Characterization of Modular Resilin-based Biomaterials with Tunable Mechanical Properties for Cartilage Engineering

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Statement of Purpose: Resilin is an elastomeric protein found in insect cuticles. It has a high strain, high fatigue life time, and high resilience.[1] Materials with high resilience are promising scaffold candidates for cartilage engineering because of the repetitive compression that cartilage undergoes. Our lab has successfully developed recombinant resilin-based proteins based on the mosquito sequence (AQTPSSKQFGAP).[2] We previously demonstrated that crosslinked hydrogels of our resilin protein (16 wt% protein with a crosslinking ratio of 5:1 of crosslinker to crosslinking site) possessed a compressive modulus of 2.4 MPa[3], which is on the same order of magnitude of natural cartilage (2.3 MPa- 15 MPa).[4] When human mesenchymal stem cells (hMSCs) were cultured on the resilin-based protein, they showed 95% viability after three days and an affinity for the resilin-based domain.[3] In the current study, we modulated the mechanical properties of the resilin-based protein by varying the protein concentration and the crosslinking ratio. We are currently using our resilin-based hydrogels to study the effects of mechanical cues on hMSC chondrogenesis and cartilage matrix formation in a 3D environment.

Methods: Resilin-based proteins were expressed using IPTG induction in a fermentor (BioFlo 110, 14 L capacity, New Brunswick). Purification of resilin-based proteins was carried out using a combination of salting out and heating. Resilin-based proteins were crosslinked with tris(hydroxymethyl)phosphine (THP, Strem Chemicals, Inc.). Crosslinking was performed *in situ* on a rheometer (AR2000, TA Instruments, New Castle, DE) using a plate-on-plate geometry. Frequency sweeps (0.1-100 rad/s at 1% strain) and strain sweeps (0.1-1000% strain at 1 rad/s frequency) were performed to determine the viscoelastic region. Dynamic time sweeps were performed with 1% strain and 1 rad/s frequency to monitor real-time gelation and determine the complex modulus. For compression tests, the top plate of the rheometer was lowered at a speed of 10 μm/s, and the resulting normal stress was measured. The compressive modulus of resilin-based proteins was determined from initial slopes (0-4% strain) of the stress-strain curve. Cell encapsulation was done by mixing hMSCs, resilin-based protein solutions, and crosslinker together. The constructs were cultured at 37°C and 5% CO_2 for 1 day before the cell viability was quantified by LIVE/DEAD® Viability/Cytotoxicity Kit for mammalian cells (Molecular Probes L-3224).

Results: Crosslinked resilin-based hydrogels were prepared at different concentrations (8-12 wt%) and a crosslinking ratio of 5:1. The complex modulus and unconfined compressive modulus were measured for each hydrogel composition Fast gelation (~5-6 min) was observed for resilin-based hydrogels, and resilin-based hydrogels formed a stable structure in about 20-30 minutes (Figure 1).

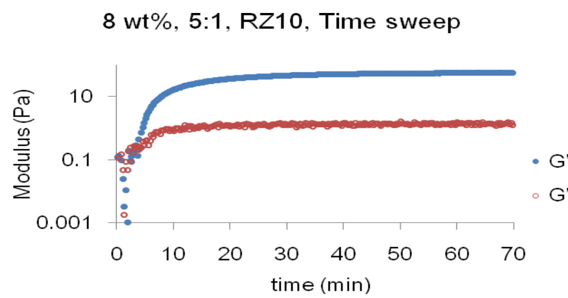

Figure 1. Dynamic time sweep of a 8 wt% resilin-based hydrogel.

Preliminary results showed that both the complex modulus and the compressive modulus increased with increasing protein concentration (Table 1).

Protein concentration (wt%)	Crosslinking ratio	Complex modulus (Pa)	Compressive modulus (kPa)
8	5:1	62.6±7.5	3.5
9	5:1	84±8	9.4±0.8
12	5:1	7400	59.3

Table 1. Mechanical properties of resilin-based hydrogels at different protein concentrations.

hMSCs encapsulated in 3D resilin-based hydrogels showed >95% viability after 1 day (Figure 2).

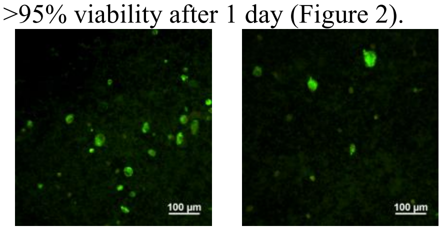

Figure 2. hMSCs showed high viability (live cells are green and dead cells are read) after being cultured for one day in the 3D resilin-based hydrogel.

Conclusions: The complex modulus and the compressive modulus of resilin-based hydrogels at different protein concentrations were determined. We observed an increase for both moduli with increasing protein concentration. We are currently examining the effect of varying the crosslinking ratio on mechanical properties and will investigate cell response to these different hydrogels.

References:

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