**Processing and Storage Effects on Poly(ethylene glycol) Hydrogel Mechanical Properties and Bioactivity**

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**Statement of Purpose**: Polyethylene glycol (PEG)-based hydrogels are attractive biomaterials for tissue engineering due to their charge neutrality, hydrophilicity, and resistance to protein adsorption. They can be engineered to exhibit a wide range of mechanical, chemical, and biological properties by adjusting fabrication parameters and methods. These scaffolds are intrinsically bioinert, but are easily modified to express bioactivity through the incorporation of specific ligands and molecules. Applications of bioactive PEG hydrogels as biomimetic components of synthetic vascular grafts have been explored to improve patency in small diameter applications.[1] A design goal is to make the graft readily available as an "off-the-shelf" product, which subjects the graft and its components to processing, sterilization, and storage for an extended period of time before use. These post-fabrication steps can alter the physical structure and *in vivo* performance of the scaffold. It is important to understand how to extend the shelf-life of the bioactive scaffold while retaining desired mechanical and biological properties after processing. In the present study, the effects of different processing methods and storage conditions on PEG hydrogels were assessed by monitoring tensile properties, compressive modulus, swelling ratio, and cell adhesion and spreading.

**Methods:** ***Hydrogel Fabrication and Processing:*** Aqueous solutions of PEG-diacrylate (PEGDA, 3.4kDa or 6kDa) were prepared at concentrations of 10, 20, or 30 wt% with photoinitiator (Irgacure 2959). Solutions were injected into molds (3mm inner diameter, 4 mm outer diameter tubes or 1.5 mm sheets) and crosslinked by exposure to UV light for 6 minutes. Discs (8 mm diameter) or rings (4 mm long) were exposed to four processing treatments: hydration in water (control); hydration, then vacuum-drying (swell-dry); vacuum-drying (dry); and lyophilization. ***Hydrogel Characterization:*** Swelling ratio was calculated by dividing equilibrium swollen mass after processing by dry mass after processing and rehydration. Compressive storage moduli of processed discs were measured with a dynamic mechanical analyzer equipped with parallel-plate compression clamps. Hydrogel rings were strained until fracture at a uniaxial strain rate of 6 mm/min to determine the tangential tensile modulus, ultimate tensile strength (UTS), and ultimate elongation (UE). ***Storage Effects on Bioactivity:*** Acrylated collagen (4 mg/mL) was photocrosslinked with 10 wt% PEG(3.4k)-diacrylamide (PEGDAA) into 0.75-mm thick hydrogel sheets (4mg/mL). Collagen-PEGDAA hydrogels were sterilized in 70% ethanol and stored for 1 week or 6 weeks. Three storage treatments were tested: lyophilized and stored dry at 20°C, lyophilized and stored dry at -20°C, or stored in PBS at 20°C. Hydrogels were rehydrated in PBS, seeded with bovine aortic endothelial cells (BAEC, 10,000 cells/cm$^2$), and incubated for 3 hours (37°C/5% $CO_2$). Cells were fixed and stained with rhodamine phalloidin and SybrGreen. Cell adhesion and spreading were analyzed using images taken on a fluorescent microscope.

**Results:** ***Processing Effects:*** There were no significant differences in the compressive moduli, swelling ratios, UTS, or UE of processed hydrogels relative to controls. Vacuum-drying generally increased the tensile modulus of PEGDA hydrogels, with significant increases for 10 and 20 wt% PEG(3.4k)DA hydrogels. Lyophilization of the 30 wt% hydrogels resulted in an increased tensile modulus for the 3.4 kDa hydrogel and a decreased tensile modulus for the 6 kDa hydrogel (**Fig. 1**). ***Storage Effects:*** Cell adhesion to collagen-PEGDAA gels was significantly reduced after 6 weeks of storage in PBS, whereas adhesion was maintained after dry storage in both temperatures (**Fig. 2**).

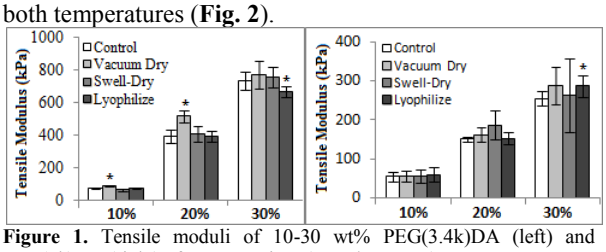


**Figure 1.** Tensile moduli of 10-30 wt% PEG(3.4k)DA (left) and PEG(6k)DA (right) after processing. ($n$=6, *p < 0.05)

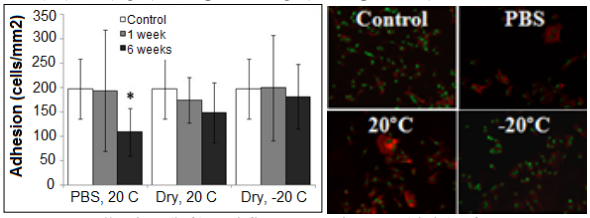


**Figure 2.** Adhesion (left) and fluorescent images (right) of BAECs on PEGDAA-collagen hydrogels after storage. ($n$=10, *p<0.05)

**Conclusions:** Processing results suggest that vacuum-drying directly after crosslinking increases the tensile modulus of PEG hydrogels. It is hypothesized that contraction during drying brings polymer chains into closer proximity and may result in crosslinking of unreacted macromer, increasing crosslink density and tensile modulus. Swelling before drying removes unreacted macromer; thus, higher tensile moduli are not observed in swell-dried samples. Lyophilization immobilizes polymer chains within the hydrogel network, which prevents reactive end-groups from interacting, but the effect may be reduced when using higher concentrations of polymer solution. Hydrolytic degradation of the ester bond on acrylated collagen followed by protein loss in solution is likely the cause of reduced bioactivity in collagen-PEGDAA hydrogels stored in PBS. Dry storage avoids hydrolytic degradation and has the potential to retain bioactivity after long-term storage. These studies suggest that hydration before processing and that dry storage conditions are acceptable post-fabrication treatments to maintain hydrogel mechanical properties and bioactivity.

**References:**

1. MB Browning. *Acta Biomater*. 2012.8(3):1010-1021.


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