Differential extracellular matrix compartmentalization in 3D bioartificial muscle engineering promotes neuromuscular junction formation

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Introduction

Neuromuscular junctions (NMJs) are critical for motor control, yet replicating their structural and functional complexity in vitro remains challenging. This study compares 3D bioartificial muscle (BAM) models differing in cellular ratio's, matrix composition (fibrin vs. collagen/Matrigel), compartment engineering strategy (one-step vs. two-step) to identify conditions that best support NMJ formation.

Methods

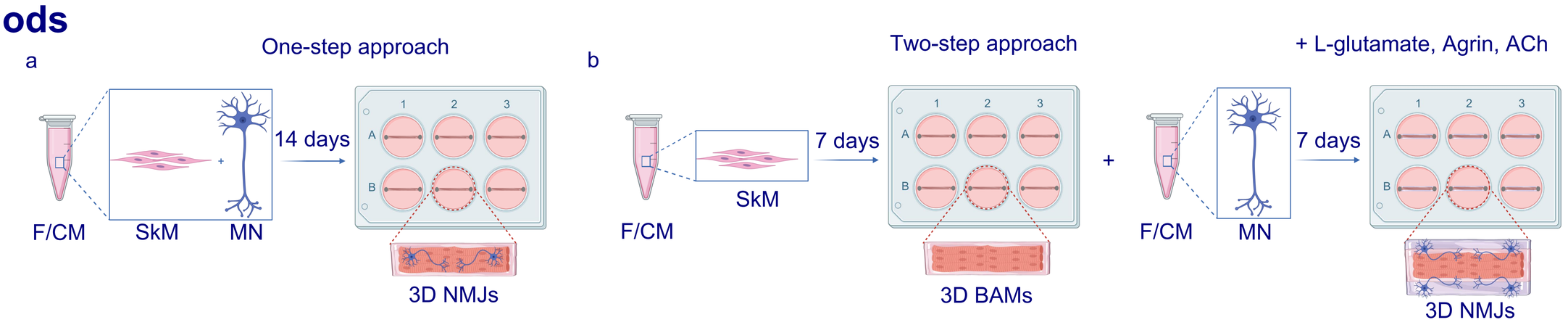


***Fig. 1**: 3D co-culture of SkMs (C2C12s) and motor neurons (MNs, NSC-34s) in a) one-step (direct co-culture for 14 days), b) two-step approach (first forming BAMs with SkM in 7 days, then adding MNs for 7 days with the addition of L-glutamate, agrin or ACh). F: Fibrin, CM: Collagen Matrigel.*

