Mechanical Analysis of Poly(Ethylene Glycol) Diacrylate and Agarose Interpenetrating Network Hydrogels

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Statement of Purpose: The combination of cytocompatibility and improved mechanical integrity with interpenetrating networks (IPNs) has recently incited much enthusiasm in the hydrogel community[1]. This study investigated how component concentrations and molecular weights influenced the mechanical properties of IPN hydrogels designed for cartilage regeneration. Two biocompatible materials – poly(ethylene glycol) diacrylate (PEG-DA) and agarose – were combined to create IPNs with greatly improved moduli and compressive failure stresses. The methods have been shown compatible with incorporating porcine chondrocytes without loss of viability[1]. This study was designed to expand the set of known viable composition alternatives by comparing component concentrations and PEG-DA molecular weights.

Methods: 2-hydroxy ethyl agarose powder (Type VII, Sigma-Aldrich, St. Louis, MO) was added to 0.01M phosphate buffered saline (PBS) to form 2% and 5% w/v solutions. The mixtures were autoclaved for 45 minutes and pipetted into cylindrical silicon rubber molds ~2 mm thick and ~5 mm in diameter. The molds were then pressed between two glass plates and cooled at 4°C for 10 minutes. Once cooled, the gels were removed from the molds and stored in PBS for a minimum of 24 hours for equilibration.

PEG-DA (molecular weight = 3400 and 6000 Da, Sunbio, Anyang City, South Korea) was added to PBS to make 10%, 15%, and 20% w/v solutions, followed by Irgacure 2959 photoinitiator (0.1% w/v). Agarose gels were then added to each solution and soaked under constant agitation on a rocker. The soak times for 3400 MW PEG-DA and 6000 MW PEG-DA were 3.5 hours and 6 hours, respectively, and were calculated from literature data on PEG diffusivity in gels to ensure adequate time for equilibration[2]. The agarose gels and PEG-DA solutions were then placed in rectangular silicon molds, pressed between optical glass microscope slides and exposed to ultraviolet light (312 nm wavelength) in a crosslinker (Spectronics Corporation, Westbury, NY) for 10 minutes. Once photopolymerized, a biopsy punch was used to create samples 3 mm in diameter and ~2 mm tall. Samples were stored in PBS for at least 24 hours before mechanical testing.

Gel diameter was measured with a micrometer under a standard stereomicroscope. Samples were then loaded onto an RSA-III dynamic mechanical analyzer (TA Instruments, New Castle, DE). Samples were compressed unconstrained to 95% of their original height, or to fracture, which was measured directly with the RSA-III. A compression rate of 0.005 mm/s, corresponding to an average of 15%/min, was used. The Young's modulus was calculated as the slope up to an x-axis value of 10% strain of the stress versus strain curve. Using the neo-Hookean model for ideal elastomers, the shear modulus was calculated as the slope of a plot of the stress versus $(\lambda - 1/\lambda^2)$, where $\lambda = L/L_0$, L=strained thickness, L_0=original thickness[1].

Results: The shear and Young's moduli for IPNs with the same agarose concentration were generally greater when the 3400 molecular weight PEG-DA was used, with this difference being more pronounced with increasing PEG-DA concentration (Tukey's post hoc test pending). An increased concentration of both agarose and PEG-DA typically led to an increase in Young's modulus.

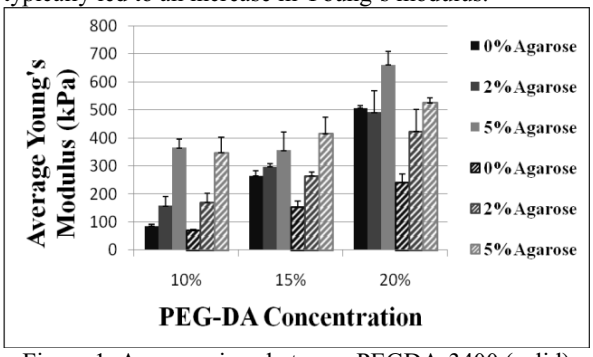

Figure 1. A comparison between PEGDA-3400 (solid) and PEGDA-6000 (striped) shows an increase in Young's Modulus with increased component concentration.

The majority of IPNs and pure PEG-DA hydrogels had an increased fracture stress – up to three times, in some cases – when the 6000 molecular weight PEG-DA was used. Moreover, this difference of fracture stress between the molecular weights often doubled with increased PEG-DA concentration. The effect of increased agarose concentration, however, was not so straightforward. The lowest concentration of PEG-DA revealed a decrease in fracture stress with the incorporation of agarose. However, higher concentrations of PEG-DA usually showed an increase in fracture stress with the addition of agarose.

Conclusions: It has been shown that the IPN has significantly better mechanical performance than that of the single component gels. Generally, increasing the concentrations of both components led to an increase in both modulus and fracture stress. However, while an increase in PEG-DA molecular weight led to slightly lower moduli, it yielded a much higher fracture stress. As an ongoing part of this study, IPNs will be selected to determine the influence of these molecular weights and concentrations on cell viability to provide a more complete picture of IPN suitability for cartilage tissue engineering.

References:

[1]DeKosky BJ. Tissue Eng. Part C. 2010: Epub
[2]Weng L. Macromol. 2005: 38, 5236-5242