Development of injectable, biodegradable and thermosensitive hydrogels that become highly flexible at body temperature and can form composites with biomolecules

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Introduction: Injectable hydrogels have been extensively explored as vehicles for drug delivery and cell transplantation. Thermosensitive and injectable hydrogels are attractive for such applications since they can strengthen upon temperature change, without requiring further chemical reactions to achieve, for instance, crosslinking.[1] Our objective was to synthesize a thermosensitive hydrogel appropriate for soft tissue engineering applications and possessing the following characteristics: injectable at room temperature, capable of binding biomolecules such as collagen or other bioactive factors, highly flexible and relatively strong in situ at body temperature. The hydrogel should posses a lower critical solution temperature (LCST) below 37C before degradation and a LCST above 37C after degradation, with soluble and non-toxic degradation products. Such a thermosensitive matrix could be employed to encapsulate and deliver cells for subsequent mechanical training in vivo or in vitro. To meet these objectives, a family of thermosensitive hydrogels was synthesized based on n-isopropylacrylamide (NIPAAm), acrylic acid, a bioconjugating functional group and a biodegradable polylactide based macromer. The resulting polymer was conjugated to varying collagen mass fractions and mechanical and cytocompatibility properties were assessed.

Methods: Hydrogels comprised of NIPAAm, acrylic acid, N-acryloxysuccinimide, and a biodegradable macromer were synthesized by free radical polymerization in dioxane at 70C followed by repeated purification steps. The biodegradable macromers were prepared by ring-opening polymerization of lactide with 2-hydroxyethyl methacrylate (HEMA) at 110C for 1.5 h.[2,3] For these macromers, several different numbers of lactide repeat units were synthesized to affect polymer degradation properties. Type I collagen (4 wt%) conjugation was conducted in 20% copolymer in phosphate buffered saline (PBS; pH=7.4) at 4C for 24 h. Hydrogels were formed at 37C and cut into 6mm x 0.5 mm discs. Rat arterial smooth muscle cells (SMCs) were statically seeded at a density of 3×10^5 /mL for cytocompatibility studies.

Results: Hydrogel structure was confirmed and characterized by FTIR, ^{1}H-NMR and differential scanning calorimetry. Copolymers were injectable at or below room temperature and formed robust hydrogels at 37C (**Fig. 1**). Depending on polymer composition, LCSTs were found to be 18-26^{o}C. After complete hydrolysis with NaOH, hydrogels were soluble in PBS at 37C with LCSTs above 41.2C. Collagen incorporation slightly increased LCSTs. Water absorption was 132-180% without collagen and increased to 330% with collagen. At 37C the hydrogels were highly flexible and relatively strong with tensile strengths from 0.30-0.90 MPa, and elongations at break from 344-1841% depending on composition and collagen content (**Figs. 1 and 2**). Hydrogel weight loss at 37C was 85-96% over 21 days and varied with polylactide content. Degradation products were shown to be non-cytotoxic. Cell adhesion on the hydrogels was 30% of that for tissue culture polystyrene (TCPS; $p<0.01$), but increased to statistically approximate TCPS after collagen incorporation.

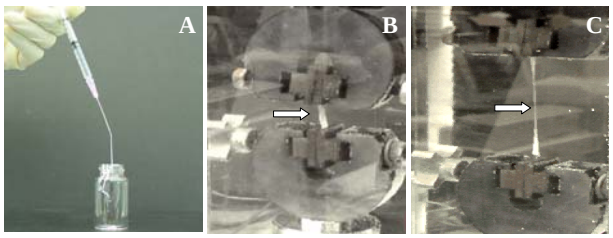

Fig. 1. Hydrogel injectability at 22C (A); tensile testing in a 37C water bath with no strain (B) and high strain (C).

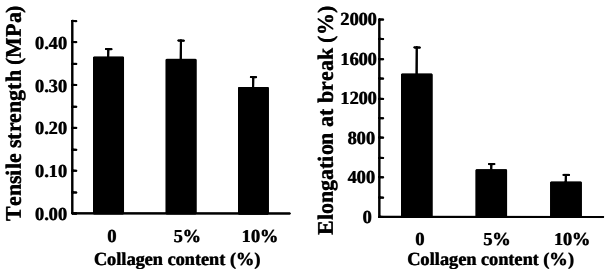

Fig. 2. Effect of collagen content on mechanical properties.

Conclusions: A family of novel injectable, biodegradable and flexible thermosensitive hydrogels has been developed that possess chemical and mechanical properties attractive for application in soft tissue engineering. Future investigations with these hydrogels will necessarily involve evaluation of cell behavior in three-dimensional constructs in vitro and in vivo as well as the examination of the effects of cyclic mechanical loading.

References:

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