Bioengineering a tendon-like substitute:
adult stem cell behavior in aligned fibrous scaffolds and stimulating culturing environments

M. T. Rodrigues[1,2]; A. I. Gonçalves[1,2]; R. L. Reis[1,2]; M. E. Gomes[1,2]
[1]3B's Research Group, Univ. of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Portugal; [2]ICVS/3B's - PT Government Associate Laboratory, Portugal

Statement of Purpose: Healthy tendons are composed of a highly oriented network of collagen fibrils and tendon resident cells. This unique structural organization provides these tissues with the necessary tensile properties to withstand constant loading from locomotion and routine movements. Also, mechanical conditioning has been described to positively affect tendon cell proliferation, differentiation and increase extracellular matrix (ECM) synthesis (1, 2). Therefore, the use of mechanical loading is expected to promote this level of organization for achieving successful tissue-engineered (TE) tendon substitutes.

In this work, we propose to bioengineer a tendon-like structure based on a biodegradable natural based polymer made of a blend of starch and polycaprolactone (SPCL) with aligned arrangements similar to the collagen fibrillar bundles in tendons, mimicking the native tissue architecture. Human adipose stem cells (hASCs) were seeded onto these SPCL aligned scaffolds and cell behavior assessed in dynamic and static conditions. Dynamic stimulation was provided by a flow perfusion bioreactor, whose effect on the proliferation and tenogenic differentiation of hASCs was analyzed after 14 days of culture.

Methods: Aligned fibrous scaffolds were obtained from SPCL (30/70% wt) melt spun fibers by a method consisting in bonding parallel and aligned fibers at 120ºC. Scaffold samples were cut into 6mm diameter discs. hASCs were isolated from lipoaspirates and expanded in basic medium (α-MEM, 10% FBS, 1% antibiotic). Primary tenoblasts obtained from surgery surplus were expanded in basic medium and used as positive controls. Cells were seeded at a density of 125,000 cells/scaffold and cultured under either static or dynamic conditions for 14 days in basic medium. In the perfusion bioreactor, a continuous medium flow rate of 2mL/min (0.1mL/min/scaffold) was established assisted by a peristaltic pump (Ismatec). Tenogenic differentiation was weekly evaluated on static and dynamic cultured constructs by SEM analysis, cell metabolic activity (MTS) and real time RT-PCR for tendon related markers detection (tenascin-C, decorin, collagen I and III). The protein expression of tenascin-C was also assessed by immunolocalization.

Results: SPCL scaffolds consisting of parallel aligned fibers were successfully produced. The fibers alignment enabled a good attachment of seeded hASCs, and promoted cells alignment according to substrate orientation, which seems to be determinant to achieve tenogenic differentiation. In fact, SEM analysis showed that cells cultured on SPCL scaffolds assumed an elongated shape, a common feature in tenoblasts, in both static and dynamic systems using basic medium. MTS assay showed an increased cell metabolic activity along the culturing time in both static and dynamic cultures.

Moreover, cells expressed tenascin-C, a protein naturally present in tendon ECM, both in static and flow perfusion conditions (Fig. 1A, B), thus suggesting tenogenic ECM formation. Real time RT-PCR results (Fig. 1C) indicated that the collagen III and decorin genes are up-regulated in static culture compared to GAPDH, the housekeeping gene. However, constructs cultured for 14 days in the flow perfusion bioreactor showed an increase in the expression levels of all genes analyzed. With the exception of decorin, all gene expression fold changes are increased in dynamic culture compared to the static environment.

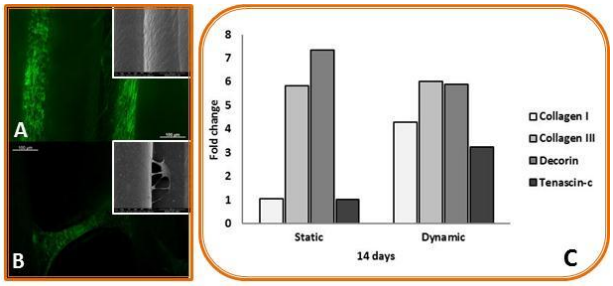

Figure 1. Tenascin-C immunofluorescence images of scaffolds at 14days in static (A) and dynamic (B) culture. C) Relative gene expression of SPCL-hASCs constructs after 14 days in static or dynamic culture.

Conclusions: The development of TE constructs based on biomimetic scaffold design and stem cells explores a promising system for regeneration with potential for tendon application. The stimulation into tenogenic lineage was demonstrated by the elongated and aligned cell morphology, the presence of a tenascin-C rich ECM and the expression of gene markers, typically associated to tendon tissues. The results indicate that the differentiation was regulated by the fiber arrangement and also by the mechanical stimulus provided by the flow perfusion bioreactor. Aligned SPCL-based scaffolds promoted the colonization and tenogenic differentiation of hASCs cultured in basal medium in static and dynamic conditions. Also, flow perfused constructs showed a higher expression of tendon related genes, namely collagen I, III and tenascin-C. Thus, scaffolds architecture and mechanical stimulation may be determinant at tendon regeneration approaches in order to closely match the properties and functionality of native tendons. Longer culture periods will be considered in future experiments in order to better understand flow perfusion stimulus on hASCs.

References: [1] Goncalves, A *et al*. Acta Biomaterialia. 2011:7: 1644-1652; [2] Doroski, DM *et al*. Tissue Eng Part A. 2010: 16: 3457-3466.