Initial Evaluation of Ingrowth into Superporous Hydrogel Scaffolds

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Statement of Purpose: Current scaffolds are insufficient due to mismatched properties, whether mechanics, degradation, transport, or cell-material interactions [1]. Few materials exhibit sufficient positive properties in balance with the limiting properties. For synthetic hydrogel-based scaffolds, cells can be readily embedded within the polymer system with minimal cell mortality; however, long-term survival is an issue due to nutrient transport. Due to this, porous hydrogel systems are being investigated [2]. Superporous (SPH) poly(ethylene glycol) diacrylate (PEGDA) hydrogel scaffolds have the potential to overcome the problems of difficulty delayed angiogenesis that are seen with current scaffolds [3]. Our group has recently reported that the PEGDA SPH scaffolds allow human mesenchymal stem cell loading, survival for up to a month, and stimuli-induced differentiation [4]. We hypothesized that the porous structure would also be favorable for cellular infiltration and angiogenesis *in vivo*.

Methods: PEGDA was synthesized from PEG (MW~4000 g/mol) as previously described [5]. To make the PEGDA SPH, polymer solution, foam stabilizer (Pluronic® F127), water, the initiator pair, N,N,N′,N′-tetra-methylethylenediamine, and ammonium persulfate, were added sequentially to a 4 mL vial. Citric acid was used for pH adjustment. Sodium bicarbonate, 200 mg, was added with constant stirring to evenly distribute the salt and evolving gas. The SPHs were then removed from the tube and allowed to swell in water before dehydrating them in ethanol and drying in a food dessicator [4]. All animal experiments were approved by the IACUC at the University of Illinois at Chicago. Experimental design for the pilot study was developed with power analysis of published data of porous polymer implants. Briefly, animals were anesthetized and an incision made. Subcutaneous pockets were opened with a blunt probe and hydrogels (either superporous or nonporous PEGDA hydrogels) were inserted. The incision was closed and the animals were monitored for four weeks and sacrificed. At sacrifice, the hydrogels were removed *en bloc* and prepared for sectioning and immunohistochemistry and other biochemical analysis.

Results: Upon implantation, all hydrogels could be palpated easily. Daily monitoring did not reveal any changes that were quantitative or any apparent toxicity. Upon sacrifice of the hydrogels were easily identifiable and recoverable. The gross appearance, **Figure 1**, revealed what was initially observed as red, vascularized superporous hydrogels (SPH) and avascular nonporous hydrogels (NPH). Cells were observed throughout the superporous PEGDA hydrogel, **Figure 2**. The staining did not appear qualitatively uniform, but this needs further analysis. Blood cells and vascular-appearing structures were observable throughout the structures, **Figure 3**.

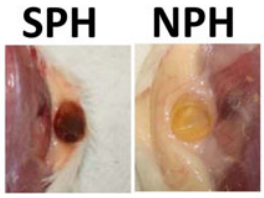

Figure 1. Gross images of (SPH) superporous hydrogel and (NPH) nonporous poly(ethylene glycol) diacrylate hydrogel synthesized after 4 weeks implanted subcutaneous in SCID mice. The skin (center of image) and muscles ("red" on outside of image) are apparent.

Upon sectioning, the nonporous PEGDA hydrogels had no vascularization observable and no cellular infiltration observable.

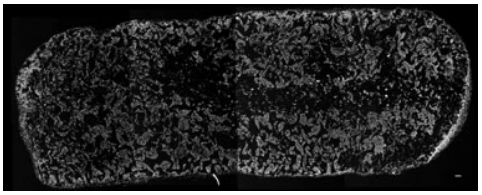

Figure 2. Combined epifluorescent images of H33258 staining within a SPH. The scale bar is 5 μm.

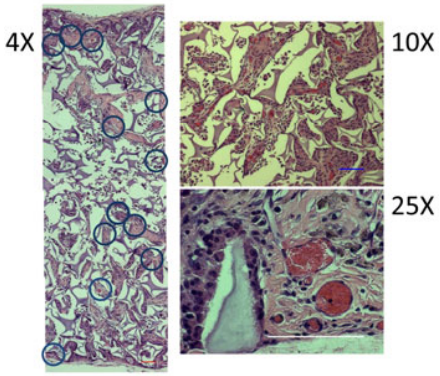

Figure 3. H&E stained sections of cells within SPH after four weeks implantation. The scale bar is 5 μm.

Conclusions: Within superporous PEGDA hydrogels, cells infiltrate and vascular networks are apparent at four weeks. This platform scaffold technology should be further examined for tissue engineering applications.

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