Posters: XXXVIII Annual ESAO & IV Biennial IFAO Congress, 9-12 October 2011, Porto, Portugal

P159 (EI0315)

THE DEGRADATION OF PORCINE ENDOGENOUS RETROVIRUSES DNA IN ACELLULAR PORCINE HEART VALVE SCAFFOLDS FIXED WITH DIFFERENT LOW TOXIC AGENTS

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Objectives: The application of tissue engineered scaffolds based on porcine heart valves is connected with danger of porcine endogenous retroviruses (PERVs) transmission because acellularization process might not remove all PERVs DNA from tissue. Chemical fixation with glutardaldehyde induces complete degradation of PERVs genetic material in porcine tissue, but this compound is too toxic for cells which will be seeded on scaffolds. Therefore the purpose of the present study was to investigate how chemical fixation with low toxic agents, i.e. two different derivatives of flavonoids – DF1 and DF2, and genipin influence on the PERVs DNA existence in acellular porcine heart valve scaffolds.
Methods: Porcine pulmonary and aortic valves were acellularized using Trypsin/EDTA and sodium sulphate. Acellular tissues were treated with 1) flavonoid derivative DF1, 2) flavonoid derivative DF2 or 3) genipin. The fixation was carried out at 20°C for 3 days. Genomic DNA was isolated from native, acellular and acellular fixed tissues by means of salting out extraction method. Quantification of PERV-A, PERV-B and PERV-C DNA was performed by real time Q-PCR technique. Native and acellular valves were used as a control.
Results: All subtypes of PERVs were detected in native porcine heart valves. Reduction of copies number of PERV-A, PERV-B and PERV-C DNA was observed in acellular porcine valves as well as in acellular valves fixed with flavonoid derivative DF1 and with genipin. PERVs DNA was completely degradated only in acellular porcine heart valves fixed with flavonoid derivative DF2.
Conclusions: Our results demonstrated that fixation of acellular porcine valves with flavonoid derivative DF2 causes completely degradation of PERVs DNA in tissues, thus the acellular porcine heart valve scaffolds fixed with low toxic agents can be used for transplantation without risk of PERVs transmission.

P160 (EI0407)

INTERACTION OF CHONDROCYTES WITH ELECTROSPUN POLYMER SCAFFOLDS DEPENDING ON THE FIBER ORIENTATION

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Objectives: Biocompatible polymer-based scaffolds with a tailaroble degradation rate and a predefined structure might provide an approach to improve cartilage repair, which is limited by the poor intrinsic healing capacity of cartilage. The aim of this study was to explore whether electro-spun polymer scaffolds with different fiber orientation could influence the growth of primary articular chondrocytes.
Methods: Electro-spun scaffolds with aligned and random fiber orientation were prepared from two degradable polymers: poly(ether)ester urethane (PDC) and poly(*p*-dioxanone) (PPDO) as well as polyetherimide (PEI) as a reference polymer, which is not intended to degrade. PDC was selected as a candidate material showing an almost linear mass loss in hydrolytic and enzymatic *in vitro* degradation experiments. Electro-spinning was conducted at ambient temperature using hexafluoro-2-propanol (HFP) as solvent for PDC and PPDO, while PEI was processed from dimethylacetamide (DMAc) solution. The electro-spun structures exhibited an average deposit thickness of 80±20μm with a single fibre diameter around 2-3μm. Primary porcine articular chondrocytes were seeded on the ethylene oxide sterilized scaffolds and analyzed for vitality, ultrastructure and type II collagen expression.
Results: Satisfactory numbers of vital chondrocytes could be detected on all electro-spun scaffolds, which were able to produce the cartilage-specific protein type II collagen. An almost flattened cell shape of the chondrocytes was observed on scaffolds with random fiber orientation, while on scaffolds with aligned fibers the chondrocytes exhibited a spherically cell shape and penetrated into the scaffold pores between the parallel fibers. Surprisingly, it was found that the chondrocytes did not align along the fiber direction.
Conclusions: Chondrocytes were able to grow on all polymer scaffolds tested and expressed the differentiation marker type II collagen. Cell morphology differed depending on the fiber orientation within the scaffolds.

