Engineering of an MMP-12 loaded bioink for suppression of fibrosis in volumetric muscle loss defects

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Introduction

Volumetric muscle loss (VML) results in irreversible skeletal muscle damage that exceeds the tissue's intrinsic regenerative capacity, owing to extensive disruption of the extracellular matrix (ECM), depletion of resident progenitor cells, and pronounced fibrotic scarring. Consequently, functional muscle architecture and contractile capacity cannot be effectively restored. Current therapeutic strategies remain insufficient, as they fail to adequately suppress fibrosis or provide the necessary biochemical and biophysical cues for organized muscle regeneration.

Matrix metalloproteinase-12 (MMP-12) is known to regulate ECM remodeling through the degradation of various ECM components (e.g., elastin and collagen IV) and has been implicated in the modulation of fibrotic microenvironments. Through these modulations, MMP-12 contributes to the attenuation of fibrosis and promotes tissue regeneration.

In this study, we investigate the therapeutic potential of an MMP-12-incorporated, cell-laden bioink for VML treatment. To recapitulate a fibrotic environment in vitro, transforming growth factor-beta 1 (TGF-β1) was employed, while optimized concentrations of MMP-12 were introduced to regulate ECM remodeling, modulate the fibrotic microenvironment, and attenuate fibrosis without inducing cytotoxic effects.

Materials and Methods

Materials:

Collagen (MSBio, South Korea), Dulbecco's Modified Eagle Medium powder (Gibco, USA), MMP-12 (Sigma Aldrich, USA) , TGF-β1 (Sigma Aldrich, USA), C2C12, hASC (ATCC, USA), Dulbecco's Modified Eagle Medium/High-glucose (Welgene, Korea) FBS (Sigma Aldrich, USA), Penicillin streptomycin (Cytiva, USA)

Methods:

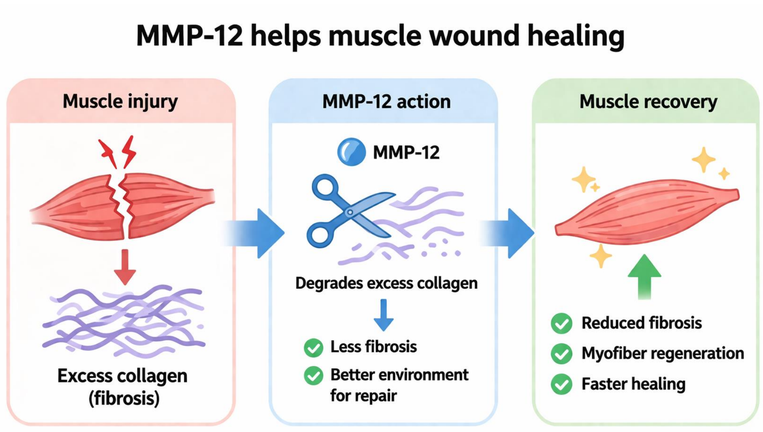


Figure 1. Schematical image describing hypothesis of MMP-12 effect on muscle regeneration.

