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CONGRESS ABSTRACTS

Oral Sessions

Session 1 - Nanosystems

OC1
SILVER NANOPARTICLES LOADED ELECTROSPUN NANOFIBERS FOR WOUND HEALING

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Introduction: The incidence of chronic wounds is likely to dramatically increase as long as the population ages due to the rising prevalence of type 2 diabetes, peripheral vascular disease and metabolic syndrome. Whereas the treatment strategies adopted for acute and limited area traumatic wounds are effective, the problems arise in the long-term care of patients with large area burns, infected and severe chronic wounds. Moreover, microbial infections negatively affect the healing process. The aim of the present work was the development of electrospun nanofibers loaded with silver nanoparticles as scaffolds to enhance cutaneous wound healing of chronic lesions and burns preventing infections.
Materials and methods: Silver nanoparticles were directly added to polymeric mixtures based on chitosan and hyaluronic acid or chitosan and chondroitin sulfate to be electrospun obtaining nanofibrous membranes. Moreover, a membrane based on chitosan and loaded with silver nanoparticles was prepared as comparison. The membranes were characterized by morphology, silver nanoparticle stability, biocompatibility (cell adhesion) and antimicrobial properties.
Results: All the membranes prepared were based on continuous and randomly oriented nanofibers having diameters in the nanometric range (500-600 nm). The polymeric mixtures did not affect silver colloid stability and the presence of silver nanoparticles was evidenced by TEM analysis. Membrane compositions and colloid did not significantly modify nanofiber morphology. Membranes were characterized by good propensity to promote cell (normal human dermal fibroblasts) adhesion, migration and proliferation: the presence of silver nanoparticles did not interfere with cytocompatibility. The microbiological evaluation evidenced that the antimicrobial properties of silver nanoparticles were preserved also entrapped into nanofibers.
Discussion: Even if further characterizations will be necessary, scaffolds based on electrospun nanofibers and loaded with silver nanoparticles demonstrated to promote fibroblast adhesion and proliferation and represent a suitable approach for the treatment of chronic wounds.

OC2
MICRO-NANO DELIVERY SYSTEMS FOR AN EFFICIENT CYSTIC FIBROSIS INFLAMMATION CONTROL

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Introduction: Cystic fibrosis (CF) is a progressive genetic disease caused by mutations in the gene that produces the CF transmembrane conductance regulator (CFTR) protein. The malfunction of the CFTR protein causes a thick build-up of mucus in the lungs that clogs the airways and traps bacteria, thus leading to infections and extensive lung damage. Nanomedicine strategies were explored for locally delivering drugs to the pulmonary system. This study aimed to develop novel drug delivery systems based on micro-nanoparticles (MNPs) for a multilevel pharmacological treatment of the CF lungs.
Materials and methods: Synthetic and natural MNPs were prepared through procedures based on water/oil emulsion techniques and precipitation polymerization. Morphological and physicochemical characterizations were carried out on all classes of MNPs using SEM, DSC, TGA and FTIR Chemical Imaging. In vitro biocompatibility tests were performed using cell line A459. Microparticles were loaded with a mucolytic agent (N-acetyl cysteine, NAC), and their release kinetics was evaluated. Mucus crossing capability of NAC-loaded microparticles was evaluated in an artificial sputum (AS) model through its rheological analysis. Synthetic MNPs were evaluated for their ability to specifically recognize an anti-inflammatory agent.
Results: MNPs showed a diameter range suitable for reaching the CF airways. Cells treated with microparticles showed no cytotoxic effect. NAC release kinetics from microparticles was rapid for inducing an early mucus degradation. The effect of the NAC-loaded microparticles on AS hydrolysis was found leading to the lowest viscosity profile. MNPs were able to in vitro rebind the anti-inflammatory agent in a specific and selective way in order to induce a drug long-term bioavailability.
Discussion: The interesting results obtained in this work allow selecting suitable MNPs as potential drug targeting systems having mucolytic and anti-inflammatory action for the regulation of CF pathological processes.

OC3
INNOVATIVE HYBRID AND INTRINSICALLY MAGNETIC NANOBEADS AS DRUG DELIVERY SYSTEM

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Introduction: The main goal of drug delivery nanosystems is the improvement of the drug efficacy, increasing the drug's concentration that goes to target site and lowering the collaterals effects. In the present work, magnetic hybrid nanobeads (MHNs) were created composed by alginate and Fe-hydroxyapatite nanoparticles that are both biocompatible and bioresorbable. Alginate derives by a brown algae and it is very investigated for its biodegradability, biocompatibility, low cost and capability of gelation with multivalent cations. Fe-hydroxyapatite is used because it allows to absorb or link on its surface a lot of target as bioactive molecules, moreover, Fe(II) and Fe(III) ions confer on apatite magnetic properties without containing secondary phases like magnetite that accumulates in the body and could have side effect on long-term. In this way, it is possible to drive nanosystem to the desired site before releasing bioactive molecules with the use of external magnetic field.
Materials and methods: Iron-doped apatite was heterogeneously nucleated on self-assembling Alg matrix by a bio-inspired mineralization process and MHNs are formed by a subsequent emulsification by oil-in-water technique and cross-linked by following adding of calcium ions. The MHNs' chemical-physical, stability and magnetic properties were evaluated before assessing the in vitro cytotoxicity.
Results: A bio-inspired mineralization approach was followed to synthesize a superparamagnetic hybrid composite consisting of Fe-doped apatite nanocrystals nucleated onto alginate polymeric matrices. An oil-in-water emulsification process following by cross-linking technique was settled to obtain egg-like hybrid composites featuring uniform size distribution and exposure of mineral phase at the nanobeads surface. The obtained MHNs exhibited biomimetic composition, adequate swelling properties and stability in physiological-like environment and superparamagnetic properties. Finally MHNs did not negatively affect the cell viability and the cell proliferation over the time.
Discussion: MHNs can be considered as promising magnetic drug delivery systems suitable for smart applications in nanomedicine.

OC4

PHOTO-THERMAL EFFECT OF PLASMONIC NANOPARTICLES INKJET-PRINTED ON LATEX COATED SUBSTRATES

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Introduction: Inkjet-printing has met important challenges to pattern a broad range of functional materials with promising biomedical application. Printing of gold nanostars (GNS) with Localized Surface Plasmon Resonance (LSPR) in "bio-transparent window" (700-1100 nm), results to surfaces with highly localized and controlled photo-thermal effect. Unlike GNS, the absorption of CuS nanoparticles in NIR region (≈900-1300 nm) is a result of d-d energy band transition of Cu^{2+} ions. In the current communication, GNS and CuS patterns were fabricated by inkjet-printing on biocompatible latex coated substrates and photo-thermal effect was studied.
Materials and methods: The stable inks were formulated by adding 1,2-ethanediol (20% vol.) and 2-propanol (10% vol.) to the aqueous PEGylated GNS solution (70% vol.) to adjust the viscosity and surface tension. GNS patterns were inkjet-printed with Dimatix Materials Printer. CuS patterns were fabricated by inkjet-printing of the copper acetate films that were subsequently exposed to hydrogen sulfide gas at controlled temperature and humidity. The photo-thermal effect was induced by irradiating at three NIR wavelengths (λ = 800 nm, λ = 940 nm and λ = 1064 nm).
Results: A steep temperature increase, followed by a plateau after ≈15-20 s was observed in all cases. A significant photo-thermal effect can be reached even under low laser intensities for all printed substrates. Beside the direct impact of laser power, the printing parameters also affect the photo-thermal efficiency. In addition, the Bodipy-thiol dye, was bound to the gold surface and its photo-thermally induced release from the patterns was demonstrated as additional effect.
Discussion: All patterns showed a significant photo-thermal effect under NIR irradiation. The cytotoxic studies indicated low toxicity of printed patterns. Further studies will be focused on the development of printed surfaces for biofilms eradication based on their localized and pronounced photo-thermal effect.

Session 2 - Mechanical Characterization

OC5

NANOMECHANICAL INVESTIGATION ACROSS OSTEOCHONDRAL INTERFACE REGENERATED BY NANO-COMPOSITE THREE-LAYERED BIOMIMETIC SCAFFOLDS

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Introduction: The bone-cartilage interfaces in human joints represent one of the main sites of failure because of the transition between highly different materials that makes the loads transfer more difficult. Orthopaedic treatment is here challenging: several surgical approaches were adopted to regenerate osteochondral articular defects, i.e. using multilayered scaffolds, but no mechanical data of the regenerated transition zone have been reported. Indeed, the mechanical properties across the osteochondral interface (OI) were investigated by nanoindentation only for native tissues, highlighting a sigmoid-like mechanical gradient across the interface between the hyaline (HC) and calcified (CC) cartilage. Here, we report the first evaluation by nanoindentation of the transition across the tidemark for tissue engineered cartilage obtained by the insertion of a three-layer scaffold in a sheep model of osteochondral critical-size defect.
Materials and methods: Biomimetic three-layer scaffolds (Finceramica, Faenza) were implanted in the femoral condyle of sheep and the osteochondral tissue was analysed at 6 months from surgery. For the nanoindentation tests, matrixes of indent lines scanning across the OI were performed on PMMA-embedded slices for both regenerated and native tissue. The elastic modulus (E) was obtained by Oliver-Pharr model for each indent. The trend of the E was fitted with a sigmoid function and the width (*W*) of the transition region was evaluated.
Results: E across the OI increased from 5 GPa (HC + PMMA) to 16 GPa (CC). Interestingly, the trend of E showed a sharp increase in correspondence of the tidemark in the engineered tissue, whereas the mechanical properties of the native cartilage increased with a more gradual transition. Accordingly, the OI of engineered tissue showed a W (~2 μm) smaller than that of native tissue (~15 μm).
Discussion: The results of the present study confirm the ability of the nanoindentation technique to quantitatively describe the mechanical gradient between confining hard (bone) and soft (cartilage) tissues. This mechanical gradient playing on the micrometer scale is envisaged to exert a key role for a correct load transfer from cartilage to bone tissue.
Acknowledgments: This study was partially supported by "5 × 1000" (2013) funding, provided by Istituto Ortopedico Rizzoli, Bologna, Italy.

OC6

COMPUTATIONAL AND EXPERIMENTAL APPROACH TO THE INVESTIGATION OF FOOT PLANTAR SOFT TISSUE MECHANICS

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Introduction: Plantar soft tissue plays a relevant mechanical role in the different phases of the step, providing a valid transfer and distribution of forces to the foot structures, mostly absorbing high strain rate loads. Aiming at the investigation of the biomechanical functionality of the plantar tissues, an integrated experimental and computational investigation is performed. Experimental data support mechanical properties identification and models assessment. Computational methods make it possible to broad results to a larger scenario, offering the potentialities for investigating different operational conditions up to foot structural scale.
Materials and methods: The plantar soft tissue is composed of adipose chambers circumferentially bounded by connective septa. Such honeycomb configuration deeply affects the compressive strength and the damping capabilities. Mechanical tests must be performed on tissue samples, as compression, shear and stress relaxation tests, to provide the basis for constitutive model definition and parameters identification. The structural behaviour can be analysed by tests on the different regions of the foot. Such experimental data are mandatory for reliability assessment of computational models. Biomedical images of foot structures are processed to provide a virtual solid and finite element models. A specific visco-hyperelastic anisotropic constitutive formulation is developed and implemented to get to the characterization of the mechanical behaviour of plantar soft tissue. The inverse analysis of experimental tests ensures identification of constitutive parameters and validation. Computational analyses of heel strike, midstance and push off phases are performed to point out the mechanical functionality of plantar soft tissue in both heel and forefoot regions.
Results: The results of the computational analyses make it possible to evaluate the actual mechanical role of the plantar soft tissue, providing a proper evaluation of foot biomechanical behaviour during the gait cycle. Moreover, computational analyses allow for the evaluation of interaction phenomena with orthosis and shoes, to estimate functionality to be considered from health and industrial point of view.
Discussion: The analysis of the biomechanical behaviour of the foot, with particular regard to plantar soft tissue, has a relevant socio-economical impact, because of the increasing evidence of foot problems related to pathology, as diabetes, obesity and aging.

OC7

INVESTIGATIONS OF THE SURFACE MODIFICATIONS DETERMINED BY THE SUPERPLASTIC FORMING ON TITANIUM BIOMEDICAL PROSTHESES

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Introduction: Investigations were conducted on two types of Titanium (Ti) alloys Ti-6Al-4V (Gr5 and Gr23), subjected to Super Plastic Forming (SPF) process to produce a cranial implant designed starting from diagnostic images of an artificial defected skull.
Materials and methods: A numerical model was used to simulate SPF implant manufacturing starting from data derived by free inflation tests. Glow Discharge Optical Emission Spectrometry (GDOES) analyses on undeformed specimens kept in air at 850°C for different time levels, nano-indentation tests on specimens subjected to SPF conditions (from both free inflated samples and the cranial prostheses) and metallographic analyses allowed to assess the mechanical alteration to the Oxygen enrichment due to the exposition at the SPF conditions. Moreover, samples from prostheses were subjected to cytotoxicity analyses.
Results: Both the alloys can reach very large strains (>600%) at the investigated temperature and the evaluated ESR Sensitivity Indexes are 0.485 for Gr5 and 0.746 for Gr23. From GDOES analyses, the oxidation rate appears to be faster for Gr23 while no specific trend to saturation was observed up to 180 min. The two alloys feature similar oxidation kinetics showing a 2 µm layer close to the surface which is enriched in oxygen (up to 2 wt.%). At higher oxidation times, diffusion acts by spreading the absorbed oxygen toward the depth. Hardness profiles obtained by nano-indentations revealed higher values at both extremities of the formed material (maximum values at the side exposed to air for both the alloys). No cell death and no qualitative difference between the morphology of the cells in contact with the Ti specimens and the control ones could be found by cytotoxicity analyses, being this aspects interpreted as an absence of cytotoxicity of the oxidized layer.
Discussion: For both the alloys the exposure time has a strong influence on oxygen-enriched layer and mechanical properties; the alloy Gr23 tends to absorb more oxygen in its surface for times greater than 18 minutes. The high values of viability for all the three considered time points guarantees that the material biocompatibility was not affected by the manufacturing process.
Acknowledgments: The paper contains results of the PRIN project "BIO-FORMING" funded by MIUR.

OC8

DERMIS CONSTITUTIVE MODEL IDENTIFICATION FROM EXPERIMENTAL STRAIN DISTRIBUTION

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Introduction: Dermis constitutive behaviour can be best approximated through a hyperelastic law. Uniaxial or biaxial tests are usually employed to identify the respective model from boundary load and displacement data, as well as specimen section. A different approach has been here followed, making use of experimentally determined full-field stress to validate finite element models where different constitutive laws have been implemented. The stochastic variability of material constitutive parameters has been taken into account fitting one single model on different specimens. As a result, the best fitting constitutive law has been identified.
Materials and methods: Large strips of dermis tissue from the lower back of a human donor were dissected along the anatomical cranio-caudal (CC) direction, obtaining four specimens per donor. Equi-biaxial tensile tests were performed through a suitable fixture, designed to be interfaced with a uniaxial testing machine (Bose Electroforce® 3200). A full-frame digital camera (Canon EOS 5D Mark II) was used to capture real-time specimen strain at 1 Hz frame-rate, while two load cells measured boundary loads. Three different material models (Ogden's that is isotropic hyperelastic, Holzapfel's, and Gasser-Ogden-Holzapfel or GOH, both anisotropic) have been implemented in FE software Abaqus Standard 6.13, and the respective parameters have been identified minimizing the objective function given by the sum of squared errors between experimentally measured and numerically calculated full-field stress. The average relative error (Err) in full-field displacement estimation has been used as an index of model goodness.
Results: Constitutive parameters have been measured for all three models as well as the respective errors. The isotropic model has proved to give a poorer performance, especially with reference to CC direction. Hozapfel's and GOH's models have given similar performances. The set up methodology has proved to be able to provide a reliable estimate of material parameters.
Discussion: A constitutive anisotropic model of the dermis has been set up from biaxial experimental tests. This model can be used for the pre-operative planning of surgeries where dermal patches need to be applied as skin substituted or for tendon augmentation.

