Spatio-temporal control of physical architecture within 3D-bioprinted constructs for enhanced cellular function

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Adequate nutrient and oxygen supply remain an issue in engineering clinically relevant sized tissue constructs. Sacrificial templating, where 3D-printed templates are embedded into matrix biomaterials and subsequently sacrificed to leave open channels[1], is an attractive approach to address this problem. However, the temporal variations in nutrient/oxygen concentrations during native tissue developmental biology[2], cannot be mimicked by current sacrificial inks. Therefore, the aim of this study is to develop sacrificial inks that allow tailorable temporal dissolution, which will affect downstream cellular function and tissue formation.

Sacrificial templates composed of gelatin (10 wt%, termed 'non-crosslinked templates') or gelatin (10wt%) supplemented with Ru (0.1 mM)/SPS (3-10 mM) co-initiators (termed 'crosslinked templates') were printed using a Bioscaffolder (Sys+Eng). Templates were subsequently embedded in a matrix biomaterial composed of allylated gelatin (Gel-AGE) with 18 mM dithiothreitol, 0.1/5 mM/mM Ru/SPS, and finally photopolymerized (30mW/cm^2, 180s, 400-450nm). During culture, the timing of channel opening as a result of template sacrificing was assessed daily (1-14 days). The effect of temporal dissolution of sacrificial inks on cell function was assessed via osteogenesis and vasculogenesis. For osteogenesis, mesenchymal stromal cells (MSCs) were encapsulated within Gel-AGE hydrogels containing crosslinked templates, and cultured under osteogenic conditions for 21 days, assessing end-point mineralization (Alizarin Red). For vasculogenesis, MSCs and GFP-labelled human umbilical vein endothelial cells (HUVECs) co-culture was further encapsulated and cultured under endothelial conditions for 10 days, assessing capillary network formation (GFP) using AngioTool[3].

Non-crosslinked templates embedded within Gel-AGE hydrogels dissolved within 4 hours, leaving open channels post dissolution. In contrast, crosslinked templates showed a delayed dissolution behaviour, with channels only opening after 4.3±0.6 to 15±1 days, dependent on the Ru/SPS concentration used. The timing of template dissolution, and subsequent channel opening, greatly affected osteogenic culture: constructs with crosslinked templates exhibited enhanced mineralization throughout the construct. In terms of vasculogenesis, constructs with crosslinked templates demonstrated increased vessel length (271.4±25.1 versus 84.9±14.4µm) and junction density (21.1±11.7 versus 91.2±33.9 junctions mm^{-1}), as compared to constructs with non-crosslinked templates.

We demonstrated that tuning the dissolution time of the sacrificial template to better mimic native tissue developmental biology, is an attractive strategy to promote tissue formation. In this study, we present a novel sacrificial bioink that allows engineering of temporal architectural cues, which will have wide application within biofabrication.

References

[1] Lim K et al. Trends Biotechnol. 2019;37(1189); [2] Mas J et.al. Int J Mol Sci. 2019;20(3390); [3] Zudaire E et al. PLos One. 2011;6(11).