Results and Discussion

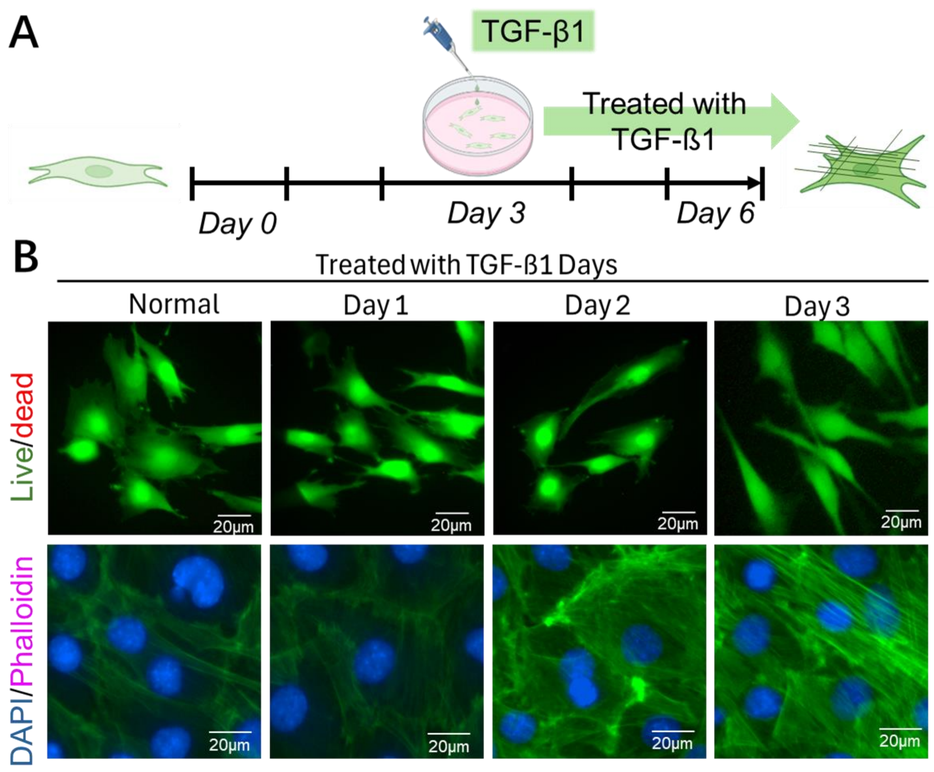


Figure 2. (A) Schematical image illustrate the process of fibrosis induction in C2C12 cells. (B) Live/dead and DAPI/Phalloidin images of fibrosis C2C12 cells according to culture duration.

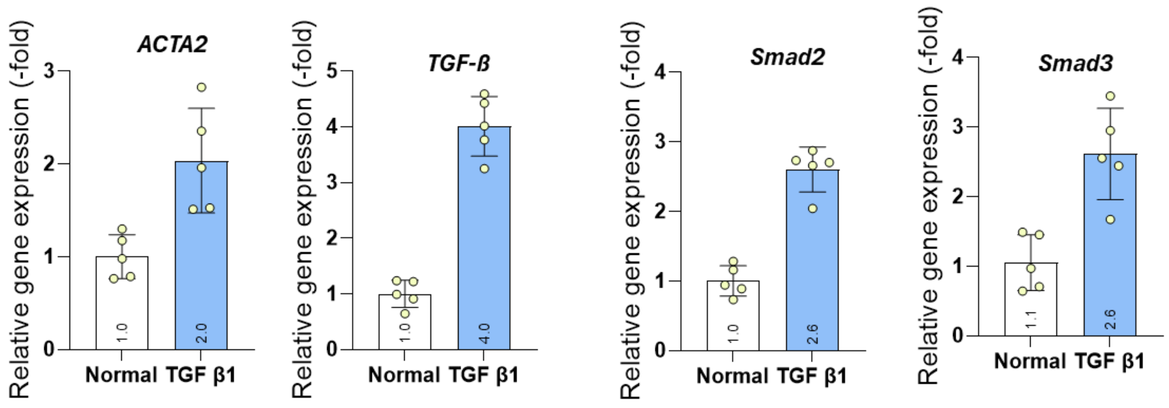


Figure 3. Gene expression results of fibrosis model related to fibrosis.

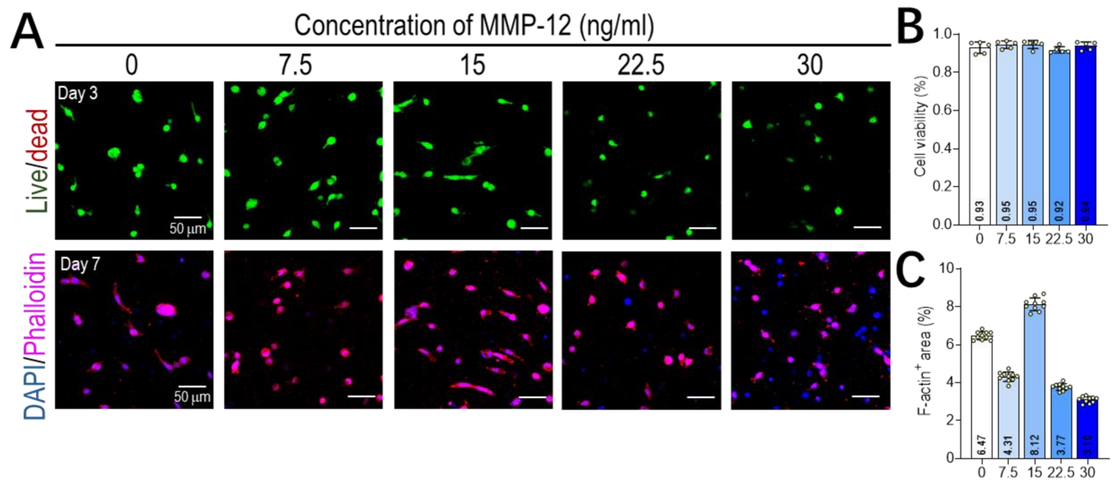


Figure 4. (A) Live/dead and DAPI/Phalloidin images of C2C12 cells with different concentration of MMP-12. (B) Cell viability and (C) F-actin positive area of C2C12 cells based on the captured images.

