In-situ Crosslinked Scaffolds of Elastin-like Polypeptide Block Copolymer for Tissue Repair

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Introduction: Hydrogels formed from elastin-like polypeptides (ELPs) and enzymatically crosslinked forms of ELP have been shown to support cartilage matrix synthesis in vitro, for both embedded primary chondrocytes and adult, adipose tissue-derived stem cells[1-3]. In a complementary approach, we recently developed a new method to chemically crosslink ELPs into hydrogels with hydroxymethylphosphines (HMPs)[4]. This method is attractive for *in situ* crosslinking of ELPs in tissue defects as it proceeds spontaneously in aqueous medium and generates only water and stable aminomethylphosphines as a by-product. These studies were carried out on an ELP that contained crosslinkable Lys residues that were distributed along the polypeptide chain[4]. In an effort to expand upon the range of mechanical properties of ELP hydrogels, we have designed ELP block copolymers comprised of crosslinkable, hydrophobic ELP blocks with Lys residues and ELP hydrophilic blocks with no crosslinking sites. In this work, we report studies of gelation kinetics, mechanical properties, and an ability to support cell viability for these HMP-crosslinked ELP block copolymer hydrogels. Results show that the mechanical properties of crosslinked ELP block copolymer hydrogels can be modulated by their architectures, and that fibroblast cell survival in these hydrogels is comparable to that of other hydrogel systems.

Materials and Methods: Using recursive directional ligation[5], ELP block copolymers were genetically designed from repetitive ELP pentapeptide sequences, Val-Pro-Gly-Xaa-Gly (Xaa is a guest residue and any amino acid other than Pro); ELP[KV_7F-72, 144] and ELP[VG_8A_7-32, 64], where the bracketed capital letters and lower numbers are the single letter amino acid codes and the ratio of the guest residues of ELP pentapeptides, and the number indicates the total number of the pentapeptides. ELP block copolymers with different numbers of blocks ranging from 2 to 4 were genetically synthesized, expressed in *E. coli,* and purified by inverse transition cycling[5]. The fully crosslinked ELP hydrogels were prepared by addition of 20 wt. % of [tris(hydroxymethyl)phosphino]-propionic acid (THPP) in PBS at pH 7.5 to 20 wt. % of ELP block copolymers in PBS at a 5-7 fold molar excess of –OH groups of THPP to $–NH_2$ groups on the ELP. Mixtures were incubated at 37 °C for 1 h. Equilibrium compressive modulus (E), complex shear modulus (|G*|), equilibrium shear modulus (μ) and loss angle (δ) were measured for the ELP gels in a torsional shear test (0.1 to 50 rad/sec, PBS at 37 °C) using a parallel plate configuration (plate diameter = 25mm). In separate experiments, ELP block copolymers were mixed with mouse fibroblasts (NIH-3T3, 10^7 cells/ml) and THPP at a 1×molar excess of –OH to $–NH_2$. Cell viability was assessed at day 0 and 3 after culture using Live/Dead and PicoGreen assay kits (Molecular Probes).

Results and Discussion: Two ELP block copolymers, a single-block, ELP[KV_7F-144] (61.1 kDa) and a tri-block, ELP[KV_7F-72]-ELP[VG_8A_7-64]-ELP[KV_7F-72] (85.1 kDa) were crosslinked with THPP by Mannich type condensation reaction. Figure 1 shows that the mechanical properties were significantly affected by the inclusion of an uncrosslinked, hydrophilic ELP[VG_8A_7-32, 64] block in the tri-block ELP as compared to the single block ELP. Values for E, |G*|, and μ of the single-block ELP gel were over 2 times higher than those of the tri-block ELP gel, with values for the loss angle (δ) that generally reflect elastic behaviors for both block copolymers (single: δ = 2.7°±0.5°; tri-block: δ = 9.5°±2.5°). The tri-block ELP gels are modestly more viscous than single-block ELP gels, likely due to the incorporation of an uncrosslinked, middle block that provides for viscous domains interspersed in the crosslinked end blocks.

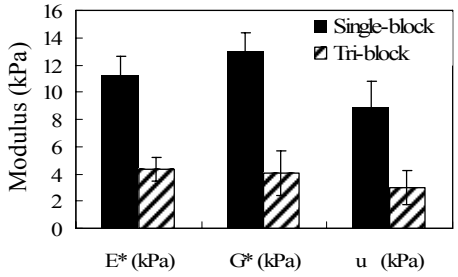

Figure 1. Mechanical properties of crosslinked single- and tri-block ELP hydrogels (mean±SD, n=4).

When fibroblasts were mixed with the ELP block copolymers and THPP during crosslinking, ELP hydrogels were formed after incubation at 37°C within several minutes. Cell viability based on DNA quantitation was found to be slightly less than 50% immediately after crosslinking, but was greater than 60% after 3 days of culture, values that compare well with embedding cells in alginate or agarose. These results may arise as the THPP is capable of reacting with cell-associated amines; however, shortly after encapsulation, the cells appear to recover or proliferate in a process that does not interfere with long-term cell survivability as shown in Figure 2. In conclusion, this study shows that the mechanical properties of ELP hydrogels that are crosslinked with THPP can be modulated by design of different ELP block copolymers with elastic and viscous domains, a feature that is likely to be useful in the design of in situ crosslinking biomaterials for tissue repair over a broad range of biological and mechanical functions.

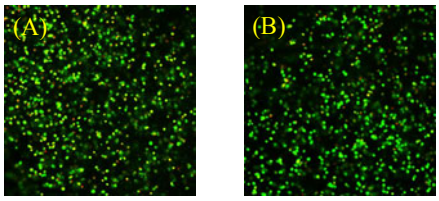

Figure 2. Live/Dead assay of fibroblasts in crosslinked (A) single- and (B) tri-block ELP hydrogels at day 3.

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References

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