Session 3 - Advanced Applications

OC9

NEW ALGINATE-MATRIGEL® COMPOSITE GELS ALLOW HIGHLY METASTATIC BREAST CANCER CELLS TO MAINTAIN THEIR AGGRESSIVE BEHAVIOUR IN A 3D ENVIRONMENT

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Introduction: It is an established fact that 3D cultures are essential for a better comprehension of cell behaviour, as they reproduce an environment more similar to the in vivo one. This is particularly important in pathological conditions, such as in cancer studies (e.g. breast cancer). At the moment no established 3D models are available to carry out standardized and reproducible studies in breast cancer, since the proposed models lack of structural integrity or bioactivity. Aim of this work is the development of a new, composite, hydrogel able to allow cells expressing some typical mechanisms of their in vivo behaviour.
Materials and methods: Three different materials, each one constituted by different concentrations of alginate (A) and Matrigel (M) (i.e. 100% A, 75%:25% A:M and 50%:50% A:M), were developed. The choice of these polymers is related to their complementary characteristics: the first one allowing obtaining structurally compact and stable-in-time 3D materials, the second one known to enhance cell biological events. Metastatic breast cancer cells (MDA-MB-231) were embedded within the gels, with a density of 1 million cells/ml. Considering, as widely proven, the link between cell morphology and malignancy, cells grown in the different materials were deeply morphologically characterized by the use of confocal microscopy.
Results: Three significant results emerged:

1. Cells maintained a spherical shape in 100% A gels, while they assumed an elongated shape in presence of Matrigel; this is in agreement with what is known in literature, in which MDA-MB-231 cells assume stellate shape, related to their malignancy;
2. Nuclei expressed both irregular shapes and poly-nuclei organization exclusively in cells with elongated shape, feature known to be linked to cell malignancy;
3. Formation of the so-called invadopodia (actin-based protrusions through which cells degrade the ExtraCellular Matrix) was exclusively observed within 50%:50% A:M gels.

Discussion: Globally, these results appear of great importance, since they provide a new 3D material (50%:50% A:M) structurally stable and able to support in vitro the aggressiveness of breast cancer cells, at the base of metastatic cascade. Thus, this model could potentially become a powerful instrument for pharmaceutical tests, more realistic than 2D cultures.

OC10

DEVELOPMENT OF ENGINEERED IRON-OXIDE NANOPARTICLES BY LENTIVIRAL VECTORS FOR TARGETED CANCER THERAPY

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Introduction: Nanomaterials conjugated with biological moieties such as antibodies, polymers or peptides appear to be suitable for both drug delivery and cancer treatment. Here, biocompatible iron oxide magnetic nanoparticles (MNPs) with/without a silica shell coupled with lentiviral vectors (LVs) are proposed as a combined therapeutic approach to specifically target gene expression in a cancer mouse model.
Materials and methods: MNPs were prepared by co-precipitation method, stabilized with citric acid, enriched with silica shell to modulate their surface reactivity. They were characterized by X-Ray diffraction, transmission electron microscopy, Vibrating Sample Magnetometer, zeta potential and cytocompatibility tests were performed on murine endothelial cells (MS1), using MNPs coupled with/out LVs. Firstly, MNPs and LV-MNPs were tail vein injected in C57BL/6 mice; then the complexes were in situ injected in tumor bearing mice with/without magnetic field application next to the tumor and biodistribution/expression studies performed by histology and immunofluorescence, using GFP as a marker transgene.
Results: 20 nm diameter MNPs were obtained with good dispersion in water, showed superparamagnetic behaviour and cytocompatibility in vitro and in vivo with/out LV association. GFP expression was detected mainly in the liver and the application of a magnetic field guided complexes in the abdomen after in vivo LV-MNPs injection. Intra-tumor LV-MNPs injection followed by magnetic plaque application showed the efficient uptake of LV-MNPs with high number of transduced cells and iron accumulation in the tumor parenchyma, with no dissemination through the body.
Discussion: These studies can significantly improve cancer therapy effectiveness with a selective and localized therapeutic transgenes delivery comparing two different types of LV-MNPs in a mouse model. Overall our data provide a first insight in using and positioning LV-MNPs in tumours, representing the basis for a new platform of personalized cancer treatment.

OC11

MODELLING THE AIRWAY MUCUS MICROENVIRONMENT TO BE APPLIED ON CYSTIC FIBROSIS THERAPY

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Introduction: Cystic fibrosis (CF) is a life-threatening genetic multiorgan disease characterized by the build-up of thick and sticky mucus, abnormal in composition and rheological properties. The inefficient mucus secretion enables the establishment of bacterial colonies that trigger chronic infection, and lately lead to lung failure. The need to characterize drug behaviour in a rapid, simple and reproducible manner has urged the development of airway mucus models. In this work, an airway mucus model composed by alginate and mucin is herein proposed aiming to model both composition and rheological properties of the pathologic CF-mucus.
Materials and methods: Alginate (from brown algae)/mucin (from porcine stomach, type III) hydrogels were produced in NaCl 7 mM. Rheological measurements were carried out to access the viscoelastic and shear thinning behaviour of the developed gels and further compared to the pathological CF-mucus. Stability analysis was also conducted to acquire using both water and PBS, at 25°C, to analyse changes on weight percentage and volumetric increase. Finally, both drug diffusion and interaction through alginate and alginate/mucin gels were carried out using aspirin, cephalexin and epirubicin, as well as gold nanoparticles (GNP) as model drugs.
Results: The viscosity of the mucus model decreases with the increasing of shear stress, with no differences observed between both mucus model and CF mucus. Additionally, no differences on the dissipative modulus were detected between the CF and model mucus, although differences were detected over storage modulus. Weight and size increased in both H_2O and PBS, at 25°C. Diffusion studies of drugs and gold nanoparticles through the gels exhibited compositional and structural dependency, thus effecting the interaction with mucin. The diffusion of drugs was also related to both alginate-drug interactions or steric barrier effect of the gel. Likewise, the diffusion of GNP was hindered by alginate-mucin gels compared to alginate gel, probably related to mesh size of the model.
Discussion: A mucus model was proposed to study drug permeability in presence of mucus secretion. This platform will serve as the basis to implement the complexity of the model in terms of components, also including the effect of bacteria.

OC12

SUPERPARAMAGNETIC IRON-OXIDE NANOPARTICLES FUNCTIONALIZED WITH CONJUGATED LINOLEIC ACID: EFFECT ON VIABILITY OF MOUSE BREAST CANCER CELLS

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Introduction: Cancer nanomedicine pays particular attention to superparamagnetic iron oxide nanoparticles (SPIONs), since, thanks to their small size, they can easily circulate in the blood escaping the capture by reticulo-endothelial cells, and selectively accumulate in the tumor microenvironment. Unluckly, unmodified native SPIONs tend to aggregate into large clusters decreasing their activity. This research aimed to improve SPIONs dispersion and anticancer potential preparing conjugated linoleic acid (CLA)-functionalized SPIONs able to reduce cancer cell viability.
Materials and methods: SPIONs were functionalized with two distinct amounts of CLA: SPIONs + CLA1 (3 µl CLA/ml SPIONs) and SPIONs + CLA2 (4.5 µl CLA/ml SPIONs). FT-IR (Fourier transform-infrared spectroscopy) analysis was used to evidence functionalization. The antitumor effect was investigated in 4T1 mouse breast cancer cells. Viability was evaluated by MTT assay after 24, 48 and 72 hours. The CLA percentage content in cellular lipids was determined by gas chromatography-mass spectrometry.
Results: FT-IR analysis confirmed that SPIONs were functionalized with CLA; SPIONs uptake was evidenced by iron staining. The viability of cells treated with SPIONs + CLA1 or SPIONs + CLA2 was lower than that of control cells and of cells treated with SPIONs alone in a dose-dependent manner. The analysis of fatty acids in lipids extracted from 4T1 cells showed that only the highest CLA dose was able to effectively increase intracellular CLA content. These results seem to indicate that the antitumor effect of functionalized SPIONs are proportional to the increase of CLA incorporation in lipids.
Discussion: SPIONs functionalized with the highest amount of CLA can be suggested as promising therapeutic carriers for treating cancers because of their better dispersion and anticancer properties.

OC13

STRUCTURAL CHARACTERIZATION OF BOVINE OVARIAN CORTICAL TISSUE AND ITS EFFECT ON OXYGEN TRANSPORT TO FOLLICLES

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Introduction: A promising regenerative strategy to exploit fertility potential of women with ovarian failure or malignancies is cryopreservation of strips of cortical tissue, thawing, in vitro culture, in vitro fertilization and re-implantation. In in vitro culture of cortical strips it is difficult to maintain follicles viable and to guide their progression to secondary stage. Perifollicular dissolved oxygen concentration (pO_2) may affect follicle viability and progression. The

development of models of oxygen transport in cortical strips is hindered by scarce quantitative information on cortical tissue structure and functions. In this work, the structure and oxygen consumption rate (OCR) of bovine cortical tissue were characterized. An oxygen transport model was developed to predict perifollicular pO_2 in cortical strips and optimize in vitro culture.
Materials and methods: Ovarian tissue was harvested from abattoir bovines and cut in 0.5 mm^3 cortical strips. Tissue structure was characterized by confocal microscopy. Images were digitalized, segmented and reconstructed in 3D with ImageJ. The number of granulosa cells (GCs) was estimated. OCR of strips was characterized by respirometry. Ovarian tissue was modelled as a stack of pseudo-homogeneous layers, varying in size, stromal cell and fiber density, in which follicles are included. Follicles were modelled as spherical impermeable oocytes surrounded by pseudo-homogeneous layers of GCs. Follicle features varied with stage. Oxygen transport was described through diffusion-reaction equations for Michaelian consumption.
Results: The structural analysis yielded exclusive information on cortex. Fiber density decreased from tunica albuginea to inner cortex. Cell density was highest in the outer cortex. Geometry of tissue layers and primordial follicles, and OCRs, were consistent with literature. Number of follicles per strip and cell density varied by a factor 30 and 2 respectively. The oxygen transport model predicted that stromal cells consume large amounts of oxygen. Significant oxygen reaches follicles from strip bottom and sides. Strip OCR depended on actual follicle number. Higher perifollicular pO_2 at decreasing strip thickness was soundly predicted.
Discussion: Quantitative characterization of structure of bovine ovarian cortex was performed. A structural model was built and used in an oxygen transport model to optimize in vitro culture conditions of ovarian cortical strips.

Session 4 - Advanced Biomaterials

OC14
EUMELANIN-BASED SUBSTRATES AS SMART MATERIALS FOR NEURONAL REGENERATION

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Introduction: The regeneration of neurite network constitutes a strategy for the treatment of neurodegenerative disorders characterized by a loss of trophic factors, but their clinical use is limited by their inability to cross the blood brain barrier. In the field of regenerative medicine, much effort has been devoted to the design of effective biomaterials for neurite regeneration. Eumelanin-based materials can find applicability in biomedicine and (bio)electronics thanks to both their physicochemical properties as well as structural features. Many attempts have been finalized to get eumelanin 2D thin–film fabrication, but any effort has failed to obtain 3D or 1D eumelanin architecture assembly. The fabrication of eumelanin-coated microfibrous structures represents a novel strategy to realize tissue-engineering scaffolds for neuronal cells growth and control by providing both mechanical support and biological signals.
Materials and methods: To realize the scaffolds, an appropriate protocol combining electrospinning, spin coating and solid-state polymerization process was established. For biological analysis, a human derived cell line from neuroblastoma was used. Cell differentiation on eumelanin microfibers both random and aligned was evaluated through GAP-43 expression, a marker of differentiating neurons, by using confocal analysis. Furthermore, cell morphology by using SEM analysis and βIII tubulin expression was tested.
Results: Biological results showed eumelanin microfibers support biological response in terms of cell survival and adhesion thus promoting over time cell differentiation toward a neuronal phenotype. In fact, GAP-43 expression over culture time confirmed differentiation processes. Furthermore, morphological studies (SEM and confocal microscopy) revealed that eumelanin microfibers were able to induce a good cellular spreading.
Discussion: Our results suggest eumelanin microfibers might be worthy of consideration for future evaluations of new therapeutic strategies for neurodegenerative diseases.

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OC15
3D PRINTING OF A TYRAMINE-MODIFIED HYALURONIC ACID WITH A DUAL CROSSLINKING MECHANISM

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Introduction: 3D printing is an additive manufacturing technique that is rapidly growing in the field of tissue engineering and regenerative medicine. Although a variety of biomaterial inks have been reported, it is challenging to match in a single material all the properties of the ideal ink. This work is focused on developing a tyramine-modified hyaluronic acid ink (HA-Tyr) with a dual crosslinking mechanism consisting of an enzymatic pre-crosslinking (horseradish peroxidase, HRP/hydrogen peroxide, H_2O_2) for optimal extrusion and visible light crosslinking (Eosin Y/green light) for shape stabilization.
Materials and methods: Tyramine was grafted to hyaluronic acid via amidation, achieving 14.5% degree of substitution. HA-Tyr was dissolved at 2.5% w/v in a buffer solution containing HRP 0.1 U/ml and Eosin Y 0.02% w/v. H_2O_2 0.17 mM was added to obtain a soft gel via enzymatic crosslinking whereas visible light (λ = 504 nm) was subsequently used to further crosslink the gel. An Anton Paar MCR-302 rheometer was used to investigate shear-thinning and viscoelastic shear moduli of the ink. Circular 3D constructs of 10 mm diameter and 3 mm height with a criss-cross structure were produced with a 3D Discovery® system (RegenHU Ltd). Different printing parameters were optimized in order to obtain the highest shape fidelity. Micro-computed tomography (μCT) was performed to assess shape fidelity.
Results: The non-crosslinked HA-Tyr showed a prevalence of viscous behaviour, turning into an elastic behaviour after enzymatic crosslinking, improving post-printing shape retention. A 4-fold increase in the storage modulus was observed performing subsequent light crosslink, allowing shape stabilization. μCT indicated printed struts of 0.88 ± 0.24 mm width and pore size of 0.68 ± 0.14 mm.
Discussion: HA-Tyr hydrogel is a viable ink in extrusion-based 3D printing. The dual crosslinking of the HA-Tyr ink allows for optimal extrusion and shape retention. Current work is focused on seeding these 3D printed constructs with human mesenchymal stem cells for tissue engineering.

OC16
APPLICATIONS OF SOL-GEL MATERIALS IN BIOMEDICAL FIELD: ZrO_2/PEG HYBRID MATERIALS

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Introduction: Glass-ceramics are an important family of materials proposed for bone repair and substitution. An ideal technique to prepare bioglass is the sol-gel methods. The chemistry of the process, indeed, leads to the formation of –OH groups on the surface of the sol-gel materials, which stimulate hydroxyapatite nucleation, promoting materials easier osseointegration. Therefore, the sol–gel materials are more bioactive and biocompatible than the materials with the same composition but prepared using other techniques. Moreover, the low processing temperatures allows of entrapping thermolabile molecules (e.g. polymers and drugs) in the inorganic matrix, producing organic-inorganic hybrids (OIHs). In the present work, the preparation of OIHs, consisting of an inorganic ZrO_2 matrix in which the polyethylene glycol (PEG) was encapsulated, is reported. The materials were proposed to prepare bioactive and biocompatible bulks, coatings on titanium implants and matrices for controlled-release of indomethacin.
Materials and methods: ZrO_2 and ZrO_2/PEG (6, 12, 24 and 50wt%) OIHs were synthesized by a sol-gel process, mixing a solution of zirconium propoxide with a solution of PEG 400. The sol was used to coat titanium grade 4 implants by dip coating technique. Moreover, a solution of ethanol/Indomethacin (5, 10 and 15wt%) was added to the ZrO_2/PEG sols to prepare drug delivery matricies. The obtained gels were charaterized by SEM, XRD, FTIR and solid-state NMR, and their bioactivity and biocompatibility were investigated by the apatite forming ability test and WST-8 assy respectively. Release kinetics was investigated by HPLC UV-Vis spectroscopy.