One-step BAMs are insufficient for NMJ formation

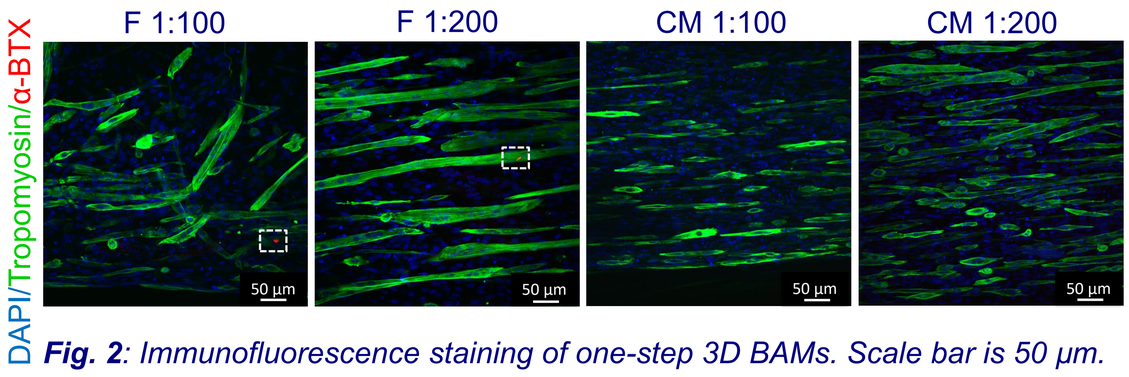


***Fig. 2**: Immunofluorescence staining of one-step 3D BAMs. Scale bar is 50 µm.*

Two-step BAMs promote NMJ formation

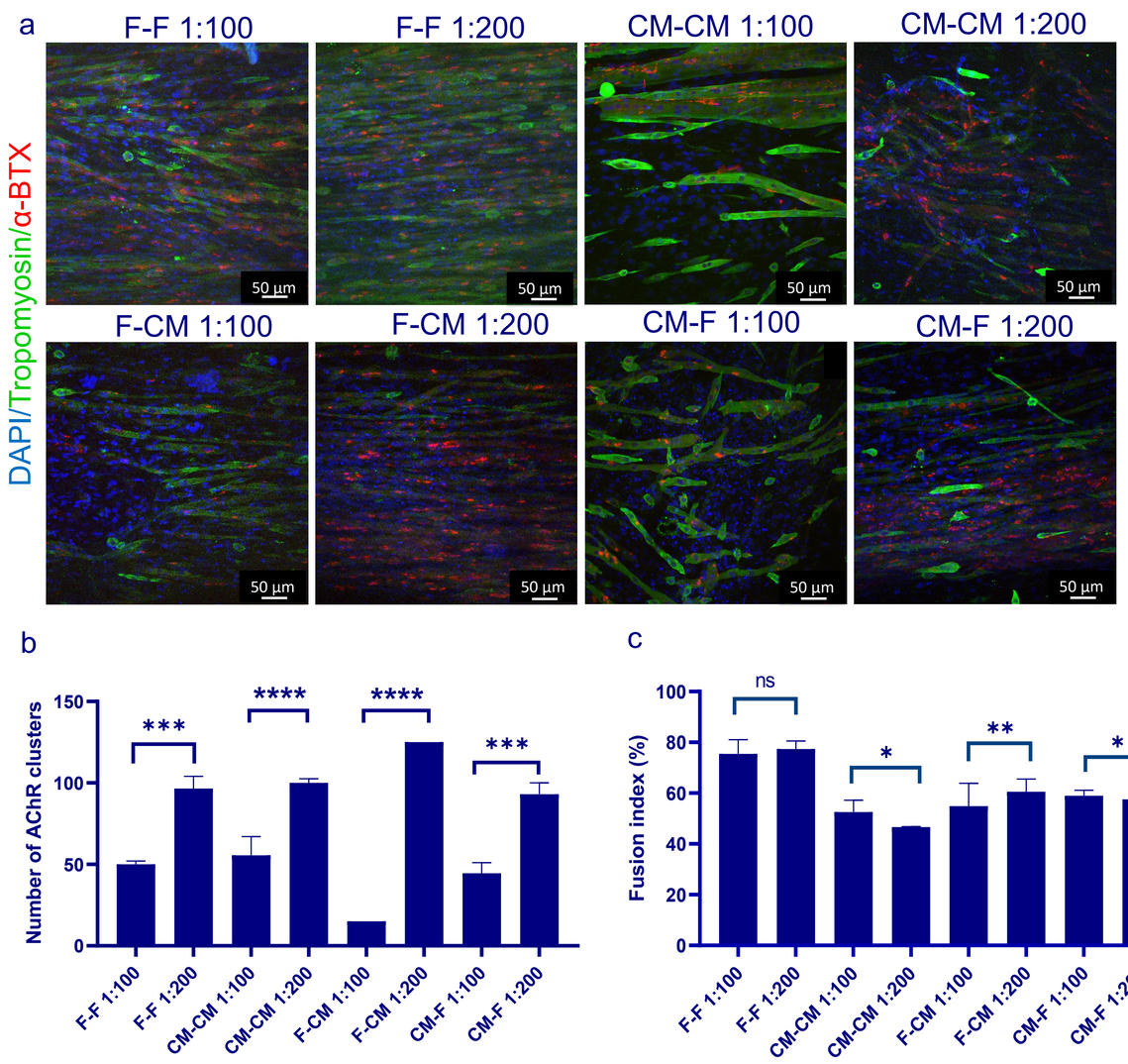


***Fig. 3**: Two-step 3D BAMs. a) Immunofluorescence staining. First letter-SkM layer, second letter-MN layer. Scale bar is 50 µm. b) Number of AChR clusters. c) Fusion index (%).*

