Heparinized PLGA-PEG-PLGA copolymer as a bioactive injectable matrix

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Statement of Purpose: Most modern tissue engineering biomaterials strive to implement a bioactive design, where cell adhesion through cell surface receptors is mediated by interactions with proteins adsorbed on the material. Accordingly, a number of studies on bioactive hydrogels have made process toward introducing ECM-like proteins and polysaccharides for improved tissue compatibilty.

Heparin (HP) has been knowns to control cellular responses by binding with various growth factors as well as ECM proteins. Biodegradable *in situ* gel-forming matrices with thermosensitivity offer several advantages over systems shaped into their final form before implantation. Especially, one of the most studied copolymer, PLGA-PEG-PLGA triblock hydrogel gains importance due to thermogelling and biocompatible properties.

In this study, heparinized PLGA-PEG-PLGA hydrogel was simply prepared via Michael type reaction and the physico-chemical properties were characterized. To further explore applications of this material in bioactive tissue-regenerative matrix, *in vitro* basic fibroblast growth factor (bFGF) release behaviors and cell study were studied.

Methods: Thiolated heparin (HP-SH) for Michael type reaction was synthesized as previously reported by Cai *et al*. PLGA-PEG-PLGA triblock copolymer was synthesized by bulk ring-opening polymerization of D,L-lactide and glycolide (molar ratio 3:1) in the presence of poly(ethylene glycol) 1K (PEG) and stannous octoate. PLGA-PEG-PLGA was end-functionalized by acryloyl chloride and triethylamine (TEA). The quantity and pKa value of free thiol groups in HP-SH was analyzed by Ellman method and UV spectrophotometer. The activity of HP-SH was evaluated by chromogenic assay. PLGA-PEG-PLGA and its diacrylate were confirmed by ^{1}H-NMR and GPC. The conjugation of HP-SH to PLGA-PEG-PLGA diacrylate was performed by simply mixing both components (HP-SH:PLGA-PEG-PLGA diacrylate; thiol:acrylate molar ratios of 2:1 and 4:1). Thermal gelling properties of heparin conjugated hydrogels were investigated by a vial-tilting method. *In vitro* bFGF release test was carried out by following 4h incubation of bFGF in heparinized polymer solution.

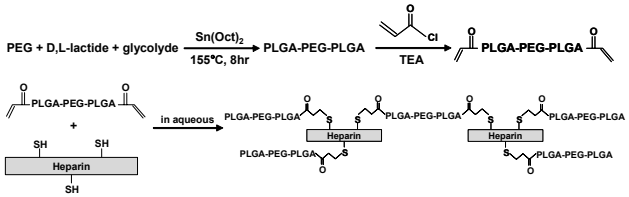

Figure 1. Synthetic scheme of heparinized PLGA-PEG-PLGA

Results/Discussion: The chemical modification of HP was successfully accomplished and confirmed by ^{1}H-NMR. The free thiols on the side chain of HP-SH were measured to be 0.7μmol/IU. The pKa value of thiol-containing molecular such as HP-SH is critical for evaluating its reactivity by Michael type reaction and the pKa value of HP-SH was approximately 10.5. The Mw of PLGA-PEG-PLGA was about 5500 (PDI 1.2). The sol-gel transition diagram of triblock copolymer reveals that 15-25% (w/v) aqueous polymer solution converts to a gel phase at 20°C. Acrylation of triblock copolymer was confirmed by ^{1}H-NMR spectrum, supporting the complete conversion to acryl group. The phase transition behavior of PLGA-PEG-PLGA diacrylate was similar to that of PLGA-PEG-PLGA.

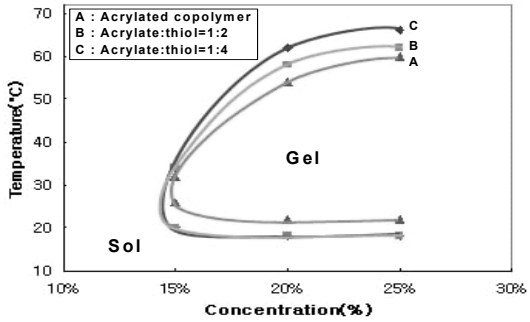

Figure 2. Phase diagram of heparinized PLGA-PEG-PLGA

Figure 2 shows the phase diagrams of heparin conjugated PLGA-PEG-PLGA with different molar ratio of HP-SH and 15-25% (w/v) of diacrylated triblock copolymer. Overall, the gel zone in the phase diagram becomes larger with increasing HP-SH content in the triblock copolymers. More detailed HP-SH effect on the gelation mechanism and in vitro bFGF release are under investigation.

Conclusions: In summary, heparinized PLGA-PEG-PLGA was prepared and showed the sol-gel transition behavior around body temperature. The addition of thiolated heparin into the polymer solution could improve the thermal stability of hydrogel through additional Michael type reaction. Therefore, heparin conjugated PLGA-PEG-PLGA hydrogel can be used for various medical applications, particularly localized delivery vehicle of heparin binding growth factors as well as a bioactive tissue-regenerative injectable matrix.

References:

[1] S. Cai, Y. Liu, X. Zheng Shu, G.D. Prestwich, Biomaterials 26, 2005; 6054-6067

[2] B. Jeong, Y. H. Bae, D. S. Lee, and S. W. Kim, Nature, 1997; 388: 860-862.

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