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

Saporito F[1], Sandri G[1], Bonferoni MC[1], Rossi S[1], Athanassiou A[2], Perotto G[2], Caramella C[1], Ferrari F[1]
[1]Department of Drug Sciences, University of Pavia, Italy; [2]Nanophysics Department, Italian Institute of Technology, Genoa, Italy

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

Colombo V[1-3], Forgione D[1,4], Gherardi M[1-3], Laurita R[1,2], Simoncelli E[2], Nassar M[5], Stancampiano A[1,2,6], Tonini R[1,4]
[1]AlmaPlasma srl, Bologna, Italy; [2]Department of Industrial Engineering, Alma Mater Studiorum, University of Bologna, Bologna, Italy; [3]CIRI - Advanced Applications in Mechanical Engineering and Materials Technology, Alma Mater Studiorum, University of Bologna, Bologna, Italy; [4]School of Dentistry, University of Brescia, Brescia, Italy; [5]Cariology and Operative Dentistry, Tokyo Medical and Dental University, Tokyo, Japan; [6]Department for Life Quality Studies, Alma Mater Studiorum, University of Bologna, Rimini, Italy

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

Bonetti L[1], Cochis A[1], Sorrentino R[1,2], Contessi N[1], Rimondini L[1,2], Farè S[1,2]
[1]Department of Chemistry, Materials and Chemical Engineering "G. Natta", Politecnico di Milano, Milan, Italy; [2]Department of Health Science, University of Piemonte Orientale "A. Avogadro", Novara, Italy; [3]National Interuniversity Consortium of Materials Science and Technology (INSTM), Florence, Italy

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
Tunesi M[1,2], Raimondi I[3], Russo T[4], Cappelletti P[5], Fracasso R[3], Pollegioni L[5,6], Ambrosio L, Gloria A[4], Albani D[3], Giordano C[1,2]
[1]Department of Chemistry, Materials and Chemical Engineering "G. Natta", Politecnico di Milano, Milan, Italy; [2]National Interuniversity Consortium of Materials Science and Technology (INSTM), Florence, Italy; [3]Department of Neuroscience, IRCCS-Istituto di Ricerche Farmacologiche "Mario Negri", Milan, Italy; [4]Institute of Polymers, Composites and Biomaterials, National Research Council, Naples, Italy; [5]Department of Biotechnology and Life Sciences, University of Insubria, Varese, Italy; [6]The Protein Factory Research Center, University of Insubria and Politecnico di Milano, Milan, Italy

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.

Session 7 - Regenerative Medicine: Hard Tissues

OC28
IN VITRO EFFECTS EXERTED BY KERATIN SCAFFOLDS IN COMBINATION WITH PEMF EXPOSURE ON HUMAN SAOS-2 OSTEOGENIC DIFFERENTIATION
Bloise N[1,2], Patrucco A[3], Cristofaro F[1,2], Viganò S[1], Fassina L[4], Tonin C[3], Visai L[1,2]
[1]Department of Molecular Medicine, Center for Health Technologies (CHT), UdR INSTM, University of Pavia, Pavia, Italy; [2]Department of Occupational Medicine, Toxicology and Environmental Risks, Istituti Clinici Scientifici Maugeri, IRCCS, Pavia, Italy; [3]CNR-ISMAC, Italian National Research Council, Institute for Macromolecular Studies, Biella, Italy; [4]Department of Electrical, Computer and Biomedical Engineering, University of Pavia and Centre for Health Technologies (CHT), Pavia, Italy

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
Bonifacio MA[1], Gentile P[2], Ferreira AM[2], Altamura E[1], Cometa S[3], De Giglio E[1]
[1]Department of Chemistry, University of Bari "Aldo Moro", Bari, Italy; [2]School of Mechanical and Systems Engineering, Newcastle University, Newcastle upon Tyne, UK; [3]Jaber Innovation s.r.l., Rome, Italy

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