From rupture to regeneration: bioengineering anisotropic tendon replacements

POLYMAT

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• INTRODUCTION •

• Background •

Tendon ruptures are one of the most common acute tendon injuries in adults. However, effectively treating full-thickness tendon ruptures is currently hindered by the lack of **resilient support materials** that can **promote tissue formation** while withstanding **physiological loading conditions**. The tendon-bone interface (**enthesis**) is the **most common site of injury** in tendons due to the presence of high stress concentrations. The mechanical properties of tendon enthesis are **anisotropic**, and the phenotype of cells within, **heterotypic**.[1]

• Aim of the project •

The present work aims to fabricate 3D printed **poly (D,L-lactide-e-caprolactone) (PLCL) scaffolds** with discrete structural zones that promote mesenchymal stem cell (MSC) differentiation towards various phenotypes found on the tendon enthesis.

• RESULTS AND DISCUSSION •

3D PRINTED SCAFFOLDS

› Diverse architectures of PLCL scaffolds support MSC proliferation.

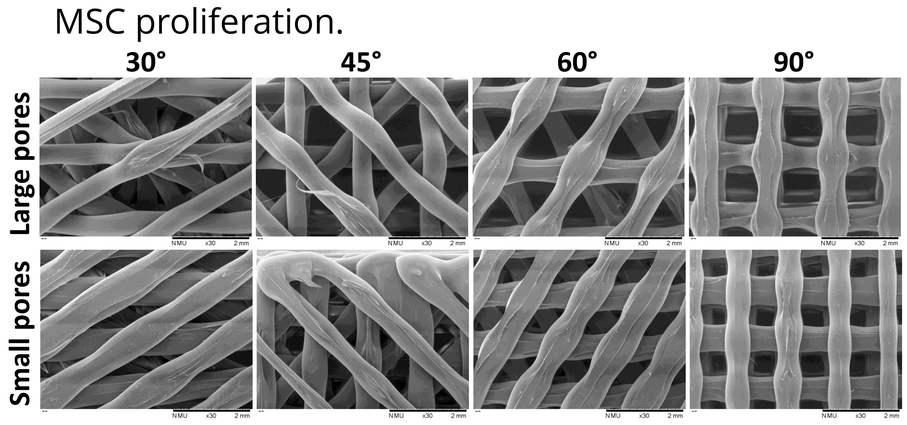


Figure 1. Top view SEM images of PLCL scaffolds with diverse pore shape (30º, 45º, 60º and 90º patterns) and dimensions (large pores, *top,* and small pores, *bottom*).

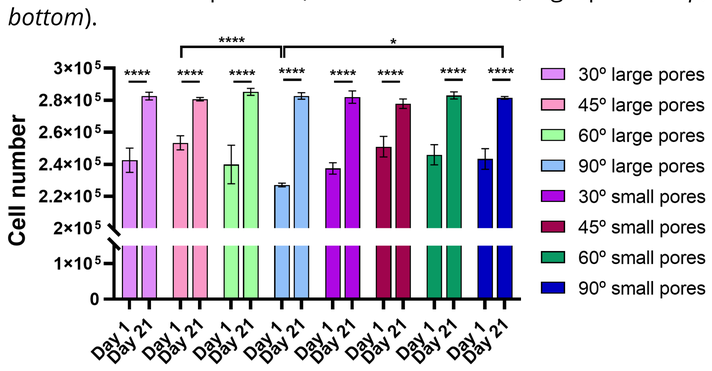


Figure 2. Cell number of MSCs calculated from the DNA content after 1 and 21 days of culture in maintenance media. Data is displayed as mean ± SD.

MECHANICAL PROPERTIES

› Scaffolds with 90º pattern exhibited the highest UTS, Young's modulus, and yield stress, being suitable to replicate the mechanical properties of **tendon**.

› Scaffolds with 30º pattern demonstrated mechanical performance consistent with **bone**.

› 45º and 60º patterns showed intermediate mechanical behavior to recreate **fibrocartilage**.

