Biomimetic PLGA 3D Scaffold Potentiate Amniotic Epithelial Stem Cells Biological Capability for Tendon Tissue Engineering Applications

INTRODUCTION: Tendon tissue engineering represents a promising solution to deal with tendinopathies and aims to develop effective implantable 3D biomimetic scaffolds with ideally native tissue's physical, mechanical, biological, and functional qualities. These constructs can be engineered with stem cells to potentiate their teno-inductive and immunomodulatory properties (El Khatib, Mauro, Di Mattia, et al., 2020; El Khatib, Mauro, Wyrwa, et al., 2020; Russo et al., 2020). In this context, amniotic epithelial stem cells (AECs) have recently received much attention in the field of regenerative medicine due to their capacity to differentiate into the tenogenic lineage and to their immunomodulatory profile (Barboni et al., 2012, 2018; Mauro et al., 2016).

The focus of this research was to create bundle tendon-like PLGA 3D scaffolds, which mimic tendon macro and micro-architecture and biomechanics, and to assess their impacts on AECs' biological potential.

METHODS: PLGA fleeces, with highly aligned fibers, were fabricated via electrospinning technique through a rotatory collector. The obtained fleeces were then wrapped manually to form 3D tendon-like scaffolds, which were evaluated in terms of structure, mechanical characteristics, and biological influence on AECs by conducting *in vitro* experiments. Indeed, ovine AECs, seeded on the PLGA 3D scaffolds and fleeces, were compared for their morphological changes and for the cytoplasmic expression of TNMD, a mature tendon protein, respect to cells cultured on Petri dishes (CTR), after 48h and 7d of culture through a confocal microscope. Moreover, the teno-differentiative potential and immunomodulatory properties of the produced constructs were assessed by analyzing the gene expression of tendon related markers (early: *SCX, late: COL1 and TNMD*) and of anti- (*IL10*) and pro- (*IL12*) inflammatory cytokines respectively. Moreover, the present research evaluated YAP protein activation in the engineered AECs through immunofluorescence assay by assessing its cellular localization.

RESULTS: The produced PLGA 3D scaffolds, analyzed though a scanning electron microscope, showed high fiber alignment, which closely resemble the architecture, both macroscopically and microscopically, and the biomechanical properties of native tendon tissue. AECs seeded on the produced constructs exhibited an elongated tenocyte-like morphology already after 24 hours, while AECs cultivated on petri dishes (CTR) retained their characteristic polygonal morphology. The engineered AECs' phenotypic change was also confirmed by visualizing the cytoplasmic expression of TNMD protein and supported by tendon-related genes (*SCX, COL1, and TNMD*) upregulation at 7-day culture respect to CTR cells ($p<0.05$), which showed no TNMD protein expression or significant increase in tendon-related genes. Moreover, AECs seeded on 3D PLGA scaffolds showed an anti-inflammatory profile, with a significant higher IL10/IL12 ratio respect to the CTR ($p<0.05$). Finally, 3D scaffolds with highly aligned fibers stimulated AECs in terms of cell cytoskeleton stress, activating their mechanosensitive YAP pathway by significantly increasing YAP nuclear localization compared to the CTR ($p<0.05$), in which YAP was instead localized in the cytoplasm.

DISCUSSION & CONCLUSIONS: Overall, these results support the biomimicry of the fabricated scaffolds in terms of structure and biomechanics and reveal their great teno/immuno-inductive potential and mechanosensing stimulus on AECs, thus standing biomimetic PLGA 3D scaffolds as a potential candidate for tendon regeneration.