Perfusable 3D Microchannel Networks Made From Sacrificial Fibers Increase Cell Viability in Gelatin Scaffolds

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Statement of Purpose: Persistent flow through multi-scale vascular networks is critical to efficient mass transport in living tissues. The slow diffusion of oxygenated media into hydrogel scaffolds limits the thickness of non-vascularized constructs to a few hundred microns. Cells that are too far from a constant supply of oxygenated media will die, resulting in a necrotic region. Thus, to engineer thick tissue, it is necessary to fabricate an artificial vasculature that supports perfusion and thus, via convection, enables sufficient delivery of oxygenated media to cells throughout the construct volume. Multiple strategies have been explored to fabricate vascular networks in artificial tissues, including 3D printing and lithographic patterning. Although significant progress has been made using these techniques to form large (artery and arteriole) channels, to date no technique has demonstrated top-down fabrication of capillary-like 3D channel networks in cell-laden hydrogels. The small size and high density of capillary vessels provide the high surface area that allows necessary exchange of soluble compounds throughout a tissue volume, and thus it is critical to develop techniques to reproduce such vessels. Here, we report the use of thermoresponsive sacrificial microfibers to fabricate 3D microvascular networks in gelatin hydrogels. Our results show that, with perfusion of cell culture medium through the microchannel network, the viability of embedded cells was greatly improved.

Methods: Red fluorescent protein-expressing human neonatal dermal fibroblasts (RFP-HNDF) were obtained from Angio-Protieomie and maintained in Dulbecco's Modified Eagle Medium (DMEM) with 1% penicillin/streptomycin and 5% fetal bovine serum (FBS). To make cell-embedded gelatin scaffolds, 10% w/v gelatin in DMEM was prepared and mixed with cells ($5x10^6$/mL) and 1% microbial transglutaminase (mTGase, Moo Gloo TI, Modernist Pantry). Gelatin solution was allowed to gel at 37ºC for 30 min before being placed in medium and cultured for 24 hr. To fabricate scaffolds with microchannels, gelatin solution containing cells and mTGase was cast into PDMS molds containing interconnected thermoresponsive fibers and allowed to gel at 37ºC. The fibers were then removed by shaking the construct in DMEM at room temperature for 1 hour to induce fiber dissolution. Scaffolds with microchannels were perfused with culture medium, while scaffolds without microchannels were maintained in static culture. After 48 hours, the microchannels were perfused with green Fluospheres (20 nm, Invitrogen). Scaffolds with and without channels were stained with Sytox Blue (Invitrogen), and imaged using confocal microscopy (LSM710, Zeiss).

Results: Vital fibroblasts were observed at the edges of the scaffold without microchannels with a larger number of dead cells in the center (Fig. 1a), indicating that limited mass transport to the scaffold core induced a marked increase in dead cells in that area. In contrast, scaffolds with perfused microchannel networks contained live cells dispersed uniformly throughout the scaffold volume (Fig. 1c). The microchannels were interconnected and thus formed a network, as demonstrated by the perfusion of green Fluospheres injected into an interfacing macrochannel in the center (Fig. 1c and d).

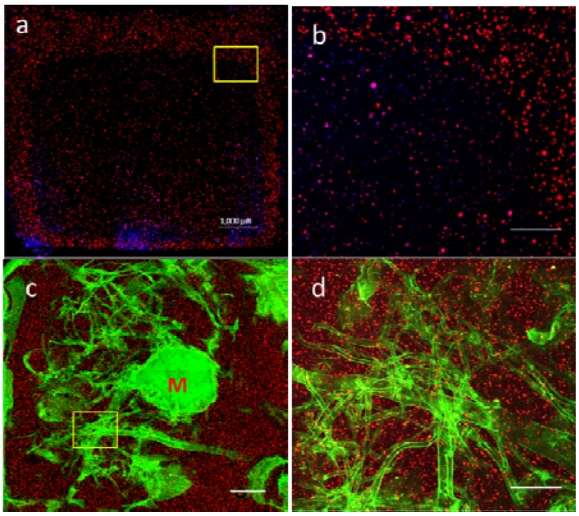

Fig. 1 The effect of perfusable microchannels on cell viability in gelatin scaffolds. (a): cells in a scaffold without microchannels. (b): enlarged area of the yellow square in (a). (c): live cells dispersed uniformly in scaffolds with perfusable microchannels with fewer dead cells compared to (a). (d): the yellow square in (c) was enlarged to show the interconnectivity and patency of the channels. Scale bars: 1mm in (a) and (c); 500µm in (b) and (d). Red: RFP-HNDF, green: Fluospheres, blue: dead cell nuclei. M represents the macrochannel.

Conclusions: This study demonstrates the feasibility of using sacrificial thermoresponsive fibers to form interconnected 3D capillary-like microchannel networks in gelatin scaffolds. Initial studies indicate improved cell viability in such perfused microfluidic hydrogels.

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