Mushroom-derived Chitosan Bioink and It's Applicates for Bone-Tendon Interface Regeneration

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Introduction

Replicating the complex, gradient architecture of the bone-tendon interface remains a challenge in 3D bioprinting. To address this, we developed a comb-integrated nozzle strategy that uses shear stress to align collagen fibrils, creating anisotropic cues that guide cellular orientation and differentiation. By optimizing printing parameters, we achieved high structural fidelity and 90% cell viability, with results showing significant upregulation of osteogenic markers compared to random-fiber controls. A key innovation is the use of an allergen-free, sustainable chitosan-collagen bioink derived from *Tricholoma matsutake*. This mushroom-based material offers superior biocompatibility and immunomodulatory properties. When combined with hydroxyapatite, the printed scaffolds effectively promoted osteogenic differentiation of hASCs and suppressed inflammatory cytokines. Overall, this shear-mediated platform provides a biomimetic and sustainable solution for the functional regeneration of bone-tendon junctions.

Materials and Methods

▪ Materials

- *Tricholoma matsutake* from south korea.
- Chitosan from shrimp shells and high molecular weight chitosan was purchased form Sigma-Aldrich.
- Porcine skin derived collagen type I from MS bio, south korea.
- Sodium dodecyl sulfate (SDS) purchased from Sigma-Aldrich.
- Human adipose stem cell (hASCs) from ATCC, USA.

▪ Methods

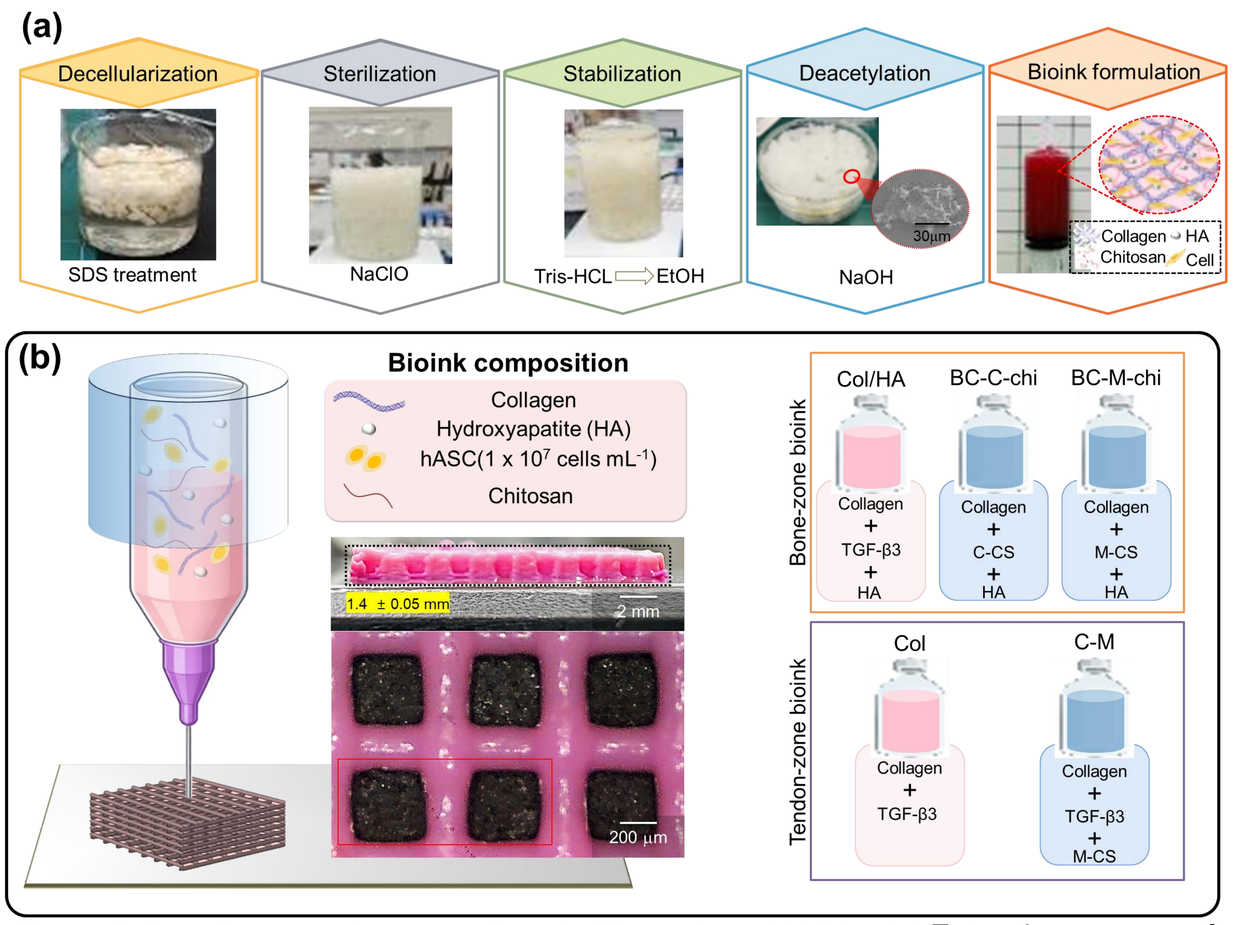


Figure 1. Overall process for preparation of chitosan-based bioinks. (a) Extraction process of M-CS. (b) Schematic of extrusion-based bioprinting for mesh structure fabrication.

