Enhancing Bone-Tendon Repair: A Biomimetic Strategy Utilizing Enthesis-Mimicking Suture Anchor-Tendon Hybrid Graft with Growth and Differentiation Factor-7 (GDF-7) for Multi-Cell Patterning

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INTRODUCTION: Rotator cuff repair remains challenging due to the need for addressing load bearing deficits in the mechanically compromised shoulder while simultaneously revitalizing injured fibrocartilage and tendon tissue. To address these, we designed a biomimetic bone-tendon graft (Suture Anchor-Tendon Hybrid Graft; SATHG) containing Growth and Differentiation Factor-7 (GDF-7) to reduce mechanical stress and enhance tissue healing at the repair site. To reduce mechanical stress, SATHG was designed to mimic geometric attributes of native entheses such as collagen fiber angulation, tissue geometric flaring, mechanical gradation, and tissue interdigitation[1-4]. To enhance fibrocartilage and tendon healing, we utilized GDF-7[5] to achieve the simultaneous differentiation of human mesenchymal stem cells (hMSCs) into fibrochondrocytes and tenocytes, enabling facile multi-cell patterning of SATHG.

METHODS: To perform FEA optimization, 3D *in silico* models of the SATHG and humeral bone were created in Solidworks (Dassault Systèmes, France) and arm abduction was simulated using a 100 N tensile force (**Figure 1**). FEA results subsequently were validated by *ex vivo* tensile testing of physical SATHG, which were fabricated from phototunable QHM polymer[6]. To perform multi-cell patterning, hMSCs were cultured as spheroids and individual cells in 200 ng/mL GDF-7 for 15 days *in vitro*. Immuno- and cytochemical staining assessment for tenocyte (SCX, TNC, and Picosirus Red) and fibrochondrocyte (COL2A1, Sox9, and Alcian Blue) differentiation was performed. To assess tissue response and *in vivo* healing, 10 rabbits underwent surgery to create a 5 mm rotator cuff defect for implantation of non-porous SATHGs followed by histological assessment (H&E and safranin-O staining) at 6 weeks post-surgery.

RESULTS: FEA showed a 43.6% reduction in peak stress concentration for the optimized SATHG design compared to the control[7]. Optimized SATHGs had low stress in central regions. *Ex vivo* tensile testing validated the design, exhibiting superior load, displacement, stiffness, and work-to-failure. Larger SATHG diameters also improved pull-out strength, indicating robust bone attachment. Immuno- and cytochemical staining assessment such as Alcian blue staining showed simultaneous fibrochondrocyte and tenocyte differentiation of hMSCs within a single construct/well when treated with GDF-7. *In vivo* studies showed unexpected damage in SATHG implants in the non-optimized, tendon-like regions but encouragingly, H&E and safranin-O staining revealed evidence of biocompatibility and enthesis regeneration (**Figure 2**).

DISCUSSION: This study demonstrated that geometric mimicry of the enthesis increased the mechanical properties (e.g., ultimate load) of a bone-tendon graft and that a single growth factor (GDF-7) induced simultaneous cell fates (tenocyte and fibrocartilage) in a spatially controlled manner within a single construct for multi-cell patterning These findings will facilitate soft-to-hard, tendon-bone tissue attachment despite their severe mechanical mismatch and promote eventual graft-host integration. However, our FEA study focused on mechanical optimization of SATHG's soft-to-hard interface and unexpected damage was observed in the graft's tendon-like regions. This hampered biomechanical evaluation and is a limitation of the current work. Further optimization of the SATHG's tendon-like regions (e.g., geometric shape and thickness) will be performed in future studies to improve mechanical performance. Taken together, this biomimetic approach holds promise for musculoskeletal repair and implant durability.

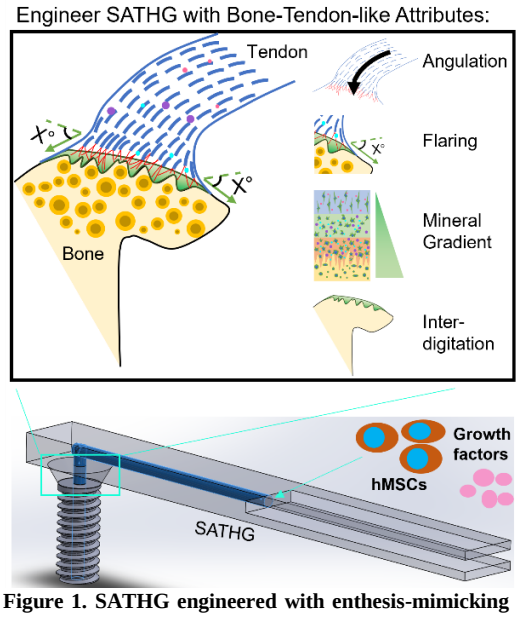

Figure 1. SATHG engineered with enthesis-mimicking attributes and containing with GDF-7.

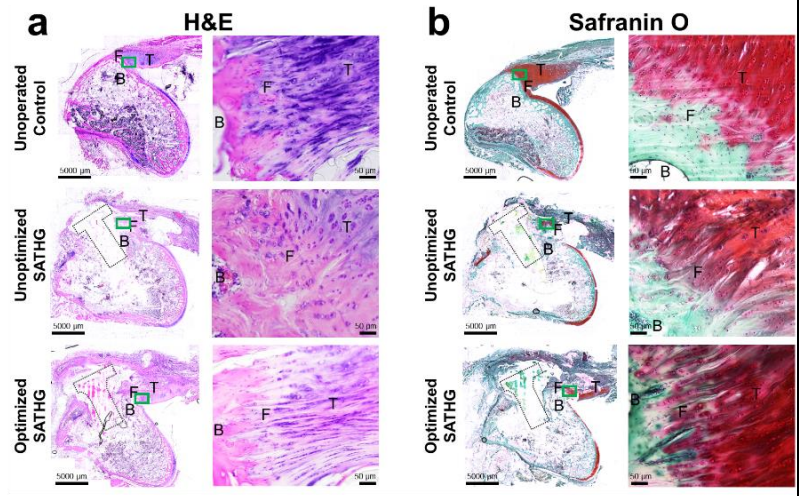

Figure 2. Histological analysis of bone-tendon integration following rotator cuff repair augmented with SATHG in a rabbit model. (a) H&E staining shows native fibrocartilage enthesis in controls and fibrocartilage-like bridging in SATHG groups at 6 weeks. (b) Safranin O staining reveals native fibrocartilaginous enthesis in controls and fibrocartilage-like enthesis regeneration (F) in SATHG groups at 6 weeks.

SIGNIFICANCE/CLINICAL RELEVANCE: A new class of medical device of suture anchor-tendon hybrid graft with GDF-7 was developed to improve bone-tendon repair via enthesis-mimicking features and via multicellular patterning, showing potential for enhancing graft long-term durability and graft-host tissue integration.

REFERENCES: [1] Lu H H, et al. *Annu Rev Biomed Eng* 2013. [2] Benjamin M, et al. *J Anat* 2006. [3] Hu, Y. et al. *Biophys J* 2015. [4] Liu, Y X, et al. *J Eng Mater Technol* 2011. [5] WANG D, et al. *Acta Biomater* 2022. [6] Ker D F E, et al. *Adv Funct Mater* 2018. [7] WANG C Y, et al. *ORS Annual Meeting* 2021.

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