Results: Experimental results showed that all synthesized materials are amorphous OIHs where PEG and Indomethacin are linked to inorganic matrix by H-bonds. All materials are bioactive and biocompatible and able to improve the biological performance of titanum implants when used as coatings. Moreover, PEG presence increases the indomethacin release, but a full release of drug is hindered when 24 and 50wt% is present due to the formation of weak interactions between indomethacin and PEG.
Discussion: The results prove that sol-gel is a versatile technique which allowed of obtaining bioactive and biocompatible OIHs materials and coatings and modified-release systems.

OC17
DEVELOPMENT OF A COMPOSITE MATERIAL WITH TUNABLE HYDROPHOBIC-OLEOPHILIC PROPERTIES

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Introduction: An appropriate sorbent material should fulfil specific requirements, such as highly hydrophobic and oleophilic surface properties, high oil-absorption capacity (thanks to a three-dimensional and porous structure), low material and processing costs, good reusability. Obviously, it should not have any negative environmental effects and should be based on renewable resources. Herein, a new composite material based on a cellulose 3d porous matrix, treated with stearic acid and expended graphite flakes, is presented. The obtained composite system exhibits an almost superhydrophibic surface with considerable values of water contact angle (WCA) and oil absorption rate (OAR).
Materials and methods: Cellulose foams used as substrates were obtained by MAIN Spa. Stearic acid, in powder, was purchased by Sigma Aldrich. Expanded graphite flakes (Grafysorber™) are obtained by Biocart Srl and dispersed with stearic acid (both 1% w/V) in acetone, after sonication for 1h. Small pieces of cellulose foams received the dispersion *via* drop casting and were heated in oven at 100°C for 45 minutes. WCA and OAR measurements were performed with a FTA1000B-B1A3310 goniometer. Morphological analysis was performed by means of a Scanning Electron Microscope (SEM) by ZEISS, EVO 40.
Results: Native cellulose foams is omniphilic. They are modified with stearic acid and graphite, leading to an almost superhydrophobic matrix (WCA 140.22 ± 2.43°) and enhancing the initial oil-absorbing character (OAR of 7.61 ± 2.43 µl/s instead of 4.62 ± 1.12 µl/s). In this way, the modified foams exhibit a selective behaviour towards water and oil, which makes them suitable to be used for the removal of oily contaminants from water. Morphological analysis reveals that the dimensions of the pores is on the order of a few hundreds of microns, matching with the sizes of recently developed functional meshes for water/oil separation. Moreover, the pristine foams exhibit completely smooth fibres surface, while that of the modified samples is rougher, due to the presence of the deposited hydrophobic stearic acid layer entrapping graphite particles.
Discussion: The presented composite is based on easily available and fully natural materials, like cellulose, the most abundant polysaccharide in nature, stearic acid and graphite, and is obtained through an easy, cost-effective and scalable fabrication process.

Session 5 - Surface Treatments and Characterization

OC18
WIDE ANGLE X-RAY SCATTERING OF DIFFERENT ISOFORMS OF TYPE-I COLLAGEN: THE IMPACT OF PROCESSING CONDITIONS ON STRUCTURAL AND MECHANICAL PROPERTIES

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Introduction: Collagen-based biomaterials are widely used to support tissue regeneration, due to their biocompatibility, biodegradability and mechanical properties. In this work, raw collagen fibers and collagen-based films were structurally characterized by wide-angle X-ray scattering (WAXS), in order to investigate the structural changes at the atomic scale after film fabrication. Mechanical properties of films were also assessed.
Materials and methods: Raw type-I collagens from bovine hide (KN, CS, SYM) and equine tendon (TYP_{en} and TYP_{ch}) were analysed. Bovine collagens were then used for fabricating air-dried films, from collagen suspensions obtained by: 1) dissolution in distilled water (HH); 2) dissolution in acidic medium (AA); 3) homogenization of acid solubilized fibers (HOM). Physico-chemical cross-linking treatments (DHT, DHT + EDC) were applied to increase the film stability. WAXS analysis was carried out at the XMI L@b (CNR-IC-Bari) on both raw materials and films, while mechanical analysis was performed at the BioSLab (DII-UniSalento).
Results: Two collagen-specific WAXS reflections were observed: the equatorial peak (d = 10.8 Å), mark of collagen lateral packing, and the meridional peak (d = 2.8 Å), mark of periodicity along the helix central axis. 2D WAXS profiles of equine collagens showed preferred orientation on both reflections, while the equatorial peak full-width-at-half-maximum (FWHM) highlighted a higher crystallinity for equine collagens, compared to bovine ones. Finally, CS showed sharp reflections ascribable to salt contamination. 1D WAXS profiles of cross-linked films showed that FWHM increases/decreases in AA/HOM respectively, suggesting a lower/higher crystallinity. Tensile tests performed on SYM films showed that the stiffness of AA/DHT films increased after the EDC treatment, while this was not noticeable in HOM/DHT samples.
Discussion: WAXS analysis showed that the rod-like helical structure of all collagen isoforms is mainly preserved after the extraction and fabrication processes, while different degrees of crystallinity and orientation were found. These differences were found to influence the scaffold mechanical properties.

OC19
DEVELOPMENT AND CHARACTERIZATION OF SURFACES FOR THIOL-MEDIATED CELL ADHESION

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Introduction: Material surface engineering can be used to modulate cell-surface interactions and to enhance certain biological functions. Of note, cells need to attach and spread on a surface in order to survive and the chemical surface functionalities and hydrophilicity strongly affect cell adhesion and functions. Although thiols play a key role in cellular uptake of thiolated molecules and particles, their role as regulators of cell adhesion is still unclear. In order to ascertain the possible influence of thiols in cell adhesion mechanisms, we herein propose the development and characterization of glass surfaces that were thiol-tethered by means of sol-gel dip coating.
Materials and methods: Sols were obtained by mixing two alkoxides, tetraethylorthosilicate (TEOS) and n-Propyltrimethoxysilane (CH_3), with different amounts (up to 60%) of (3-Mercaptopropyl)trimethoxysilane (MSH). The resulting sols were deposited onto glass surfaces by dip coating at controlled speed, allowing the deposition of thin films. The surfaces were first characterized in terms of wettability by contact angle analysis, while thickness and roughness were evaluated by profilometer. Finally, a preliminary in vitro characterization using HeLa cell line was carried out in order to highlight the possible role of surface thiols in cell adhesion mechanisms.
Results: We thus obtained functionalized surfaces showing different thiol content (from 0 to 60%) that depended on the MSH concentration in the starting sols. Contact angle analysis showed that TEOS- and CH3-coated surfaces had different wettability, according to the thiol content of the coatings: TEOS-based surfaces showed a decreased wettability by increasing the MSH content in sol, while an opposite behavior was observed for CH3-coated surfaces. Profilometry analysis revealed that TEOS-based coatings had a thickness between 200 and 300 nm, while CH3 coatings between 100 and 200 nm. Preliminary results obtained using HeLa cells showed different cell adhesion depending on the surface wettability that relies on the amount of MSH in the starting sols.
Discussion: We obtained the functionalization of surfaces with thiol groups by means of sol-gel dip coating. The amount of surface thiols can be properly modified by sol composition and, according to preliminary in vitro studies, it seems to affect cells adhesion mechanisms.

OC20

MOLECULAR DYNAMICS STUDY OF AN ALBUMIN SUBDOMAIN ADSORPTION ON DIFFERENT CRYSTALLOGRAPHIC SURFACES OF ANATASE TiO_2

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Introduction: The interaction strength of protein adsorption and the possible conformational changes induced by the biomaterial substrate can affect the functionality of adsorbed proteins, then their functionality and finally cell adhesion and spreading. In previous work, a theoretical study based on Molecular Mechanics (MM) and Molecular Dynamics (MD) methods, the adsorption of an albumin subdomain on the surface of TiO2 polymorphs (rutile, anatase, and brookite) was studied.
Materials and methods: Using the same methodology, the adsorption of the same albumin subdomain on different crystallographic faces of anatase is presented. The exposed surfaces taken into account are the most stable (1 0 1) crystallographic face and the stable surfaces (0 0 1) face, that exposes hydroxyl groups, the (0 0 -1) face exposing Ti atoms and the (1 0 0) face exposing oxygen and titanium atoms in a peculiar surface order.
Results: The results show that in the initial adsorption stage the albumin subdomain shows an interaction energy E_{int}, normalized by the number of aminoacids in contact with the surface (i.e., at a distance of 6 Å), that is comprised between 40 and 46 kJ/mol when the exposed surface does not contain hydroxyl groups. On the contrary, the E_{int} obtained for the hydroxylated (0 0 1) face amounts to about 28 kJ/mol. Afterwards, MD simulations were carried out for all the above-mentioned surfaces, starting both from the most stable initial geometry and from the less stable one. After these MD runs, in general some surface spreading of the modeled subdomain of this *soft* protein was found. However, when the surface contains hydroxyl groups the overall structure remained globular, whereas on the non-hydroxylated surfaces a much larger surface spreading was achieved, with a partially ordered arrangement of distant parts of the backbone in parallel strands.
Discussion: These results are in essential agreement with those obtained in our previous work considering the adsorption of the same albumin subdomain on graphite surface and on carbon SWNTs, where surface spreading and parallel ordering was found, and conversely on poly(vinyl alcohol) where the surface spreading was basically absent and the protein subdomain kept a globular shape.

OC21

NEW RESEARCH TOOLS FOR THE FIELD OF COLD ATMOSPHERIC PLASMA APPLICATIONS

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Introduction: In recent years, increasing research efforts have been devoted toward the use of Cold Atmospheric Plasma (CAP) as a promising tool for a wide range of biomedical applications on thermosensitive materials, thanks to the synergistic effects of its physical and chemical active agents and to the possibility of tuning them for specific uses.
Materials and methods: Alma*PLUS* (Plasma Laboratory Unified System) by AlmaPlasma srl, is a unique system that integrates all components required to explore a great variety of CAP-assisted processes in terms of fundamental research. The variety of compatible plasma sources grants Alma*PLUS* ample versatility. Through a user-friendly interface the operator can define specific geometric patterns for the treatments, select electrical parameters for plasma generation and control the flows of gases and liquid precursors for the deposition of coatings; the system allows easy saving and recall of all process parameters ensuring treatment repeatability.
Results: Alma*PLUS* can be used for the treatment of solids and liquids (water, cell culture medium, PBS) for different purposes: surface functionalization, crosslinking of polymeric mats, increase of biocompatibility, degradation of pollutants, bacterial decontamination, treatment of eukaryotic cells with plasma activated culture medium, improvement of the electrospinnability of polymeric solutions. Alma*PLUS* can also support plasma treatments of ex-vivo and in-vitro 2D/3D cell cultures, through dedicated patterning for different types of multiwell culture plates. Within this perspective, the second system presented, Alma*MED* by AlmaPlasma srl, is a prototype specifically designed for the treatment of biological materials, but more generally suitable for the treatment of solids and liquids. Alma*MED* is a tabletop system composed by a hand-heldable DBD-jet source, a dedicated pulse generator, a small disposable gas (He) tank, an intuitive user interface, disposable dispenser tips and autoclavable parts.
Discussion: Alma*MED* is a viable tool to further investigate the fields of chronic wounds disinfection, immune system stimulation and antitumor treatments. The shape and electrical insulation of the DBD-Jet source allow safely reaching and treating even remote areas of the oral cavity. Preliminary results demonstrate Alma*MED* potential in dental applications such as the decontamination of infected root canals and the enhancement of the adhesive performance of dental restoration systems.

OC22

EFFECTS OF PAM ON EUKARYOTIC CELLS GROWN ON PCL SCAFFOLDS FOR TISSUE ENGINEERING

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Introduction: Cold plasmas are continuously developed for Biomaterials Engineering and in Plasma Medicine for therapeutic treatments. Although a complete understanding has still to emerge on the application of atmospheric pressure (AP) plasmas on eukaryotic cells, reactive oxygen and nitrogen species (RONS) generated in Plasma Activated Media (PAM) gained attention as key compounds responsible in influencing and addressing specific biological effects.
Materials and methods: Air cold plasma working at AP was utilized, for generating RONS in Dulbecco's Modified Eagles Medium (DMEM); superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), nitrates (NO_3^-) and nitrites (NO_2^-) were detected. PAM was applied to ovine Bone Marrow Stem Cells (BMSC). For better mimic the in vivo biological environment, both native and plasma-modified PCL scaffolds were used as three dimensional (3D) supports for cell cultures.
Results: BMSCs grown on plasma-coated scaffolds tolerated better PAM and grow healthy with respect to those on native scaffolds.
Discussion: This preliminary finding deserves more investigations (e.g., intracellular RONS detection, use of different substrates); however, it clearly indicates that PAM could be conveniently used for stimulating and/or driving the cellular colonization of polymeric scaffolds in Tissue Engineering and Regenerative Medicine and properly tuning cell responses on surfaces.
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OC23

NON-EQUILIBRIUM ATMOSPHERIC PRESSURE PLASMA ASSISTED FUNCTIONALIZATION OF ELECTROSPUN MATS FOR THE SELECTIVE CAPTURE OF ADIPOSE-DERIVED MESENCHYMAL STROMAL/STEM CELLS

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Introduction: Recently, the covalent immobilization onto polymeric surfaces of bioactive compounds has been investigated. It has been demonstrated that aliphatic polyesters present excellent biocompatibility and bulk properties; however, polyesters do not have a significant amount of surface functional groups, therefore, a surface treatment prior to the conjugation is recommended. Non-equilibrium atmospheric pressure plasma turns out to be an effective technology for the introduction of functional groups. The work reports on the use of a non-equilibrium atmospheric pressure plasma for the introduction of carboxyl groups on the electrospun poly-L-lactic acid (PLLA) mats; enabling the bioconjugation of antibody anti-CD10 by exploiting the N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide (EDC)/N-Hydroxysuccinimide (NHS) chemistry. The suitability of the produced mats to capture mesenchymal stromal/stem cells (MSC) from lipoaspirates has been investigated.
Materials and methods: A homemade electrospinning apparatus was used to fabricate the PLLA mat. A 13% w/v PLLA solution in 65:35 v/v DCM:DMF mixture was electrospun by applying 20 kV voltage, 20 cm needle to collector distance, using 1.2 mL/h solution flow rate. The plasma functionalization was performed with an atmospheric pressure Dielectric Barrier Discharge (DBD) plasma source driven by a microsecond pulses generator; peak voltage, frequency and treatment time were fixed at 13.5 kV, 500 Hz and 6 min, respectively. Functionalized PLLA mats groups were activated with EDC/sNHS and incubated with anti-CD10. Bioconjugation was performed after introduction of a diaminic linker. The efficacy on antibody conjugation was evaluated by a semi-quantitative determination of the quantity of fluorescent spots visualized by fluorescence microscopy-Zen software analysis (10× objective).
Results: SEM analysis highlighted that plasma did not induce any morphological change of mat; the water contact angle (WCA) measurements reported that while a constant WCA of 120° was obtained for pristine PLLA mat, an almost instantaneous penetration of water was registered after the functionalization. The Zen software analysis reveals a diffuse and homogenous distribution of the antibody, demonstrating a good optimization of the process.
Discussion: The work highlights the possibility to employ a non-equilibrium atmospheric pressure plasma process for antibody conjugation onto PLLA electrospun fibers. Furthermore, the potential suitability of the produced mats to capture MSC has also been evaluated.

