°C. The printed structures have been investigated by scanning electron microscopy (SEM) and Dynamic mechanical analysis (DMA).

## Results and discussion

SEM analyses highlighted a well-organized structure with a fiber diameter of  $\approx 200$   $\mu\text{m}$  and a porosity of  $\approx 700$   $\mu\text{m}$ . The second step process does not alter the 3D structure of the scaffold that retains their original shape. Mechanical properties of DN scaffolds showed



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almost a 10-time increase of the storage modulus, if compared to neat structures. The cell viability expressed in terms of percentage of cells on material surface than control (tissue culture plates), after 24 h of culture time, was analyzed. The results demonstrated a good cell viability and proliferation in the first 24 h of culture.

### **Conclusion**

The DN scaffolds showed enhanced mechanical properties if compared with the neat MEHA and MAHA structures. Results strongly suggest that the 3D structures have a great potential as load-bearing materials for biomedical applications.

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### **Acknowledgments**

The authors would like to thank PRIN2017 "SAPIENT - A system approach for identifying connective tissue degeneration in diabetic analogues", Grant N° 2017CBHCWF and Progetto Premiale di AREA SCIENCE PARK "OPEN LAB 2 - A System of Open Research Facilities" for financial support.



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# Antimicrobial nanoparticles-loaded microspheres intended for the treatment of infected wounds

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**Introduction** Infections in non-healing wounds remain one of the major challenges<sup>1</sup>. In this work, antibacterial nanoparticles (CuO and ZnO NPs) were encapsulated in spray-dried microparticles based on polysaccharides, in order to combine antimicrobial properties and wound healing properties to prevent/treat wound infection that could delay and impair the healing process.

**Experimental methods** Briefly, two different polymeric blends were prepared by mixing maltodextrin (MD) or dextran (DX) with a mixture of amino acids (Thr, Cys and Gly) in aqueous solution and citric acid was used as cross-linking agent. Analogously to unloaded solutions, ZnO or CuO NPs were loaded to each solution. Microparticles were prepared using a Buchi 190 mini spray drier and the microparticles were then cross-linked by heating. The morphology of the microparticles were analyzed by means of SEM and particle size distribution was determined using Malvern Mastersizer 3000E granulometer. The crush test was carried out by means of a TA.XT plus Texture Analyzer and the biocompatibility of microparticles was evaluated using normal human dermal fibroblasts.

**Results and discussion** The morphological analysis shows that all the microparticles were spherical and with smooth surfaces, and the loading of the NPs did not modify their morphology. Moreover, the crosslinking by heating did not significantly change the microparticles morphology and particle size distribution. In particular, the microparticles were characterized by narrow size distributions, as highlighted by the SPAN Index values, and particle size analyses indicated that microparticles had  $d[4,3]$  values of 19–26  $\mu\text{m}$ . The hydration of microparticles were expressed as Swelling Index (SI): MD microspheres showed SI values (0.9–1.2) remarkable lower than the microspheres containing DX (1.8–3.5). The crushing test shows that the NPs loaded microparticles, in comparison with unloaded microparticles, were less elastic while no significant differences were observed on the rupture forces. Moreover, unloaded microparticles were characterized by high biocompatibility and, the encapsulation of NPs in microparticles increased the cytocompatibility compared to the free NPs, suggesting that the systems were able to prevent the negative effect of CuO and ZnO towards the fibroblasts.



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**Conclusion** The spray-dryer was successfully used to obtain microparticles with regular shape and smooth surface. Due to their capability to support fibroblasts proliferation and to prevent CuO and ZnO cytotoxicity, these microparticles are promising to prevent/treat nonhealing wound infection.

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**Acknowledgments** This work was funded by Horizon 2020 (Grant Agreement No 81460).



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# Superabsorbent hydrogel stiffness impacts intestinal tissue homeostasis in ex-vivo organ culture system

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**Introduction** Recent studies have demonstrated the benefits of dietary fibers on gastrointestinal health by, among other effects, regulating intestinal transit and controlling absorption of different nutrients. Nevertheless, the modern western lifestyle, characterized by a low fiber dietary intake, represents a growing health risk, being associated with the development of metabolic disorders, cardiovascular diseases and cancer.

Therefore, finding a way to upturn everyday fiber intake can be a helpful alternative to maintain gut health and prevent the onset of gut-related disorders.

A newly designed platform of naturally-derived superabsorbent hydrogel having elastic and physical properties similar to those of ingestible fibers can potentially be used as a dietary supplement.

