Dextran-tyramine Hydrogels for Cartilage Repair: Enhanced Chondrogenicity by Engraftment with Hyaluronic Acid and Heparin

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Introduction

Injectable scaffolds based on *in situ* gelating hydrogels are promising biomaterials for application in regeneration of diseased cartilage. They allow the simultaneous incorporation of cells and/or bioactive components. The high water content of these hydrogels favors the exchange of nutrients and metabolites while mimicking the extracellular matrix (ECM) (Yi, H., et al. Acta Biomateriala, 2007). Previously, we have shown the potential of novel *in situ* forming dextran hydrogels based on dextran-tyramine conjugates (Dex-TA). These hydrogels are rapidly formed under physiological conditions using horseradish peroxidase (HRP) and hydrogen peroxide (H_2O_2). With respect of mechanical properties, swelling and degradation behavior, Dex-TA hydrogels have favorable characteristics, but biocompatibility can be improved. In this study, we have prepared composite hydrogels consisting of Dex-TA engrafted with hyaluronic acid (HA) and mixtures of Dex-TA and heparin in different ratios. To evaluate if these smart hybrid gels lead to enhanced chondrogenicity, chondrocytes were incorporated to assess cell survival and matrix production.

Materials and Methods

Polymer synthesis: Dex-TA conjugates linked by a urethane bond were synthesized as previously reported (Jin, R., et al. Biomaterials, 2007). Heparin-tyramine conjugates (Hep-TA) were synthesized by the coupling reaction of tyramine amine groups to heparin carboxylic acid groups using EDAC/NHS activation. Copolymers of HA grafted with Dex-TA (HA-*g*-Dex-TA) were synthesized by a coupling reaction of Dex-TA-NH2 with HA using EDAC/NHS as coupling reagent. A HRP/TA ratio of 0.25 mg/mmol and H_2O_2/TA molar ratios of 0.2 were applied in the preparation of the hydrogels. *In vitro* biological evaluation: Cell viability was evaluated with a live-dead assay and quantified by CyQuant. Collagen type II and Aggrecan mRNA expression was assessed. ECM production was analyzed by histology and quantification of sulphated glycosaminoglycans (GAGs).

Results and Discussion

The results showed that hybrid hydrogels of Dex-TA and Hep-TA or HA successfully formed within 1 minute. Cells were incorporated without a sign of cytotoxicity. The results showed that the hydrogel with a Dex-TA/Hep-TA weight ratio of 50/50 showed the best chondrocyte viability, increased cell proliferation, and enhanced ECM production. Compared to Dex-TA hydrogels, the biomimetic HA-*g*-Dex-TA hydrogels induced an enhanced cell proliferation and ECM deposition (increased GAGs and collagen production).

Conclusion

HA-*g*-Dex-TA hybrids, designed to resemble the macrostructure of proteoglycans, showed a better stability than HA-TA hydrogels and an improved chondrocyte performance than Dex-TA hydrogels. The hybrid hydrogels of Dex-TA/Hep-TA facilitated higher chondrocyte proliferation and enhanced ECM production, when compared to Dex-TA. The presence of Dex-TA in Hep-TA hydrogels significantly improved the mechanical properties of Hep-TA hydrogels, decreased swelling and increased degradation time. Thus, these novel dextran-based smart hydrogel systems show promising characteristics for various applications in cartilage reconstruction.

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