F-CM BAMs are responsive to NMJ modulators

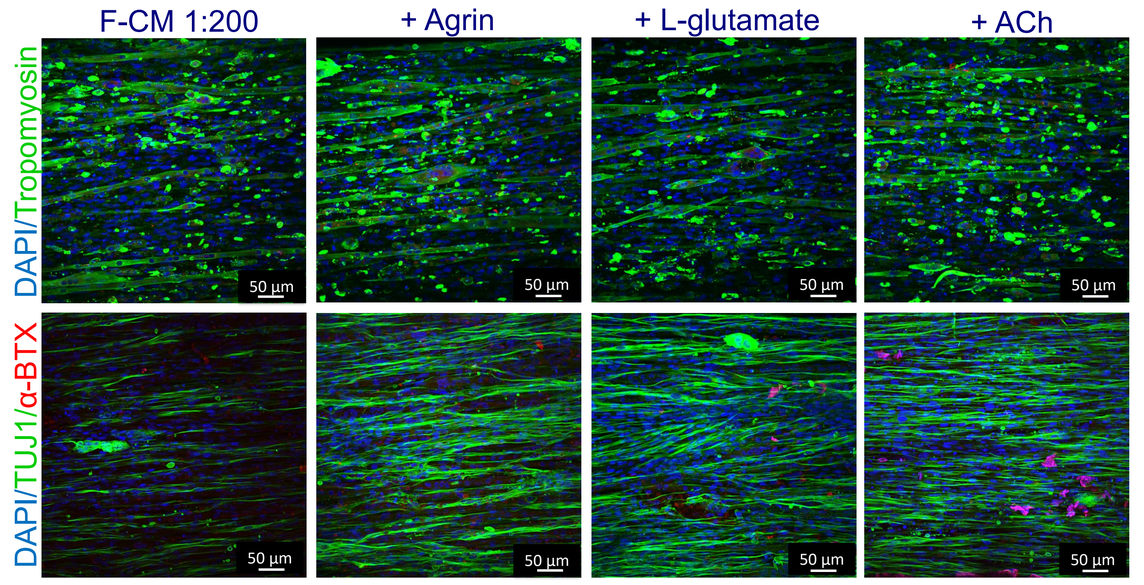


***Fig. 4**: Immunofluorescence staining of non-stimulated and agrin, L-glutamate and ACh stimulated F-CM BAMs. Scale bar is 50 µm.*

Future Perspectives

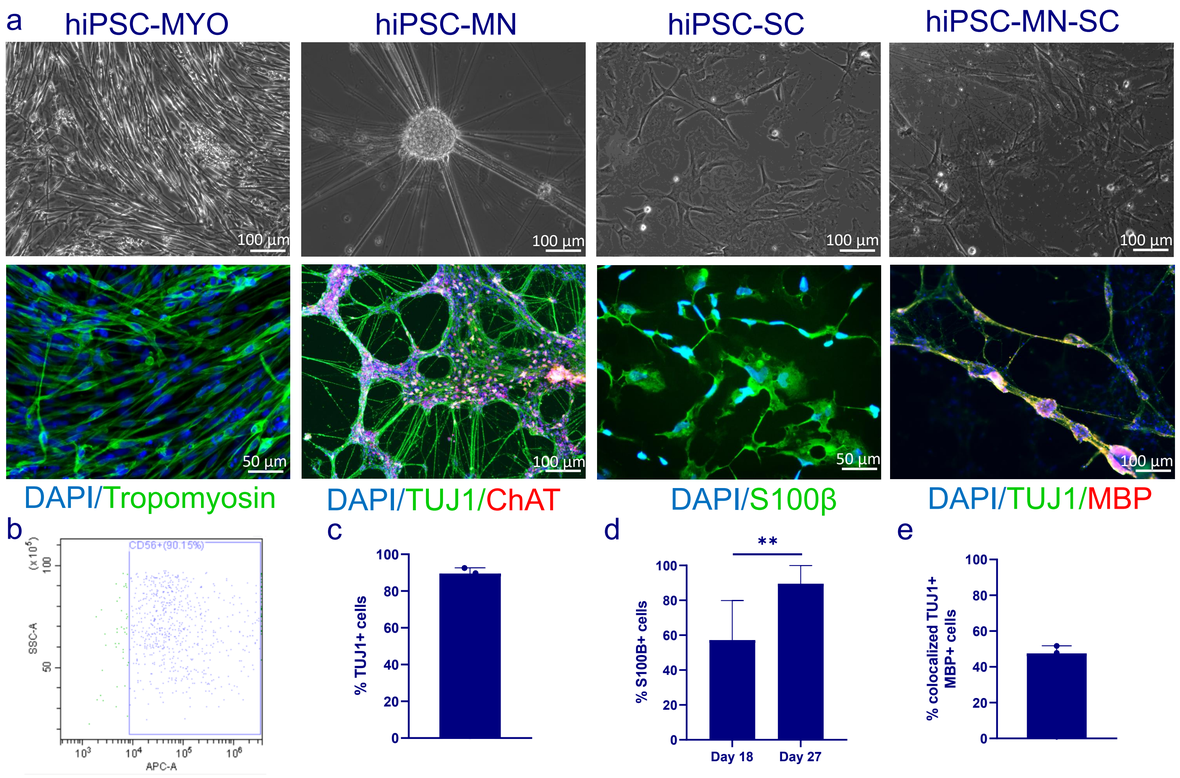


***Fig. 5**: a) Myoblasts (MYO), motor neurons (MN) and Schwann cells (SC) differentiated from human induced pluripotent stem cells (hiPSCs) and co-culture of hiPSC-MN and SCs. b) CD56+ FACS analysis of hiPSC-MYO. Quantification of c) TUJ1, d) S100β and e) TUJ1-MBP positive cells.*

Conclusion

Matrix composition, compartmenting strategy, and culture duration synergistically influence NMJ development. The two-step 3D BAM model provides a promising platform for investigating NMJ formation. Future studies will focus on improving functional readouts and integrating human-derived cells to increase translational relevance.

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