Composites of Elastin-Like Polypeptide, Collagen, and Bioglass: Mechanical and Cell Culture Properties

Tyler Wheeler, Nathanael Sbravati, and Amol V. Janorkar
Biomedical Materials Science, School of Dentistry, Univ of Mississippi Med Center, 2500 N State St, Jackson, MS 39216

Statement of Purpose: Collagen is the most commonly used extra-cellular matrix protein for tissue engineering applications. Unfortunately, collagen hydrogels display poor mechanical properties. Here, we report on the preparation and characterization of a novel multi-component composite system that incorporates a stimulus-responsive smart polymer (elastin-like polypeptide, ELP), biodegradable ceramic (45s5 bioglass), and minimal amount (~ 20% w/w) of collagen as network former. The major component, ELP, is genetically engineered to provide precise control on its properties and exhibits an inverse phase transition behavior in response to changes in its environment. We hypothesized that incorporation of ELP and bioglass would improve mechanical properties of the composites.

Methods: *Hydrogel Preparation*. Rat tail collagen type I was from Invitrogen. ELP was prepared as described.[1] ELP-bioglass-collagen gels were formed by mixing ELP and collagen (ELP:collagen = 2.5:1; 8 mg collagen) in PBS, adding 5 mg bioglass (Mo-Sci Corp), and then incubating at 37°C. The control ELP-collagen composite gels (ELP:collagen = 2.5:1) were prepared similarly.
Mechanical Testing. Dog-bone shaped specimens (n = 6) were prepared by forming the hydrogels into custom-made molds and tested by the uniaxial tensile testing at an extension rate of 12.7 mm/min on a Sintech 2/G Materials Testing System (MTS) using a pre-load of 0.22 N.
Cell Culture. To demonstrate the ability to encapsulate, grow, and differentiate cells within our hydrogels, 50,000 MC3T3-E1 cells (ATCC) per hydrogel were incorporated during gelation and cultured over a period of 3 weeks in alpha-modified Eagle's minimum essential medium with 10% FBS. After 24 h, the cells were supplemented with 10 mM β-glycerophosphate and 50 µg/mL ascorbic acid.
Cell Culture Characterization. Viability was assessed using live/dead assay (Invitrogen). Differentiation was assessed by alkaline phosphatase (ALP; BioAssay Systems) and osteocalcin production (OCN; Biomedical Tech). Mineral deposition visualized by Alizarin red Stain (Millipore). Manufacturers' protocols were followed. All assays were performed on day 7, 14, and 21.
Statistical Analysis. ANOVA and Games-Howell post hoc test for unequal variances was performed. Results reported as mean ± 95% confidence intervals.

Results: Figure 1 shows the average values of tensile strength, elastic modulus, and toughness for the ELP-collagen and ELP-bioglass-collagen hydrogels. The tensile strength and elastic modulus (Fig. 1) nearly doubled between ELP-collagen and ELP-bioglass-collagen hydrogels ($p \leq 0.05$), while the toughness did not show any statistically significant increase ($p > 0.05$).
The normal differentiation process of pre-osteoblastic cells begins with an increase in cell density, followed by an increase in various protein levels including the alkaline phosphatase (ALP) and osteocalcin (OCN), further continuing with mineralization of the matrix secreted by the cells.[2] Live/Dead assay done on day 21 (Fig. 1a,c) showed high number of live cells within all hydrogels, attesting their biocompatibility. Addition of bioglass in the composite nearly doubled the ALP activity compared to ELP-collagen gels ($p \leq 0.05$), while OCN production and mineralization (Fig. 1b,d) were equivalent.

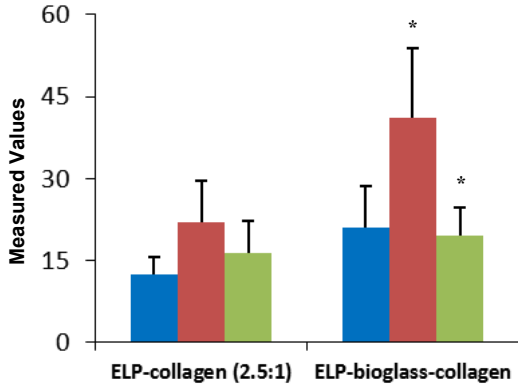

Fig 1. Effect of bioglass addition on the mechanical properties of the hydrated composites. ■: Tensile strength (x 2 kPa); ■: elastic modulus (kPa); and ■: toughness (x 5000 N-mm/mm^3). * $p \leq 0.05$ against ELP-collagen. Error bars = 95% confidence intervals.

Hydrogel	ALP (µmol/L-min)/(µg/mL)	OCN (ng/mL)
ELP-collagen	0.7 ± 0.2	1.7 ± 0.2
ELP-bioglass-collagen	1.4 ± 0.2*	1.7 ± 0.1

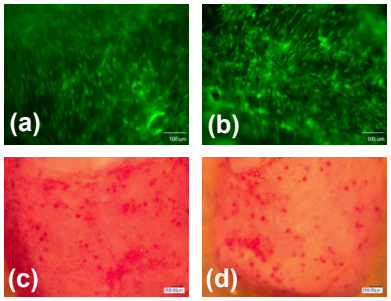

Fig 2. ALP activity and OCN production for cells cultured within composite hydrogels. * $p \leq 0.05$ against ELP-collagen. Live/Dead and Alizarin red staining for (a,c) ELP-collagen and (b,d) ELP-bioglass-collagen hydrogels. All results on day 21.

Conclusions: We have successfully demonstrated the preparation of ELP-bioglass-collagen composite scaffolds. Importantly, our preliminary testing showed improvements in the mechanical properties after addition of the bioglass. MC3T3-E1 cells cultured on our scaffolds followed the normal differentiation process.

References: [1] Janorkar A, et al. Biomaterials 2008;29:625-32.
[2] Burdick JA, et al. J Control Release 2002;83:53-63.