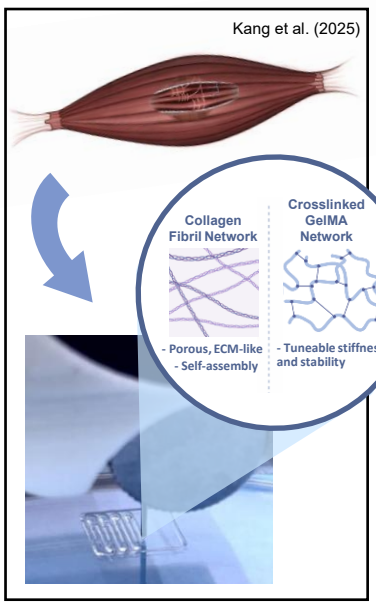
Bioink Development and Characterisation for 3D Bioprinting of Muscle Spindle Models

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INTRODUCTION



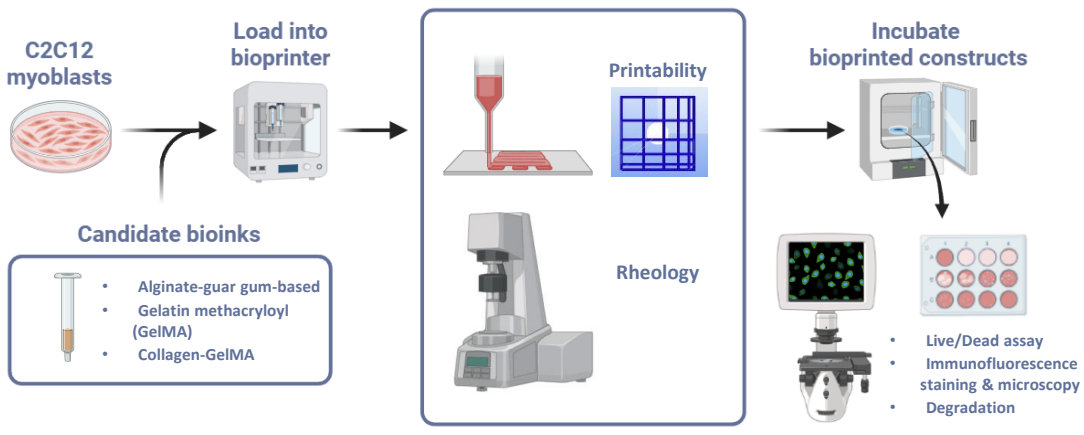
Muscle spindles are key proprioceptive mechanoreceptors in skeletal muscles, composed of specialised intrafusal fibres that regulate kinaesthetic sensations and reflex actions. They are frequently affected in neuromuscular diseases, causing loss of proprioceptive feedback, balance problems and motor deficits[1].

Currently, no established strategies exist to reconstruct the sensory circuit or spindle itself. Existing models poorly replicate the intrafusal-specific morphology and molecular profiles due to a simplified extra-cellular matrix (ECM) microenvironment and intrinsic architecture[2]. Emerging 3D bioprinting technology overcome these issues and provides suitable technology for muscle spindle model development.

OBJECTIVE

Our work addresses the limitations of traditional tissue engineering approaches by developing **physiologically relevant, 3D bioprinted muscle spindle models**, providing **enhanced structural fidelity**, **cell alignment**, **distribution** and **microgeometries**. It was hypothesised that recreating a native-like ECM within a spatially defined scaffold will promote spindle fibre maturation.

METHOD



Optimised formulations with cells were bioprinted via extrusion bioprinting using the CELLINK BioX3 to generate spatially defined constructs alongside acellular and 2D controls. Constructs were cultured under identical differentiation conditions, with subsets treated with neuregulin-1 (NRG-1) to promote intrafusal fibre specification. Analyses were performed at day 4, 7, 14 and 21.