Results and Discussion

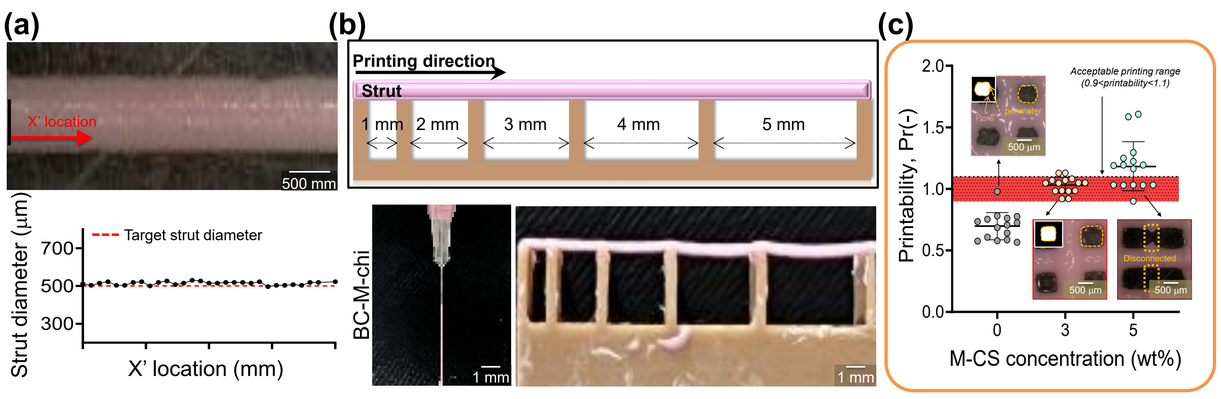


Figure 2. Analysis of printability. (a) High-magnification view of the filament surface. (b) Schematic of the experimental setup for span distance variations. Optical images of the extrusion nozzle and the finalized bridging structure across varying support distances.

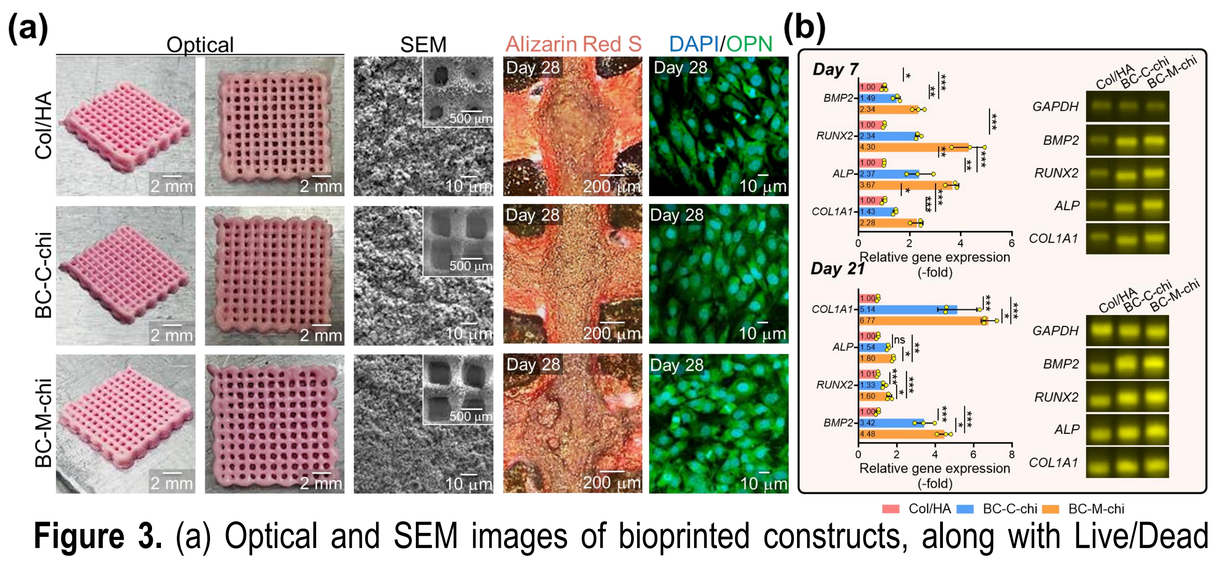


Figure 3. (a) Optical and SEM images of bioprinted constructs, along with Live/Dead and DAPI/F-actin staining. Alizarin Red S (ARS) staining and immunohistochemical analysis of OPN at day 28. (b) Relative osteogenic gene expression at days 7 and 21, along with corresponding agarose gel electrophoresis images, demonstrating enhanced osteogenesis in BC-M-chi.

