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

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Statement of Purpose: Osteoarthritis is a degenerative disease that affects articular cartilage. An estimated 38 million American adults are projected to suffer from osteoarthritis by the year 2030.[1] Thus, it is important to develop effective tissue engineering scaffolds to replace or regenerate cartilage. Our lab has successfully developed modular recombinant proteins based on resilin, an elastomeric protein found in insect cuticles. Natural resilin is known for its high resilience, which makes it a promising scaffold for cartilage engineering because of the repetitive compression that cartilage undergoes. 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,[2] which is on the same order of magnitude of natural cartilage (2.3-15 MPa).[3] 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.[2] In the current study, we modulated the mechanical properties of the resilin-based protein by varying the protein concentrations and the crosslinking ratios. 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 one day before the cell viability was quantified using a LIVE/DEAD® Viability/Cytotoxicity Kit for mammalian cells (Molecular Probes L-3224).

Results: Crosslinked resilin-based hydrogels were prepared at different concentrations (8-14 wt%) and different crosslinking ratios (0.5:1, 1:1, and 5:1). The complex modulus and unconfined compressive modulus were measured for each hydrogel composition (Table 1). Preliminary results showed that gelation time can be modulated from 30 to 5 minutes by increasing the crosslinking ratio (data not shown). Both the complex and compressive moduli increased with increasing protein concentration at a fixed crosslinking ratio (Table 1). In addition, the complex modulus increased with increasing crosslinking ratio for hydrogels at a fixed protein concentration (Figure 1).

Protein concentration (wt%)	Complex modulus (Pa)	Compressive modulus (Pa)
8	56±6.9	5±1.3
9	85±7.8	9±0.8
10	88±9.7	6±2.1
12	7398	59
14	16704	3000

Table 1. Mechanical properties of resilin-based hydrogels at different protein concentrations with a crosslinking ratio of 5:1.

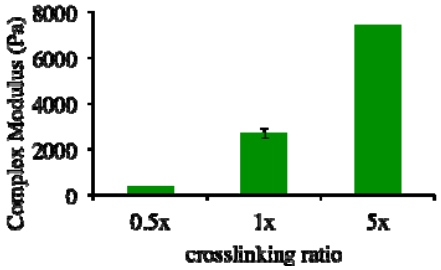

Figure 1. Complex modulus of 12 wt% resilin-based hydrogels at different crosslinking ratios.

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

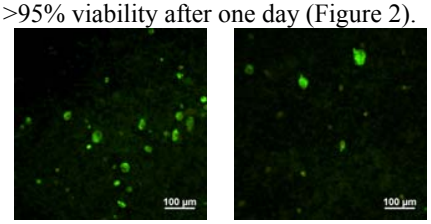

Figure 2. hMSCs showed high viability (live cells are green and dead cells are red) after being cultured for one day in the 3D resilin-based hydrogel.

Conclusions: The complex modulus and the compressive modulus of resilin-based hydrogels at different protein concentrations and crosslinking ratios were determined. We observed an increase for both moduli with increasing protein concentration and crosslinking ratio. We are currently characterizing physical properties such as water content and swelling ratio of resilin-based hydrogels and will investigate cell response to these different hydrogels.

References:

1. Centers for Disease Control, http://www.cdc.gov.
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3. Black J.; Hastings G. W. *Handbook of Biomaterials Properties*, 1st ed; Chapman & Hall: London, 1998.