Smart bioactive 3D printed scaffolds and mesenchymal stem cells for tendon regeneration

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INTRODUCTION: Tendons are specialized fibrous organs with specific mechanical properties. If treated improperly, tendon tears can lead to significant functional impairment. The surgical treatment of tendon tears can be challenging even for the most experienced surgeon, especially in a massive tissue gap. One of the main issues in tendon healing is the low damage-induced extracellular matrix production within these tissues. Also, tissue gaps and mechanical stresses further decrease the chances of adequate healing.
Modern bioengineering could provide solutions to increase the healing rates of fibrous organs such as tendons and ligaments.
Our project aimed to develop a smart, bioactive, implantable 3D-printed scaffold that can reproduce human tendons' structural and functional properties using FDA-approved materials and seeded with human MSCs (hBM-MSCs).

METHODS: Our research consisted of finding and evaluating the capacity of different scaffolds to maintain human MSCs' vitality, allow their adhesion, and promote their differentiation to tenocytes. We tested 158 tridimensional printed scaffolds made with either biological or synthetic biocompatible polymers, including Type-A pig skin collagen, Gelatin methacryloyl (GelMA), Poly-L-lactic acid (PLLA), electrospun Polylactic-co-glycolic acid (PLGA), and Polycaprolactone (PCL). All the scaffolds were treated with the same amount of hBM-MSCs.
We microscopically evaluated each scaffold material's capacity to maintain cell vitality, allow their adhesion, and later their proliferation. We also evaluated the grade of differentiation to tenocytes or osteocytes within 7, 14, and 21 days of culture using colorimetry, immunofluorescence, and genetic expression tests (including specific primers for genes including SCX, COL1A1, NANOG, and MKI67).
We also tried to replicate the interaction between the scaffolds and the human immune system, seeding scaffolds with HL60 cells and evaluating NET formations.
RESULTS: All the tested scaffolds could allow good cellular adhesion. In immunofluorescence and gene expression tests, electrospun PLGA was the best substrate for MSC proliferation, followed by PCL. Within 21 days, MSCs in electrospun PLGA scaffolds showed an increase in type 1 collagen deposition (COL1A1). Their significant reduction of proliferation (MKI76) and stemness (NANOG) genes, associated with a significant increase of the scleraxis transcription factor (SCX) in the TENO2-differentiated sample, also confirmed the successful differentiation into tenocytes. On the other hand, 3D-printed PCL scaffolds promoted osteoblastic differentiation, with increased calcium deposition and overexpression of genes, including TSPO, CYP11A1, CYP17A1, 3b-HSD, and 17b-HSD.
NET did not significantly reduce MSCs' proliferation, suggesting that the human immune system could tolerate these implants if they were used in the surgical field in the near future.
DISCUSSION AND CONCLUSION: Bioengineering can provide new solutions to the treatment of massive tendon tears. Biocompatible artificial tendons with variable quantities of PLGA and PCL loaded with patient's MSCs could represent a solution to mimic both tendons and entheses, increasing the chances of successful healing.