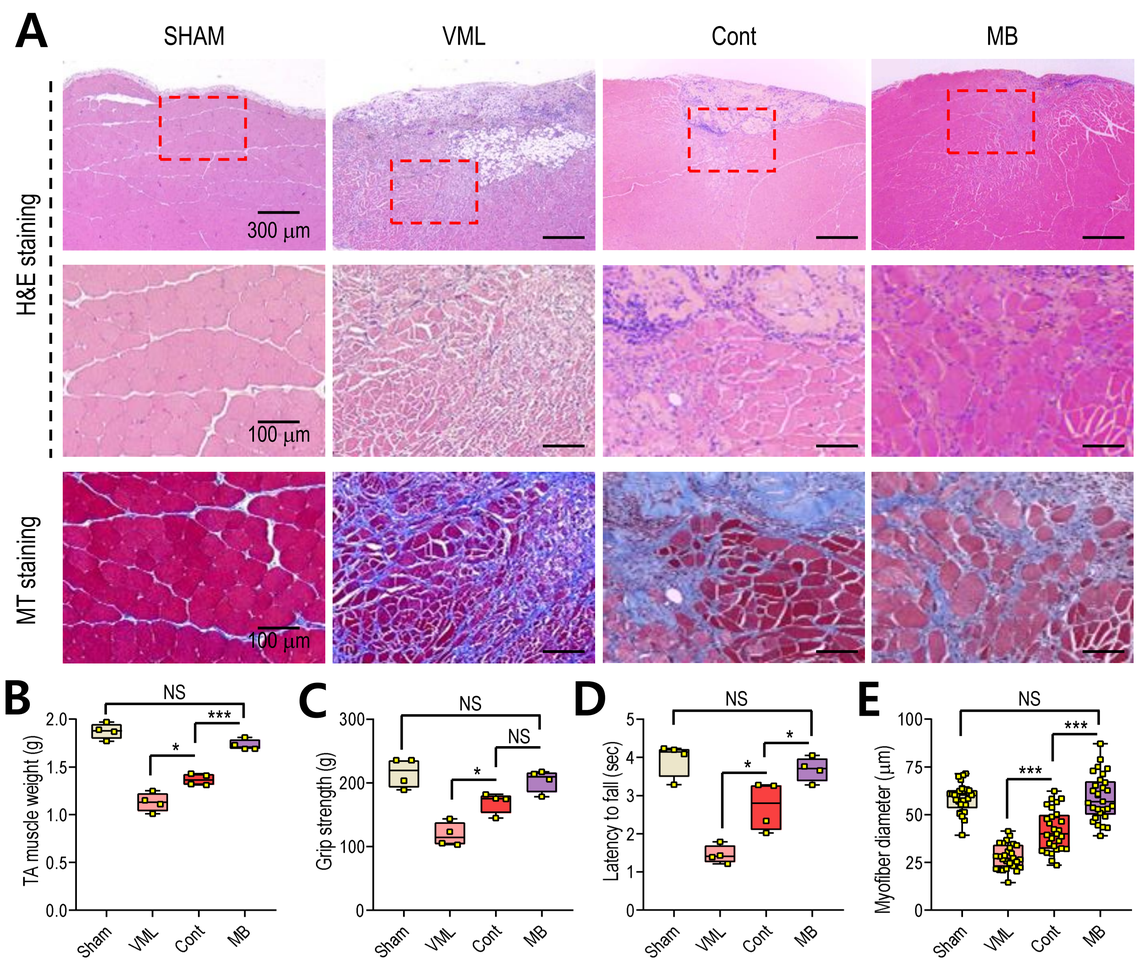


Figure 5. (A) Hematoxylin and eosin (H&E) and Masson's trichrome (MT)-stained images. (B) TA muscle weight (n = 5), (C) grip strength (n = 5), (D) latency to fall (n = 5), and (E) myofiber diameters (n = 20).

Conclusion

The MMP-12 loaded cell-laden bioprinted constructs enhanced muscle regeneration without cytotoxicity. In a mouse VML model, the constructs improved muscle recovery, showing reduced fibrosis, improved myofiber alignment, increased muscle mass, and larger myofiber diameters. Collectively, these results indicate that the MMP-12 laden bioink combined with alignment-inducing bioprinting is a promising strategy for functional muscle reconstruction in VML defects.

Acknowledgement

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