Skeletal muscle spheroids as building blocks for structured cultivated meat

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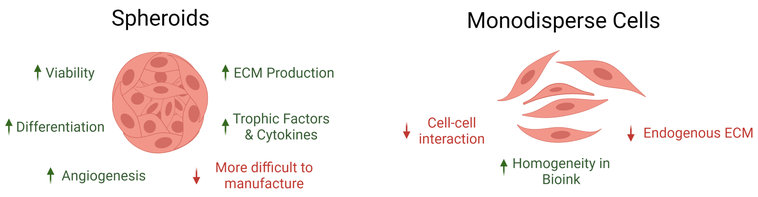
Intro to Spheroids & Bioprinting



Spheroids, or cell aggregates, are promising building blocks for tissue engineering due to their enhanced ability to mimic the physiological cellular environment *in vivo* compared to monodisperse cells.

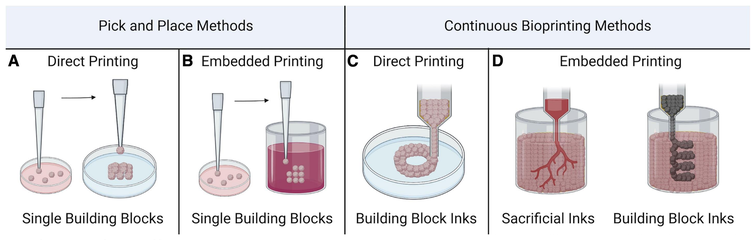


Figure adapted[1]

Bioprinting enables patterning of multiple cellular and acellular components needed to replicate complex tissue structures.

Hypothesis

We hypothesized that skeletal muscle cell spheroids will function as potent building blocks of muscle tissue when embedded in 3D microenvironments.

Spheroid & Bioink Formation

Spheroid Formation and Characterization

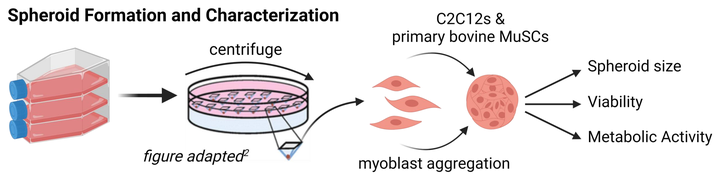
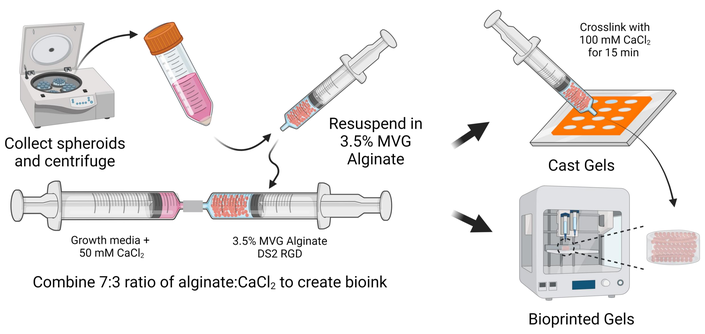


figure adapted[2]