Session 6 - Regenerative Medicine: Soft Tissues

OC24
HYALURONAN-BASED DERMAL FILLERS: THE KEY DETERMINANTS OF CLINICAL PERFORMANCE

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Introduction: Hyaluronan (HA)-based dermal fillers are continuously being developed and launched on the market. At the same time, scientific efforts to provide biophysical and biochemical data for these gels and to correlate these data with clinical outcomes are also intensifying. HA-based dermal fillers are HA hydrogel microparticles suspended in physiological or phosphate-buffered solution. For most of the available products, HA hydrogel is obtained by crosslinking the biopolymer with 1,4-butandioldiglycidyl ether. It has been found that different manufacturing procedures result in different HA crosslinking degree and hydrogel particle size. These features, together with the final gel concentration, are responsible for different swelling capacity, rheological parameters, sensitivity to degradation, finally affecting the clinical performance (injectability, application site, tissue integration pattern, filling capacity, duration of the effect etc.). Here, chemico-physical data for diverse available fillers are provided and the correlation with the clinical use and outcomes is discussed.
Materials and methods: Fillers were evaluated in terms of swelling degree, rheological properties, soluble HA content, crosslinker amount in the network, sensitivity to in vitro enzymatic hydrolysis. The swelling capacity was evaluated by means of gravimetric measurements. Rheological measurements were carried out using a Physica MCR301 oscillatory rheometer (Anton Paar, Germany). For the degradation studies, bovine testicular hyaluronidase was used. Some products were evaluated for their biological response using an in vitro 3D skin model.
Results: The fillers were non equilibrium gels also showing different water uptake capacities. A shear thinning behaviour was found for all the products examined and the rigidity varied over a wide range. Diverse resistance to the BTH action was found. Biological results proved gels ability to promote skin restoration.
Discussion: Overall, collected data consistently correlated with 1) the crosslinking degree and the insoluble hydrogel concentration indicating that these are the two main parameters to set in order to tune the fillers properties and with 2) the clinical indications of use from the manufacturers thus confirming that biophysical data are a valuable support in dermal fillers use. Collected data also allowed a prediction of the relative tissue integration pattern for the diverse gels.

OC25
POLYLYSINE ENRICHED DECELLULARIZED MATRICES: A PROMISING APPROACH FOR VASCULAR SURGERY

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Introduction: Cardiovascular diseases are a leading cause of death worldwide. Current clinical approaches show poor efficiency in the replacement of small-caliber arteries (<6 mm). The use of decellularized scaffolds has shown good prospects in various applications for regenerative medicine. The purpose of this work is to obtain a scaffold chemically enriched with polylysine. This acts as a cross-linker making the scaffold more resistant from the mechanical point of view, without altering biocompatibility and hemocompatibility properties.
Materials and methods: The matrices were obtained by decellularization and enrichment with polylysine (sueGraft®) of porcine arteries (femoral and carotid). In order to verify the effectiveness of the decellularization process, DAPI, hematoxylin/eosin staining, and quantification of residual DNA were performed. After culture of endothelial cells on the enriched matrix, biocompatibility of the material was verified. In order to measure elasticity, burst pressure and degradation in working condition, mechanical tests were performed. Finally, several parameters related to hemocompatibility of the scaffold were evaluated.
Results: DAPI and hematoxylin/eosin staining confirmed the effectiveness of the decellularization method. The quantification of the DNA test showed that the amount of residual DNA was significantly reduced compared to untreated control. Values obtained resulted much lower than the threshold values reported in literature. Cells grown on polylysine-enriched matrices showed excellent biocompatibility. The analysis of the Young moduli showed that stiffness value of the enriched matrix is not significantly different to native vessel. Burst pressure test showed strengthening of the polylysine-enriched matrix, which can withstand higher pressures compared to native vessel. Matrix degradation test showed that the polylysine-enriched vessel has almost no weight loss, which indicates an absent degradation. Concerning hemocompatibility, the evaluated parameters suggest that polylysine-enriched matrices increase clotting time.
Discussion: The matrices obtained by decellularization of blood vessels and enriched with polylysine show an excellent biocompatibility, promising mechanical properties and improved hemocompatibility properties for the intended use as vascular substitutes. Based on these results, matrices enriched with polylysine are a promising approach for vascular substitution.
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OC26
IMPROVED CALCIUM PHOSPHATE NANOPARTICLES FOR TARGETED CARDIAC DRUG DELIVERY

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OC27

ELECTROSPINNING OF A BIOMIMETIC AND BIORESORBABLE MONOLITHIC AORTIC VALVE SCAFFOLD

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Introduction: Mechanical and biological prosthetic heart valves are currently implanted to restore the proper blood hemodynamics when the native valves fail. Despite their widespread use, these devices present some disadvantages, including hemolysis or calcification, and, above all, they are, evidently, unable to grow, repair and remodel. In this scenario, tissue-engineered heart valves represent a very attractive alternative approach. Here, we evaluated the possibility to design a monolithic aortic valve by electrospinning of natural occurring ECM polymers, with the aim to construct a scaffold closely resembling the architecture of native valve tissue.
Materials and methods: Gelatin Type A (10% w/v) and gelatin/elastin blend (9:1 ratio, 22% w/v) were electrospun from 90% acetic acid and 20% poly-ε-caprolactone (PCL, used as reference) from chloroform/DMF mixture (9:1). A custom-made, aortic valve-shaped aluminium collector was used for valve fabrication. Gelatin-based fibers were cross-linked with carbodiimide according to standard protocols. Electrospun valves materials were characterized morphologically by electron microscopy, mechanically, in a dynamic-mechanical analyser and biologically by seeding L929 fibroblast and EAhy926 endothelial cells. A preliminary evaluation of electrospun valve behaviour in the systole phase was also performed in a pulse duplicator device.
Results: After an accurate optimization of electrospinning parameters, homogeneous fibrous structures were obtained and morphological evaluation revealed beads-free fibers with average diameter of 1.6 µm and 0.23 µm for PCL and gelatin scaffolds respectively. Mechanical characterization confirmed that gelatin and gelatin/elastin nanofibers could better mimic biological ECM mechanical behaviour compared to the much stiffer PCL. All materials resulted non-cytotoxic and in cytocompatibility test slightly higher cell viability was found on gelatin-based scaffolds. In the preliminary functional assessment, synchronous opening of leaflets was observed.
Discussion: The development of a tissue engineered heart valve still remains a challenge. However, electrospun ECM molecules have great potentials as they can combine the strength of highly oriented polymers, with excellent cell compatibility, mechanical compliance and cell mediated remodelling. Although much effort is still needed, this work represents a step forward in demonstrating the feasibility of this approach.

Fire Session

F1

DEVELOPMENT OF A THERMOSENSITIVE FORMULATION FOR THE TREATMENT OF VULVOVAGINAL CANDIDOSIS RECURRENCES

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Introduction: Vulvovaginal candidosis is an infection of the vaginal mucosa, caused by *Candida spp.*, which represents one of the most frequent causes of gynaecologic counselling. Vaginal probiotics, such as *Lactobacillus spp* are able to directly compete with pathogens for both nutrients and adhesion sites at the mucosal surface, moreover to lower intravaginal pH, thus establishing a hostile environment for pathogen growth.
The present work aims to develop an in situ gel forming vehicle for the vaginal administration of probiotics. *Lactobacillus gasseri*, one of the dominant endogenous *Lactobacillus spp*, was selected as active ingredient.
Materials and methods: Poloxamer 407 (P407) and methylcellulose 4KM (MC) were considered as thermosensitive polymers, xyloglucan (XYL) as stabilizer agent. Polymer mixtures as such and after dilution in simulated vaginal fluid (SVF) were subjected to rheological analysis (viscosity, viscoelasticity, thixotropy). Vehicle mucoadhesive properties were evaluated by means of a tensile tester, using porcine vaginal mucosa as biological substrate. The compatibility of *L. gasseri* with vehicle was evaluated by means of viability test.
Results: The association 1.5% w/w MC - 15% w/w P407 showed an increase in gelation extent at 37°C even after dilution in SVF (10:1 and 10:1.5 w/w ratios). In presence of low concentration of XYL (0.25% w/w), MC kept the capability to gelify at 37°C even at lower concentration (0.75% w/w) after dilution with SVF. Vehicles had a thixotropic behaviour functional to an easy administration. Viability tests performed up to 24 hours on *L. gasseri* demonstrated that the polymers employed did not disturb microorganism growth.
Discussion: The developed formulations are able to gelify at 37°C upon dilution in SVF and to preserve *Lactobacillus* viability. Moreover they show mucoadhesive properties. They are promising candidates for the local delivery of *L. gasseri* in the treatment of vulvovaginal candidosis.

F2

DEVELOPMENT OF SILK FIBROIN FILMS FOR WOUND DRESSING

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Introduction: Skin injuries represent a severe health problem. Smart wound dressings can promote the wound healing process. Silk fibroin is a biopolymer with many advantageous properties, very appealing for application in tissue engineering because of the capability to enhance adhesion, growth and differentiation of cells.
Materials and methods: Cocoons were boiled in aqueous solution of sodium carbonate to remove sericin. Degummed silk fibroin fibres were dissolved in a solution of calcium chloride, ethanol and water. The fibroin solution was dialyzed and mixed with glucose at different weight ratios. The mixed solutions were poured into polystyrene plates and dried for formation of films. Some films were also immersed in methanol solution to induce crystallization. Silk fibroin films (SF) and glucose modified silk films (GMSF) were characterized by Fourier Transform Infrared Spectroscopy and Differential Scanning Calorimetry. Water absorption capacity, mechanical properties, biocompatibility and regenerative properties of silk fibroin films were analysed.
Results: After methanol treatment, silk fibroin films resulted brittle whilst glucose modified silk films resulted insoluble in water and more flexible. The infrared spectra and thermal analysis indicated the formation of β-sheet structures in GMSF and a shift in degradation peak. The data demonstrated an increase in water adsorption of GMSF due to the hygroscopic property of glucose molecules. The incorporation of glucose reduced the tensile strength and increased the elongation at break. MTT assay and scratch assay confirmed the biocompatibility and the regenerative properties of silk fibroin films.
Discussion: The results obtained demonstrated that the addition of glucose has induced crystallization and enhanced flexibility of silk fibroin films. GMSF showed improvement water uptake and increased cell viability. Scratch assay demonstrated that silk fibroin induced higher cell migration capability. Silk fibroin supplemented with glucose also had a positive effect in promoting the wound closure. Silk fibroin films demonstrated a potential for application in wound healing due to the biocompatibility and regenerative properties.

F3

SCAFFOLDS BASED ON ELECTROSPUN NANOFIBERS FOR WOUND HEALING

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Introduction: Electrospinning is a one-step method to manufacture (nano) fibers having diameters ranging from 20 nm up to 1 mm and more. Scaffolds based on nanofibrous membranes could allow protecting chronic skin lesion them from microbial contamination and could induce cell

adhesion and growth. The aim of the present work was the development of electrospun nanofibers based membranes as scaffolds to enhance cutaneous wound healing of chronic lesions and burns. The nanofibers were prepared starting from aqueous polymeric solutions to obtain insoluble membranes in aqueous fluids able to act as a support for cell growth, migration and proliferation.
Materials and methods: Polymeric mixtures based on chitosan and hyaluronic acid or chitosan and chondroitin sulfate were electrospun to obtain nanofibrous membranes. Moreover, a membrane based on chitosan was prepared as comparison. The membranes were characterized by morphology, nanofiber size and mechanical properties. Furthermore, safety and efficacy were studied by means of an in vivo murine model.
Results: All the membranes prepared were based on continuous and randomly oriented nanofibers having diameters in the nanometric range (500-600 nm). The membrane compositions did not significantly modify nanofiber morphology. Membranes were characterized by force at break and elongation suitable for skin application both in dry and wet conditions. The in vivo results suggested that all the membrane were biocompatible without evidence of adverse effects. Moreover, all the scaffolds allowed a wound closure within 18 days while the untreated lesions did not show a complete re-epithelialization in the same time frame.
Discussion: The nanofibrous membrane showed suitable properties for cutaneous application as scaffolds. Glycosaminoglycans seem to enhance healing process. Even if further characterizations will be mandatory, scaffolds based on electrospun nanofibers demonstrated to be an effective tool to speed up skin reparation.

F4

ELECTROSPUN GELATIN/CHONDROITIN SULFATE NANOFIBROUS MEMBRANES FOR THE TREATMENT OF MYOCARDIAL INFARCTION

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Introduction: ECM of the heart is made up of collagen type I and III, which are produced by cardiac fibroblasts and vary in their physical properties and determines the mechanical strength. Thus, in case of myocardial infarction, functional cardiomyocytes are replaced by a fibrotic or "scar tissue" caused by an increase in collagen expression leading to enhanced tissue stiffness with an elastic modulus much higher than that of typical myocardium. Collagen type I is most favored for producing therapeutic platforms and their denaturated product gelatin has been competitively used as biomaterial.
Materials and methods: Gelatin B (20%) was dissolved in acetic acid solution (20%) with or without the addition of chondroitin sulfate (2%), used in order to improve growth factors interaction. Solutions were stirred for 1 h at 40°C. The electrical conductivity of gelatin solutions was measured by using a conductometer and the surface tension by means of a tensiometer. The viscosity measurements were performed using a rotational rheometer.
Applied voltage, syringe-collector distance and flow rate were modulated to obtain nanofibrous membranes, crosslinked by heating (150°C for 2 h). Aqueous solubility of nanofibers, before and after crosslinking, was evaluated. Mechanical and chemical-physical properties and morphology were characterized. Finally, the capability of gelatin nanofibers to increase cell proliferation was evaluated by means of MTT test 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide).
Results: Gelatin solutions were characterized by good electrical conductivity as well as surface tension and together with the right entanglement concentration allowed to obtained nanofibrous membranes characterized by good mechanical properties as well as uniform morphology and low fiber diameter. After crosslinking treatment, it was possible to obtain insoluble fibers. The membranes were biocompatible and allowed the in vitro fibroblasts adhesion/proliferation.
Discussion: Even further evaluations are needed, electrospun gelatin/chondroitin sulfate nanofibers seem promising to enhance cell proliferation.

F5

COLD ATMOSPHERIC PRESSURE PLASMA TREATMENT TO IMPROVE THE BONDING STRENGTH OF DENTIN-ADHESIVE SYSTEM INTERFACE IN DENTAL COMPOSITE RESTORATION

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Introduction: Debonding is the main reason for dental composite restoration failures and the need for better adhesive performances is prompting the research on innovative solutions. This study investigates the potential of Cold Atmospheric Plasma (CAP) treatments of dentine to enhance the bonding strength of the dentin-adhesive system interface.
Materials and methods: Sixty extracted monocanalar teeth were standardized (crown sectioning and root canal shaping), then embedded in epoxy resin using a custom molding procedure ensuring accurate alignment during *push-out* tests. The dentin surface was treated with different chelating agents (EDTA or phytic acid) and then treated with CAP for 180s. Afterwards, a self-etch adhesive system (Clearfil-SEBond2) was applied before the luting cement (Clearfil-DC-CorePlus) was used to seal the root canal; finally both components were light cured. After storage in water (24h, 37°C), the teeth were sectioned in 2 mm thick slices. Bonding strength was finally evaluated by means of *push-out* tests. CAP treatment was performed using Alma*MED* by AlmaPlasma srl, a tabletop system designed for biomedical applications and composed by a hand-heldable DBD-jet source with disposable dispenser tips and autoclavable parts, a dedicated pulse generator, a small disposable gas tank and an intuitive user interface; the device shape and electrical insulation allow safely reaching and treating even remote areas of the oral cavity.
Results: Compared to control, *push-out* results show a significant enhancement of the bonding strength when CAP is applied on dentin after the chelating agents EDTA (+131.9 ± 4.1%) or phytic acid (+148.0 ± 26.6%); SEM analysis and contact angle measurements (performed on dentine with both water and the etchant-primer component of the adhesive system) show an increase, with respect to control, of the amount of dentinal tubules filled with the adhesive resin and support the hypothesis of a bonding strength improvement mainly driven by the plasma-induced increase in dentin wettability.
Discussion: Presented results demonstrate that CAP is a feasible option for enhancing the performances of the dental adhesive systems and, when combined with already published ones demonstrating the efficacy of the DBD-jet source in bacterial decontamination of the root canal, shorten the gap between laboratory experience and CAP application in real-life procedures.

