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Engineered fiber-based anisotropic microenvironments for 2D and 3D *in vitro* models of skeletal muscle tissues

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The functionality of skeletal muscle tissue (SMT) hinges on its hierarchical anisotropic microstructure. Conventional 2D cell cultures are unable to closely replicate SMT *in vitro*, driving the research towards more reliable and predictive *in vitro* models [1]. Biomimetic fiber-based scaffolds may provide additional topographical cues supporting SMT *in vitro* engineering, however so far optimal biomaterials and methods for easy control of 3D fiber organization are missing. This work addressed such limitations by designing 2D and 3D *in vitro* models of SMT using natural polymers and advanced fabrication techniques.

2D longitudinally-aligned gelatin nanofibers were prepared by electrospinning (A-Gel). A-Gel topographical cues triggered the formation of aligned elongated C2C12 myotubes, showing myosin-positive myofibers, sarcomere-like organization and high fusion index and myotube lengths.

To reproduce more complex 3D SMTs, innovative fiber-reinforced C2C12 cell-laden bioinks were designed by embedding fragmented A-Gel into an interpenetrating network of Alginate and Gelatin. Multi-scale aligned 3D bioprinted fibrous constructs, fabricated by micro-extrusion printing, supported C2C12 differentiation into muscle fibres arranged anisotropically in mature bundles.

In conclusion, the formation of highly organized, densely packed myofibers on 2D and within 3D anisotropic scaffolds was triggered by their tissue-like chemical, topographic and mechanical cues. Modelling robustness will be improved by culturing human induced pluripotent stem cell-derived myogenic cells. Upon further validation, *in vitro* models of SMT could be exploited to support drug preclinical testing, in agreement with the 3R principle.

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Reference

[1] Zhuang, P., An, J., Chua, C. K. et al. (2018). *Mater Des 193*, 108794. doi:10.1016/j.matdes.2020.108794

Conflict of interest

The authors declare no conflicts of interest.

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