RESULTS

1. Biomaterial screening

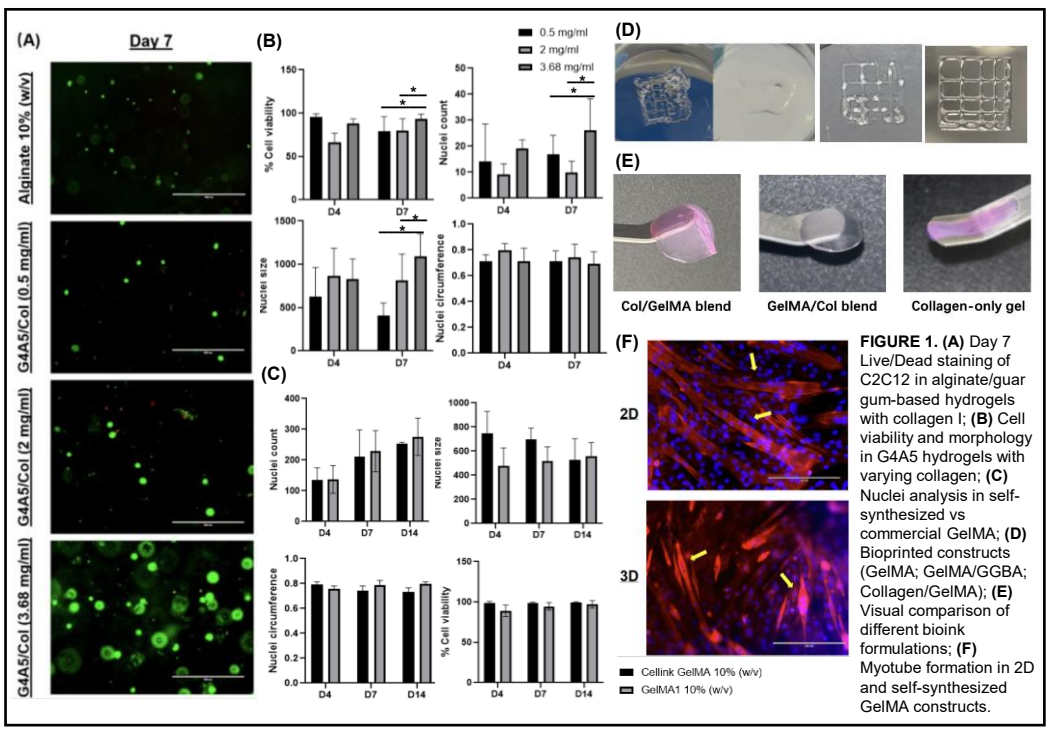


FIGURE 1. (A) Day 7 Live/Dead staining of C2C12 in alginate/guar gum-based hydrogels with collagen I; **(B)** Cell viability and morphology in G4A5 hydrogels with varying collagen; **(C)** Nuclei analysis in self-synthesized vs commercial GelMA; **(D)** Bioprinted constructs (GelMA; GelMA/GGBA; Collagen/GelMA); **(E)** Visual comparison of different bioink formulations; **(F)** Myotube formation in 2D and self-synthesized GelMA constructs.

RESULTS

2. Bioink characterisation

- Collagen-GelMA blends displayed improved viscoelastic properties while maintaining shear-thinning behaviour suitable for extrusion bioprinting.
- **GelMA-mediated mechanics**: higher GelMA content increased stiffness and higher viscosity, indicative of stronger physical crosslinking within the hydrogel matrix.
- **Collagen-mediated compliance**: increasing collagen content reduced overall stiffness and promoted a more compliant, elastic behaviour.
- **Tunability**: precise modulation of rheological and mechanical properties via adjustment of the collagen-GelMA ratio enables tailoring to native skeletal muscle mechanics

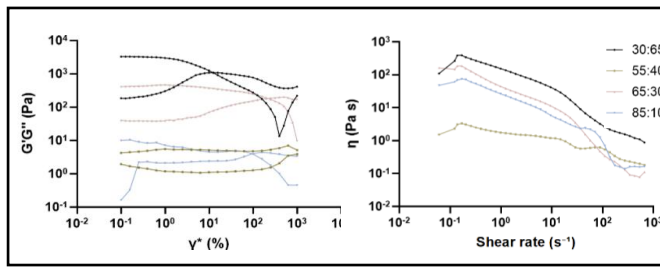


FIGURE 4. Rheological properties and printability of Collagen-GelMA bioinks. Bioink formulations are labeled as Collagen:GelMA ratio with UV exposure time.

(Left) Storage and loss modulus plot as a function of shear strain at 37°C;

(Right) Viscosity variation as a function of shear rate at 37°C.

- Collagen-GelMA hybrid bioinks demonstrated excellent cytocompatibility and stability over 21 days.
- Immunofluorescence staining of F-actin and myosin heavy chains (MHC) confirmed myogenic differentiation.
- Quantitative analysis showed increased myotube formation, greater myotube width, and enlarged myonuclear domain between day 14 and 21, indicating myotube maturation.

