A 3D TENDON BIOMIMETIC SCAFFOLD WITH POTENTIATED BIOLOGICAL PERFORMANCE ON AMNIOTIC EPITHELIAL STEM CELLS FOR TENDON TISSUE ENGINEERING APPLICATIONS

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Introduction: Advanced strategies in tendon Tissue Engineering might overcome the unsatisfactory results of conventional treatments for tendinopathies [1]. It aims at designing 3D tendon biomimetic scaffolds with adequate physical, mechanical, and biological properties of the native tissue. In this context, 3D tendon biomimetic poly(lactide-co-glycolic) acid (PLGA) scaffolds with highly aligned fibers were fabricated and engineered with Amniotic Epithelial Stem Cells (AECs), to verify their teno-regenerative and immunomodulatory potential.

Methods: Electrospinning technique has been used to produce PLGA fleeces with highly aligned fibers. 3D scaffolds, obtained by wrapping manually the fabricated PLGA fleeces, were characterized for their ultrastructure and mechanical properties. The teno- and immuno-inductive potentials of PLGA fleeces and 3D scaffolds have been assessed on AECs by analyzing YAP, a mechanotransducer protein, TNMD protein, a mature tendon marker, and tendon-related (*SCX*, *COL1* and *TNMD*) and interleukins (*IL-10* and *IL-12*) gene expressions after 48h and 7d culture. AECs cultivated on petri dishes were used as control (CTR).

Results: The fabricated PLGA 3D scaffolds mimic the structure of native tendons, characterized by high fiber alignment and significantly higher biomechanical properties compared to PLGA fleeces ($p<0.05$). AECs engineered within PLGA fleeces and 3D scaffolds changed their cobble-stone morphology, acquiring a spindle tenocyte-like one already after 48h culture, confirmed by the decrease in cell nuclei aspect ratio with significantly lower values for 3D scaffolds ($p<0.05$). The teno-inductive potential of the fabricated materials was confirmed by analyzing TNMD protein expression and tendon related genes. TNMD was already expressed after 48h culture within the cytoplasm of AECs engineered within PLGA fleeces and 3D scaffolds. Moreover, all tendon markers were significantly upregulated by the cells seeded on both PLGA materials respect to CTR ($p<0.05$), especially after 7d culture ($p<0.05$). The immuno-inductive potential of PLGA materials on AECs was confirmed by the significant expression of IL-10, an anti-inflammatory cytokine, with boosted upregulation for 3D scaffolds at 7d ($p<0.05$). Instead, the expression of IL-12, a pro-inflammatory cytokine, was maintained to its basal levels within 3D scaffolds. The IL-10/IL-12 ratio was especially in favor of 3D scaffolds ($p<0.05$). The effect of fiber topography on AECs' biology was assessed by analyzing YAP protein expression. Differently to CTR where YAP was localized within cells' cytoplasm, PLGA fleeces and 3D scaffolds induced YAP localization within AECs' nuclei with significant enhanced activation on 3D scaffolds compared to fleeces ($p<0.05$). Indeed, 3D scaffolds exerted an enhanced activation of YAP mechanotransducer on AECs'.

Conclusions: The obtained results demonstrated that the fabricated 3D scaffolds mimic native tendon tissue ultrastructure and biomechanics and exhibit high teno- and immuno-inductive potential on AECs, making them a potential candidate to be applied for surgical purposes in tendon regeneration.

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References:

1. Russo V., El Khatib M. et al. Cells, 11, 266 (2022)

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