Therefore, the aim of our work is to test the effect of naturally-derived superabsorbent hydrogels within a range of different elastic modules on intestinal tissue homeostasis.

**Experimental methods** We took advantage of an ex-vivo organ culture system to evaluate the ability of superabsorbent hydrogel with different elastic properties ( $G'$  stiffness), varying from Gel 01 with 846 Pa, Gel 02 with 1292 Pa, Gel 03 with 6550 Pa, to Gel 04 with 13481 Pa to preserve tissue integrity and homeostasis. We assessed intestinal mucus layer integrity (Alcian Blue staining) and tissue proliferative niche through immunohistochemistry for Ki67. Colon tissue explants were obtained from healthy mice (C57BL6/J, 8 weeks of age) and incubated for 2 hours with the different stimuli in a controlled environment (37°C 5% CO<sub>2</sub>).

**Results and discussion** Intestinal tissue integrity of colon explants was better preserved when incubated with Gel 02 and Gel 03.

In comparison to samples incubated with control, those exposed to Gel 02 and 03 showed a greater mucus layer thickness, deeper colon crypts and a better distribution of acidic and neutral mucins. Conversely, these effects were not observed when tissues were treated with extremely high or low stiff hydrogels (Gel 01 and Gel 04).



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Besides, Ki67 staining revealed that tissue treated with Gel 02 and Gel 03 revealed an increased number of proliferative cells (PBS vs. Gel 02:  $10,2 \pm 9,5$  vs.  $24,2 \pm 9,6$   $p=0,006$ ; PBS vs. Gel 03:  $10,2 \pm 9,5$  vs.  $20,7 \pm 8,7$ ,  $p=0,044$ . One-way ANOVA, Tukey post-test).

**Conclusion** We demonstrated that, ex-vivo, hydrogel 02 and hydrogel 03 had the best physical properties to preserve intestinal mucus homeostasis and proliferative ability. Taken together these results underline the essential function covered by the elastic modules in fiber mimicking compounds in the preservation of tissue homeostasis. Therefore, matching the elastic properties of the tissue with those of the superabsorbent hydrogel results essential for the maintenance of intestinal tissue health and homeostasis.



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# Bioactivation of gelatin-based scaffolds to enhance at nanoscale level bone tissue regeneration

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**Introduction** In bone tissue engineering, porous 3D scaffolds play a critical role in new tissue formation for their similar structure to natural bone. Indeed, the function of scaffold should be to provide a 3D spatial and temporal structure to guide cell infiltration and proliferation, leading to a new tissue. In this study, a biodegradable and biocompatible protein such as gelatin was chosen for scaffold development [1]. The presence of chemical groups on the polymer chain allows the bio-activation of scaffolds by specific signals able to trigger the cellular behavior in terms of proliferation and osteogenic differentiation of human cells. These types of scaffold modifications provide biochemical cues for promoting stem cell osteogenic commitment. Here, two different bio-activation routes of gelatin-based scaffolds were pursued through the functionalization with organic and inorganic signals, to enhance at nanoscale level bone tissue regeneration. Then, the effect of inorganic functionalization by biomimetic approach on mechanical properties and on in vitro biological behavior was evaluated through proliferation and early osteogenic differentiation studies by using human mesenchymal stem cells (MSCs).

**Experimental methods** The crosslinking of Gelatin was performed by soaking porous lyophilized scaffolds, at different time points (1, 3 and 6 h) at room temperature, in acetone-water solution (4:1v/v) containing a water-soluble EDC, followed by incubation at 4 °C for 24 h. Bio-mineralized scaffolds with bioactive solid signals on the gelatin scaffold surfaces, were obtained by using simulated body fluid solutions (5 x SBFs). Meanwhile, the organic functionalization of the scaffolds was performed by covalent immobilization of BMP-2 like-peptide. The peptide was characterized by analytical High Performance Liquid Chromatography (HPLC, Agilent) and mass spectrometry (micro-TOF; Bruker). Mechanical properties of scaffolds, before and after biomimetic treatment, were evaluated by compression tests. Furthermore, to identify the functional groups ATR-FT IR spectroscopy was implemented. The in vitro peptide release profile from gelatin scaffolds was studied by HPLC as reported in a previous study [2]. Cell metabolic activity was analyzed by using Alamar Blue assay. Meanwhile, the alkaline phosphatase activity (ALP) of cells seeded onto scaffolds before and after inorganic treatment and organic functionalization was determined at different days of in vitro cell culture.