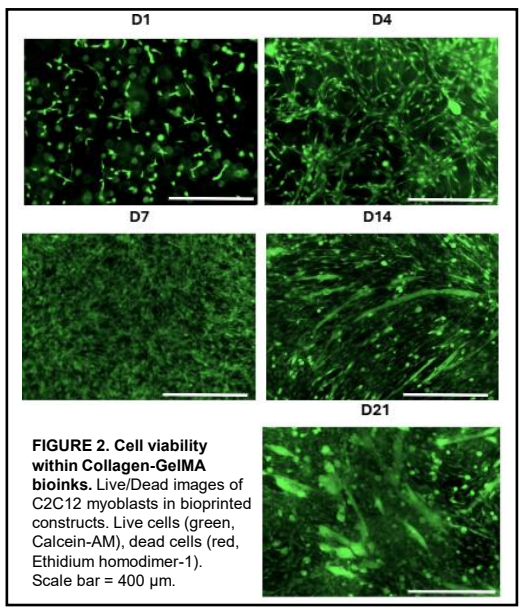


FIGURE 2. Cell viability within Collagen-GelMA bioinks. Live/Dead images of C2C12 myoblasts in bioprinted constructs. Live cells (green, Calcein-AM), dead cells (red, Ethidium homodimer-1). Scale bar = 400 µm.

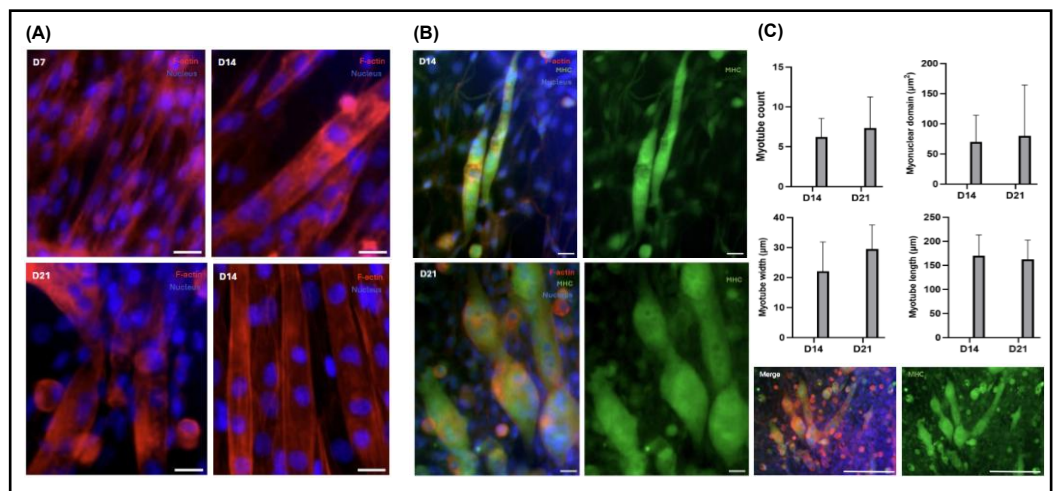


FIGURE 3. Myogenic differentiation and myotube formation within Collagen-GelMA bioinks. Cytoskeleton staining of C2C12s in **(A)** bioprinted constructs at D7, 14, 21 and (Botton-right) 2D control at D14. Scale bar = 20 µm; **(B)** Immunofluorescence staining for MHC (green). Scale bar = 20 µm; **(C)** Quantitative analysis of myotube formation derived from MHC+ structures. (n=9, p<0.05).

DISCUSSION & CONCLUSION

- This work develops a tissue-mimetic, compliant collagen-GelMA bioink targeting intrafusal fibre specification and physiologically relevant mechanics, while remaining robust for handling and 3D bioprinting, addressing a major gap in neuromuscular modelling.
- Ongoing work aims to demonstrate NRG-1-driven intrafusal-like features within 3D bioprinted constructs, with future PCR-based validation of spindle-specific genes.
- Long-term, we aim to engineer a functional spindle unit with key sensory features and translational potential, enabling more accurate *in vitro* studies of sensory-motor systems and disease mechanisms.

REFERENCE. 1. Kroger, S. and B. Watkins, *Muscle spindle function in healthy and diseased muscle.* Skeletal Muscle, 2021. **11**(1): p. 3.
2. Kang Y, Kalaskar DM, Player DJ. In vitro models of muscle spindles: From traditional methods to 3D bioprinting strategies. J Tissue Eng. 2025;16:20417314251343388.