Comparison of Compressive Properties and Cell Viability of Species-Specific Methacrylated Collagen Hydrogels

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Statement of Purpose: Collagen type I is often used to generate biomimetic hydrogels due to its excellent biocompatibility and availability of cell adhesion sites.[1] However, weak mechanical properties and expedited enzymatic degradation are major limitations.[2] While chemical crosslinking using genipin or carbodiimide (i.e., EDC-NHS) crosslinkers has been commonly utilized to improve the mechanical properties of collagen hydrogels, cell encapsulation within the hydrogels is not feasible due to toxicity of the crosslinking solution.[3] Recent studies have shown that modification of collagen structure via introduction of methacrylate groups allows for photochemical crosslinking of collagen hydrogels with improved mechanical properties and high cell viability.[4,5] Further, species-specific variation in the amino acid composition of collagen is known to significantly impact cellular response.[6] The goal of this study is to assess the effect of three different collagen species (bovine, human, and rat) and two different photoinitiators (Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) and Irgacure 2959 (I-2959)) on compressive properties of methacrylated collagen (CMA) hydrogels, and human mesenchymal stem cell (hMSC) viability and metabolic activity.

Methods: Type I collagen from different species (bovine, human, rat) was methacrylated by reacting the free amines with methacrylic anhydride by following a previously published protocol.[7] Briefly, Na_2HPO_4 buffer was added to the acid soluble collagen to neutralize the pH followed by the dropwise addition of methacrylic anhydride under constant stirring. After completion of the reaction, the CMA solution was dialyzed against 10 mM hydrochloric acid and lyophilized until use. To prepare the CMA hydrogels, chilled neutralized CMA solution (6 mg/ml) was mixed with a photoinitiator (i.e., I-2959 or LAP; 0.1% w/v), added to the circular rubber washers, and exposed to UV light (17 mW/cm^2) for 2 min to induce gelation. Uniaxial compression tests (10 µm/sec) were performed using Cellscale Microtester (N=5/group) and the compressive modulus was calculated by taking the slope of the stress-strain curve between 0%-10% strain region. For the cell studies, hMSCs were encapsulated within CMA hydrogels and cultured in α-MEM growth medium (with 10% FBS) for 7 days. The effect of different collagen species and photoinitiator type on cell viability (N=3/group) was assessed using live-dead assay. Further, cell metabolic activity (N=4/group) was investigated using Alamar blue assay. Statistical significance was determined using one-way ANOVA with Tukey posthoc test.

Results: Compression test results showed species-based differences in the compressive modulus of I-2959 crosslinked CMA hydrogels whereas LAP crosslinked hydrogels were comparable. Specifically, the compressive modulus of bovine CMA hydrogels was significantly higher than human CMA and rat CMA hydrogels (Fig. 1A). Results from Alamar blue assay showed that cell metabolic activity increased with time for all hydrogels (Fig. 1B). When comparing between different photoinitiators, cell metabolic activity in rat LAP hydrogels was significantly than rat I-2959 hydrogels at day 4 and day 7. In addition, cells in bovine LAP hydrogels showed significantly higher metabolic activity compared to bovine I-2959 hydrogels at day 7. Further, species-based differences in cell metabolic activity were observed in LAP hydrogels wherein cell metabolic activity was significantly higher in rat CMA hydrogels compared to bovine CMA hydrogels. Qualitative assessment of cell viability images showed higher viability in LAP hydrogels compared to I-2959 hydrogels (Fig. 1C-1H).

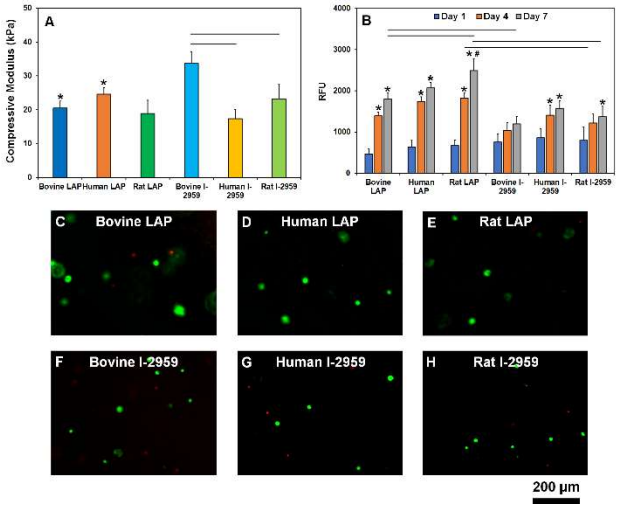

Figure 1: (A) Compressive Modulus of CMA hydrogels (* denotes $p < 0.05$ when comparing different photoinitiators and horizontal line indicates $p < 0.05$ between connecting groups), **(B)** Cell metabolic activity of CMA hydrogels using Alamar Blue assay (* denotes $p < 0.05$ when comparing with day 1, # denotes $p < 0.05$ when comparing with day 4, and horizontal line indicates $p < 0.05$ between connecting groups), **(C-H)** Live-dead assay to assess the viability of hMSCs at day 1.

Conclusions: Results from this study demonstrate that LAP photoinitiator is more cytocompatible than I-2959 which is in agreement with prior work using a different polymeric hydrogel system.[8] Further, preliminary findings from the current study are indicative of species-based differences in compressive properties and cellular response of CMA hydrogels. In conclusion, careful selection of collagen species and photocrosslinking conditions may be warranted for generation of collagen-based hydrogels for tissue engineering applications.

References: [1] Diamantides et al., Biofabrication, 2017; [2] Omobono et al., J. Biomed. Mater. Res. A, 2020; [3] Macaya et al., Adv. Func. Mater., 2011; [4] Gaudet et al., Biointerphases, 2012; [5] Kajave et al., Mater.Sci. Eng., 2020, [6] Lin et al., Food Chem., 2006, [7] Jongprasitkul et al., Biomacromolecules, 2021, [8] Xu et al., Biomedical Materials, 2020.