Evaluation of the Potential of Polycaprolactone (PCL) and Starch Polycaprolactone (SPCL) Nanofiber Meshes for Cartilage Tissue Engineering Approaches

Marta Alves da Silva[1,2], A. Crawford[2], J. Mundy[2], A. Martins[1], J.V. Araújo[1], P.V. Hatton[2], R. L. Reis[1], N. Neves[1]
[1]3B´S Research Group, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[2]Center for Biomaterials and Tissue Engineering, University of Sheffield, Sheffield S10 2TA, United Kingdom

Statement of Purpose:
In most tissue engineering applications, the scaffold performs a critical role. By electrospinning it is possible to produce fiber based materials, with fiber diameters ranging from submicron to several nanometers. It is believed that its morphology can mimic the extracelular environment existing in most human tissues, including bone and cartilage. Additionally, nanofibrous scaffolds possess a high surface area to volume ratio, which is believed to enhance cell adhesion. Based on this, it is expected that nanofibrous scaffolds should serve as a better environment for cell attachment, proliferation and activity than traditional scaffolds [1].

Articular hyaline cartilage is an avascular tissue that covers the joint and acts as a load-bearing structure. It is composed by chondrocytes and extracelular matrix (ECM). The structure of ECM is composed mainly by glycosaminoglycans, proteoglycans and collagen type II [2].

The present work aims at evaluating the potential of PCL and novel SPCL nanofiber meshes for a cartilage tissue engineering approach, using bovine articular chondrocytes.

Methods:
Polymeric solutions of polycaprolactone (PCL) and starch-compounded polycaprolactone (SPCL) were electrospun using a flat collector. A tension of 9.5 kV and a n-to-ground collector distance of 20 cm were established as optimized processing conditions.

Bovine articular chondrocytes were isolated from the metacarpophalangeal joint of calfs, as previously reported [3]. Cells were seeded dynamically onto PCL and SPCL nanofiber meshes, in a rotator inside the incubator, for 72 hours. Afterwards, cells were transferred to Petri dishes and kept in culture for 28 days. Samples were collected at 1, 2, 3 and 4 weeks of culture. Constructs were characterized by scanning electron microscopy (SEM), staining procedures (Hematoxylin-Eosin, Toluidine Blue and Alamar Blue), immunolocalisation of collagens type I and II and dimethylmethylene blue (DMB) assay for glycosaminoglycans(GAGs) quantification.

Results/Discussion:
Results show that cells are viable throughout the time course of the experiment, either in PCL or SPCL nanofiber meshes with 50-100 µm of thickness. SEM results for both materials show an extensive colonization of the whole nanofiber meshes, and the chondrocytes present the typical spherical morphology. H&E staining confirmed those observations (Figures 1A and 1C), evidencing the interpenetration of cells within the nanofiber meshes. Toluidine Blue (Figures 1C and 1D), Alcian Blue and immunolocalization of collagen type I and collagen type II revealed the formation of ECM by the cells attached
to the materials, and detected both GAGs and collagens. It was also detected more collagen Type I in the sections from SPCL nanofiber meshes than in the sections from the PCL ones, indicating formation of fibrocartilage.

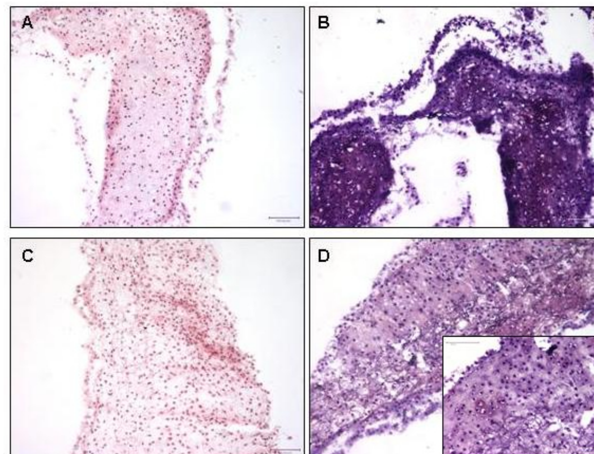

Figure 1. Histologycal sections of PCL (A,B) and SPCL (C,D) nanofiber meshes sedeed with bovine aricular chondrocytes. A, C – Hematoxylin-eosin staining. B, D – Toluidine Blue staining.

Conclusions:
The results show the effective formation of ECM in both types of nanofiber meshes. Both PCL and SPCL nanofiber meshes obtained by electrospinning seem suitable for the production of engineered cartilage. Further work will be performed to optimize the culture conditions and enhance the production of ECM.

References:
1. Huang, Z-M. *et al.*. Composites Science and Technology. 2003; 63,2223
2. Almarza *et al.*. Ann Biomed Eng. 2004. Jan;32(1):2-17
3. Crawford *et al.*. Methods Mol Biol.2004

Acknowledgements
This work was carried out under the scope of the European NoE EXPERTISSUES (NMP3-CT-2004-500283) and IP GENOSTEM (LSHB-CT-2003-503161), Marie Curie Actions – ALEA JACTA EST (MEST-CT-2004-008104), Portuguese FCT projects CARTISCAFF (POCI/SAU-BMA/58991/2004) and NATURALLY NANO(POCTI/EME/58982/2004). Portuguese Foundation for Science and Technology (FCT) for the grant SFRH/BD/24382/2005 attributed to A. Martins.