Degradable GAG-based Microparticles with Tunable Sulfation for Growth Factor Delivery within MSC Aggregates

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Statement of Purpose: Mesenchymal stem cells (MSCs) are a promising cell type to be used in injectable therapies to promote bone regeneration due to their ability to undergo osteogenic differentiation. However, maintaining cell viability while encouraging differentiation in MSC-based therapies remains a challenge. To increase cell viability, MSCs can be cultured as aggregates (spheroids), which can be injected through standard needles (Lee 2009). To promote robust osteodifferentiation, growth factors such as bone morphogenic protein-2 (BMP-2) are often included in MSC culture (Even 2012). For homogenous BMP-2 delivery to MSC aggregates, we have developed a system whereby BMP-2 is loaded onto degradable microparticles (MPs) and incorporated into MSC spheroids during formation. In order to achieve a more tunable platform for growth factor delivery, we have explored altering sulfation levels of the integrated GAGs (changes affinity to BMP-2) and/or GAG concentrations in MPs (changes degradation kinetics). It is hypothesized that a decrease in sulfation degree will lead to faster and more complete growth factor release from degradable MPs, while a decrease in GAG content will lead to slower MP degradation and growth factor release.

Methods: MPs were prepared through water and oil emulsion with modified 4-arm PEG acrylate (PEG-4Ac), heparin (highly negative GAG species), and 25 mM of a hydrolytically degradable cross-linker, dithiothreitol (DTT). To alter sulfation pattern, three heparin derivatives, N-desulfated (Hep^{-N}), 6O,N-desulfated ($Hep^{-N,-6O}$) and fully desulfated (Hep-) were prepared through solvolytic desulfation. Sulfation levels were quantified using a 1,9-dimethylmethylene blue (DMMB) assay. All heparin derivatives were functionalized with thiol groups through cystamine/hydroxybenzotriazole/1-ethyl-3-(3-dimethyl-aminopropyl)-carbodiimide reactions. To alter degradation, MPs were formulated with 1 wt% heparin and 99 wt% PEG-4Ac (1% heparin MPs) or 10 wt% heparin and 90 wt% PEG-4Ac (10% heparin MPs).

Growth factor loading was examined by incubating 0.1 mg 10% heparin MPs and 30 ng BMP-2 in PBS solution for 16 hours. BMP-2 release from MPs was determined by analyzing BMP-2 levels in supernatant at 16 hours, Day 1, 4, 7, 10, and 14 via ELISA. BMP-2 bioactivity was assessed via C2C12 cell alkaline phosphatase (ALP) activity assay. MP degradation was monitored in PBS solution and in MSC spheroids (700 cells/spheroid; 3:1 MP to cell ratio) cultured in αMEM with 10% FBS, 1% antimitotic/antibiotic, and 1% L-glutamate over 14 days.

Results: MP growth factor loading was dependent on heparin sulfation pattern (Figure 1A). Fully-sulfated heparin loaded ~80% of the BMP-2 added to solution, whereas desulfated heparin derivatives loaded ~25%-40% of BMP-2. Maximum release of all groups occurred by ~Day 7, likely due to MP degradation by Day 8. Fully-sulfated heparin MPs released ~40-50% loaded BMP-2, whereas Hep^{-N} released ~50-60% loaded BMP-2 over 14 days (Figure 1B), suggesting that less-sulfated heparin leads to more complete BMP-2 release. Due to the limited BMP-2 loading onto $Hep^{-N,-6O}$ and Hep- MPs, little release was seen over 14 days. C2C12 ALP activity indicated that BMP-2 loaded onto Hep and Hep^{-N} MPs retained similar bioactivity to soluble BMP-2 (Figure 1C), whereas no bioactivity was seen from $Hep^{-N,-6O}$ and Hep- MPs due to lack of initial BMP-2 loading (data not shown).

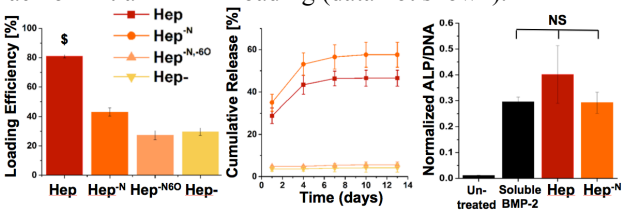

Figure 1. Loading efficiency of BMP-2 into 10% heparin MPs comprised of different heparin derivatives (A), Release curve of BMP-2 from MPs over 14 days (B), ALP activity of C2C12 cells after incubation with BMP-2 released from MPs (C). Untreated group is media control without MPs or BMP-2 and soluble BMP-2 group is 30 ng BMP-2 in cell culture media. $ indicates significant difference to all other groups, ANOVA, $p<0.05$.

MP degradation was dependent on the heparin to PEG-4Ac ratio within MPs. Lowering GAG concentration leads to decreased negative charge and water attraction to MPs, thereby slowing hydrolytic degradation (Elsabahy 2013). In solution, 10% heparin MPs degraded before Day 8 whereas 1% heparin MPs remained over 17 days. In MSC spheroids, 10% heparin MPs and 1% heparin MPs were incorporated at Day 1. 10% and 1% heparin MPs appeared to have degraded by Day 7 and 14 respectively (Figure 2).

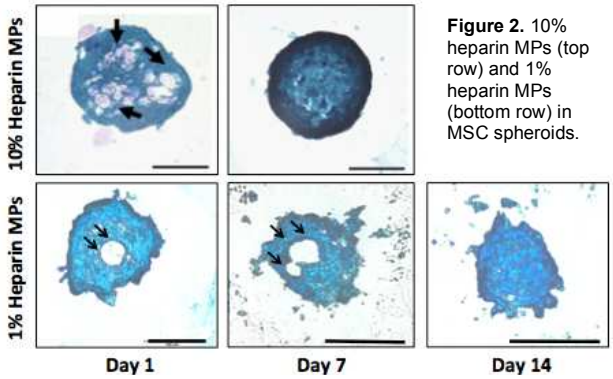

Figure 2. 10% heparin MPs (top row) and 1% heparin MPs (bottom row) in MSC spheroids.

Conclusion: We have fabricated novel degradable GAG-based MPs using heparin derivatives of varying sulfation patterns, which alters BMP-2 affinity, and of varying GAG:PEG-4Ac/DTT ratios, which alters MP degradation. In this way, we have developed a highly tailorable platform for temporal control of growth factor delivery. Incorporation of these materials within MSC spheroids offers a novel means to direct osteodifferentiation before and after MSC spheroid delivery to sites of bone injury.

[1] Lee W. Biomaterials 2009(30:5505-5013) [2] Even J. J Am Ac Ortho Sur 2012(20:547-52) [3] Elsabahy M. Sci Reports 2013(3:3313).