Directed assembly of cell-laden microgels for building porous three-dimensional tissue constructs

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Statement of Purpose: The organization of cells within a well-defined microenvironment is important in generating the resulting tissue function. However, the cellular organization within biodegradable scaffolds often does not resemble those of native tissues. In this study, we present directed assembly of microgels to organize cells for building porous 3D tissue constructs. Cell-laden microgels were generated by molding photocrosslinkable polyethylene glycol diacrylate (PEGDA) within a poly(dimethyl siloxane) (PDMS) stencil. The resulting microgels were subsequently packed as individual layers on a glass substrate by removing the excess pre-polymer solution around the microgels. These clusters were crosslinked and stacked on one another to fabricate thick 3D constructs that were greater than 1 cm in width and 3 mm in thickness. To generate pores within the engineered structures, sodium alginate microgels were integrated in the engineered constructs and used as a sacrificial template. These pores may be potentially useful for fabricating a vascular network to supply oxygen and nutrients to the engineered tissue constructs. This simple and versatile building approach may be a useful tool for various 3D tissue culture and engineering applications.

Methods: To fabricate PEGDA microgels, pre-polymer solution was prepared by dissolving 0.5% photoinitiator (Irgacure 2959), and 20% PEGDA (Mw 1,000 Da) in Dulbecco's Phosphate Buffered Saline (DPBS). A drop containing PEGDA pre-polymer and photoinitiator was pipetted onto an PDMS stencil. The UV light was irradiated for 28 sec. To direct the assembly, the pre-polymer solution was added to microgels and removed by a pipette and an absorbent material. A small agitation was applied to the entire system to reduce the local defects and overlapping of the microgels. Microgel assemblies were exposed to secondary UV for 6 sec. To fabricate alginate microgels as a sacrificial template, 2.0% sodium alginate was dissolved in 0.9% NaCl. The suspension was poured into the patterned PDMS stencil. The stencil was immersed in a solution of 100 mM $CaCl_2$. To direct the assembly of microgels of PEGDA and alginate, microgel assemblies were exposed to secondary UV for 6 sec. To elute sodium alginate microgel units, the assembled microgels were immersed in NaCl solution containing 5.0% sodium triphosphate (TPP) for 90 min. To fabricate the 3D constructs, individual microgel cluster layers were stacked in a layer-by-layer manner with pre-polymer solution added between each layer. Pre-polymer solution was removed by a pipette and an absorbent material. To stabilize the assembled units, construct formed were exposed to UV for 6 sec. Mouse fibroblast (NIH-3T3) and hepatocarcinoma (HepG2) cell-laden microgel assemblies were generated according to the conditions in the above-mentioned experiments. Cell viability was evaluated using live/dead (EthD-1/Calcein) assay kit.

Results: Figures 1A and 1B show images of fabricated PEGDA microgel clusters in the form of individual monolayers (Fig. 1A) and stacked layers (Fig. 1B). To assemble the microgels into an individual monolayer, the removal of excess pre-polymer was used to drive the compaction process on a hydrophilic glass surface. These results indicate that this technique can be used to fabricate multimillimeter 3D tissue constructs by stacking layers on each other. Figure 1C shows fluorescence image of the cluster consisting of the PEGDA (red) and alginate (green) microgels. As can be seen in Fig. 1D, the alginate microgel in the cluster is completely dissolved after 90 min of the TPP treatment to produce a pore. We also investigated the impact of the TPP treatment on the cell viability. There was no significant decrease in the cell viability even after 90 min of the TPP treatment. These results indicate that the alginate microgels can be used as a sacrificial material for generating pores within cell-laden microgel based constructs.

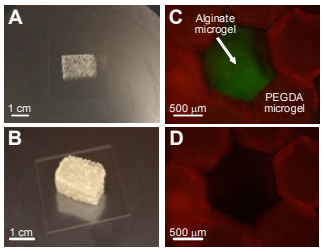

Figure 1. Photograph of assembled PEGDA microgel layer after (A) secondary and (B) tertiary crosslinking. Images of a microgel layer made from PEGDA and alginate microgels in TPP solution at (C) 0 min and (D) 90 min.

Conclusions: We have demonstrated fabrication of porous 3D tissue constructs by building multilayered microgel clusters using a directed assembly technique. This technique can be used to create tightly packed multicomponent tissue-like 3D constructs with homogeneously distribution of cells. In addition, microgel blocks fabricated by sodium alginate were employed to generate porous structure within the 3D tissue-like construct. The ability to precisely control the cell distribution and porous structure within assembled tissue-like constructs could greatly improve engineered tissue function and morphology. The proposed technique could create single layer sheet or could further be stacked to create multilayer 3D tissue constructs and this directed assembly approach can potentially become a powerful and scalable approach to generate multicomponent 3D tissue constructs in millimeter scale. These techniques would be helpful for scale up of tissue engineering using biocompatible hydrogels in the future.

References: 1) Khademhosseini A. et al. Proc Natl Acad Sci 2006;103:2480-2487. 2) Yanan D. et al. Proc Natl Acad Sci 2008;105:9522-9527.