Molecule Release from Proteolytically Degradable Hyaluronic Acid Hydrogels for Improved Osteogenesis

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Statement of Purpose: Every year millions of patients undergo surgical procedures to promote bone regeneration. Currently, the clinical gold standard to promote bone repair remains autograft bone. Disadvantages of this treatment include limited tissue supply, donor site morbidity, and poor integration [1]. The use of bone morphogenic proteins (BMPs) shows promise in therapies for improving bone regeneration [2]; however, high supraphysiological concentrations required for a desired osteoinductive effect, costs, and patient variability have prevented the full advantages of BMP-based therapeutics from being realized [1]. Thus, one strategy is to deliver synergistic molecules with BMP to enhance efficacy and lower doses. To accomplish this, we developed a hyaluronic acid (HA) hydrogel delivery system where gel degradation and molecule release are controlled via local proteolytic activity.

Methods: A modified Boyden chamber assay was used to determine the chemotactic activities of stromal-derived factor-1 alpha (SDF-1α) and HA using bone marrow derived cells (BMCs). A monoclonal antibody to CD44 (for HA specific blocking) and a CXCR4 antagonist (for SDF-1α specific blocking) were used to determine the individual effects of both HA and SDF-1α on cell migration. Maleimide-modified HA (MaHA) was synthesized via a 2-step protocol: (1) synthesis of the tetrabutylammonium salt of HA (HA-TBA) as described previously [3]; (2) coupling of 2-aminoethylmaleimide to HA-TBA in the presence of benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (BOP) in DMSO, followed by dialysis and lyophilization. Maleimide modification was assessed with ^{1}H NMR. For gelation, MaHA was dissolved in phosphate buffered saline (PBS) with or without growth factors. Cell adhesive (GCGYG*RGDS*PG) and matrix metalloprotease (MMP) sensitive (GCRDGPQG↓IWGQDRCG) peptides were added and reacted according to a Michael Type addition reaction, allowing for cell-mediated hydrogel degradation. MaHA hydrogel degradation was performed in 2U/ml collagenase (non-specific MMP degradation) at 37°C and compared to degradation in PBS. Hydrogel degradation was evaluated using an uronic acid assay.

Results: In this work, a matrix metalloprotease (MMP)-sensitive HA-based hydrogel scaffold was used for potential synergistic molecule delivery towards improving BMP-induced osteogenesis. SDF-1α has been shown to play an important role in stem cell trafficking [4] and HA hydrogels are known to increase extracellular matrix production [5]. The chemotactic activities of both SDF-1α and HA were measured using a modified Boyden chamber assay and are shown in Figure 1. Both SDF-1α and HA induced chemotaxis of BMCs, which was blocked with the addition of either CXCR4 or CD44 interactions, respectively. These results motivate the use of both SDF-1α and HA in hydrogel design.

To design a material with cell-mediated molecule release, MaHA was synthesized and used as a platform for incorporation of proteolytically degradable crosslinks and cell-adhesive peptides via a Michael Type addition reaction (Figure 2a). Maleimide functionalization was varied based on the maleimide to HA-TBA ratio during coupling. Hydrogel degradation studies indicate rapid degradation in the presence of MMPs (collagenase) and little degradation without MMPs as shown in Figure 2b.

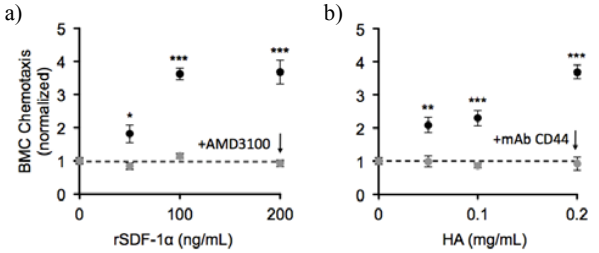

Figure 1. Chemotactic activity of rSDF-1α and HA macromer. (a) SDF-1α stimulated significant dose-dependent chemotaxis of unfractionated BMCs and was blocked with a CXCR4 antagonist, AMD3100. (b) HA macromer stimulated similar dose-dependent chemotaxis of BMCs and was blocked with a monoclonal antibody to CD44. $P <$ *0.05, **0.01, and ***0.001.

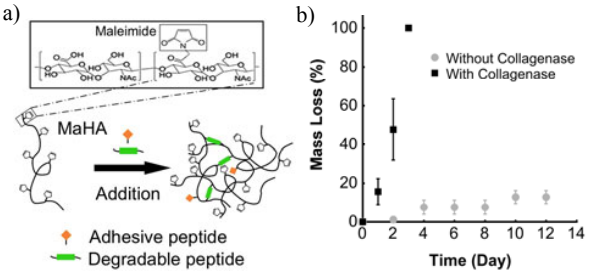

Figure 2. (a) Schematic of MaHA hydrogel formation, where MaHA is cross-linked with a thiol-terminated MMP-cleavable peptide using a Michael Type addition reaction. (b) MaHA hydrogels were degraded in solutions with and without collagenase, indicating primarily proteolytic degradation over the time frame studied.

Conclusions: Towards the development of materials that can release multiple factors through cell-mediated mechanisms to enhance osteogenesis, HA hydrogels were designed that incorporate proteolytically degradable crosslinks and adhesive peptides. SDF-1α and HA were both found to contribute to cell migration, motivating their use in the development of therapies for improved tissue repair. Current work is evaluating the synergistic delivery of SDF-1α and BMP2 using an *in vivo* cranial defect rat model on BMP-induced osteogenesis.

References: [1]M. Stevens. Materials Today. 2008.; [2]H. Seeherman, J. M. Wozney. Cytokine Growth Factor Rev. 2005: 16(3), 329-45.; [3]S. Khetan, J. Katz, J. Burdick. Soft Matter. 2009: 5, 1601-6.; [4]E. L. S. Fong, C. K. Chan, S. B. Goodman. Biomaterials. 2011: 32(2), 395-409.; [5]H. S. Yoo, E. A. Lee, J. J. Yoon, T. G. Park. Biomaterials. 2005: 26(14), 1925-33.