Bioprinted living tissue constructs with layer-specific growth factors for Rotator Cuff Enthesis Healing

Lang Bai[1], Zhanhai Yin[2], Jiankang He[3]
[1]Orthopaedics, The First Affiliated Hospital of Xi'an Jiaotong University, [2]Orthopaedics, [3]State Key Laboratory for Manufacturing Systems Engineering

INTRODUCTION:

The healing of rotator cuff injuries poses significant challenges, primarily due to the complexity of recreating the native tendon-to-bone interface, characterized by highly organized structural and compositional gradients. Addressing this, our innovative approach leverages bioprinted living tissue constructs, incorporating layer-specific growth factors (GFs) to facilitate enthesis regeneration. This method aims to guide in situ zonal differentiation of stem cells, closely mirroring the natural enthesis tissue architecture.

METHODS:

The methodology involves several steps: preparation of growth factor-loaded microspheres, 3D bioprinting of living tissue constructs with layer-specific GFs, and characterization of these constructs. Human umbilical cord mesenchymal stem cells (hUCMSCs) were used, encapsulated in a hydrogel matrix with embedded microspheres containing different GFs (BMP-2, TGF-β, and bFGF) corresponding to the needs of bone, cartilage, and tendon tissues, respectively. The printed constructs underwent various analyses to assess their mechanical properties, GF release profiles, cellular viability, proliferation, and differentiation in vitro. An animal model of rotator cuff tear was used to evaluate the healing effectiveness of the constructs in vivo.

RESULTS:

***In Vitro* Outcomes**

Growth Factor Release and Mechanical Properties

The bioprinted constructs demonstrated controlled release of growth factors over 42 days, crucial for sustained stem cell differentiation. Mechanical testing indicated that the constructs possess sufficient strength and flexibility to support post-implantation activities without immediate degradation, showing a gradual decrease in mass over 18 days, indicating a suitable degradation rate for tissue integration.

Cellular Response

hUCMSCs within the constructs maintained high viability (>90%) up to 7 days post-printing. Cell proliferation assays showed a consistent increase in cell numbers across all construct types, indicating that the constructs provide a conducive environment for cell growth. The differentiation assays revealed significant upregulation of tenogenic, chondrogenic, and osteogenic markers in corresponding layers of the constructs, demonstrating successful directed differentiation of hUCMSCs towards desired lineage-specific phenotypes.

***In Vivo* Healing Efficacy**

Biomechanical Restoration

Constructs implanted in rabbit models of rotator cuff injury showed progressive improvement in biomechanical properties over 12 weeks. The ultimate tensile strength of tissues repaired with the bioprinted constructs approached those of native tissues, indicating functional restoration of the tendon-to-bone interface.

Histological and Immunohistochemical Analysis

Histological examinations revealed organized collagen deposition and alignment, particularly in constructs with layer-specific GFs. Immunohistochemical staining for Col-I, ACAN, and OCN demonstrated successful regeneration of tendon, cartilage, and bone tissues, respectively. The formation of a gradient interface of fibrocartilage structures with aligned collagen fibrils was observed, mimicking the natural enthesis structure.

Gene Expression Analysis

RT-qPCR analysis supported the histological findings, showing increased expression of tenogenic, chondrogenic, and osteogenic markers in implanted constructs, indicative of successful enthesis regeneration. The gene expression profiles corroborated with the structural and functional improvements observed in the repaired tissues.

DISCUSSION AND CONCLUSION: **Discussion**

The study demonstrates the potential of bioprinted constructs with layer-specific growth factors (GFs) for enthesis regeneration. The controlled release of GFs and the structural design of the constructs facilitated targeted stem cell differentiation and tissue integration, closely mimicking the natural enthesis. Compared to traditional repair methods, these constructs showed improved biomechanical properties and biological function, indicating a significant advancement in tissue engineering techniques.

Conclusion

This research confirms that bioprinted living tissue constructs with layer-specific GFs can effectively regenerate the enthesis, offering a promising strategy for repairing complex tissue interfaces. The success of this approach highlights the importance of biomimetic design in tissue engineering and sets the stage for future studies on long-term outcomes and clinical applications, potentially revolutionizing treatments for rotator cuff injuries and other complex tissue repairs.