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Optimization and establishment of a co-culture model to study cellular interactions in tendon-to-bone interface

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Tendon detachment from its bony insertion is one of the most frequent injuries occurring in the musculoskeletal interface, constituting an unmet challenge in orthopaedics. Tendon-to-bone integration occurs at the enthesis, which is characterized by a complex structure organized in a gradient of cells and microenvironments. Hence, the maintenance of a heterotypic cellular niche is critical for tissue functionality and homeostasis. Replicating this unique complexity constitutes a challenge when addressing tendon-to-bone regeneration and interfacial tissue engineering strategies. Currently, mechanisms presiding to tendon-to-bone interface healing are not yet fully understood, particularly the interactions between tendon and bone cells in the orchestration of interfacial repair versus regeneration. Therefore, this study focused on the hypothesis that interactions between human tendon-derived stem cells (hTDCs) and pre-osteoblasts (pre-OB) can initiate a cascade of events, potentially leading to interfacial regeneration. Thus, hTDCs and pre-OB (pre-differentiated human adipose-derived stem cells) were used. Herein, five different ratios between basal and osteogenic media (100:0,75:25,50:50,25:75,0:100) were assessed to estimate their influence on cell behaviour and identify the ideal parameters for simultaneously supporting tenogenic and osteogenic differentiation before establishing a co-culture. Tenogenic and osteogenic differentiation were assessed through the expression of tendon and bone markers, mineralization (alizarin red, AZ) and alkaline phosphatase (ALP) quantification. Results showed that hTDCs exhibited osteogenic differentiation potential when cultured in the presence of osteogenic media, as demonstrated by an increase in ALP activity and mineralization. Pre-OB expressed osteogenic markers (OCN,OPN) in all media conditions confirming osteogenic commitment, which was simultaneously confirmed by ALP levels and AZ staining. Thus, three different conditions (100:0, 50:50, 0:100) were chosen for further studies in a direct contact co-culture system. Similarly to single cultures, a significant proliferation was observed in all conditions and mineralization was increased as soon as 7 days of culture. Additionally, osteogenic, tenogenic and interface-relevant markers will be assessed to study the effect of co-culture on phenotype maintenance. In summary, the present work addresses major limitations to clinical translation of cell-based therapies aiming at promoting interfacial regeneration. Particularly, we explored the influence of culture media on the maintenance of tenogenic and osteogenic niches, taking a basic and critical step towards the establishment of more complex cell-based systems.

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