Spheroid encapsulation in alginate bioink

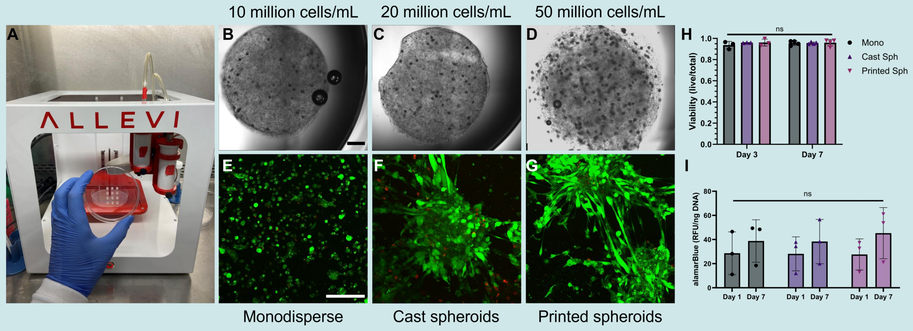


Combine 7:3 ratio of alginate:$CaCl_2$ to create bioink

References

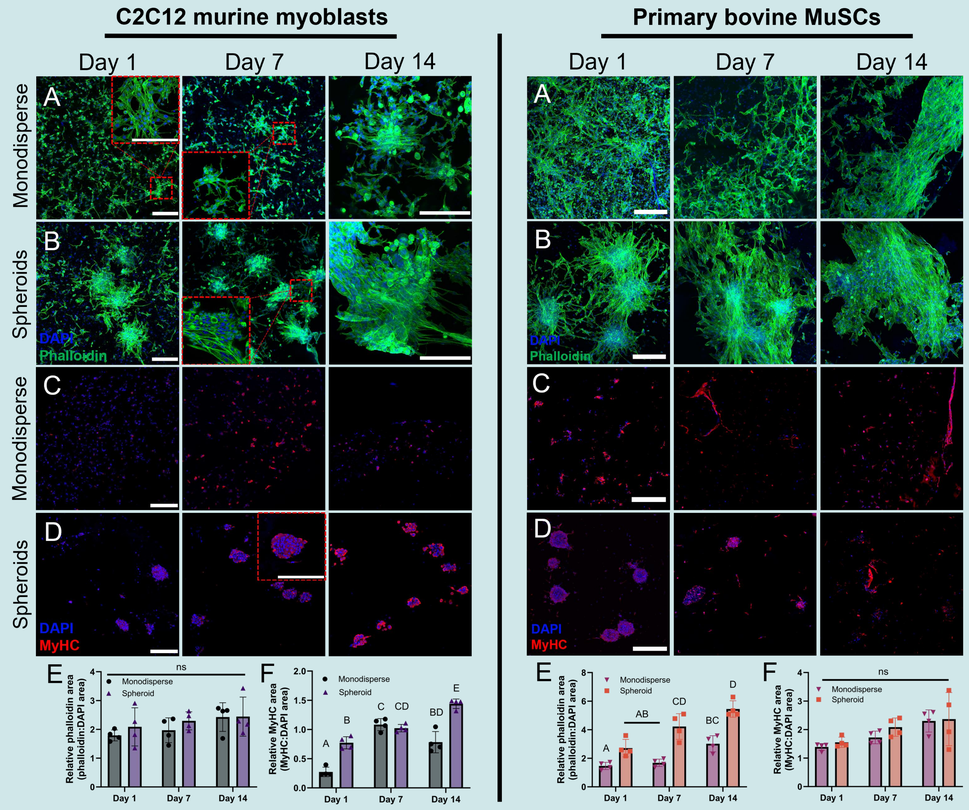
1. Wolf, K. J., Weiss, J. D., Uzel, S. G. M., Skylar-Scott, M. A. & Lewis, J. A. Biomanufacturing human tissues via organ building blocks. *Cell Stem Cell* **29**, 667–677 (2022)
2. Vorwald, C. E., Ho, S. S., Whitehead, J. & Leach, J. K. High-Throughput Formation of Mesenchymal Stem Cell Spheroids and Entrapment in Alginate Hydrogels. *Methods Mol. Biol.* **1758**, 139–149 (2018)

Spheroids remain intact and viable after bioprinting



(A) Photo of bioprinted C2C12 samples. **(B-D)** Bioprinted alginate disks containing increasing C2C12 spheroid densities (scale bar = 1 mm). **(E-G)** Live/dead confocal images 3 days after printing with 50 million cells/mL (scale bar = 150 µm). **(H)** Quantification of cell viability 3 and 7 days after printing (n=3). **(I)** Metabolic activity of samples 1 and 7 days after printing (n=3).

