PARACRINE EFFECTS OF MACROPHAGE PHENOTYPE ON TENDON TISSUE REMODELING

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Introduction

Tendinopathy is characterized by tissue degeneration and the transformation of the normally aligned extracellular matrix (ECM) towards a disorganized ECM. Simultaneously, polarized tenocytes change into randomly oriented tenocytes [3]. Restoration of the healthy tissue organization is an important challenge to restore its function. Macrophages are thought to be one of the key regulators during remodeling, and their polarization into a spectrum of phenotypes is hypothesized to play an important role in both tissue remodeling and fibrosis [2], being influenced by environmental cues such as cytokines and topographies [1]. The interplay between macrophages and tenocytes, and how this influences tendon remodeling remains unknown. In order to get a better understanding of the processes involved, we aim to elucidate the effect of paracrine signaling of distinct macrophage phenotypes on tendon-like tissue remodeling.

Methods

3D constrained microtissue platforms were used *in vitro* to create aligned tendon-like tissues consisting of tenocytes in a collagen type I gel (Figure 1). The microtissues were cultured in conditioned media (CM) from macrophages in three different biochemically induced polarization states, (IFN-γ+LPS stimulated M1, IL-4+IL-13 stimulated M2a, and IL-10 stimulated M2c). Microtissue contraction, tenocyte gene expression, cellular orientation, and collagen organization were analyzed to get more insight in the remodeling behavior.

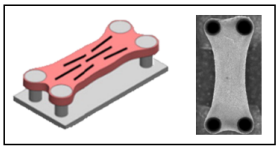

Figure 1. 3D microtissue model using constraints [3]

Results

Macrophage-secreted factors showed to influence tenocyte tissue remodeling by differences in actin orientation (Figure 2A), collagen organization (Figure 2B), and gene expression (Figure 2C). Culturing in M1-CM resulted in less anisotropically orientated actin and collagen, and less expression of remodeling genes compared to M2a- and M2c-CM. Culturing in M2a- and M2c-CM resulted in a similar orientation of actin and collagen, while higher remodeling gene expression in M2c-conditioned samples, and higher collagen expression in M2a-conditioned samples was observed.

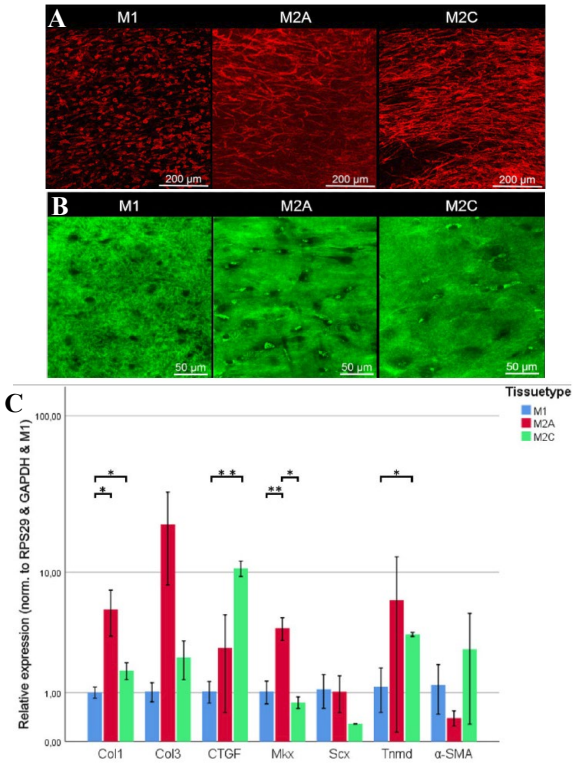

Figure 2. (A) phalloidin staining actin stress fibers; (B) CNA35 staining collagen fibers; (C) qPCR of Col1 (collagen type 1), Col3 (collagen type 3), CTGF (connective tissue growth factor), Mkx (mohawk), Scx (scleraxis), Tnmd (tenomodulin), α-SMA (α-smooth muscle actin).

Discussion

Macrophage-secreted factors were shown to influence tenocyte tissue remodeling in terms of orientation and tenogenic marker genes. M1-CM resulted in less anisotropic tissue orientation and lower expression of tissue remodeling genes, indicating less tissue remodeling, compared to M2a- and M2c-CM samples. No clear differences between M2a- and M2c-CM samples could be found. Next, a transwell co-culture will be performed to further examine the paracrine interplay between macrophage phenotypes and tenocytes during remodeling, and to identify which paracrine factors (e.g. cytokines) are the main modulators. Further unraveling of the interplay between macrophages and tenocytes will provide us with new targets to steer functional tendon healing.

References

[1] Luu et al, ACS Appl Mater Interfaces, 7:28665-28672, 2015. [2] Wissing et al, NPJ Regen Med, 2, 2017. [3] Foolen et al, Matrix Biology, 65:14-29, 2018.