P161 (EI0291)

IONIC LIQUIDS IN NOVEL PROCESSING WAYS TO OBTAIN CHITOSAN/SILK FIBROIN HYDROGELS FOR SKIN TISSUE ENGINEERING

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Objectives: The main goal of this work renders the application of green chemistry principles, namely the use of ionic liquids (ILs) and biorenewable sources, such as chitosan (CHT) and silk fibroin (SF), to newly process hydrogel-based constructs. The combination of this polysaccharide and protein may mimic the naturally occurring tissue environment. Although the solubilization of both materials in ILs has been studied individually, this work reports, for the first time, the use of ILs as a common solvent, for blended CHT/SF hydrogels production. These systems offer the advantage of being homogeneous and of presenting easy and short dissolution time of both biomacromolecules. Moreover, the intrinsic properties of these biopolymers are expected to accelerate the regeneration of chronic skin wounds.
Methods: Hydrogels were obtained by dissolving CHT and SF in 1-butyl-3-methylimidazolium acetate, [bmim][Ac] (4wt%) at different ratios. The systems were gelified and ILs removal was performed by soxhlet extraction with ethanol. The effect of the chitosan source and CHT/SF ratio on consistency, crystallinity, protein adsorption and mechanical properties was evaluated. Moreover, the ability of the developed materials to support adhesion and proliferation of human dermal fibroblasts (hDFb) was assessed up to 21 days of culture.
Results: The findings suggest that [bmim][Ac] allowed the production of CTH/SF hydrogels with a soft and rubbery consistency, microporous surface, good protein adsorption and viscoelastic behavior. Additionally, *in vitro* biological performance revealed a positive influence over adhesion, viability and proliferation of hDFb.
Conclusions: The use of [bmim][Ac] as a common solvent provided a versatile approach to obtain CHT/SF hydrogels with interesting properties and with potential to sustain dermal fibroblasts outgrowth. This work constitutes a strong basis for future healing studies of chronic skin wounds.

P162 (EI0279)

MICROVESICLES DERIVED FROM HUMAN ADULT MESENCHYMAL STEM CELLS PROTECT AGAINST ISCHEMIA-REPERFUSION-INDUCED ACUTE AND CRHONIC KIDNEY INJURY

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Objectives: Several studies demonstrated that mesenchymal stem cells (MSCs) reverses acute kidney injury (AKI) in different experimental models by a paracrine mechanisms rather then by MSC transdifferentiation. We recently demonstrated that microvesicles (MVs) released from MSCs may account for this paracrine mechanism by an horizontal transfer of mRNA and microRNA.
Methods: MVs were purified from MSC supernatants and were injected intravenously in rats (30mg/rat) immediately after monolateral nephrectomy and renal artery and vein occlusion for 45 minutes. To evaluate the MV effects on AKI induce by IRI, the animals were divided into different groups: normal rats (n=4), sham operated rats (n=6), IRI rats (n=6), IRI+MV (n=6), IRI+RNase-MV (n=6) and all animals were sacrificed at day 2 after operation. To evaluate the CKD induced by IRI, the rats were divided into different groups: sham operated rats (n=6), IRI rats (n=6), IRI+MV (n=6) and all animal were sacrificed 6 months after the operation.
Results: We found that a single administration of MVs, immediately after induction of ischemia-reperfusion injury, protects rats from AKI by inhibiting apoptosis and stimulating tubular epithelial cell proliferation. The MVs also significantly reduced the impairment of renal function induced by ischemia reperfusion injury. Pre-treatment of MVs with RNase to inactivate their RNA cargo, abrogated these protective effects. Moreover, MVs protected from chronic kidney disease observed at 6 months in control rats with ischemia reperfusion injury.
Conclusions: MVs released from MSCs protect from ischemia reperfusion induced AKI and chronic renal injury, suggesting that MVs could be exploited as a potential new therapeutic approach.