Recruitment of Endogenous Stem Cells for Tissue Repair

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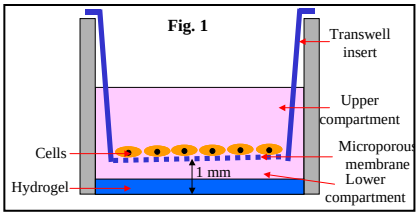
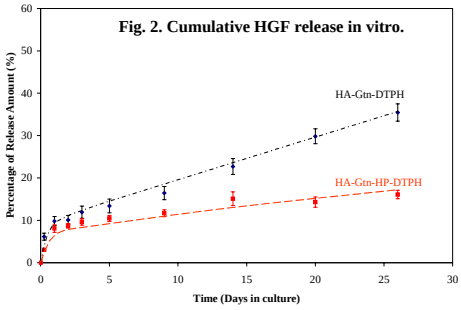
Background: Conventional concept of stem cell therapy is based upon the isolation of stem cells from patients, in vitro propagation, and then implant back to the patients, which is associated with many problems particularly during the in vitro manipulation process. In contrast, it is easier, safer, and more efficient to attract endogenous stem cells and precursor cells to the defect site for de novo tissue regeneration. Studies have shown that hepatocyte growth factor (HGF), a pleiotropic cytokine of mesenchymal origin, exerts strong chemoattractant effect on mesenchymal stem cells (MSCs) [1]. Exposure of MSCs to HGF induced migration of MSCs in vitro. However, like most proteins, HGF undergoes enzymatic digestion in the body, resulting in a short lifetime of HGF in a soluble form. To maintain the therapeutic level of HGF at the defect site necessary for endogenous stem cell recruitment, sustained, long-term, and localized delivery of HGF is essential. ECM-derived polymers with a wide array of physiological functions represent ideal substrates for HGF delivery and stem cell recruitment. In addition, heparin in the ECM has been demonstrated to protect growth factors from enzymatic degradation and thermal denaturation, and extend their releasing durations in vivo while retaining their bioactivity [2]. Recently, an injectable, in situ-crosslinkable ECM-based hydrogel was developed by crosslinking thiol-modified hyaluronan (HA) and gelatin (Gtn) using poly(ethylene glycol) diacrylate (PEGDA) [3]. In this study, we utilized this hydrogel for controlled release of HGF to recruit human bone marrow mesenchymal stem cells (hMSCs) to the scaffold in vitro.

Methods: Thiolated chemically-modified HA (CMHA-S), thiol-modified gelatin (Gtn-DPTH), thiol-modified heparin (HP-DTPH) and PEGDA were kindly provided by Glycosan BioSystems Inc. (Salt Lake City, UT). In vitro release of HGF: HGF was allowed to release from the hydrogels at 37°C into a PBS buffer solution supplemented with 1% BSA, 1mM EDTA, and 10μg/ml heparin. hMSC migration: $5X10^3$ cells in 200μl culture media were added to the upper compartment of each transwell. The lower compartment was filled with 200μl hydrogel with or without HGF, and 400μl culture media (Fig.1). At 8 or 48 hours, cells and ECM on the top surface of the filter were wiped off with cotton swabs. Cells that had migrated into lower compartment and attached to the lower surface of filter were stained with Alexa 546-phalloidin and Draq 5.



Results/Discussion: Cumulative in vitro HGF release: Over 26 days in vitro, the HA-Gtn hydrogels without heparin released a total of 35% of the loaded HGF, as compared to 18% by the HA-Gtn-HP hydrogels. Addition of heparin to the hydrogels significantly prolonged the releasing duration of the loaded HGF (Fig. 2). hMSC migration: hMSCs migrated to the lower surface of the membrane in all groups (Fig.3 A-D) except in significantly lower numbers in the no-HGF control group (Fig.3 A). Compared to the soluble HGF group (Fig.3 F), a large number of cells were recruited to the HGF-loaded hydrogels without HP (Fig.3 G), but not to the HGF-loaded hydrogels with HP (Fig.3 H), suggesting that sustained delivery of HGF at therapeutic concentrations (as made possible by the hydrogels) is able to recruit stem cells to the scaffold, and that heparin plays a major role in controlling HGF releasing rate. The lack of stem cell recruitment in the HGF-loaded HA-Gtn-HP hydrogels (Fig.3 H) is perhaps due to the slower release amount from hydrogel with HP, which results in HGF release that is only sufficient to induce hMSC migration to the lower surface of the membrane, but not stem cell recruitment to the hydrogel scaffold. Increase the HGF loading in HP-coating gel would be able to overcome this problem. This also indicates that stem cell recruitment to scaffolds is HGF concentration dependent.



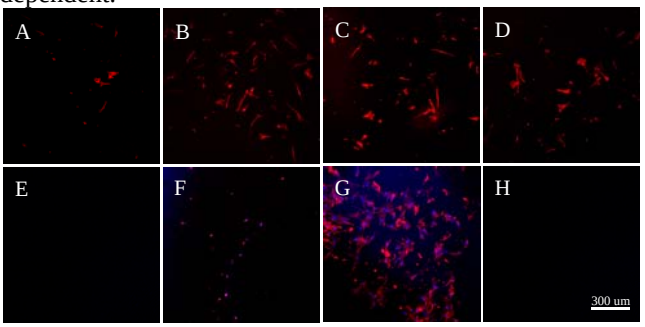


Fig. 3. Cells attached to the lower surface of the membrane (A-D) and recruited to the hydrogels (E-H). A,E) no HGF control; B,F) solubilized HGF (20ng/ml) in culture media; C,G) 500ng HGF loaded in 200μl HA-Gtn hydrogel; D,H) 500ng HGF loaded in 200μl HA-Gtn-HP hydrogel.

Conclusions: Sustained, and localized release of HGF from scaffold is able to induce stem cell migration and recruitment to the scaffold. Heparin in the scaffold controls HGF release rate. Studies in progress are optimizing heparin concentration in the hydrogel for HGF releasing profile that best promotes stem cell recruitment. Future study involves stem cell recruitment into the scaffolds with controlled release of HGF in vivo.

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