F6

3D PRINTING OF METHYLCELLULOSE *THERMO-RESPONSIVE* HYDROGELS FOR REGENERATIVE MEDICINE

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Introduction: A possible strategy in regenerative medicine is cell sheet engineering consisting in developing smart culture surfaces, which allow to obtain intact cell sheets. The main goal of this work is the realization and characterization of methylcellulose (MC) based hydrogels and their 3D printing via extrusion-based bioprinting. In particular, the prepared substrates were tested with two cellular phenotypes for the realization of cell sheets.
Materials and methods: Hydrogels were prepared by mixing MC powder in saline solutions of Na_2SO_4 and PBS. In order to extrude the MC based hydrogels, a Kiwi 4D printer (Sharebot, Nibionno, LC, IT) was used. MC based hydrogels where characterized from the rheological point of view using a rotational rheometer to investigate possible modification induced by the extrusion in the 3D printing. For cellular in vitro tests three kinds of samples (bulk,

ring and print-ring) were examined. Bulk and ring samples were realized by putting MC solutions, respectively, in a 24 wells TCPS and in PDMS supports. The print-ring samples were obtained by printing MC based hydrogels inside of PDMS supports, using the Kiwi 4D printer. In vitro tests were performed with murine embryonic fibroblasts (NIH/3T3) and endothelial murine cells (MS1) so to obtain cell sheets characterized by cell viability, immunofluorescence analysis.
Results: The extrusion process reduces the LCST of the MC based hydrogels; moreover, after extrusion, the hydrogels show a degree of swelling in water higher than that of non-printed hydrogels. With respect to the obtaining of cell sheets, in particular, the technique of 3D printing was proved to be the best strategy for obtaining ring-shaped cell sheets, comparing this technique with the use of specific supports. A very interesting result derives from cell orientation showed by ring-shaped cell sheets, also confirmed by the degree of circularity of the nuclei in the cell sheets. The nuclei present on the ring-shaped cell sheets (ring and print-ring) are more elongated compared to those present on the sheets detached by bulk hydrogels.
Discussion: 3D printing process appears adequate for the preparation of cell sheet with different shape for the regeneration of complex tissues.

F7
COLLAGEN/HYALURONIC ACID-BASED HYDROGELS FOR THE DELIVERY OF NEUROPROTECTIVE PROTEIN HSP70 IN PARKISNON'S DISEASE
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Introduction: Parkinson's disease (PD) is a central nervous system disorder characterized by the progressive loss of dopaminergic neurons. Starting from their biocompatibility and applications in neural tissue engineering, we have proposed collagen (COLL)/hyaluronic acid (HA)-based semi-interpenetrating polymer networks (semi-IPNs) as Hsp70 (a 70 kDa neuroprotective heat shock protein) release systems to face PD-related neurodegeneration.
Materials and methods: In this work (Fondazione Cariplo, grant 2011-0335) COLL/HA semi-IPNs were prepared by promoting COLL fibrillogenesis in the presence of HA (Mw = 100 g·mol^{-1}) and eventually loaded with gelatin micro/nanoparticles (25 μg/ml). Dynamic moduli were evaluated by small amplitude oscillatory shear tests, viscosity was assessed by steady shear measurements and injectability through a 30G needle was confirmed by an INSTRON 5566 testing machine. Tat-fused human Hsp70 (TAT-Hsp70) was expressed in *E. coli* and loaded in the semi-IPNs (350 μg/ml). Hydrogel suitability to deliver TAT-Hsp70 was investigated in an in vitro model of neurotoxicity based on SH-SY5Y cells and 6-hydroxydopamine (6-OHDA). Hydrogel biological performance was studied in vitro with SH-SY5Y cells in both 2D and 3D by MTS assay and in mouse models by the air pouch model. Finally, COLL/HA composites were injected in mouse striatum and the inflammatory response was evaluated by glial fibrillary acidic protein (GFPA) and CD11b staining after 3 and 7 days.
Results: Small amplitude oscillatory shear tests have shown that the proposed semi-IPNs share a gel-like behaviour. Steady shear tests have indicated a shear thinning behaviour, while injectability tests have suggested that they are easily injectable and their load-displacement curves are similar. A purity greater than 95% was achieved for TAT-Hsp70, with a yield of 6.3 mg/l culture medium. TAT-Hsp70 released from both semi-IPNs was able to counteract 6-OHDA degeneration in the set up model of neurotoxicity. Results from MTS assay have confirmed that both matrices are highly biocompatible with neuronal-like cells and in vivo tests have indicated that the inflammatory response elicited by COLL/HA and COLL/HA/TAT-Hsp70 composites is negligible.
Discussion: Globally, these results suggest that the rheological and biological properties of the proposed COLL/HA composites are suitable for the final application.

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OC28
IN VITRO EFFECTS EXERTED BY KERATIN SCAFFOLDS IN COMBINATION WITH PEMF EXPOSURE ON HUMAN SAOS-2 OSTEOGENIC DIFFERENTIATION
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Introduction: Considerable efforts have been devoted toward uncovering the best approach in reconstructive bone surgery. A wide number of synthetic and natural scaffolds are known to be effective on cellular attachment, adherence, proliferation and differentiation towards osteogenic lineage. Furthermore, in literature there are evidences that proliferation and differentiation of various cultured stem cells can also be increased by the exposure to pulsed electromagnetic field (PEMF). Recently, we used sheep's wool as a natural source to prepare keratin microfibril sponges for scaffolding, whose unique structure, with controlled-size macro-porosity, made them suitable matrix for in vitro osteoblast adhesion and colonization. In this study, we aimed to electromagnetically stimulate human SAOS-2 cells seeded on porous wool keratin scaffolds to differentiate to osteoblasts and evaluate the deposition of a calcified bone matrix.
Materials and methods: Cells were seeded on keratin scaffolds and their differentiation was evaluated in the presence (PEMF-treated) or absence (PEMF-untreated) of daily PEMF exposure (magnetic field: 2 mT, amplitude: 5 mV), either with or without osteogenic factors. After 21 days of culture, the expression of genes involved in osteogenic differentiation was investigated by qRT-PCR. In addition, we evaluated the levels of osteogenic proteins by ELISA assay and we quantified calcium deposits by calcium-cresolphthalein complexone method.
Results: The results showed that in comparison to PEMF-untreated cultures, the PEMF stimulus induced significant changes in the expression of the typically osteogenic markers on keratin-seeded cells. With respect to untreated scaffolds, PEMF exposure in combination with osteogenic medium, increased on the keratin scaffolds the content of bone extracellular proteins and calcium deposits. All together, these data demonstrated the capability of PEMF-treatment to promote on keratin scaffolds the deposition of newly formed bone mineral matrix and, therefore, making them more suitable as biomaterial for cell colonization and bone differentiation.
Discussion: this study should be considered a preliminary in vitro investigation to setup further experiments aim to stimulate the conversion of bone marrow mesenchymal cells to the osteogenic phenotype on keratin substrates. This strategy might be a promising application in bone regenerative medicine.

OC29
PCL-REINFORCED HYDROGEL SCAFFOLDS BASED ON GELLAN GUM AND HALLOYSITE FOR BONE TISSUE REGENERATION
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Introduction: A novel three-dimensional (3D) construct was prepared for bone tissue engineering, consisting in a 3D-printed poly(ε-caprolactone) (PCL)-micro- and macro-channeled scaffold and a composite hydrogel, based on gellan gum (GG), glycerol and halloysite nanotubes (HNT) previously developed to improve the mechanical features and GG cytocompatibility. The GG-based gel was impregnated into the PCL construct, fabricated by Fused Deposition Modelling, with a biomimetic design simulating the osteon architecture. The proposed hybrid construct combined the physico-chemical and mechanical advantages of a 3D-printed polymer with a cell-friendly gel microenvironment.
Materials and methods: *3D-printed scaffolds*: filament spools (1.75 mm diameter) were made using a microfilament extruder (Rondol) and PCL pellets

as base material (3 mm diameter, 70-90kDa molecular weight, Sigma). The PCL scaffold was 3D-printed by Fused Deposition Modeling (Makerbot replicator 2X) (printer settings: temperature 140°C, speed 25 mm/min). *Hydrogel*: GG powder (2%w/v) was added to a heated glycerol solution under stirring. An aqueous suspension of HNT was mixed with GG to obtain the composite hydrogel, which was cross-linked by the external gelation method. The optimised mixture of the hydrogel with cells was pipetted dropwise within the PCL scaffolds. The cytocompatibility was studied seeding fibroblasts on the gels surface and encapsulating them into the hydrogels.
Results: The physico-chemical characterizations provided insights into the hydrogel morphology and composition. The mechanical features of the gel could be tuned varying the HNT amount, which also affected cell response. Indeed, the presence of HNT enhanced fibroblasts viability and metabolic activity.
Discussion: Live/Dead analysis demonstrated the successful infusion of GG-based hydrogel and cells into the PCL structure. High cellular viability was maintained throughout the printed structure. In future, the opportunity to functionalise the HNT surface and their mesoporous lumen with bioactive molecules able to elicit in situ advantageous cell responses will be exploited to treat complex bone defects.

OC30
INCREASE IN SURFACE HARDNESS OF TI6AL4V FOR APPLICATION IN ARTHROPROSTHETIC JOINTS

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Introduction: Ti6Al4V alloy is a biocompatible material, with a good corrosion resistance and low Young modulus. For these properties, it is used for un-cemented stems of hip joints and tibia components of knee joints, but due to low fretting and wear resistance it cannot replace CoCrMo alloy in hip joint's heads and femoral components of knee joints and it cannot be used in cemented hip in place of stainless steel. The goal of this research is to improve bio-tribological characteristics of Ti6Al4V alloy by a surface ceramic conversion treatment.
Materials and methods: A titanium boride coating was obtained on Ti6Al4V disks by a thermal treatment in a mix of salts containing a boron source. Temperatures of 1050-900-800-750°C and durations of 4.5-3.5-2.5h were tested, in order to obtain coatings with suitable surface mechanical and tribological properties.
In order to characterize the coatings optical microscope observations, FES-EM-EDS, XPS and XRD analyses, micro/nano-indentation and scratch tests were carried out. Finally, surface charge and protein absorption were investigated by means of electrokinetic measurements.
Results: The coating consists of an upper compact layer and a lower layer of whiskers of titanium boride. Chemical composition, thickness and hardness of the coatings depend on temperature and duration of the thermal treatment. Analyses show that treatment temperature has a much greater effect than treatment time. Coated alloy presents peculiar surface charge, functional groups and protein absorption ability compared to uncoated one.
Discussion: Temperatures of 750-800°C allow to obtain a hard coating of several microns, without changing the substrate microstructure significantly. Increasing the temperature above 900°C there is an increasing of coating adhesion but also a considerable changing in the bulk microstructure and so in the mechanical properties of the alloy. At the moment the main issues are adhesion and a good polishing protocol to reach a suitable final roughness without removing the coating.

OC31
BIOREACTOR MECHANICALLY GUIDED 3D MESENCHYMAL STEM CELL CHONDROGENESIS IN A NOVEL THERMO-REVERSIBLE METHYLCELLULOSE-BASED HYDROGEL

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Introduction: Autologous chondrocyte implantation for cartilage repair represents a challenge due to chondrocytes' poor expansion capacity in vitro. Mesenchymal stem cells (MSCs) can differentiate into chondrocytes, while mechanical loading has been proposed as alternative strategy to induce chondrogenesis, thus excluding the use of exogenous factors. Here, a novel 8% w/v methylcellulose 0.05M Na_2SO_4 thermo-reversible hydrogel (MC-hydrogel) was used as a 3D injectable matrix for bioreactor guided MSCs chondrogenesis in combination with a porous polyurethane (PU) scaffold.
Materials and methods: MC-hydrogel was obtained by dispersion technique and mechanically characterized. Biocompatibility was in vivo evaluated by spleen lymphocytes stimulating index (SI) and histology. To induce in vitro chondrogenesis, MSCs were seeded into the hydrogel solution retained within a porous PU; PU-MC composites were subjected to a combination of compression and shear forces for 21 days in a custom made bioreactor. All in vitro studies were performed in absence of exogenous transforming growth factor β. Chondrogenesis was confirmed by RT-PCR, biochemical and histochemical assays. Statistical analysis was performed using one-way ANOVA followed by *t-test*.
Results: MC hydrogel exhibited reversible thermo-responsive properties as confirmed by the storage shear modulus (G') and the loss shear modulus (G'') sol-gel transition. Moreover, in the heating and cooling tests, a hysteresis was observed. Swelling and degradation assays showed a significant increase of hydrogel volume ($p<0.05$) but a low bulk degradation (<20%). In vivo implant did not reveal any immunological reaction or fibrous tissue formation. Bioreactor-loaded PU-MC composites PCR analysis showed a higher expression of chondrogenic genes when compared with the unloaded controls ($p<0.05$). Accordingly, COL 2 ratio was significant ($p<0.05$) in comparison with COL 1 and COL 10. Finally, biochemical analysis confirmed that loaded specimens released a significant higher amount of glycosaminoglycans (GAG) in the medium ($p<0.05$) during loading cycle. Similarly, loaded PU-MC composites displayed a higher GAG amount in comparison to the control even when GAG/DNA ratio was evaluated ($p<0.05$).
Discussion: Due to the thermo-responsive behavior and its biocompatibility, MC hydrogel represents a good candidate to act as vehicle for cell delivery for tissue engineering purposes.

OC32
CONTROLLING SOFT TISSUE ADHESION ON TITANIUM SURFACES BY NANOGROOVES AND KERATIN NANOFIBRES

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Introduction: Despite of wide research on surface modifications of titanium for bone contact applications, few works consider its interaction with soft tissues, which is of interest, for example, for the transmucosal dental implants. Topography and roughness play a crucial role both in bacterial and cell adhesion, moreover specific chemical and biological stimuli can be sent to the cells in order to modulate their response. A lower Ra limit of 0.2 µm has been defined in literature in order to avoid an increased bacterial contamination. Fibroblasts preferentially adhere on polished substrates and can be guided by surface grooves. Keratin is a well-known chemical stimulus for fibroblasts. On this basis, the specific aim of this research work is to modify titanium surfaces by means of nanogrooves and keratin nanofibers, in order to drive gingival fibroblasts alignment and proliferation through topographical and chemical stimuli without increasing bacterial adhesion.
Materials and methods: Oriented nanogrooves were obtained on c.p. titanium by a mechanical route (abrasive papers) or by means of Electron Beam (EB) structuring maintaining the final surface roughness lower than 0.2 µm. Keratin was extracted from wool and nanofibers were deposited onto mirror polished/roughened titanium substrates by electrospinning both as fibers randomly oriented or aligned to the grooves. The viability/orientation of gingival fibroblasts were investigated on the modified surfaces together with bacterial (*S. aureus*) adhesion and biofilm formation.

Results: Oriented nanogrooves (Ra 0.1-0.2 µm) were successfully obtained on titanium substrates both by mechanical and EB routes. Keratin nanofibers were deposited on the titanium surfaces without significantly altering surface roughness and they resulted stable up to 1 month of soaking in water. Gingival fibroblasts were able to adhere and to get a specific orientation along the grooves and they were positively affected by keratin. None of the tested surfaces increased bacterial adhesion compared to a mirror polished control.
Discussion: These results suggest nanogrooves and keratin nanofibers as promising modification strategies for the surface of transmucosal dental implants (collar) in order to obtain an effective gum sealing and reduce bacterial penetration. Moreover, the proposed strategy is in line with a sustainable employment of resources.

