Acknowledgment: the Grant Agency of the Ministry of Health of the Czech Republic (NT/13297-4), the Ministry of Education, Youth and Sports of the Czech Republic (EE2.3.30.0029), the European Regional Development Fund (the project ''BIOCEV - Biotechnology and Biomedicine Centre of the Academy of Sciences and Charles University'' (CZ.1.05/1.1.00/02.0109)

Production of Elastic Chitosan/Chondroitin Sulphate Multilayer Films For Tissue Regeneration

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Membrane-like scaffolds are suitable to induce regeneration in many and different anatomic sites, such as periodontal membrane, skin, liver and cardiac tissues. In some circumstances, the films should adapt to geometrical changes of the attached tissues, such as in cardiac or blood vessel tissue engineering applications. In this context, we developed stretchable two-dimensional multilayer constructs through the assembling of two natural-based polyelectrolytes, chitosan (CHT) and chondroitin sulphate (CS), using the layer-by-layer methodology. The morphology, topography and the transparency of the films were evaluated. The influence of genipin, a natural-derived cross-linker agent, was also investigated in the control of the mechanical properties of the CHT/CS films. The water uptake ability can be tailored by changing the cross-linker concentration, which influenced the young modulus and ultimate tensile strength. The maximum extension tends to decrease with the increase of genipin concentration, compromising the elastic properties of CHT/CS films: nevertheless using lower cross-linker contents, the ultimate tensile stress is similar to the films not cross-linked but exhibiting a significant higher modulus. The *in vitro* biological assays showed better L929 cell adhesion and proliferation when using the cross-linked membranes and confirmed the non-cytotoxicity of the CHT/CS films. The developed free-standing biomimetic multilayer could be designed to fulfill specific therapeutic requirements by tuning properties such as swelling, mechanical and biological performances.

Acknowledgments: Maria Sousa wants to acknowledge the Portuguese Foundation for Science and Technology (FCT) for financial support through SFRH/BD/97606/2013 grant.

Comparison of Urine Stem Cell and Adipose Stem Cell

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Adipose tissue derived stem cells (ADSCs) would be an attractive autologous cell source. However, ADSCs require invasive procedures, and has potential complications. Recently, urine derived stem cells (USCs) have been proposed as an alternative stem cell source. In the current study, we compare USCs and ADSCs from same patient on stem cell characteristics and capacity to differentiate into various cell lineages to provide a useful guideline for selecting the appropriate type of cell source for use in clinical application. The urine samples were collected from upper urinary track by catheterization, and adipose tissue was obtained from subcutaneous fat tissue from the same patient (n = 10). Cell proliferation, colony formation, cell surface markers expression, immune modulation, chromosome stability and multi-lineage differentiation capability were analyzed for each USCs and ADSCs at cell passage 3, 5, and 7. USCs showed high cell proliferation rate, enhanced colony forming ability, strong positive for stem cell markers expression, high efficiency for inhibition of immune cell activation compared to ADSCs at cell passage 3, 5, and 7. In chromosome stability analysis, both cells showed normal karyotype through all passages. In analysis of multi-lineage capability, myogenic, neurogenic and endogenic differentiation rate of USCs were higher, and osteogenic, adipogenic and chondrogenic differentiation rate were lower than ADSCs. USCs possessed superior cell proliferative ability, immune suppressive effect, and myogenic, neurogenic and endothelial differentiation potency compared to ADSCs. (2014R1A1A3049460)

Targeting Biomimetic 3D Soft-Hard Tissue Interfaces: Formation of a Calcified Spherical Shell with a Double Diffusion Bioreactor

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Introduction: Entheses are the interfaces between bone and soft tissues, such as articular cartilage, meniscus and labrum fibrocartilage, intervertebral disc, tendon, and ligament. Formation of such interfaces normally involves local biochemical processes combining calcium and phosphate to form hydroxyapatite-reinforced materials. In contrast, engineering of such interfaces involves both the initiation and diffusion-limited growth of hydroxyapatite-like structures. Previous studies established the ability of flat-ended double diffusion bioreactors, providing calcium and phosphate from opposite sources, to form a calcified flat structure within hydrogels and at tissue interfaces. The objective of the present study was to test if such a reactor could be extended to a 3D configuration to generate such interfaces in a more complex geometry, specifically a spherical shell.

Methods & Results: Diffusion-reaction modeling in spherical coordinates allowed determination of a general relationship between bath concentrations, location of the formed calcified interface, and the rate of hydroxyapatite formation.

Experiments on a 3D-printed prototype and a CNC-machined spherically symmetric double-diffusion bioreactor incubated for four days, and micro-computed tomography analysis of the resultant material, indicated the ability to rapidly form an internal radiopaque shell, with a thickness of $\sim$100 um and positioned at a distance of $\sim$2–4 mm from the internal and external hydrogel surfaces.

Discussion: These results indicate that a double diffusion bioreactor, for forming calcified tissue plates and interfaces, can be extended from creating flat geometries to partial-spherical shell geometries, which are typical of certain entheses. Further control of the sample boundary conditions may enable creation of even more complex plates and interfaces.

Real-time Visualization of Blood Flow for Monitoring in Regenerative Surgery using a Smartphone Application

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Background: Free flap monitoring is of paramount importance to ensure that the vascular supply is not compromised over the postoperative course. Early detection of arterial or venous failure facilitates salvage. The ideal monitoring system has been described as continuous, non-invasive, quantitative, easy to administer, highly sensitive, and highly specific. A variety of currently used technologies provide proxies to assess flap perfusion, however are expensive and fall short of the ideals. Other attempts at using smartphones for microsurgical free flaps do not provide real-time monitoring.

Methods: We have developed an Android-based smartphone application that utilizes the Eulerian Video Magnification (EVM) [3] algorithm to directly visualize skin perfusion of non-buried free flaps in real-time and provides immediate analysis of tissue. The EVM algorithm allows us to amplify small changes that are not discernable to the human eye, such as the change in color of skin in systole and diastole. Our application has modified this algorithm to assess arterial