DEVELOPMENT OF A BIOENGINEERED THREE-DIMENSIONAL SCAFFOLD ABLE TO COMMIT STEM CELLS TOWARD TENOGENIC PHENOTYPE

Abstract

The term tendinopathy is a generic descriptor of tendon disorders. Spontaneous adult tendon healing results in scar tissue formation and fibrosis, with impaired biological and mechanical properties. Adult tendons have a limited natural healing capacity, and often respond poorly to current treatments, including exercise, drug delivery and surgical procedures. To treat tendon diseases and support tendon regeneration, cell-based therapy and tissue engineering approaches are considered options. None can however yet be considered conclusive in their restoration of a safe and successful long-term solution for full microarchitecture and biomechanical tissue recovery.

This thesis describes the work to develop an innovative bioengineered multiphasic three-dimensional scaffold, seeking to reproduce the complex microenvironment able to support tenogenic differentiation. Mesenchymal stem cells derived from human bone marrow (hBM-MSCs) are one of the main stem cells sources used in tissue-engineering protocols while extra-embryonic cord-derived, including from Wharton's Jelly (hWJ-MSCs), are emerging as useful alternatives.

To explore the tenogenic plasticity of hBM-MSCs and hWJ-MSCs, we first conducted a pilot study treating both cell types with different doses of human Growth Differentiation Factor-5 (hGDF-5), a growth factor which induces tenogenic differentiation, improving the outcome of tendon repair. hGDF-5 induced the expression of genes (SCX-A, COL1A1, TNC, DCN, TNMD) and proteins (type I collagen, tenomodulin) linked to the neo-tendon phenotype in a time and concentration-dependent manner. The concentration of 100 ng/mL was the most effective for both stem cells types, coupled with specific alignment and shape modification. However, compared to hBM-MSCs, hWJ-MSCs showed higher proliferation rate and earlier up-regulation of tenogenic markers. Since the modulation of the inflammatory response is reported to be necessary in tendon healing and

regeneration, we analyzed the gene expression of pro-inflammatory (IL-6, TNF, IL-12A, IL-1β) and anti-inflammatory (IL-10, TGF-β1) cytokines, observing an anti-inflammatory immunomodulatory response of both stem cells, related to their tenogenic commitment.

In a following study, we focused on a three-dimensional (3D) scaffold, which we named HY-FIB, incorporating a force-transmission band of braided hyaluronate embedded in a cell localizing fibrin hydrogel and poly-lactic-co-glycolic acid (PLGA) nanocarriers as transient components for growth factor controlled delivery. The tenogenic supporting capacity of HY-FIB on hBM-MSCs was explored under static conditions and under bioreactor induced cyclic strain conditions. Mechanical signaling, conveyed by HY-FIB to hBM-MSCs, promoted upregulation of tendon related genes (SCX-A, COL1A1, DCN) and proteins (type I collagen). Cells also showed pro-inflammatory (IL-6, TNF, IL-12A, IL-1β) and anti-inflammatory (IL-10, TGF-β1) cytokine gene expressions, with a significant increase of anti-inflammatory ones in dynamic conditions.

Since these results provided strong evidence in support of the HY-FIB system and its interaction with cells, we enhanced the system fabricating PLGA nanocarriers, engineered to ensure a controlled delivery of hGDF-5.
PLGA nanocarriers, with controlled size and suitable encapsulation efficiency, were produced using Supercritical Emulsion Extraction (SEE) technology and were added to the fibrin hydrogel to provide a controlled growth factor delivery. The effect of biochemical and mechanical stimuli was studied on hWJ-MSCs, showing a significantly increased expression of tenogenic markers.

The described approach opened exciting perspectives for future development of engineered tendon tissue substitutes. HY-FIB was an excellent system for the sustained release of biochemical signals and a strategic approach to develop *in vitro* 3D bioengineered models to understand specific behaviour of tendon healing and regeneration.