Heparin-mimicking reverse thermal gel for the treatment of myocardial ischemia

Melissa R Laughter[1], Brisa P Castellanos[2], David J Lee[1], Anna L Nelson[1], James R Bardill[1], DaeWon Park[1,*]
[1]Department of Bioengineering, University of Colorado, Anschutz Medical Campus, Denver, CO, USA
[2]Division of Cardiology, University of Colorado, Anschutz Medical Campus, Denver, CO, USA

Statement of Purpose: Myocardial infarction (MI) still remains a leading cause of morbidity and mortality worldwide despite many advances in treatments [1]. The current standard of care for MI aims for early reperfusion of the occluded vessels to prevent further cell death using surgical or pharmacological agents [2]. Growth factors (GF), such as basic fibroblast GF (bFGF) and vascular endothelial GF (VEGF), have been shown to induce therapeutic angiogenesis and augmentation of normal collateral development, moving them to the forefront of potential new treatment options for MI [3] [4]. Although promising, initial studies show that GFs administered directly by injection to the treatment site present physiochemical instability, rapid diffusion, and a short half-life. To achieve effective and controlled therapeutic angiogenesis, a delivery system sustaining GF expression in the specific zone of interest while maintaining GF bioactivity must be designed. Heparin, a naturally sulfonated biopolymer with an intrinsic negative charge, is known to store, protect and stabilize proteins in extracellular matrix [5] [6]. However, heparin as a GF delivery system is difficult to modify and presents batch-to-batch variability in structure and biocompatibility [7]. To overcome these limitations, we have developed a novel heparin-mimicking reverse thermal gel (S-RTG) that may successfully bind GFs while preserving bioactivity. A significant advantage of this system is that it can change phases from solution to physical gel simply by exposure to an increased (e.g. body) temperature. This would allow direct injection at target area with minimal surgical intervention. We **hypothesize** that the injectable S-RTG will mimic heparin function, binding and preserving the bioactivity of angiogenic GFs, providing localized and sustained release of GFs, and promoting localized functional vascularization with substantial functional recovery from MI.

Methods: We began by synthesizing and characterizing a functionalizable biomimetic polymer backbone, poly(serinol hexamethylene urea) (PSHU, Mw: 10,500) using urea, N-BOC-serinol, and hexamethylene diisocyanate. NH_2 groups on the PSHU backbone (after PNIPAAM conjugation) will be functionalized with sulfonate groups at different theoretical percentages. Sulfonation will be conducted based on the Wu et al. sulfonating protocol using 1,3-propane sultone and *tert*-butoxide as a catalyst [8]. We conducted a preliminary release study using 15 wt% PSHU-PNIPAM-Sul-100 loaded with 500ng bFGF and analyzed the results using an enzyme-linked immunosorbent assay (ELISA).

Results: Both 1H NMR and FT-IR (data not shown) were used to confirm the overall polymer structure and ensure the presence of free functionalizable amines on the backbone. Poly(N-isopropyl acrylamide) (PNIPAAm, Mw: 11,000) was conjugated to PSHU to form the PSHU-PNIPAAm copolymer. Next, we confirmed the polymer structure and successful sulfonation using fourier transform infrared spectroscopy (FT-IR) (data not shown), nuclear magnetic resonance (1H NMR) (data not shown), and elemental analysis (Figure 1). During the FGF release test, we were able to see from this study that the

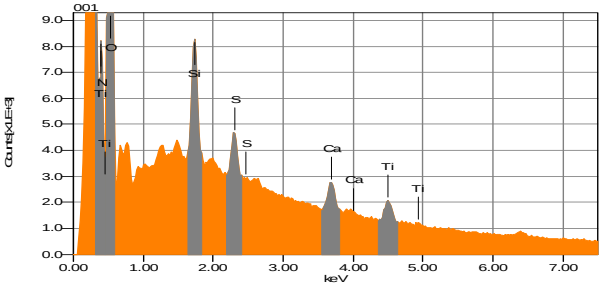

Figure 1. *Elemental analysis of PSHU-NIPAAm-Sul-100 showing the appearance of a sulfate groups on the polymer backbone.*

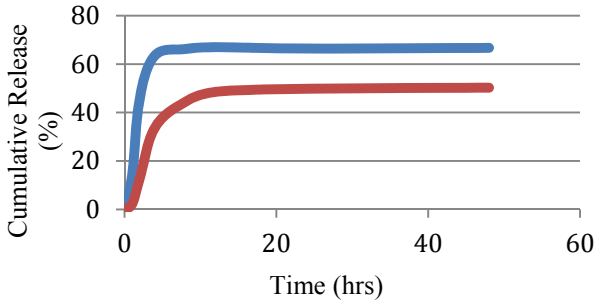

Figure 2. *bFGF release profile of sulfonated polymer with (red) and polymer with sulfonation (blue).*

sulfonated polymer system (PSHU-PNIPAM-Sul-100) did not show as large of a burst release as the non-sulfonated polymer system (PSHU-PNIPAM) (Figure 2).

Conclusion : These results indicate that the addition of the sulfonate groups, may prevent the bFGF from being released all at once. However, we can also see from that most of the bFGF was released from both systems in less than 3 days. Damaged cardiac tissues must be exposed to bFGF for a long timeframe to have any significant effects [9]. Thus the duration of bFGF release from the polymer system must be increased for this application. We plan to increase the amount of sulfonate groups on the polymer backbone by altering the reaction conditions and initiator amounts. After repeating the release test with an increased amount of sulfonate groups, we plan to induce MI in a mouse model and inject this GF-polymer system within the infarct site of the heart wall. With the help of collaborators in the cardiothoracic surgery department at UC Denver, we will analyze this polymer system's ability to revascularize damaged cardiac tissue and improve heart function.

1. Jawad, H. *et al.* Myocardial tissue engineering : a review. 327–342 (2007).doi:10.1002/term

2. Segers, V. F. M. & Lee, R. T. Protein therapeutics for cardiac regeneration after myocardial infarction. *Journal of cardiovascular translational research* **3**, 469–77 (2010).

3. Freedman, S. B. & Isner, J. M. Therapeutic angiogenesis for ischemic cardiovascular disease. *Journal of molecular and cellular cardiology* **33**, 379–93 (2001).

4. Yancopoulos, G. D. *et al.* Vascular-specific growth factors and blood vessel formation. **407**, (2000).

5. Park, H. D. *et al.* Anticoagulant activity of sulfonated polyrotaxanes as blood-compatible materials. (2001).doi:10.1002/jbm.10054

6. Bentz, H. & Estridge, T. D. Collagen and heparin matrices for growth factor delivery. **49**, 291–298 (1997).

7. Nguyen, T. H. *et al.* A heparin-mimicking polymer conjugate stabilizes basic fibroblast growth factor. *Nature chemistry* **5**, 221–7 (2013).

8. Wu, L., Jasinski, J. & Krishnan, S. Copolymer Coatings : A Comparison of the Surface Properties and Antibiofouling Behavior. (2011).doi:10.1002/app

9. Tayalia, P. & Mooney, D. J. Controlled growth factor delivery for tissue engineering. *Advanced materials (Deerfield Beach, Fla.)* **21**, 3269–85 (2009).