OC33

ELECTROPHORETIC DEPOSITION OF CHITOSAN/BIOGLASS® COMPOSITE SCAFFOLDS WITH ORIENTED MICRO PATTERNS: FABRICATION PROCESS AND IN VITRO BIOLOGICAL PROPERTIES

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Introduction: Electrophoretic deposition (EPD) is a powerful bottom-up technology that can be advantageously exploited to design biopolymer-based scaffolds. Here, we present the first successful effort to fabricate self-standing Chitosan (CS)/Bioglass (BG) scaffolds with regularly-oriented micro-channels.
Materials and methods: Fabrication of composite scaffolds was performed via EPD in baths of 1 mg mL-1 CH (Sigma-Aldrich 417963) in 1% vol. acetic acid (Sigma-Aldrich 320099), added with 2 sizes of Bioglass® 45S5 (BG) powders (median particle size of 30 and 3.0 µm, 0.3 mg mL-1). Micro-patterned Ti substrates of circular holes (Ø = 500 µm, distance = 200 µm, square lattice) were used as cathode. Freeze-dried scaffolds were peeled off the substrate and analyzed in terms of chemico-physical and morphological properties. Moreover, each scaffold has been incubated in 10 mL cm-2 of Simulated Body Fluid (SBF, 37°C, up to 28 days) for mineralization studies. SAOS-2 cells (human osteosarcoma cell line) assessment was performed for both cytotoxicity and cytocompatibility tests.
Results: Obtained scaffolds were manually peeled off from the substrate: they resulted in regularly-oriented micro-channels with diameter ϕ = 380 ± 50 µm and inter-channel spacing of d = 600 ± 40 µm, same scale of Haversian canal diameter and osteon units' spacing. SEM micrographs showed well-dispersed BG particles inside CH matrix. In-vitro analysis of scaffolds in SBF reveals up to 150% mass gain, related to deposition of hydroxyapatite (HA). According to indirect cytotoxicity and cytocompatibility tests, no evidence of cytotoxic effects deriving from dissolved ionic species was observed.
Discussion: Highly oriented micro-channel CS/BG composite scaffolds with high bioactivity has been successfully produced using a replica EPD approach, BG particles being homogeneously distributed inside the CS matrix. A thick layer of HA formed through 3 distinct stages for in vitro treatment of the samples in SBF. HA deposition was homogenous all over the surface and in a long time can also fill the open porosities. Furthermore, no evidence of cytotoxic effects deriving from dissolved ionic species was observed in experiments performed.

Session 8 - Antibacterial Strategies

OC34

DAC® GEL A HYALURONAN BASED HYDROGEL ANTIBIOTIC-LOADED AGAINST BIOFILM FORMATION: NEW CLINICAL PERSPECTIVE IN THE PREVENTION OF PERIPROSTHETIC JOINT INFECTION

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Introduction: Periprosthetic Joint Infection (PJI) after hip and knee arthroplasty is a serious concern in orthopaedics and considered the most devastating complication with significant burden for patients and health care system. The incidence of infection of primary joint arthroplasties is 1%-2%, but rate as high as 5.6% in certain risk population was seen. Infection is the cause of 14.8% of Total Hip Arthroplasty revisions and the most common cause of Total Knee Arthroplasty revisions (25.2%). Many risk factors are related to PJI onset, including immune system deficiencies, malignancy, and previous surgery. Prevention remains the least expensive approach against PJI, therefore, the attainment of effective and easy-to-use methods to elude PJI is definitely the most rational methodology. Nevertheless, systemic antibiotic therapy does not eradicate the problem, in fact, the success rate ranging 10%-25%. Here we present an innovative approach to prevent PJI through a procedure that combines a gel-barrier effect with a local delivery of antibiotic.
Materials and methods: DAC® is a Hyaluronan (HY)-Poly D-L-lactic acid (PLA) derivative gel designed as a physical barrier that associated with antibiotic inhibits bacterial adhesion over an orthopedic implant. This approach provides a hydrophilic barrier to "win the race to the surface" against bacteria, avoiding the biofilm formation. In vitro studies demonstrated that the gel provides a hydrophilic change over the implant surface, inhibiting the switch of bacteria from planktonic to sessile form. The clinical effectiveness of DAC® was assessed in a trial on 380 patients. Patients undergoing either primary or revision for hip (n = 298) and knee (n = 82) arthroplasty were subsequently randomized to receive an implant coated with DAC® (treatment) or uncoated implant (control). Radiography, laboratory tests, infection rate, were evaluated from 6 to 24 months follow-up.
Results: All patients tolerated well the surgery and no adverse events related to the use of DAC® gel were observed. Infection rate was significantly lower in the DAC® group (0.6%) vs. control group (6%); furthermore, a good osteointegration was seen at x-Ray findings in DAC® gel group.
Discussion: Local application of antibiotic-loaded DAC® hydrogel may represent a safe and effective tool in the prevention of PJI.

OC35

SILVER NANOPARTICLES WITH PECTIN: IDEAL GREEN BIOMATERIAL FOR ANTI-BACTERIAL AND ANTI-BIOFILM APPLICATIONS

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Introduction: Synthesis and biomedical properties of silver nanoparticles (AgNP) have been extensively investigated over the past decades. Their use as additives for wound-healing biomaterials endowed with antibacterial properties has also been documented. A key factor for biocompatibility is the amount of Ag^+ ion released by AgNP. A further requirement, together with biocompatible reductant agents, is a narrow dimensional distribution, a typical bottleneck of many synthesis procedures. Here, we report an efficient, simple, green synthesis method of AgNP with citrus peel pectin (p-AgNP), used both as a reductant and coating agent.
Materials and methods: Pectin from citrus peel (0.5%, 1%, 2%) was dissolved at 60°C. Upon cooling, $AgNO_3$ (final concentration of 1mM) and immediately after 0.5M NaOH were added. Vigorous stirring was continued for 12/24 hours. *E. coli* PHL628 and *S. epidermidis* RP62A were used as model Gram – and + strains, respectively. Minimum Inhibitory Concentration (MIC) was determined in planktonic condition, as well as effect of p-AgNP was analysed before and after biofilm formation. 3-[4,5-dimethylthiazol-2-yl]-2,5diphenyltetrazoliumbromide (MTT) test was used to assess viability of bacteria. Confocal Laser Scanning Microscopy on bacterial biofilms was also performed.
Results: The new synthesis method provided ~100% reduction of Ag^+ to Ag0, together with fast and straightforward procedure. The generated nanocomposite displayed excellent long term stability, narrow dimensional distribution and low Ag^+ release. Despite this, excellent MIC values were reported for both strains, close to or lower than $AgNO_3$ ones. This effect is due to the weak interaction between p-AgNP pectin coating and bacterial surface. Similarly good properties were reported for their activity on biofilms in the two experimental settings analysed. In both planktonic and biofilm conditions, *E. coli* showed to be more affected, owing to its documented higher Ag^+ sensitivity compared to *S. epidermidis*.

Discussion: We have designed a new, simple, green method to generate stable, dimensionally uniform AgNP, with pectin being both reducing agents during synthesis and coating of AgNPs themselves. Our p-AgNP are the best compromise between good antibacterial and anti-biofilm effect and possible cytocompatibility issues, the former normally requiring sustained Ag^+ release, that is detrimental for human applications because of cytotoxicity.

OC36
GELATIN BASED NANOFIBERS FUNCTIONALIZED WITH ANTIBACTERIAL AGENTS FOR TISSUE ENGINEERING APPLICATIONS

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Introduction: Polymeric nanofibers that mimic the structure and function of the native extracellular matrix are of great interest in tissue engineering as scaffolding materials. Gelatin (GL) is one of the most promising biomaterials due to its biocompatibility and biodegradability. In this work, GL crosslinked nanofibers with improved antimicrobial properties were prepared via electrospinning technique to reduce the bacterial proliferation in infected injuries. Silver nanoparticles (AgNPs) and gentamicin sulphate (GS) were loaded into the GL nanofibrous matrices to impart antibacterial properties on a wild range of strains.
Materials and methods: GL/AgNPs and GL/GS nanofibers were developed using an electrospinning apparatus. For GL/AgNPs, 2.5% or 5% w/w $AgNO_3$ was dissolved in demineralised water, followed by GL addition to the solution (15% wt/v) and kept under stirring for 18 h. GS loaded nanofibers were prepared by dissolving GL and GS in distilled water to obtain blends with weight ratios of GS to GL of 2.5, 5, 7.5 and 10. Finally, an appropriate amount of GPTMS was added to GL/$AgNO_3$ and GL/GS solutions. The spinning conditions were optimized to obtain homogeneous nanofiber morphology: 50°C, 30 kV and 15 µl/min. Complete morphological and physicochemical characterization was performed on samples. Antibacterial tests were carried out on developed nanofibers using both Gram-positive and Gram-negative strains.
Results: Ag nanoparticles (AgNPs) formation was observed by transmission electron microscopy. AgNPs/GL and GS/GL smooth fibers with fiber size in the range of 200-300 nm were obtained. Energy-dispersive X-ray spectroscopy confirmed the presence of AgNPs and GS homogeneously distributed into the nanofibers. Lastly, both the typologies of functionalized nanofibers showed the antibacterial activity against *S. Aureus*, *E. Coli* and *P. Aeruginosa*.
Discussion: GL based nanofibers with improved antimicrobial properties were fabricated by electrospinning techniques avoiding the use of toxic solvents. These nanofibrous matrices are promising to combine tissue regeneration and antibacterial activities in wound healing.

OC37
FUNCTIONAL MIMETICS OF ANTIMICROBIAL PEPTIDES: NOVEL AMPHIPATHIC POLYURETHANE TO COMBAT GRAM-POSITIVE BACTERIA

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Introduction: The Gram-positive bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis* are two of the most common causes of medical device-associated infections. The persistence of staphylococcal infections related to foreign bodies is due to biofilm formation that cause chronic colonization and may lead to temporarily occurring infections because they show increased tolerance to antibiotics, disinfectant chemicals and body's defense system. Hence, it is urgent to develop new strategies to combat such bacteria. Our strategy is to develop a novel polyurethane based system mimicking the mode of action of antimicrobial peptides.
Materials and methods: N-isopropylacrylamide monomer was grafted from a polyurethane backbone. Further, a hydrophilic ionic liquid monomer, was sequentially polymerized to the poly(N-isopropylacrylamide) block. The chemical structure of the synthesized polymer was confirmed by FTIR and NMR spectroscopies. The characterization of the size and morphology was performed with cryo-TEM, cryo-SEM, dynamic light scattering and zeta potential. Antimicrobial susceptibility was determined by the broth microdilution method and cytocompatibility evaluation was done on the L929 cell line by the Multiplex assay.
Results: The polyurethane based system was successfully synthesized. The colloidal particles adopt a shape in which clustering of hydrophobic domains of polyurethane based system globules and hydrophilic cationic polyionic liquid segments are spatially organized in separate distinct domains. The electrostatic interactions stemming from the charged quaternary ammonium nitrogen of the polyionic liquid segment allows to interact with anionic the bacterial cytoplasmic membrane, and the hydrophobic globules of polyurethane based system segments forms pores and consequently disintegrating the cell membrane of the pathogenic gram positive bacteria. Antimicrobial studies on *S. aureus* and *S. epidermidis* were performed. Interestingly, the polyurethane based system colloidal particles showed an extraordinary improvement against both strains. Preliminary tests on fibroblasts showed that the system is safe on fibroblasts with concentrations above the MIC value.
Discussion: The developed amphipathic polyurethane system is inducing antibacterial effect against staphylococci bacteria. Further experiments are in progress to elucidate the mode of action of the developed polymeric system.
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OC38
BACTERIOSTATIC SURFACE TREATMENTS FOR TITANIUM IMPLATABLE DEVICES

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Introduction: The aim of the present study was the development and in vitro characterization of antibacterial treatments on titanium for implantable devices. The goal was to provide antimicrobial capabilities to the surface with no or low adverse effects for eukaryotic cells. Silver (Ag^{1+}) and Gallium (Ga^{3+}) are the chosen antibacterial ions to incorporate into the titanium oxide layer.
Materials and methods: Anodic Spark Deposition (ASD) technique was considered to modify the structure and composition of the surface titanium oxide. The electrochemical treatments were performed in two solutions: one containing Beta-glycerophosphate (b-GP) and Calcium Acetate (CA) salts, one based on oxalic acid. Addition of antibacterial ions Ag^{1+} and/or Ga^{3+} was considered. The surface composition, structure and morphology was investigated through XPS, ICP-OES, XRD, GDOES, SEM, EDS, AFM, contact angle and nanoindentation measurements. Microbiological characterization was performed on different bacterial strains by XTT, Alamar Blue, Live/dead assay and SEM observation. Biological characterization was performed using human osteoblasts and human osteoblastic progenitors and different biological assays.
Results: The many chemical and microstructural characterization tests and analyses allowed to identify the best ASD treatment in terms of antibacterial efficiency, mechanical stability and cytocompatibility on eukaryotic cells. Microbiological analyses identified treatments containing gallium as the ones with the highest bacteriostatic effect. The treatments also showed good cytocompatibility and an improved osteoblastic differentiation compared to not treated titanium.
Discussion: Among all experiments on ASD treatments, one treatment containing Ga^{+3} ions, and one containing $Ag^+ + Ga^{3+}$ were identified and selected as best effective treatments capable to provide bacteriostatic properties to titanium implantable devices.

Poster Session

P1

OSTEOCHONDRAL LESIONS: AN INNOVATIVE COLLAGEN-BASED SCAFFOLD BIOMIMICKING TISSUE-SPECIFIC CHARACTERISTICS FOR IMPROVED REGENERATION EFFICACY

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Introduction: Treatment of osteochondral lesions is still challenging due to defect extension from cartilage to the underlying subchondral bone. Current techniques based on pure collagen sponges or autologous graft (mosaicoplasty) have strong limitations due to improper tissue response and donor site morbidity, respectively. Scaffold engineering based on biomimetic approaches has the potential to improve regeneration efficacy via optimization of biochemical and biomechanical characteristics. The aim of this project was to characterize a novel collagen-based scaffold with improved biofunctional properties via the physical combination of HAβTCP granules and collagen thus biochemically mimicking the interested target tissues.
Materials and methods: A porous, collagen-based scaffold containing HAβTCP granules (CollagenHA) was prepared in accordance with a manufacturing procedure patented by Novagenit Srl. The resulting biphasic scaffold was analysed with respect to its tridimensional as well as surface structure, porosity, biocompatibility and biofunctionality. Cell seeding experiments were carried out to determine the loading capacity of the scaffold mimicking applicational procedures as applied in the OR theatre. Finally, experiments with perfusion bioreactors were designed and preliminarily tested with different cell lines in order to simulate in vitro the physiological conditions after implantation.
Results: Histological staining of CollagenHA demonstrated that manufacturing conditions were able to fully encapsulate HAβTCP granules thus preventing their dispersal during implantation. Due to its biphasic nature, a tissue-specific orientation of the scaffold into the osteochondral defect is easily possible. Furthermore, SEM analysis confirmed a suitable porosity of the collagenous part and a highly structured surface of the granules. Specific cell seeding channels were produced onto the surface in order to enhance cell loading of the scaffold. Cell viability tests using murine fibroblasts showed a high biocompatibility profile as well as good preliminary biofunctionality in terms of cellular adhesion on both, collagen and granules. Bioreactor experiments using two different perfusion systems were carried out co-culturing chondrocytes and osteoblasts onto the same scaffold.
Discussion: Animal studies in rabbits are on-going using CollagenHA in combination with Bone Marrow Concentrate (BMC) in an osteochondral defect model.

P2

DEVELOPMENT OF SOLID LIPID NANOPARTICLES (SLN) FOR INTESTINAL LYMPHATIC DELIVERY: FORMULATIVE STUDIES

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Introduction: The lymphatic system can be used as a new way for the delivery of therapeutic substances with low bioavailability. The phytoestrogen Genistein was chosen as a drug model and formulative studies were carried out to develop SLN to target intestinal lymphatic vessels in order to improve Genistein bioavailability.
Materials and methods: The first part of the work has been characterized by preliminary studies, during which some formulation parameters have been modified. The "hot homogenization process" was applied to prepare SLN. The formulations were characterized in vitro by evaluating: SLN Size and Polydispersity Index; Stability test; SLN Genistein Content and Drug Permeation; In vitro chylomicrons formation.
Results: These parameters are identified as suitable for SLN preparation: homogenization with the probe sonicator for 12 min and Tween 80 concentration of 0.5% w/v. All formulations have a particle size of about 300 nm that is suitable for the intestinal lymphatic targeting.
The stability test shows that the SLN size properties are stable during the 30 days. Regardless the quantity of GEN loaded, the drug loading efficiency was about 100%. GEN permeated amount from GEN-loaded SLN at pH 6.8, is very low. This hypnotized behaviour is according to lipid nanoparticles structure; this result could suggest their easy and likely passage through the lymphatic system. The in vitro formation of chylomicrons was evaluated. The particle size analysis confirms a probable interaction between the SLN and the lipid components (phospholipids and cholesterol); this could indicate the formation of chylomicrons-like structures. Further studies are currently in progress.
Discussion: From the results, it can be concluded that the SLN can be considered a promising formulation that is worth of further studying to develop a suitable system for the intestinal lymphatic delivery of unstable and poorly bioavailable drugs.

