# PC12 Behavior in Modular Poly(Ethylene Glycol) Scaffolds: Effects of Stiffness and Protein Concentration

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**Statement of Purpose:** For the past decade, many research labs have focused on optimizing neural cell behavior by controlling the chemical and mechanical properties of scaffolds [1-4]. However, it has proven difficult to decouple the chemical and mechanical cues within poly(ethylene glycol) (PEG) hydrogels with typical fabrication techniques. Although plain PEG gels only provide mechanical support for cells, covalent attachment of proteins and peptide sequences to hydrogels has been shown to alter both the chemical and mechanical properties of gels [2, 5]. For this study, a modular scaffold, assembled from polymer microgels and protein, created a 3D scaffold exhibiting decoupled mechanical and chemical properties. In addition, the assembly of a modular scaffold may be gentler on the encapsulated cells than with free radical polymerization in bulk PEG hydrogels.

**Methods:** PEG-diacrylate (PEG-DA) was synthesized as previously described [6] and the acryl-PEG-glycine conjugate was formed by binding acryl-PEG-NHS to glycine [7]. PEG microgels, formed via precipitation polymerization, were fabricated using PEG-DA and acryl-PEG-glycine, using a protocol similar that developed by Nichols et al [8]. Modular scaffolds were subsequently formed by compacting EDC/NHS activated microspheres with PEG-4arm-amine and 0, 1, 10, or 100 μg/mL collagen. Bulk PEG gels and collagen gels were fabricated as previously described [2, 9] for controls. Oscillatory shear rheometry was utilized to measure the G*, or mechanical stiffness, of the gels. PC12 cell behavior was then investigated in all gel types, examining both cell aggregation and viability in the macrogels.

**Results:** PEG-glycine microgels had an average diameter of 1.59 ± 0.14 μm, with a polydispersity of 1.08. Using a dextran gradient, the microgel density was determined to be 1.018-1.020 g/cm$^3$. The incorporation of acryl-PEG-glycine into the microgels was visually confirmed via successful binding fluorescent amine-modified latex beads to activated microgels. Examination of G*, at 10 rad/sec, demonstrated that the mechanical stiffness of the PEG macrogels was not significantly different with or without collagen. Macrogels containing 0 and 100 μg/mL collagen exhibited average stiffness of 121.38 ± 2.31 and 130.66 ± 4.49 Pa, respectively (Figure 1). Examination of PC12 cells in macrogels demonstrated that aggregate size increased as both collagen concentration and culture time increased. This trend was also occurred in the bulk PEG gels; however, the aggregate size was decreased in bulk gels as compared to both macrogels and collagen gels. Viability assays demonstrated that cell viability increased with increased collagen in both macrogels and bulk PEG gels on both days 2 and 4.

**Conclusions:** Using both phase contrast microscopy and dynamic light scattering, the average diameter of the PEG microgels was found to be approximately 1.6 μm. The relatively low polydispersity of the microgels indicates that these microspheres can be fabricated with a very narrow size range, as typically seen in microgels formed using a precipitation polymerization technique [10]. Mechanical evaluation of the PEG gels demonstrated that as the concentration of collagen increased, macrogel stiffness remained constant. One possible reason for this is that the addition of protein to the macrogel serves only to crosslink the gel, rather than altering the chain lengthening process, as seen in bulk PEG gels [2]. Neural response was found to be increased as collagen concentration increased within gels, a trend which has been previously reported [2]. PC12 aggregate size was increased in macrogels as compared to bulk PEG gels. In addition, macrogels exhibited cell viability levels larger than those seen in bulk PEG gels. Both of these results indicate that macrogel assembly may be gentler on encapsulated cells than in bulk gels. The use of modular scaffolds for use in tissue engineering applications is very exciting and further investigation of these materials for tissue engineering applications is necessary.

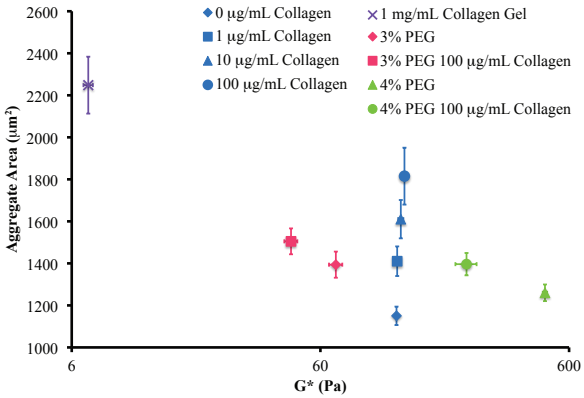


**Figure 1.** Average size of PC12 aggregates in macrogels, bulk PEG gels, and collagen gels as compared to the mechanical stiffness (G*) of the gels. Error bars represent standard error. For G*, n≥5 gels; for aggregate size, n≥63 aggregates.

**References:**

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