Nano-fiber reinforced hydrogels for 3D bioprinting of anisotropic tissue

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Anisotropic features play a vital role in the functionality of most mammalian tissues, like muscles and nerves, providing optimal directional-specific physical properties (mechanical, electrical, optical). Tissue alignment arises from the oriented deposition of ECM filaments and/or cells and has been proven to guide cell differentiation, proliferation and functional organization. To achieve *in vitro* models of engineered anisotropic human tissues, a key goal is to replicate the orientation of ECM fibres within a 3D microenvironment.

This work aimed at developing a 3D *in vitro* platform to model anisotropic tissue through the synergistic combination of electrospinning with 3D microextrusion bioprinting. Specifically, an advanced fibrous bioink was developed incorporating gelatin/polycaprolactone (Gel/PCL) nanofibers (NFs) within Alginate/modified-Gelatin (Alg/GelM) hydrogels, and inducing NFs aligned by extrusion-induced shear stresses.

Aligned Gel/PCL NFs were produced by electrospinning, varying Gel:PCL ratio (100:0, 80:20 and 50:50) and characterized by SEM analysis, showing a nanometric diameter (500-160 nm) dependent on Gel concentration. Mechanical fragmentation through homogenization allowed to achieve fragmented NFs (f-NFs) with average length lower than 50 μm. A double crosslinked Alg/GelM bioink was optimized, combining physical (Alg/calcium ions) with enzymatic (GelM/transglutaminase) crosslinking. Incorporation of f-NFs, even at low concentrations, within the bioink increased hydrogel viscosity, rheological and mechanical properties, achieving stiffness values in the range of anisotropic soft tissues. 3D printed fibrous bioinks showed excellent shape fidelity and high f-NFs alignment degree along micro-extrusion direction. Finally, 3D fibrous hydrogels embedded with C2C12 mouse myoblast cells showed high cell viability and alignment 7 days post-printing.

In conclusion, we successfully designed advanced fibrous bioinks for prospective *in vitro* engineering of anisotropic tissues. We are currently investigating the suitability of this platform to induce *in vitro* maturation of anisotropic tissues, including cardiac, skeletal muscle and vascular tissue.

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