Controlled Growth Factor Delivery Using Enzymatically Degradable Hydrogels For Improved Bone Formation

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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. Stromal cell-derived factor-1 alpha (SDF-1α) has been shown to play an important role in stem cell trafficking [3] and hyaluronic acid (HA) hydrogels are known to increase extracellular matrix production [4]. Additionally, our lab has previously shown that HA is capable of inducing cell chemotaxis through the CD44 receptor. This motivates the use of both SDF-1α and HA in hydrogel design toward increasing cell migration to the injury site.

Methods: Maleimide-modified HA (MaHA) was synthesized, dissolved in phosphate buffered saline (PBS), and cross-linked with a difunctional matrix metalloprotease (MMP)-sensitive peptide using a Michael Type addition reaction. Hydrogel mechanics and reaction time were determined as a function of cross-linking density using dynamic mechanical analysis and rheometry, respectively. MaHA hydrogels loaded with either 100 ng/scaffold of SDF-1α or BMP2 were degraded in 10, 2, or 1 U/ml collagenase (non-specific MMPs) at 37°C and compared to degradation in PBS. Hydrogel degradation and growth factor release was evaluated using an uronic acid assay and ELISA, respectively. An in vivo critical-sized cranial defect rat model was used to assess BMP-induced osteogenesis, where micro-CT and histology were analyzed after six weeks.

Results: In this work, an MMP-sensitive HA-based hydrogel scaffold was used for molecule delivery towards improving BMP-induced osteogenesis. To design a material with cell-mediated molecule release, MaHA was synthesized and used as a platform for incorporation of proteolytically degradable cross-links and cell-adhesive peptides via an addition reaction (Figure 1a). Compressive and rheology studies indicate higher modulus and faster reaction times with higher cross-linking densities. Hydrogel degradation occurred rapidly in the presence of MMPs, with little degradation without MMPs. Furthermore, BMP2 (Figure 1b) and SDF-1α (Figure 1c) release corresponded closely with hydrogel degradation curves, where limited release occurred through diffusion in the absence of MMPs. Growth factor release rate was a function of molecule size especially in the absence of collagenase, where SDF-1α (8 kDa) releases faster compared to BMP2 (dimer, 30-38 kDa).

A critical-sized cranial defect was used to determine the effect of growth factor delivery on bone formation in vivo. Micro-CT results are shown in Figure 2, where typical 3D reconstructions for an empty defect, hydrogel only, hydrogel with BMP2, and hydrogel with BMP2 and SDF-1α are shown in Figure 2a. A statistically significant increase in total bone volume was observed for hydrogels with combined SDF-1α and BMP2 delivery (Figure 2b).

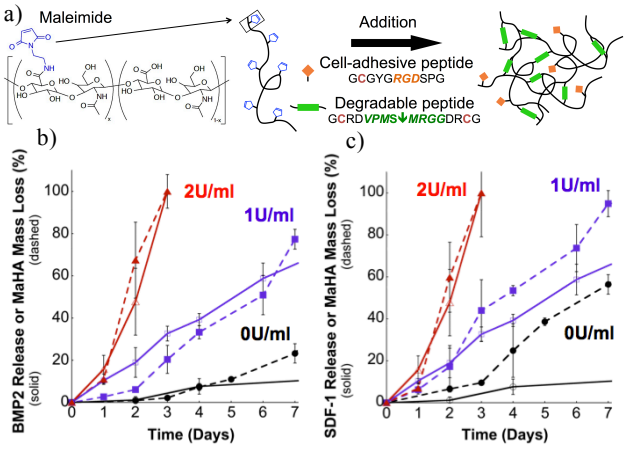

Figure 1. (a) Schematic of MaHA hydrogel formation, where MaHA is cross-linked with a thiol-terminated MMP-cleavable peptide using a Michael Type addition reaction. MaHA hydrogels loaded with (b) BMP2 and (c) SDF-1α were degraded in solutions with and without collagenase, indicating primarily proteolytic degradation over the time frame studied. Both hydrogel degradation (dashed line) and growth factor release (solid line) are shown.

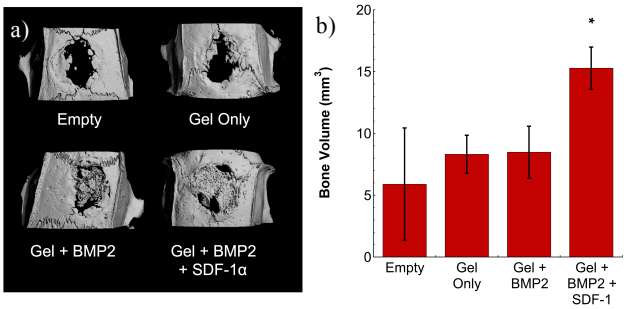

Figure 2. A critical-sized cranial defect was used to assess bone formation where (a) 3D reconstructions and (b) average total bone volume indicate increased osteogenesis with combined growth factor delivery.

Conclusions: Towards the development of materials that can release multiple factors through cell-mediated mechanisms to enhance osteogenesis, finely tunable HA hydrogels were designed that incorporate proteolytically degradable cross-links and adhesive peptides. Growth factor release occurred with hydrogel degradation, where the combined delivery of SDF-1α and BMP2 improved bone formation in vivo.

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