Synthesis of Superporous Hydrogel Scaffolds for Tissue Engineering

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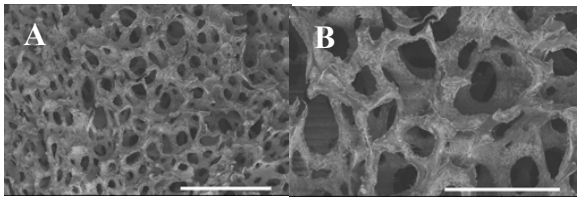
Statement of Purpose: Current synthetic tissue engineering scaffolds have two major shortcomings: difficulty seeding cells into the scaffold and delayed angiogenesis in scaffolds [1]. Research is increasingly focused on creating porous scaffolds which will allow cell attachment and promote tissue growth. Poly(acrylic acid-co-acrylamide) superporous hydrogels (SPH) previously synthesized have been used as gastric retentive devices [2]. Using the same principle of gas foaming, we have synthesized poly (ethylene glycol) diacrylate (PEGDA) superporous hydrogels. These SPHs have an inter-connected pore network that causes them to swell to equilibrium in aqueous solutions within a matter of few minutes while still retaining their three dimensional structure. The highly porous structure which increases surface area available for cell adhesion is also promising for use as a tissue engineered polymer-cell construct as it does not require the use of organic solvents.

Methods: All chemicals were purchased from Sigma-Aldrich Chemical Company as reagent grade and used without purification unless otherwise indicated. Poly (ethylene glycol) diacrylate (PEGDA) was synthesized from Poly(ethylene glycol) 4000 as previously described by Sawhney et al.[3]. To make the PEGDA SPH, polymer solution, foam stabilizer (Pluronic® F127), water, the initiator pair, N,N,N′,N′-tetramethylethylenediamine (TEMED) and ammonium persulfate (both from Fisher Scientific, Pittsburgh, PA), were added sequentially to a 4 mL vial. Acrylic acid (Fisher Scientific, Pittsburgh, PA) was used for pH adjustment. Sodium bicarbonate, 200 mg (Fisher Scientific, Pittsburgh, PA), 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. Dried SPH samples were cut using a scalpel and gold coated. Scanning electron microscopy (SEM) was used to see surface morphology. NIH-3T3 cells (ATCC) were grown in DMEM supplemented with 10% fetal bovine serum. Cells were seeded within the partially dehydrated SPH by immersion within a fresh cell suspension. Visualization of live cells was done using calcein AM® (Invitrogen Coorporation, Carlsbad, CA).

Results/Discussion: The interior surface of the dry SPH showed the presence of interconnected pores ranging from 100 -250 µm, Figure 1. The extensive capillary network so formed by these pores enabled fast uptake of water into the hydrogels. This was evident from the swelling ratio which is the ratio of the weight of the swollen hydrogel to the weight of the dried hydrogel. The swelling ratio for the non-porous sample was 10 after 14 hrs while the SPH reached a swelling ratio of 40 within 20 seconds. Thus, it was expected that cells could be rapidly seeded into the pore network.



igure 1. Scanning electron micrographs of the interior of PEGDA SPHs at a (A) magnification of 40X and scale bar of 1mm and a (B) magnification of 100X and scale bar of 500 µm.

NIH-3T3 cell uptake showed rapid uptake of the cells. As evident from live cell staining with calcein AM®, the cells were alive after a week of seeding and more in number in the SPH (Figure 2A) as compared to the non porous gel (Figure 2B) which was seeded with the same cell density.

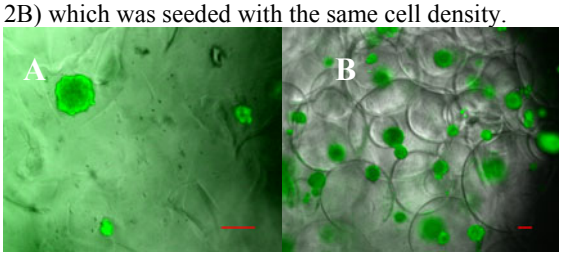


Figure 2. Micrographs of NIH-3T3 seeded within (A) PEGDA SPHs, magnification 4X and scale bar of 100 µm and (B) on nonporous PEGDA hydrogels, magnification 10X and scale bar of 100 µm.

Conclusions: We have successfully shown that PEGDA hydrogels can be synthesized with a large highly, inter-connected pore structure. These hydrogels can imbibe large volumes of aqueous fluid and reach equilibrium within a matter of few minutes. The superporous and superabsorbant nature of the PEGDA hydrogels enables rapid uptake of cells and allows cells to attach to the interior porous structure. While the cells can easily penetrate the pores, the interconnected pores facilitate nutrient exchange within cells in the construct. These hydrogels can be easily produced and can further be surface modified for specific cell attachment and cell differentiation.

References:

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