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**Results and discussion** The scaffold composition and crosslinking time influenced the scaffold performances in terms of physico-chemical, morphological and mechanical behavior. Furthermore, both bioactive signals were able to improve in vitro biological activities at different time. In particular, biomimetic approach improved cell attachment and early osteogenic differentiation at short time, meanwhile BMP-2 peptide decoration operated in vitro as bioactive signal at long time, so influencing the cellular behavior in terms of early osteogenic differentiation.

**Conclusion** The study reported the development and bio-functionalization of gelatin-based scaffolds by using two different approaches: inorganic and organic bioactive signals decoration. However, these two approaches allowed to study the possibility to functionalize at nanoscale level polymeric scaffolds by tuning the biological response at short and long time of MSCs.

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**Acknowledgments** This study was supported through funds provided by Progetto MIUR PRIN 2017 - SAPIENT, Grant N. 2017CBHCWF.



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# Comparison of Physical Properties of an Oral Non-systemic Hydrogel and Common Fiber Supplements

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**Introduction** Plenity is a non-systemic oral superabsorbent hydrogel (OSH) made of cross-linked carboxymethylcellulose and citric acid for weight management. OSH acts primarily by absorbing water to form non-aggregating, firm, elastic, gel-like particles that occupy stomach and small intestine volume. This study evaluates physical property differences between OSH and common fiber supplements.

**Experimental methods** OSH and the following viscous and non-viscous fibers were included: guar gum, glucomannan, psyllium, methylcellulose, wheat dextrin, inulin, and calcium polycarbophil. Samples in a 1:1 ratio were hydrated in a 1:8 mixture of simulated gastric fluid and water to mimic early digestion. The resultant suspensions were drained and weighed to calculate media uptake ratio (MUR) (ie, absorption capability). Rheological analysis was also performed on drained samples to determine the elastic modulus  $G'$  (ie, elasticity). Results were expressed as mean (with standard deviation) of three replicates and differences between groups assessed using one-way ANOVA.

**Results and discussion** Glucomannan and guar gum had the highest MUR ( $138.3 \pm 2.1$  and  $134.7 \pm 1.2$  g/g, respectively), followed by OSH ( $76.8 \pm 0.6$ ), psyllium ( $17.7 \pm 0.6$ ), and methylcellulose ( $2.7 \pm 0.6$ ) ( $p < 0.01$  for all comparisons). Wheat dextrin, inulin and calcium polycarbophil did not exhibit any absorption capability. OSH exhibited the highest  $G'$  ( $1451.0 \pm 64.8$  Pa), followed by psyllium ( $145.3 \pm 13.2$ ), glucomannan ( $9.0 \pm 0.2$ ) and guar gum ( $4.4 \pm 3.1$ ) ( $p < 0.001$  for all comparisons). Due to their solubility, the elasticity of all other fibers could not be assessed.

**Conclusion** OSH exhibited a unique combination of high absorption (77 g/g) and high elasticity (10-fold increase over all tested fiber supplements). Viscous fibers showed high absorption but poor elasticity, while remaining fibers did not exhibit any absorption or elasticity.

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# Nanostructured medicated scaffolds for the local treatment of bone metastasis

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**Introduction** Bone metastasis represent a major concern in case of advanced cancer, especially prostate, breast and lung cancer, and are responsible for many complications. The onset of bone metastases leads to the weakening of the tissue, resulting in poor healing capacity and risk of fracture. Therefore, the prevention and treatment of such metastatic bone fractures is highly desired. Nowadays, the use of bone grafts represents a valuable approach when bone regeneration is required. Autologous bone is considered the gold standard, despite the risk of complications, while allographic transplants are associated to considerable immunogenic rejection. Therefore, in the last decades synthetic scaffolds with osteogenic and osteointegrative potentials have been developed for the regeneration of bone tissue.

This work aims to design a controlled anticancer drug-release from two types of scaffolds for bone tissue engineering: the first scaffold, called hybrid-biomimetic scaffold (from Finceramica SpA), included biomineralized collagen-based scaffold with ionically multi-substituted apatitic nanophases; the second scaffold is a biomorphic hydroxyapatite scaffold (from Greenbone Ortho SpA) with wood-derived hierarchical structure.

**Experimental methods** Hybrid scaffolds were obtained through the biomineralization process, inspired by the Nature, in which hydroxyapatite crystals are nucleated and grown on collagen fibrils, the organic phase strictly controls and constrains the hydroxyapatitic crystals' growth conferring them the low crystallinity and morphology typical of the natural bone. Finally, these scaffolds were cross-linked to decrease the degradation and freeze-dried to create a highly interconnected porous structure essential for cell colonization and new tissue formation.