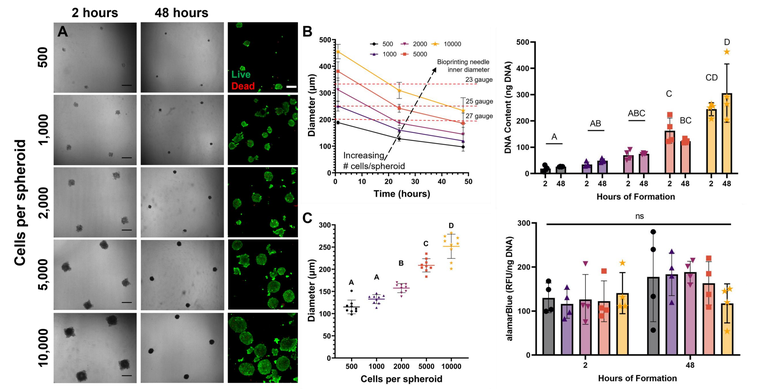
Spheroids fuse into larger tissue structures over 14 days. C2C12 spheroids upregulate MyHC expression and bovine MuSC spheroids show enhanced spreading compared to monodisperse.



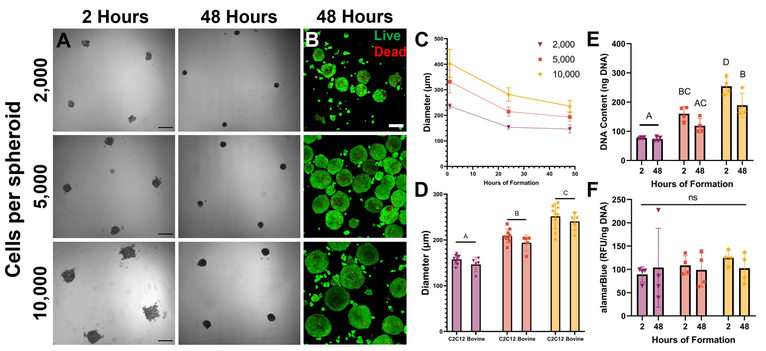
Confocal z-stack projection micrographs of **(A)** monodisperse cells and **(B)** spheroids bioprinted in alginate bioink and differentiated over 14 days (scale bars = 200 µm). DAPI (blue) stain for nucleus and Phalloidin (green) for F-actin. Immunostaining of bioprinted **(C)** monodispersed cells and **(D)** spheroids with DAPI (blue) and myosin heavy chain (red) over 14 days (scale bars = 200 µm). Quantification of the ratio of **(E)** phalloidin:DAPI and **(F)** MyHC:DAPI stain area over 14 days (n=4).

C2C12 & Bovine Spheroid Characterization

Groups with statistically significant differences do not share the same letters



C2C12 spheroids compact over 48 hours. (A) Brightfield microscopy of spheroids containing 500, 1,000, 2,000, 5,000, and 10,000 cells in agarose microwells (scale bars = 500 µm). **(B)** Confocal z-stack max projections of live/dead assay at 48 hours. **(C-D)** Quantification of spheroid diameter (n=4, 9). **(E)** Quantification of DNA content and (F) metabolic activity during spheroid formation (n=4).



Bovine MuSCs form spheroids similar to C2C12s. (A) Bright field images of primary bovine MuSC spheroids over 48 hours (scale bars = 500 µm). **(B)** Live/dead confocal images of spheroids with increasing cell number after 48 hours of formation (scale bar = 150 µm). **(C-D)** Quantification of spheroid diameter (n=5) **(E)** DNA content and **(F)** metabolic activity of bovine spheroids (n=4).

Conclusions

- **Overall, these data indicate that spheroids can generate 3D tissue at least as effectively as monodisperse cells** and, perhaps, show additional promise given enhanced tunability of spheroids
- C2C12 and primary bovine MuSC spheroid formation is similar
- **Spheroids remain intact and viable after the bioprinting process**
- Spheroids spread, fuse, and exhibit myogenic differentiation markers
- Compared to monodisperse cells, **C2C12 spheroids show enhanced MyHC expression** while **bovine MuSC spheroids exhibit increased spreading**

ACKNOWLEDGEMENTS

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