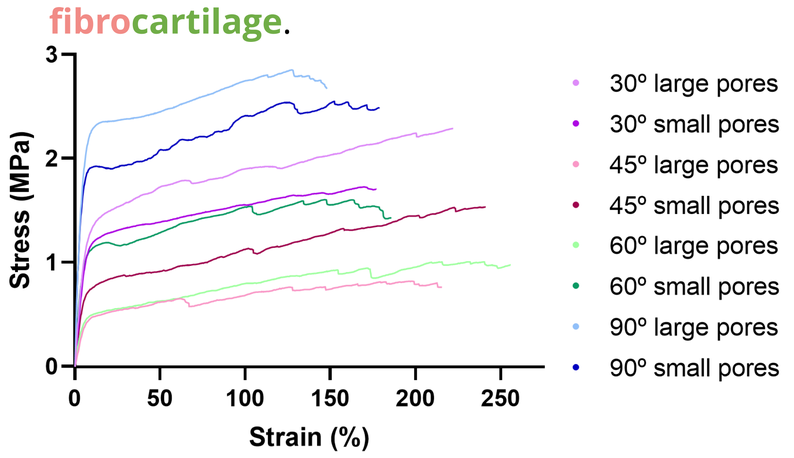


Figure 3. Representative stress-strain curves of PLCL scaffolds with diverse architectures subjected to uniaxial strain.

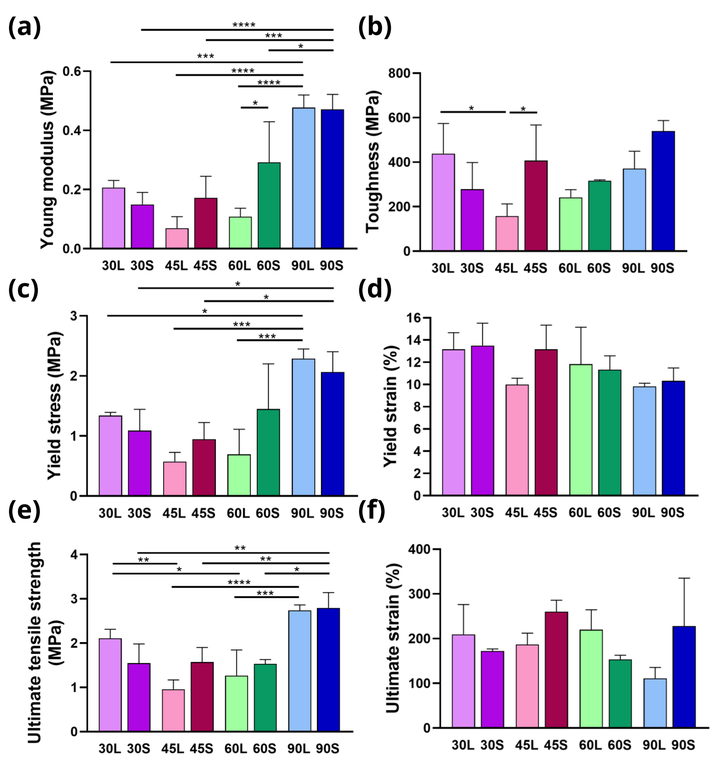


Figure 4. Tensile properties of PLCL scaffolds with diverse architectures. **(a)** Young's moduli, **(b)** toughness, **(c)** yield stress, **(d)** yield strain, **(e)** ultimate tensile strength, and **(f)** ultimate strain. Data is displayed as mean ± SD.

TISSUE FORMATION IN SCAFFOLDS WITH DIFFERENT ARCHITECTURES

› PLCL scaffolds combined with GDF-7 and ASAP induce the expression of characteristic tenogenic markers in seeded MSCs, and the deposition of a tendon-like matrix with abundant GAGs, collagen I, collagen III and fibronectin.

› The patterns selected based on the mechanical performance of PLCL scaffolds are good candidates for tendon tissue formation.

