Gelatin methacrylate (GelMA) and agarose hydrogels as a smart platform for bioprinting biomimetic vascular networks in 3D tissue engineering constructs

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Statement of Purpose: Tissue engineering represents one of the most impacting concepts emerging from the recent rapid advancement of the field of biomedical engineering. Despite such rapid progression, the implementation of technologies that offer a viable solution for the development of clinically relevant and/or fully functional organoid constructs remains rather elusive to date [1]. Important limitations of current tissue engineering approaches include: (1) the ability to mimic the complex and precise multi-cellular heterotypic microenvironments encountered in the human body, and (2) the control over dynamic mechanisms of nutrient delivery, which rely heavily on complex systems of micro-circulation and vascularization [2].

Two emerging technologies that have the potential to directly address these limitations are *bioprinting* and *microfluidics*. Bioprinting allows for the precise positioning of multiple cell lines embedded within biologically relevant ECM surrogates at specific 3D architectural arrangements. Microfluidics, on the other hand, facilitates continuous delivery of nutrients within organoid constructs while promoting the diffusion of heterotypic soluble factors that better mimic in-vivo biological systems. Therefore, exciting tissue engineering alternatives may emerge with the combination of these advantageous technologies. Here we propose a novel platform based on the utilization of two chemically dissimilar hydrogels, namely gelatin methacrylate (gelMA) and agarose, which allow for a multitude of fabrication techniques ranging from rapid engineering of bioprinted microfluidic channels in 3D tissue engineering constructs, up to improved bioprinting of cell-laden interpenetrating polymer network (IPN) hydrogels.

Methods: To illustrate the flexibility and advantageous properties of the gelMA/agarose platform, three sets of experiments were performed. Firstly, we demonstrate that thermo-cured (10 s, 4ºC) agarose gel (Novogel, San Diego, CA) does not adhere to gelMA both before and after UV exposure of the latter, which allowed us to use the agarose material to bioprint (Organovo, San Diego, CA) a biomimetic microvascular network template that was subsequently embedded in 3T3/fibroblasts-laden gelMA hydrogel. After UV cross-linking gelMA (30 s) the agarose was gently pulled out of the construct and a patent biomimetic vascular network remained. The channels were then perfused with endothelial cells (HUVECs, $5x10^5$) (fig 1). This system was compared to cell-laden gelMA blocks without the pre-vascular network. Secondly, larger multilayered hydrogel tubular constructs were created to illustrate the potential of this technique to engineer multi-layered tubular constructs (fig 2a). Finally, IPN hydrogels constituted of gelMA and agarose at ratios of 2:1, 1:1 and 1:2 were created. NIH 3T3 fibroblast cells were encapsulated in the hydrogels and bioprinted at different morphologies. The bioprinting quality, the gel mass swelling ratio, degradation and 3D cell viability of the respective hydrogel combinations were recorded (fig 2b).

Results:

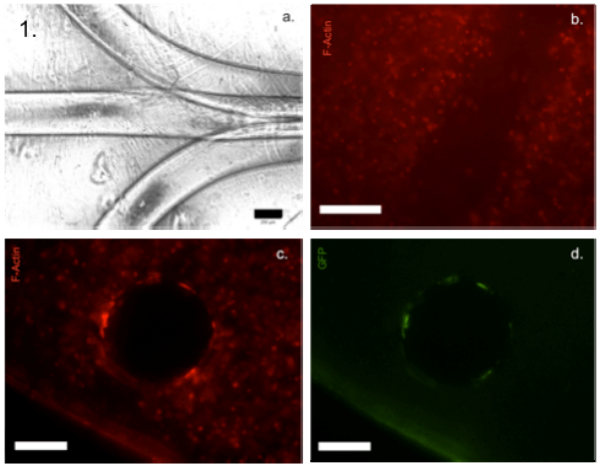

Fig 1*a* illustrates the resulting micro-channel network created within the cell-laden gelMA after removal of the bioprinted agarose. 1*b* shows a fluorescence image of the structured organization of 3T3s surrounding the micro-channel, which is visualized in cross section in 1*c*. In fig. 1*d,* GFP expressing HUVECs formed an organized ring-like coating in the microchannels, resembling the natural structure of human capillaries. Interestingly, the near absolute phase separation observed in initial experiments vanished when the gels were mixed at the pre-polymer stage. The swelling ratio of the different compositions of the IPN hydrogel was not significantly changed, while the inclusion of agarose yielded a protective effect on the degradation properties of gelMA. Despite the lack of RGD moieties in the agarose gel, the improved cell-adhesive properties of gelMA promoted the viability of cells in all different ratios of the IPN to either close to or above 80%, as illustrated in fig 2b. In summary, the GelMA/agarose platform is a powerful yet simple solution for multiple complex tissue engineering tasks. **Reference:** *[1] PNAS. Khademhosseini A et al 2006. 103:8 | [2] Bae H et al. Sci Transl Med. 2012 14;4.*