Directed assembly of cell-laden microgels for fabrication of 3D tissue constructs

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Statement of Purpose: We present a bottom-up approach to direct the assembly of cell-laden microgels to generate tissue constructs with tunable microarchitecture and complexity. This bottom-up approach for the directed assembly of cell-laden microgels provides a powerful and highly scalable approach to form biomimetic 3D tissue constructs and opens a new paradigm for directing the assembly of mesoscale materials.

Methods: Poly(ethylene glycol) (PEG) microgels were synthesized by using photolithography, transferred into hydrophobic mineral oil phase and assembled upon application of a controlled agitation force. The effect of agitation rate and time, as well as the addition of surfactants were investigated by using rectangular microgels. The microgel assemblies formed in mineral oil were exposed to a secondary UV crosslinking for 4s to stabilize the resulting structures. To generate cell-laden microgels, NIH-3T3 cells were encapsulated within the prepolymer solution at a concentration of $1x10^7$ cells/ml. Cell viability was characterized by incubating cells with live/dead dyes.

Results: Assembly of the cell-encapsulating microgel units was directed by the tendency of multi-phase liquid-liquid systems to minimize exposed surface areas, combined with mechanical stirring energy (Fig. 1). Four types of microgel assemblies were observed, namely linear, branched, random and offset (Fig. 1). Several parameters that influence the assembly process, such as agitation strength and time, surface tension of the liquid-liquid interface, and microgel size were analyzed. Increased agitation strength and higher surface tensions enhanced the efficiency of the assembly process. It was found that faster agitation, as indicated by higher Reynolds numbers, generated a larger fraction of linear assemblies (up to 30% at 15s). We observed that in most cases the average chain length of the linear microgel assemblies was approximately 3. This is expected since given the microgel dimensions (400(l)x400(w)x150(h) μm) that stacks of 3 vertically aligned microgels represented aspect ratios of near 1.

We also found that by altering the aspect ratio of the gels, the average chain length of resulting assemblies could be controlled, with higher aspect ratios resulting in a larger number of packed gels per assembly due to the minimization of exposed surface area to the oil. Finally, we showed that these assembled hydrogels can be stabilized, with a secondary crosslink, and harvested while still preserving cell viability. Secondary crosslinking with UV exposure time longer than 4s was sufficient to stabilize the microgel assemblies. To validate the use of the microscale hydrogel assembly process developed here for biological applications, we encapsulated cells within PEG microgels and analyzed the effects of the directed assembly process on the cell viability. We observed that within these hydrogels a high fraction of the cells remained viable immediately after cell encapsulation. Furthermore, we observed that the assembly process can be used to induce directed assembly of cell-laden microgels while maintaining high cell viability. To further characterize this process we analyzed the effects of each step on the microgel assembly process (agitation rate: Re=3, agitation time: 1min). We observed that the prepolymer solution and the initial crosslinking step did not result in a significant amount of cell death to the encapsulated NIH-3T3 fibroblasts. However, a slight loss of cell viability was observed during the agitation step while the hydrogels were immersed in the hydrophobic phase.

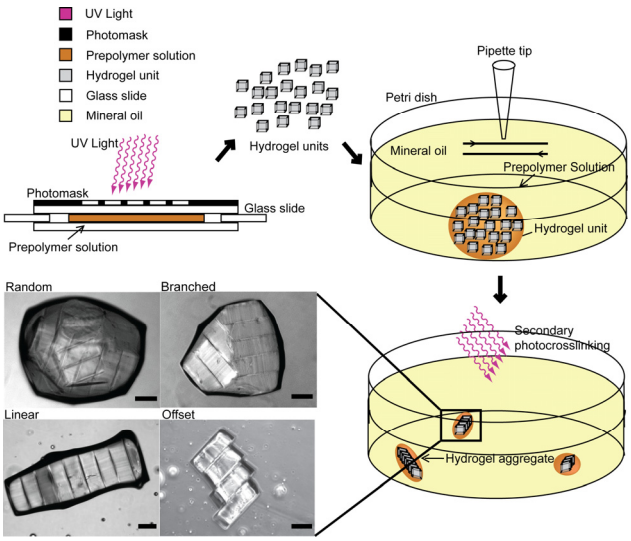

Figure 1: Overview of the hydrogel fabrication and assembly process. First, the hydrogel units are fabricated and collected into a droplet of hydrophilic prepolymer, then placed into oil. This droplet of hydrogels is then agitated, causing the formation of the resulting assemblies. Examples of the observed aggregates (random, branched, linear and offset) are shown on the bottom left.

Conclusions: We present a new method for directing the assembly of mesoscale materials. This bottom-up approach for the directed assembly of cell-laden microgels provides a fast, versatile and highly scalable approach to form 3D tissue constructs with organized microarchitectures.

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