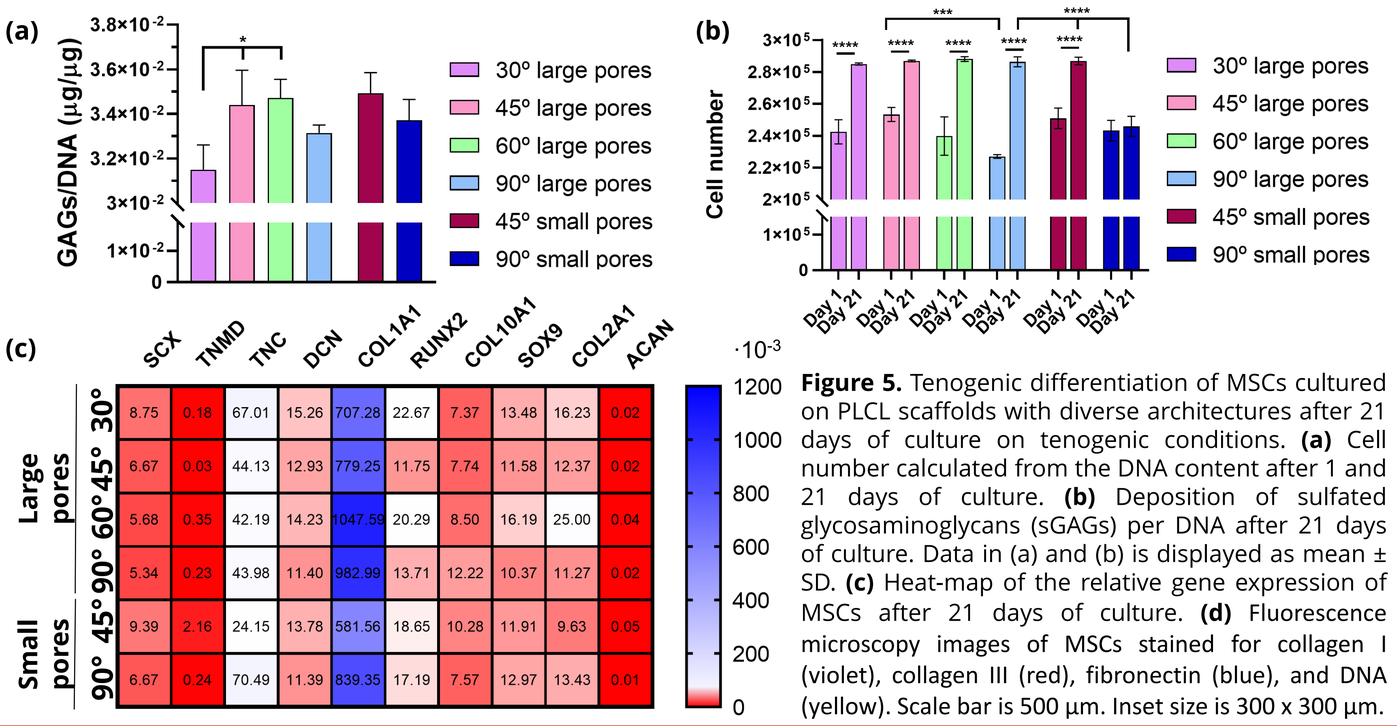


Figure 5. Tenogenic differentiation of MSCs cultured on PLCL scaffolds with diverse architectures after 21 days of culture on tenogenic conditions. **(a)** Cell number calculated from the DNA content after 1 and 21 days of culture. **(b)** Deposition of sulfated glycosaminoglycans (sGAGs) per DNA after 21 days of culture. Data in (a) and (b) is displayed as mean ± SD. **(c)** Heat-map of the relative gene expression of MSCs after 21 days of culture. **(d)** Fluorescence microscopy images of MSCs stained for collagen I (violet), collagen III (red), fibronectin (blue), and DNA (yellow). Scale bar is 500 µm. Inset size is 300 x 300 µm.

• CONCLUSIONS, FUTURE WORK AND REFERENCES •

› PLCL scaffolds 3D printed with specific architectures can recreate the mechanical properties of each individual layer in the native enthesis.

› The designed structures provide a supportive microenvironment for MSC proliferation, tenogenic differentiation and fibrillar matrix formation.

• Future work • Fabricate gradient scaffolds that, in combination with specific biochemical cues, will push the differentiation of MSCs towards the tenogenic, osteogenic and chondrogenic lineages.

[1] Camarero-Espinosa, S. et al., *Adv. Healthcare Mater.* **2023**, *12*, 2203023

• ACKNOWLEDGEMENTS •

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