Biomorphic scaffolds were obtained by a multi-step process that consist in the conversion of wood hierarchical structures into biomimetic HA scaffolds; the multi-step processes involve a series of complex chemical process including pyrolysis and heterogeneous gas/solid and liquid/solid reactions.

The two devices were functionalized with Everolimus, a molecule with capacity of bone resorption inhibition by mTOR cascade blockage, used in clinical practice for the treatment



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of bone cancer.

The drug-release profiles and biological effects on breast cancer cell lines were evaluated. Cancer cells were seeded, first on 2D and treated with Everolimus released from hybrid and biomorphic medicated scaffolds. After, the cells were seeded directly on the two different medicated scaffolds.

The effect of Everolimus treatment was also evaluated on PBMCs in osteoclastic differentiation.

**Results and discussion** A decrease in cell growth at 72h of 10-15% in a triple negative breast cancer cell line, and of about 25-30% in a ER+ breast cancer cell line were observed. The number of mature osteoclasts in the treated samples was lower compared to the untreated positive control (PBMCs treated with differentiating factors) and to the negative control (PBMCs without differentiating factors). This result shows how the Everolimus provides its biological effect also on the osteoclastic component, decreasing its vitality and therefore its differentiation capacity.

The medicated hybrid-biomimetic and biomorphic devices showed sustained drug release within 7-14 days, possibly due to the close interaction between the drug and the apatitic phase. In addition, the released concentrations were also close to therapeutically adequate doses [1,2], resulting in cell death ranging from 10 to 30% according to different Everolimus concentration.

**Conclusion** These results showed promising features of the medicated devices for bone cancer treatment, in addition to bone regeneration.

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**Acknowledgments** The authors would like to thank the EMILIA-ROMAGNA REGION program POR- FESR 2014-2020 supporting the project DINAMICA PG/2018/632022.



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# Multichannel PLGA-based scaffolds for the treatment of peripheral nerve injuries

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**Introduction** Peripheral nerve injury is one of the most debilitating pathologies that severely impairs patients' life.

The aim of the present work was to develop a therapeutic platform able to bridge the lesion gap, supporting cells during the regeneration process and acting as carrier of neuroprotective compounds. A multichannel poly (lactic-co-glycolic acid) (PLGA)-based scaffold (MC-Sc) was prepared.

**Experimental methods** A polymeric aqueous solution, containing 1% w/w alginate and two different grades of poly(ethylene oxide) (low, 1% and high, 2.2% (w/w)), was prepared. The polymeric solution was electrospun with both plate and rotating collectors to obtain random and aligned-interconnected Fbs. Fbs were immersed in various PLGA coating solutions to obtain coated Fbs (c-Fbs) via solvent casting. The addition of poly(D,L-lactide) (PDLLA) and glycerol to PLGA solution was considered to improve scaffold mechanical properties. Finally, c-Fbs were soaked in water and a MC-Sc was achieved. Fbs, c-Fbs and MC-Sc morphology, mechanical properties and wettability were evaluated.

**Results and discussion** Homogeneous micrometric electrospun Fbs were obtained. Two different set-ups of electrospinning apparatus were employed: 1) Plate collector: random Fbs, Fbs<sub>R</sub>, (mean diameter 22 μm); 2) Rotating collector: aligned-interconnected Fbs, Fbs<sub>A</sub> (mean diameter 10 μm). Size analysis on Fbs<sub>R</sub> and Fbs<sub>A</sub> coated with different solutions showed a significant increase of mean diameter of c-Fbs. Then c-Fbs were subjected to tensile test to assess the improvement of mechanical properties of different c-Fbs, compared to Fbs. The most promising c-Fbs, Fbs<sub>R</sub> and Fbs<sub>A</sub>, were selected for the prosecution of the work. MC-Sc with channels of 5-18 μm mean diameter were obtained. The results of contact angle measurements confirmed the successful coating of Fbs and the presence of channels improved scaffold wettability.

**Conclusion** The multi-step process developed has proved to be simple and effective, resulting in the formation of a promising innovative biodegradable micro-scale multi-channel scaffold, composed by a PLGA-based framework. Biocompatible matrices, based on collagen or alginate, containing neuroprotective compounds, will be employed as fillers for the multi-channel scaffold.



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Thanks!



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