Encapsulation of Cells by Microfluidics and Diffusive Michael-type Gelation of Synthetic Microgels

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Statement of Purpose: Encapsulation of cells in hydrogels allows for presentation of a highly controlled, tissue-mimetic environment. Previously we have developed a synthetic PEG-MAL hydrogel system that allows for incorporation of protease degradable or non-degradable crosslinks, as well as functionalization with bio-active ligands and growth factors in a plug-and-play fashion [1]. In this system, peptides or small molecules are used to crosslink hydrogels using cyto-compatible maleimide Michael-type addition reactions. Microgel encapsulation of cells has been extensively studied for the immuno-isolation of cells, while allowing transport of signaling molecules and nutrients across the hydrogel barrier. Microgel encapsulation in bio-polymers (e.g., alginate), the approach used by most investigators, is more easily implemented because crosslinking can occur by placing gel in ion solution. While on-chip generation of cell-laden microgels has been demonstrated [2], the chemistry used does not allow for easy inclusion of growth factors or protease-degradable peptide crosslinks. We have developed a flow-focusing microfluidic system capable of generating functionalized PEG-MAL microgels on-chip. Macromer droplets gelled by the diffusion of the crosslinker present in the surrounding oil phase. These microgels have numerous potential applications, including drug delivery and immunoisolation of cells. In this study, we generated RGD functionalized, cell-laden microgels on chip and demonstrated the maintenance of cell viability for 7 days.

Methods: Soft lithography was used to generate PDMS microfluidic flow focusing devices in which microgels were generated and crosslinked. For the continuous phase, a 20 mg/mL solution of dithiothreitol (DTT) in PBS was emulsified in 2% SPAN80/mineral oil solution in a 1:15 ratio. 5% 4-arm PEG-MAL (20kDa) functionalized with 2mM RGD containing NIH-3T3 fibroblasts was isolated from the continuous phase through the flow focusing device by co-flowing a 2% SPAN80/mineral oil on either side of the macromer solution. After droplet generation, emulsified DTT diffused into droplets, rapidly forming crosslinked microgels. Microgels were collected and washed 5 times with culture media before being suspended in fresh media and cultured at 37°C and 95% relative humidity. Cells were stained for viability (Calcein AM, TOTO3) on days 1 and 7 after encapsulation, and fraction of live cells remaining was determined by fluorescence microscopy. Cells cultured on tissue culture plastic were incubated with 70% ethanol for 30 minutes before undergoing identical staining procedure as a control for cell death.

Results: Our results (shown in Figure 1 and Table 1) indicate that encapsulation of NIH-3T3 fibroblasts in DTT crosslinked microgels with flow focusing does not affect cell viability over 7 days. All encapsulated cells imaged over the course of the experiment showed fluorescent signal from live cell stain Calcein AM, and no signal was visible for DNA binding dye TOTO3. Ethanol treated cells did fluoresce as expected for TOTO3, indicating that the stain was functioning properly.

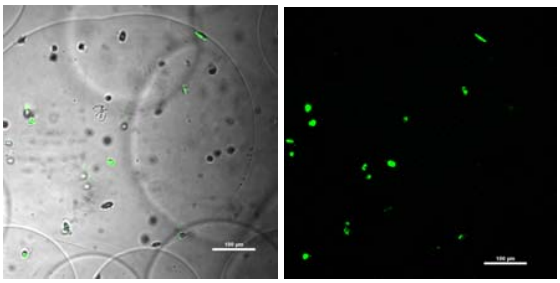

Figure 1. Calcein AM/TOTO3 staining on day 7 shows no loss in cell viability. Transmitted light is included in left image to show microgels, while only fluorescence signal is shown in right image. (scale bar 100 μm)

Time Point	Total Cells Counted	% Cells Viable
Day 1	76	100%
Day 7	150	100%
Dead Control	100	0%

Table 1. Based on Calcein AM/TOTO3 staining, NIH-3T3 fibroblasts encapsulated using this scheme demonstrate no loss in viability after 7 days.

Conclusions: In summary, our preliminary work shows that generation of synthetic microgels using a flow focusing microfluidic device, and utilizing diffusion of crosslinker from continuous phase emulsion is possible. Specifically, this study suggests that long-term maintenance of viability is possible for cells encapsulated using this method. Future work should address the ability of these microcapsules to prevent immuno-rejection of transplanted cells. Additionally, alternative applications of synthetic microgels should be investigated, including drug delivery optimization using protease degradable crosslinks.

References:

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