Gelatin-based Thiol/Disulfide Degradable Hydrogels to Encapsulate Cells

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Statement of Purpose: A current and critical challenge in medicine and biology is understanding and replicating the array of cues that are responsible for controlling stem cell behavior. A major bottleneck is that it remains unclear how synergies and hierarchies between multiple niche signals affect the heterogeneity of stem cell response. Synthetic niches consisting of three-dimensional (3D) biomaterials have the potential to provide structural and molecular cues to guide and trigger a desired stem cell behavior. Thus, it is paramount to develop functional synthetic niches and technologies that allow the temporal tracking of niche remodeling and stem cell fate heterogeneity.

Here, we report a gelatin-based hydrogel, adapting maleimide-thiol crosslinking chemistries from a PEG-based system[1] to generate libraries of miniaturized synthetic niches surrounding stem cells with defined cellular, structural, and biomolecular signals *via* a droplet microfluidic technique. This will enable the incorporation of instructive biomaterial networks within the individual droplets to make tractable a wide range of questions regarding how niche signals shape hematopoietic cell identity. Extensions of this effort will bring new precision to the design of synthetic stem cell niches to address a wider variety of traumatic injuries and degenerative diseases.

Methods: PEG-based microdroplets were produced using PDMS microfluidic flow focusing devices with a nozzle width of 200 μm.[1] Four-arm-PEG units modified with maleimide groups were crosslinked with dithiothreitol (DTT) *via* Michael-type addition. Thiolated gelatin (GelSH) was synthesized by modifying the ε-amine-sidegroups of lysine and hydroxylysine using 2 molar equivalents (assuming 0.35 mmol of amines per gram of gelatin) of Traut's reagent (2-iminothiolane hydro-chloride, Aldrich).[2] The degree of thiolation of gelatin was calculated by using Ellman's reagent (5,50-dithio-bis-(2-nitrobenzoic acid), Fisher), measuring the absorption at 412 nm. Linear PEG units (MW 3,500 Da) modified with maleimide groups were used to crosslink thiolated gelatin. Porcine adipose-derived stem cells (ASCs) were encapsulated at a density of 1.4 – 14 x 10^6 cells/mL.

Results: PEG-based microdroplets of 60 and 200 μm in diameter were produced using the same microfluidic device. Preliminary encapsulation of cells using adipose-derived stem cells (ASCs) was successful and were imaged following LIVE/DEAD viability assays (Figure 1b). The degree of gelatin thiolation was calculated to be 6 μmol of active thiols per 100 mg of gelatin sample. GelSH was crosslinked with a PEG-maleimide unit in under 30 seconds at RT (Figure 1d). In addition, a disulfide-containing bisacrylamide crosslinker rapidly degraded upon the addition of DTT (not shown); however, the crosslinking took several hours at RT. Ongoing efforts include 1) incorporation of supporting niche cells, and 2) developing a disulfide-containing crosslinker with maleimide groups to take advantage of both degradation and fast crosslinking kinetics with GelSH (Figure 1e).

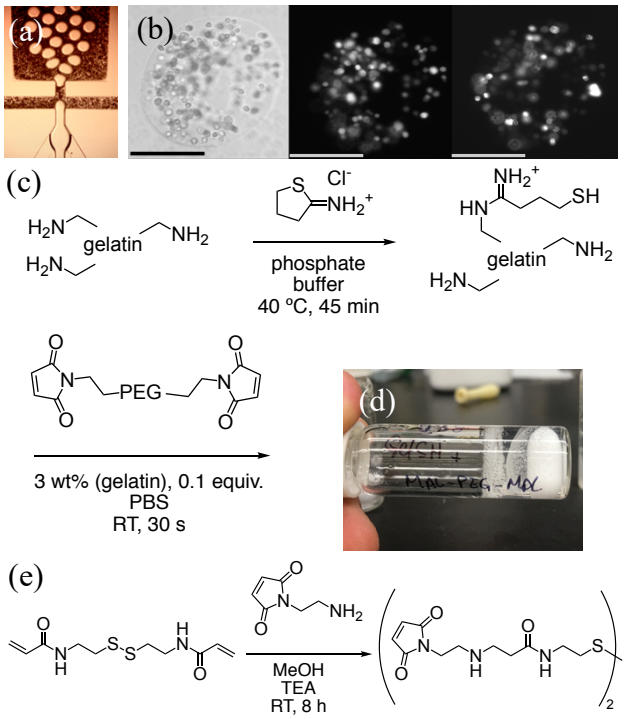

Figure 1: (a) A microfluidic device with a 200-μm nozzle producing PEG-based microdroplets of *ca.* 200 μm in diameter. (b) Images of encapsulated ASCs within microdroplets (left: bright field; middle: live; right: dead); scale bar 0.2 mm. (c) Synthetic scheme for gelatin-based hydrogel microgels using maleimide-thiol crosslinking chemistry. (d) Thiolated gelatin-based hydrogel with a PEG-maleimide crosslinker. (e) Proposed degradable maleimide-disulfide crosslinker.

Conclusions: We showed the encapsulation of ASCs into hydrogel microdroplets. Preliminary studies show the crosslinking of thiolated gelatin with a PEG-maleimide crosslinker. Current efforts are focused on the encapsulation of hematopoietic stem cells (HSCs). These are the prototypic mammalian stem cell, with a long-history of clinical use, enabling the investigation of critical questions with direct clinical results. Designed to also contain supporting niche cells, mesenchymal stromal cells (MSCs) can be leveraged to study heterotypic cultures in HSC lineage decisions. This new hydrogel system will be optimized to show encapsulation of HSCs, and the proposed maleimide-disulfide crosslinker will allow for the controlled degradation to extract cells and perform various studies. Furthermore, this hydrogel system can also be scaled to larger macrogels and bioinks to investigate matrix remodeling and study the changes in cell population based on structural and biomolecular gradients that can be incorporated.

References:

(1) Headen, D. M. *et al*. *Adv. Mater.* **2014**; *26*:19:3003.
(2) Van Vlierberghe, S. *et al*., *European Polymer Journal* **2011**;*47*:5: 1039.