P3

IN VIVO MODEL OF GUIDED BONE REGENERATION IN RAT CRITICAL SIZE DEFECT WITH A PORCINE COLLAGEN MEMBRANE AND A BOVINE BONE MINERAL GRAFT

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Introduction: The aim of the study is the histological and immunohistochemical characterization of the biological response in terms of inflammation, bone healing and membrane degradation of a rat calvarial critical-size defect.
Materials and methods: Eighteen adult Wistar rats, allocated to 7, 14, 30 days healing periods, were considered. A 5.00 mm diameter critical-size defect in the parietal bone was performed. In agreement with the Guided Bone Regeneration (GBR) principle, the critical-size defects were treated with collagen membrane in the intracranial side, then a deproteinized bovine bone mineral substitute was loosely compacted, finally a second collagen membrane was maintained in position by suture. Specimens were processed for histology to evaluate general and specific tissue reaction, newly bone formation, degradation and remodeling pattern of the membranes. Immunofluorescence analyses of specific markers were combined with histological readouts.
Results: At 7 days, a hemorrhage infiltrate was observed, due to the surgical trauma. Only few inflammatory cells were observed over the three healing periods, and the immunofluorescence emphasized the absence of inflammatory markers (Interleukin-1β and TNF-α). Collagen membrane showed a progressive degradation from 7 to 30 days and a concurrent remodeling, even if the membranes still worked as barrier at 30 days.
As evidence of degradation, immunofluorescence revealed MMPs and Tissue Inhibitor of Metalloproteinases (TIMP) activity within the collagen membrane. High vascularization and new bone formation achieved the highest results at 30 days. Bone deposition and initial bone mineralization were confirmed by the immunofluorescence detection of osteocalcin, osteopontin and bone sialoproteins.
Discussion: Our results are in agreement with previous literature that reported no signs of inflammatory reactions for non-crosslinked collagen membrane in comparison to cross-linked membranes. The MMPs were active players in the membrane degradation process. Membranes morphological changes proved the integration with the nearby tissues and an active remodeling activity. The increase of vascularization was reported to have a positive effect on the quality of newly bone formed. The GBR technique successfully promoted bone regeneration. Both the implanted biomaterials resulted well-tolerated, with an excellent tissue compatibility. The degradation rate did not interfere with the barrier function of the membrane and with the bone regeneration process.

P4

DRUG DELIVERY OF AMPICILLIN FROM PCL-BASED HYBRIDS SYNTHESIZED BY SOL-GEL PROCESSING

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Introduction: Organic-inorganic hybrids (OIHs) are biphasic materials, where the organic and inorganic phase are mixed at the nm to sub-µm scales. There is considerable interest in OIHs prepared via the sol-gel process for biomedical applications. The aim of the present work has been the sol-gel synthesis, characterization and bioactivity study of PCL/ZrO_2 and PCL/TiO_2 hybrid materials containing ampicillin to be used as drug delivery systems. The local delivery of drug has the benefit of providing the desired constant drug concentrations at the delivery site. The release kinetics from the amorphous bioactive hybrid materials was analyzed as a function of the polymer amount.
Materials and methods: Both OIHs systems (containing 6, 12 and 24wt% PCL) were prepared by means of sol-gel process, using zirconium propoxide and titanium butoxide as precursor of ZrO_2 and TiO_2, respectively. Finally, a solution of PCL in chloroform and of sodium ampicillin in ethanol (5wt%) was added to both the sols. The nature and the microstructure of samples were confirmed by XRD, FTIR, SEM and AFM analyses. In order to study their bioactivity, the samples were soaked in a simulated body fluid (SBF). The study of ampicillin release measurements was carried out by means of UV-VIS spectroscopy.
Results: The formation of H-bonds between the organic and inorganic phases in both hybrid systems was proved by FTIR measurements. XRD analysis showed that both hybrids exhibit broad humps characteristic of amorphous materials. SEM and AFM analyses confirmed that all hybrids are homogenous nanocomposites. The amount of apatite deposited on sample surfaces recorded after SBF test increases with the PCL content. The EDS confirms that the observed layer is composed of calcium and phosphate. The release kinetics study demonstrates that the investigated materials supply high doses of the antibiotic during the first hours, then a slow drug release is observed, because ampicillin is entrapped within the clusters of material. Ampicillin release is quite lower when PCL content increase.
Discussion: Sol-gel method allowed of synthesizing bioactive OIHs containing Ampicillin potentially suitable as matrices for controlled release of drugs.

P5
INORGANIC PHOSPHATE AND CANCER: ITS EFFECTS IN OSTEOSARCOMA, BREAST AND PANCREATIC CANCER CELLS
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Introduction: Inorganic phosphate (Pi) is an essential nutrient to living organisms. It represents an abundant dietary element. However, many chronic diseases, including cardiovascular diseases, obesity and even cancer have been associated with high-P intakes and high-serum Pi concentrations. In addition, very recently, interstitial inorganic phosphate has been proposed as a Tumor Microenvironment Marker for tumor progression. Notably, Pi is a relevant component of various biomaterials, such as Ca-P nanoparticles and its release can affect Pi concentration at local sites. Relevantly, Pi is emerging as an important signalling molecule capable of modulating multiple cellular functions by altering signal transduction pathways, gene expression and protein abundance in many cell types.
Materials and methods: Various osteosarcoma, breast cancer and pancreatic cancer cell lines were used and cellular effects by Pi have been investigated, carrying out flow cytometry-based assays of cell-cycle progression and cell death, would-healing and MTT assays, direct cell number counting and immunoblotting experiments.
Results: The results show that Pi inhibits proliferation and aggressiveness of human osteosarcoma U2OS cells (but not of p53 defective Saos-2 and MG-63 cells) identifying adenylate cyclase, beta3 integrin, Rap1, ERK1/2 as proteins whose expression and function are relevantly affected in response to Pi. Moreover, we describe also that Pi sensitizes osteosarcoma cells to doxorubicin in a p53-dependent manner and through a mechanism involving ERK1/2 down-regulation. Additionally, we provide evidence of Pi acting as a novel signalling molecule capable of eliciting a strong antiproliferative action in triple-negative MDA-MB-231 breast cancer cells (but not in MCF-7 estrogen receptor positive cells) and of enhancing the doxorubicin-induced cytotoxicity via a mechanism involving ERK1/2 and STAT3 down-regulation. Interestingly, we have initial evidence that Pi does not have antiproliferative action in pancreatic cancer cells.
Discussion: The finding that Pi can have antiproliferative effects on some cancer cell types, depending on cell status and genetic background and achieve additive cytotoxic effects when combined with doxorubicin, illustrates its potential for clinical applications and suggesting that up-regulating Pi levels at local sites, also by using phosphate containing nanoparticles, might contribute to the development of novel and cheap therapeutic strategies in some tumors.

P6
CHEMICALLY CROSSLINKED GELATIN MICROSPHERES FOR CELL DELIVERY
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Introduction: Cell therapy is a regenerative medicine approach where cells can be injected in pathological tissues to be regenerated by microcapsules or microspheres. Advantages of microspheres include a higher area for cell growth and better supply of nutrients to cells. Here, we propose chemically crosslinked gelatin hydrogel microspheres as vehicles for cell delivery.
Materials and methods: Gelatin microspheres (MS) were prepared by a Michael-type addition crosslinking reaction of gelatin (type A from porcine skin) and methylene-bis-acrylamide (MBA), as crosslinker. A mixture of gelatin and MBA crosslinker was dropped at 50°C in soybean oil under stirring; after 24 h, crosslinked MS were collected using a filtered syringe (Ø = 35 µm), washed with acetone and disinfected with 70% ethanol. MS weight and dimensional variation was examined by swelling MS in distilled water at 37°C. The crosslinking degree was measured by ninhydrin assay, by comparing the number of free amino groups of gelatin MS before and after the crosslinking reaction. In vitro tests were performed using L929 cell line. Indirect cytotoxicity tests were performed to investigate the possible release of cytotoxic compound from the MS. Direct cell seeding was performed by swelling anhydrous MS in a cell suspension; cell adhesion on microspheres was evaluated by optical microscopy and cell viability qualitatively investigated by LIVE/DEAD staining.
Results: The mean diameter of the collected anhydrous MS was 89.79 ± 27.11 µm. During the swelling, MS quickly increased their weight in the first 6 h, reaching a weight variation plateau after 24 h of swelling; the average diameter of the MS increased by 130%, reaching a stable value (150 µm) after 24 h. The crosslinking degree measured by ninhydrin assay was 86.3 ± 0.1%. The viability of cells cultured with medium eluates extracted until 7 days of contact with the MS was >90%. Cells adhered to MS and uniformly colonized the MS surface after 3 days of culture; LIVE/DEAD staining proved that >90% cells were viable.
Discussion: Gelatin MS were successfully produced, obtaining a uniform microsphere population with limited dimensional variability. MS supported viable cells adhesion, making them MS optimal candidates as carrier for cell delivery.

P7
STRONTIUM-CONTAINING NANOPARTICLES IMPROVE BONE FORMATION
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Introduction: The goal of osteoporosis treatments is decrease the fracture risk. In last years, studies focalized on role of Sr–based drugs as anti-osteoporotic agents as Sr showed to induce opposite effects on bone resorption and formation. The Sr action was demonstrated in pharmacological studies and in vitro studies on bone cells. Lately, the drug delivery method presented many cytotoxicity problems. For this reason, we developed a method of Sr-enriched hydroxyapatite (Sr-Hap) synthesis to incorporate Sr in the crystal structure of Hap.
Materials and methods: Ca-Hap and Sr-Hap were synthesized using a sol-gel technique and Bovine Serum Albumin was used as dispersant agent to obtain

nanoparticles (NPs) suspension (Ca-HAp_NPs, Sr-HAp_NPs). We studied the effect of nanoparticles on cell differentiation using SAOS-2 osteoblasts cell line cultured for 21 days in presence and absence of NPs. Cell proliferation was evaluated using MTT test and calcium deposition by Alizarin red staining. We measured ALP enzyme activity and evaluated expression of genes related to bone matrix proteins (*ALP*, *COL1A1*, *OCN* and *DCN*).

Results: After 21 days, SAOS-2 cells treated with NPs suspension containing Sr showed a higher rate of proliferation compared to cells untreated or treated with Ca-Hap_NPs. Cells cultured with osteogenic factors and treated with Sr-Hap_NPs showed higher ALP activity and calcified bone matrix. The expression of *ALP*, *COL1A1*, *OCN, DCN* resulted up-regulated in cells treated with Sr-Hap_NPs compared to those untreated or treated with Ca-Hap_NPs. These data suggest the positive effect of Sr-Hap_NPs on osteoblast differentiation and bone matrix deposition. Sr-Hap_NPs increased cell proliferation and differentiation toward osteoblasts. In synergy with osteogenic factors, Sr-Hap_NPs were able to improve the bone matrix deposition, in terms of both mineral deposits and proteins.

Discussion: Due to the ease synthesis, stability over the time and their osteoinductive properties, Sr-Hap_NPs could be a useful system for the delivery of strontium to bone tissue for osteoporosis treatment.

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P8

DEVELOPMENT OF AN EXPERIMENTAL SET-UP TO CHARACTERIZE THE DISTRIBUTION OF MATTER IN RADIAL FLOW PACKED BED BIOREACTORS (RPBBS) EQUIPPED WITH POROUS HOLLOW BIOCERAMIC SCAFFOLDS FOR BONE TISSUE ENGINEERING (TE)

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Introduction: Uniform supply of oxygen and nutrients to osteogenic cells in porous hollow scaffolds is fundamental for uniform construct maturation in rPBBs. Inappropriate scaffold transport properties, bioreactor geometry and operation favour formation of stagnation zones and preferential pathways yielding poor oxygen and nutrients supply to cells. Optimized set-ups for tracer experiments to assess the distribution of matter in the rPBB small scale are not available. In this work, a set-up was developed to perform tracer experiments on rPBBs equipped with porous hollow bioceramic scaffolds with controlled microstructure as a tool to optimize geometry and operation of rPBBs for bone TE.

Materials and methods: Clinical-scale hollow scaffolds with porosity >85% and full pore interconnectivity were designed with CAD. Scaffolds were produced in b-TCP by lithography-based ceramic manufacturing (Lithoz GmbH, Vienna, Austria) and loaded in rPBB prototypes. The set-up for tracer experiments consisted of two reservoirs, a 3-way valve, a peristaltic pump connected to the rPBB, and a flow-through cuvette positioned in a UV-Vis spectrophotometer. rPBBs were challenged with a Trypan blue concentration step at flow rates typical of bone TE. Tracer concentration leaving the rPBB was estimated on-line spectrophotometrically at 590 nm. Trypan distribution in time was visualized by time-lapse photometry at 30 fps. Circuit geometry was optimized to minimize effects of the set-up dynamics. rPBB dynamics was characterized with ideal transport models.

Results: In the optimal test set-up, faster dynamics of the tracer concentration challenge than rPBB confirmed the assumption of ideal step and enabled analysis in the time domain. Dynamics of inlet and outlet tubing was minimized and modeled as an ideal lag. rPBB response was reproducible and depended on inlet flow rate. The distribution of matter was modeled in terms of a well-mixed tank communicating with stagnating reservoir of flow-rate dependent volumes. Visualization experiments were consistent with the model analysis of tracer experiments under all conditions.

Discussion: An optimal set-up was developed yielding robust characterization of the distribution of matter in rPBBs, to help select porous hollow scaffolds and optimize geometry and operation of rPBBs for bone TE.

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P9

MICRO-STRUCTURAL SIMILARITY OF BIOCERAMIC SCAFFOLDS TO HUMAN CANCELLOUS BONE

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Introduction: Bioceramics are clinically used as bone substitutes in the form of small solid blocks, granules, injectable cements and three-dimensional (3D) porous blocks (scaffolds). Scaffolds that mimic the micro-architecture of bone promote osteogenic cell migration and re-organization as in the natural tissue, and better osteointegration in vivo. However, scaffold and bone micro-structures are often compared in a qualitative fashion which makes the comparison difficult and unreliable. Following and approach previously reported for hydroxyapatite scaffolds, in this work we quantitatively compared the micro-structure of glass-ceramic foams to that of human trabecular bone based on data from X-ray micro-computed tomography (micro-CT).

Materials and methods: 3D glass-derived scaffolds having different nominal porosities were fabricated by sponge replication from foams with different porous structure (45 and 30 ppi). The scaffolds were analysed non-destructively by X-ray micro-CT (1174 SkyScan, Bruker, Kontich, Belgium) and characterized in terms of six parameters (total porosity ε, pore interconnectivity I_p, mean pore size , specific surface area a_v, connectivity density β and degree of anisotropy *DA*). The micro-structural similarity of the scaffolds to one another and to human bone was quantitatively assessed on the basis of such features, also by making use of a multiparametric score.

Results: The values of the six micro-architectural features in the scaffolds fabricated suggest that the sponge replica method may be controlled to produce porous bioceramic scaffolds with different porous micro-structures and to optimize their micro-architecture for mimicking specific bone tissues of the patients. The quantitative score used to evaluate the similarity of the different scaffold batches showed that scaffolds were consistently strongly different from one another and each scaffold matched human trabecular bone from different harvesting sites.

Discussion: Glass-ceramic scaffolds with two different nominal porosities were produced by polymer sponge replication, and their 3D micro-architecture was characterized by X-ray micro-CT. A quantitative score was used to evaluate the similarity of the different scaffold batches. Scaffold features compared well with human trabecular bone from different harvesting sites, thereby suggesting the use of this approach to assess reliably the suitability of porous scaffolds for bone tissue engineering applications.

P10

SURFACE FUNCTIONALIZATION OF BIOMATERIALS WITH NATURAL POLYPHENOLS

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Introduction: There is an increasing interest in the use of polyphenols for their antioxidant, antitumor, antibacterial and anti-inflammatory properties and their provenance from a sustainable use of resources. The main limit to their effective application in the medical field is related to their poor biostability/bioavailability, as well as to the lack of systematic scientific research. Natural extracts have been widely investigated, but few attempts of their combination with carriers can be found. The aim of this research is the coupling natural polyphenols with biomaterials for modulating their biological response.

