

Novel Poly(ethylene glycol) Cloud Point Microspheres Used To Form Macroporous Scaffolds

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Introduction: We have developed a novel, surfactant-free and organic solvent-free method to produce poly(ethylene glycol) (PEG) microspheres. The PEG microspheres can be synthesized with a variety of functionalities and are well suited for the production of modular scaffolds for tissue engineering. The microspheres are formed by crosslinking PEG microgels above the cloud point (CP) of PEG, which may be dramatically lowered by adding kosmotropic salts.¹ Above the CP, spherical PEG droplets form, which may be stabilized by rapid crosslinking of the PEG. We produced small, stable microspheres and large, degradable microspheres, which were mixed and crosslinked to form macroporous, 100% PEG scaffolds in which fibroblasts remained viable for greater than three weeks.

Methods: *Microgel syntheses:* Poly(ethylene glycol)-octavinylsulfone (PEG-OVS), PEG-octaamine (PEG-OAm), PEG-octaacrylate (PEG-OAc), and PEG-tetraacrylate (PEG-TAc) were synthesized from either 8- or 4-arm PEG MW 10K. Solutions of PEG-OAm were reacted with PEG-OVS or PEG-OAc (200 mg/mL in DPBS, pH 7.4) at 37°C until microgels were observed by dynamic light scattering ($d_{PCS} = 100$ nm).

Microsphere fabrication: Microgel solutions were diluted to 20 mg/mL with 1.5 M sodium sulfate in DPBS and DPBS to produce a desired concentration of sodium sulfate. Samples were then heated above the LCST on a heating block. Scaffolds were formed by incubating microsphere mixtures at 37°C and seeded by injection of 3T3 fibroblasts and then centrifugation at 200 rcf.

Sphere sizing and Zeta Potential: ImageJ software was used to analyze photomicrographs of microspheres to determine their diameters. Zeta potentials of 3.3 mg/mL solutions of microspheres in 10 mM phosphate buffers with pH 5-10 were assessed with a 90S Particle Sizer (Brookhaven Instruments).

Results: Above its CP in water, PEG forms a biphasic solution with spherical domains. Over time, the PEG domains appear to coalesce by Ostwald ripening. If reactive PEGs are used, such as PEG-OVS and PEG-OAm, the spherical domains rapidly become stable microspheres. However, microsphere diameters are strongly influenced by the rate of the crosslinking reaction – slower crosslinking leads to larger microspheres. Factors such as pH, temperature, microgel size and number of arms on the PEG affect the time to gelation. Thus control over microsphere size can be achieved. At lower pHs, the concentration of reactive $-NH_2$ groups decreases, slowing the reaction and leading to larger microspheres (Figure 1). The time to the gel point scales with $[-NH_2]$ and the mean diameter will grow with time^{1/3}, if particle coalescence is by Ostwald ripening.² These relationships predict the model curve shown on Figure 1.

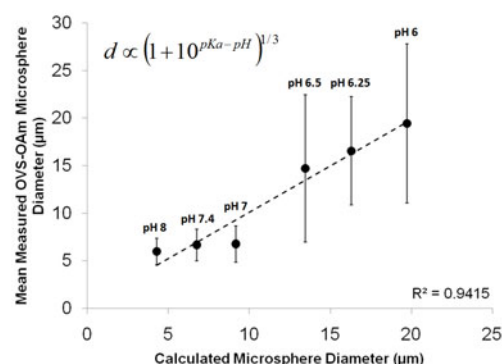


Figure 1: Measured vs. predicted OVS-OAm microsphere diameters for a variety of pHs.

Molar ratios of amines to VS or Ac groups in microgels also affect microsphere size. Zeta potential measurements demonstrated that this changes the charge density. Increased charge served to slow coalescence, decreasing microsphere size. Higher temperatures increased the reaction rate and led to smaller microspheres. Fewer arms on the PEG required a higher conversion of functional groups at the gel point, which presumably led to larger microspheres.

With the ability to control microsphere size, we produced macroporous scaffolds by mixing and crosslinking PEG-OVS/PEG-OAm and PEG-TAc/PEG-OAm microspheres. The acrylate microspheres dissolve when heated overnight at pH 7.4 or more rapidly at elevated pH. By using very large acrylate microspheres and very small vinylsulfone microspheres, connected macropores could be formed in the scaffolds (Figure 2). Fibroblasts were injected into the scaffolds and grown for longer than three weeks.

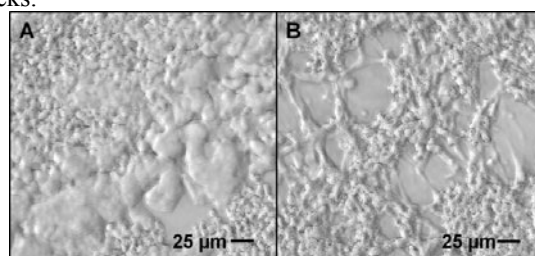


Figure 2: OVS-OAm/TAc-OAm scaffold (A) before and (B) after Ac hydrolysis via NaOH to produce pores.

Conclusions: By manipulating the CP and crosslinking kinetics of reactive PEG, stable, all-PEG microspheres were formed that could be further crosslinked to design bimodular scaffolds with large, connected pores. Due to their high PEG content, these materials may be useful for a variety of scaffold applications.

References:

1. Bailey FE. J App Poly Sci. 1959;1:56-62.
2. Lifshitz JL. J Phys Chem Solids. 1961;19:35.