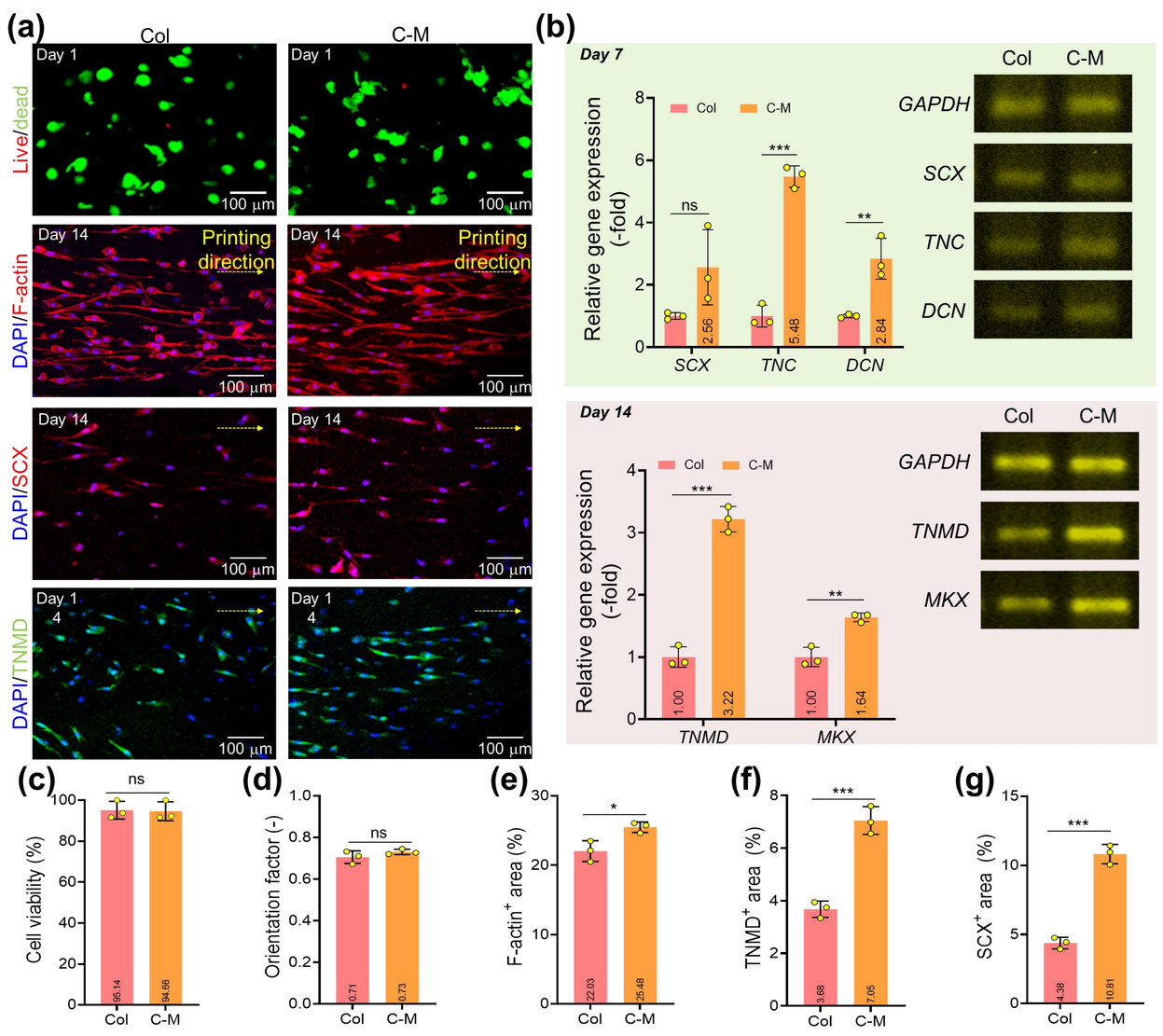


Figure 4. Evaluation of M-CS bioink for tendon regeneration. (a) Live/Dead staining at day 1, and DAPI/F-actin and immunofluorescence staining (DAPI/SCX and DAPI/TNMD) at day 14. (b) Relative tenogenic gene expression of early-stage markers (SCX, TNC, and DCN) and late-stage markers (TNMD and MKX) at days 7 and 14, respectively, with corresponding agarose gel electrophoresis images for qualitative validation. (c) Quantitative analysis of cell viability. (d) Quantitative analysis of cell alignment using the orientation factor (f). (e–g) Quantification of F-actin-, TNMD-, and SCX-positive areas.

Conclusion

This study highlights the development of a multifunctional collagen/M-CS bioink designed for the dual regeneration of bone and tendon tissues. By establishing a redox-stable microenvironment, M-CS enhances cellular mechanotransduction and proliferation, driving superior osteogenesis and angiogenesis *via* synergistic signaling pathways. Additionally, the bioink supports tenogenic differentiation by promoting cytoskeletal remodeling and anisotropic organization. These results position M-CS as a vital bioactive platform that provides the structural and biochemical cues necessary for the functional repair of complex, anisotropic musculoskeletal tissues.

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