Materials and methods: Polyphenols were extracted from green tea (TPH) and red grapes (GPH) by conventional solvent extraction method and directly grafted to the hydroxyl groups on the surface of the selected biomaterials: bioactive glasses and Ti6Al4V alloy chemically treated to be bioactive. The opportunity to exploit grafted polyphenols for in situ reduction of silver nanoparticles (Ag-NPs) was also explored.

XPS, fluorescence microscopy and Folyn&Ciocalteu (F&C) test were employed in order to verify polyphenols presence and redox activity on biomaterial surfaces. Ag-NPs precipitation was studied by XPS and FESEM. The

selective antitumor activity of polyphenols upon grafting was investigated on safe/tumor osteoblast cells. The antibacterial activity of Ag-treated surfaces was studied (*S. aureus*).
Results: The grafting conditions were optimized in order to preserve the molecular activity and maximize the grafting efficiency. The presence of polyphenols on the functionalized materials was verified by XPS, a uniform distribution of the biomolecules was observed by fluorescence microscopy and the redox activity was measured through the F&C test. A selective cytotoxic activity for tumor cells of polyphenols-grafted glasses was evidenced as well as RONS production and permanent DNA damage. On the contrary, an anti-inflammatory action was evidenced for the safe cells. These results demonstrate the possibility to graft natural polyphenols onto biomaterials maintaining their redox activity and biological properties. The formation of Ag-NPs on functionalized biomaterials was verified as well as their antibacterial activity.
Discussion: This research suggests the grafting of natural polyphenols to bioactive materials as promising solutions for bone contact applications in critical situations (cancer treatment, high infection risk).

P11
TOCOPHEROL NANOEMULSION STABILIZED WITH CHITOSAN OLEATE: KERATINOCYTE PROLIFERATION AND SPRAY DRYING TO CUTANEOUS POWDER
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Introduction: Given its poor solubility in water, alpha tocopherol was encapsulated by means of a hydrophobically modified (HM) chitosan derivative obtaining a nanoemulsion (NE) aimed to topical application in wounds and burns. The HM chitosan was obtained by ionic interaction between chitosan and oleic acid, and it was demonstrated capable to stabilize o/w nanoemulsions. In this work an alpha tocopherol loaded nanoemulsion was studied on keratinocytes evaluating the proliferative effect. To improve stability of tocopherol, NE was subject to spray drying to obtain a powder.
Materials and methods: The antioxidant was loaded in a nanoemulsion stabilized by chitosan-oleate (CS-OA) through a solvent evaporation technique. Proliferation test was performed on keratinocytes maintained in culture for 24 h and 7 days, by adding bromodeoxyuridine (BrDU) before cell fixation. Spray dryer Buchi B-191 was used for the atomization of the nanoemulsion. 10% of mannitol was added as bulk agent. The inlet temperature was 150°C and the nozzle pressure was 600 m^3/h. Relevance of inlet and aspiration as factors were evaluated by Central Composite Design. The response variables were process yield and percentage of alpha tocopherol residue in the powder. Tocopherol stability was evaluated by HPLC.
Results: The resulting NE dispersion presents dimensions of about 221 ± 7.1 nm. NE treatment increase and speed up the proliferation of the cells. Tocopherol content decreased in NE aqueous dispersion at room temperature to about 60% in one month. The spray drying process resulted in a powder loaded with NE with a 38% yield. A significant increase ($P<0.05$) in the yield (%) with the variation of the factor levels, both aspiration and inlet speed, was observed, while no influence of the studied parameters on antioxidant percentage could be observed.
Discussion: The optimized powder contained 87% of the theoretical tocopherol content and showed improved chemical stability.

P12
COLD ATMOSPHERIC PRESSURE PLASMA FOR THE CROSSLINKING OF ELECTROSPUN GELATIN MATS AND GELATIN FILMS CONTAINING DRUGS
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Introduction: The work reports an innovative approach to crosslink gelatin electrospun mats directly in the solid state, through the exposure to cold atmospheric pressure plasma (CAP). The characterization of treated materials has been carried out to investigate the effects of CAP. Furthermore, the effects of plasma-assisted crosslinking have been also investigated for gelatin films containing econazole.
Materials and methods: Treatments were performed with a Dielectric Barrier Discharge (DBD) plasma source driven by a microsecond pulse generator. For electrospun mats, plasma was generated using a 20 kHz sinusoidal waveform with 12 kV peak voltage; treatments were performed for 5, 10 or 20 min and then samples were rinsed for 20 s in double distilled water (DDW) or phosphate buffer (PB). Gelatin films containing econazole were crosslinked by setting peak voltage and frequency at 15 kV and 500 Hz, respectively; treatment times of 3 min/surface and 5 min/surface were tested.
Results: Concerning electrospun mats, 5 min treatment did not induce any increase in stability, conversely, a treatment time of 10 minutes avoided solubilisation, although fibrous structure is lost and the porous mat become a film when immersed in DDW, while morphology is partially maintained after soaking in PB. The increase of treatment time up to 20 minutes provided further improvement of mat stability and a high retention of fibres morphology. The extent of crosslinking was determined evaluating the number of moles of unreacted ε-amino groups per gram of gelatin. The number of ε-amino groups of gelatin samples did not change before and after plasma treatment. Conversely, a significant decrease of ε-amino groups was observed for 20 min treated mats, with an associated increase of crosslinking degree from 0% to 61%. The proposed plasma assisted process is suitable for the crosslinking of gelatin films containing econazole: the stability of the films in aqueous environment was improved by the plasma exposure and no alterations were induced to the drug structure. Biological tests highlighted the antifungal activity of the plasma crosslinked films containing econazole.
Discussion: The data show that CAP can induce the crosslinking of water-soluble polymers without the use of conventional chemical agents.

P13
DEVELOPMENT OF CHITOSAN COATED PLGA NANOPARTICLES LOADED WITH RESVERATROL
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Introduction: Hydrophobically modified chitosan derivatives have been studied in literature to prepare polymeric micelles. Previous researches demonstrated the suitability of chitosan oleate (CS-OA), an amphiphilic chitosan salt, in the stabilization of o/w nanoemulsions (1,2). Amphiphilic polymers, as stabilizers of nanoemulsions have the advantage of associating steric stabilization to interfacial activity.
Materials and methods: Aim of this study was to prepare PLGA nanoparticles by an emulsion evaporation method based on PLGA solution in ethyl acetate and by using CS-OA as stabilizer of the emulsion. This approach results, upon solvent evaporation, in nanoparticles coated with chitosan. Resveratrol is a natural polyphenol with relevant biological properties such as pharmacological cardio- and neuroprotective activities. Its pharmacokinetic behaviour is less favourable because it has poor bioavailability due to low water solubility. Nanoparticle carriers can be useful to improve bio-availability of poorly soluble hydrophobic drugs.
Results: PLGA (Resomer 503) and resveratrol were dissolved in ethyl acetate and homogenized in water as dispersing phase by Ultra Turrax using chitosan oleate as stabilizer. Ethyl acetate was removed under stirring at 40°C. The PLGA nanoparticles were characterized by particle size and polydispersion index and resulted to have dimensions of about 300 nm. Zeta potential was positive; confirming that chitosan, thanks to the amphiphilic modification obtained with oleic acid interaction, was distributed at the oil/water interface and remained at the surface of the nanoparticles during the preparation of the emulsion and during the solvent evaporation phase. Drug entrapment efficiency assessment resulted 60%, showing that Resveratrol was efficiently loaded inside the hydrophobic polymer core.

Discussion: PLGA nanoparticles are easily obtained by stabilization with chitosan oleate. Chitosan coating could be useful for its mucoadhesion behaviour; moreover it can be exploited for further surface modifications, to improve cell recognition.

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MACROPOROUS TCP/TIO$_2$ SCAFFOLDS WITH ENHANCED FRACTURE STRENGTH

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Introduction: A bioactive tricalcium phosphate/titania ceramic dense composites was synthesized by pressure-less air sintering of mixed hydroxyapatite and titania (TiO_2) powders. Titania particles provided a toughening effect to the calcium-phosphate matrix and a reinforcement in fracture strength, compared to sintered hydroxyapatite bodies characterized by similar relative density. In consequence, the present work proposes a modified foaming process to produce porous HA and composite TCP/TiO_2 scaffolds in relatively short time, where the pore extent and organization could be tailored in a reliable and repeatable manner to offer wide, pervious pathways to cells and fluids, in association with high mechanical strength, to meet specific demands in bone surgery.
Materials and methods: HA powder was calcined and sieved under 150 µm, and used to prepare HA scaffolds or to be mixed with TiO_2 nanoparticles to obtain TCP/TiO_2 scaffolds. A high-energy ball milling process was used to prepare ceramic suspensions for the foaming process. The foamed suspensions were poured in paper moulds and dried at r.t. to obtain stable ceramic foams. HA and TCP/TiO_2 scaffolds with different porosity were prepared by varying the air volume into the jar before the last stirring. Finally, a high-temperature thermal treatment was used to consolidate the green bodies.
Results: XRD analysis revealed that, whereas HA scaffolds maintained phase purity after sintering, the composite scaffolds underwent a sequence of solid state reactions yielding a composite made of TCP, TiO_2 and small amount of calcium titanate ($CaTiO_3$). It was possible to tailor the mechanical properties by driving the extent of pore formation during the process. Cell tests reported good cytocompatibility and cell viability, with good cell morphology on the porous scaffolds.
Discussion: The obtained results suggested that the use of a direct foaming method and planetary ball milling, could result in a simple and quick approach to generate porous bioactive scaffolds with improved mechanical performance. Statistical evaluation of the mechanical properties enabled to validate the proposed synthesis method compared to repeatable and reliable production of highly porous scaffolds. A simple model of the process parameters also pointed out the feasibility of flexible design of porous scaffolds even for load-bearing applications.

P15

HUMAN MESENCHYMAL STEM CELLS (HMSCS) EFFECT ONTO PRIMARY HUMAN KERATINOCYTES WOUND HEALING AND POSSIBLE REGENERATIVE APPLICATIONS

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Introduction: Scar formation is a common and unwanted consequence in the skin wound healing process, outcome that rarely occurs in the mucosa. The main mechanisms that regulate this process are still largely unknown. However, since recent research works have highlighted the role/influence of human mesenchymal stem cells (hMSCs) in several regenerative processes, we supposed a different behaviour for keratinocytes from heterogeneous-origin in the wound healing response. The main goals of the study are thus to*: i)* assess the wound healing capabilities of human oral- (HOKs) and epidermal- keratinocytes (HEKs) in response to the stimuli induced by hMSCs and *ii)* apply the acquired knowledge in the development of new regenerative biomedical tools.
Materials and methods: HOKs and HEKs were seeded into 24 well-plates and allowed to adhere; once confluent, each monolayer was scratch-wounded and the supernatant was replaced with hMSCs conditioned medium. Wound closure was then monitored under the optical microscope and images were captured after 0, 6 and 24 hours. To confirm hMSCs influence towards epithelium formation and wound healing, 3D organotypic epithelial raft cultures were made with either HOKs or HEKs in the presence or not of hMSCs conditioned medium. In order to assess epithelia formation, 3D cultures were then morphologically and histologically evaluated by means of haematoxylin/eosin (H/E), type I collagen and BrdU stainings.
Results: According to the literature, preliminary results obtained from the monolayer cultures showed how hMSCs are able to better interact and strongly enhance the proliferation and migration of HOKs respect to HEKs. Further experiments are still in course to clarify the diverse pathways induced by hMSCs during the formation/healing processes of 3D epithelial cultures composed by HOKs or HEKs.
Discussion: In vitro reconstructed mucosa and skin 3D systems are showing their ability to reproduce at best the tissue specific wound repair response, thus allowing to clarify the intrinsic differences in terms of repair capabilities of keratinocytes from various origin. This knowledge could add insights in the improvement and development of new biomedical tools able to reproduce, also in the skin, the same fast and efficient wound healing processes observed in the mucosa context.

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BIOMIMETIC SCAFFOLDS NANOFUNCTIONALIZED BY MOLECULAR IMPRINTING TECHNOLOGY FOR MYOCARDIAL REPAIR

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Introduction: Molecular imprinting nanotechnology has been recently proposed as a functionalization strategy in the development of bioactive scaffolds. In this work, molecularly imprinted particles (MIP) with recognition properties towards the stromal derived factor-1 (SDF-1) were synthesized, characterized and used for scaffold functionalization. Functionalized scaffolds are expected to favour the migration of cardiac progenitor cells (CPC) at the site of injury and promote myocardium healing.
Materials and methods: Scaffolds in the form of sponges were fabricated by freeze-drying, using an alginate/gelatin/elastin blend. MIP were obtained by precipitation polymerization, using the SDF-1 molecule as template, in the presence of a cross-linker. Sponges were functionalized by MIP deposition. Morphological, physicochemical and functional analyses were performed. A preliminary biological characterization using CPC was also carried out.
Results: The obtained particles were well separated, with a spherical shape and an average diameter of 0.9 µm. Template removal from the particles was not complete (78.1%), probably as a consequence of the high cross-linking degree and the hydrogen bond interactions occurring between the monomer and the template. Recognition tests showed that MIP were able to recognize and rebind the template, with a recognition factor of 2.4. MIP were also able to distinguish between the template and an analogue molecule, with a selectivity factor (2.3) and a specific selectivity factor (1.9) both higher than 1. Infrared Chemical Imaging analysis of the functionalized sponges pointed out a sufficiently homogeneous MIP distribution. Recognition tests performed on MIP-modified scaffolds showed that the deposition on polymeric sponges did not alter the specific recognition and binding behaviour of MIP. In vitro CPC culture tests showed that cell adherence was promoted by MIP functionalization.
Discussion: Results obtained in the present study suggest that alginate/gelatin/elastin sponges, functionalized by MIP with recognition properties towards SDF-1, could be successfully used in cell-based therapies of the infarcted heart.

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CHITOSAN COATED ALGINATE-BASED NANOFIBERS AS IMPLANTABLE SCAFFOLD TO SUPPORT AXON REGENERATION

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Introduction: The main issue in the treatment of spinal cord injuries is the development of a therapeutic platform, able to ensure a controlled local drug delivery, as well as the promotion of neurite outgrowth. In the present work, multilayer nanofibers were prepared using both natural and synthetic polymers *via* electrospinning. These systems were constituted by an internal core covered by overlapped layers of cationic and anionic polymers to achieve a controlled biodegradation.

Materials and methods: Mixtures of alginate/dextran (ALG/DEX) and alginate/poly(ethylene oxide) (ALG/PEO), at different concentrations and ratios, were prepared in deionized water. Poloxamer 407 (P407) and/or Triton X-100 (T100) were added at different concentrations, ranging from 0.5 to 2.0% w/w. Polymer mixtures were characterized for rheological properties, surface tension and conductivity and, then, electrospun; different experimental conditions (applied voltage, spinneret-collector distance, flow) were set to optimize the process. Nanofibers were cross-linked using $CaCl_2$ solutions and soaked in a chitosan (CS) solution; further coatings were performed using ALG/CS solutions. Coated nanofibers were subjected to SEM and FTIR analyses.

Results: Bead-free fibers were obtained for: 0.5-1.5% w/w ALG, 25-30% w/w DEX and 1.5% PEO, in presence of 1.5-2% w/w P407 and/or 0.5% w/w T100. Optimal experimental conditions were found: 20 kV voltage, 15-20 cm distance, 0.4-0.8 ml/h flow. ALG/DEX fibers were characterized by diameters ranging from 250 to 750 nm, depending on the surfactant concentration, while ALG/PEO mixtures produced fibers with a diameter >1 µm.

Discussion: The surface tension, viscosity, conductivity and concentration of polymer mixtures play an important role in the production of homogeneous bead-free fibers. In vitro studies are in progress to investigate the system capability to substitute extracellular matrix, supporting axon regeneration. The developed ALG-based nanofibers represent promising candidates for the delivery of drugs, bioactive compounds and/or cells at the site of spinal cord injury.