Controlled release of bFGF from chemically-crosslinked chitosan/Pluronic hydrogel containing heparin

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Statement of Purpose: Controlling release of growth factors from hydrogels has been challenging because encapsulated proteins often showed initial burst release profiles due to fast diffusion process. Furthermore, the released growth factors often showed decreased bioactivity due to instability of growth factors. Heparin widely used to release heparin-binding growth factor such as basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF). When bFGF bind to heparin, heparin enhances the half-life and activity of bFGF, also, protects bFGF from inactivation by acid and heat [1,2]. Furthermore, heparin-binding bFGF has a high-affinity to bFGF receptor because heparin plays as a cofactor to promote a binding of bFGF and bFGF receptor [3,4]. Chitosan, a positively-charged polysaccharide, has a wide application to biomedical fields because of superior biocompatibility. In this study, a hydrogel composed of chitosan and Pluronic was prepared and effects of the chitosan/Pluronic hydrogel containing heparin on bFGF release were investigated to control release of the growth factor from the hydrogel.

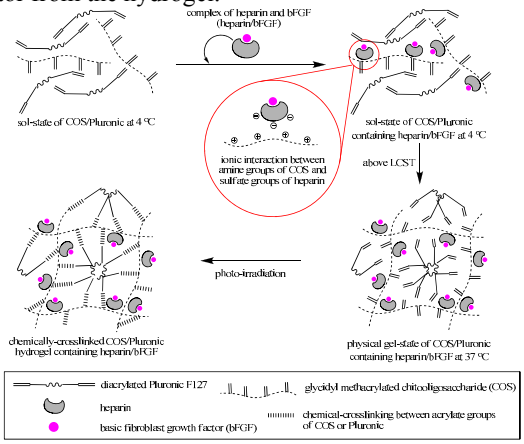

Figure 1. Schematic diagram of preparing chemically-crosslinked COS/Pluronic hydrogels containing heparin/bFGF.

Methods: Glycidyl methacrylated chitooligosaccharide (COS, Mw 10.0kDa) and di-acrylated Pluronic F127 ($(PEO)_{99}(PPO)_{69}(PEO)_{99}$, Mw 12.7kDa) were co-dissolved in deionized water to prepare 23.16% (w/v) hydrogels containing 0, 20, and 50%(w/w) of COS. A photo-initiator, Irgacure2959 (0.01%, w/w), was added to the polymer mixture. In order to prepare final concentration of 22%(w/v) for precursor hydrogel solutions containing protein solution, 190µl of the homogeneous polymer solutions were mixed with 10µl of mixture of recombinant human bFGF (1µg, Mw 17.2kDa) and heparin (Mw 4.3kDa) (molar ratio of bFG:heparin; 1:10). The precursor hydrogel solutions were poured in a siliconized 24-well plate and incubated at 37 °C for 1 h to prepare physical hydrogel. The hydrogels containing bFGF/heparin were photo-irradiated by long wave UV for 15s to photo-crosslink the COS/Pluronic hydrogels. A light source operated from 320 nm to 500 nm was located 1.5 cm from the top of the hydrogel and the output was fixed at 18 $W \cdot cm^{-2}$. In order to measure mass erosion of COS/Pluronic hydrogels and release profile of bFGF from COS/Pluronic hydrogels *in vitro*, detached hydrogels from the 24-well plate were transferred to siliconized 50-ml conical tubes and thoroughly washed with 10 ml of PBS (pH 7.4) at 250 rpm for 5 min. Hydrogels were incubated in siliconized 50-ml conical tubes with 10 ml of PBS (pH7.4) at 250 rpm, 37 °C. Released bFGF from the hydrogel was measured by ELISA.

Results: Due to the electrostatic interaction between positively charged COS and negatively charged heparin of a complex of heparin and bFGF, the loading efficiency of bFGF in a hydrogel was increased along with the increment of blend ratios of COS from 0% to 50%. Furthermore, when a content of COS was 50%, the degradation rate of hydrogel was slower than those of 0% and 20% COS and the initial burst release of bFGF was reduced by 2.4-fold and 1.4-fold compared to those at 0% and 20%, respectively.

Table 1. Loading efficiencies of bFGF in COS/Pluronic hydrogels (n=3).

blend ratio of COS/Pluronic	0:10	2:8	5:5
loading efficiency (%)	85.9±0.6	89.9±0.7	94.6±2.4
loading amount (µg/device)	0.86±0.06	0.90±0.06	0.95±0.24

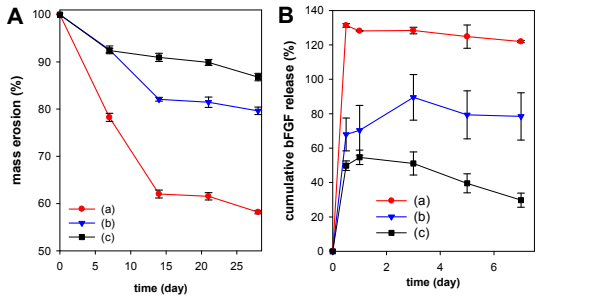

Figure 2. (A) Mass erosion of COS/Pluronic hydrogels (n=6). (B) Release profiles of bFGF from COS/Pluronic hydrogels for 7 days (n=3). Blend ratios of COS to Pluronic; (a) 0:10, (b) 2:8, and (c) 5:5.

Conclusions: Encapsulation of bFGF in the hydrogel was controllable by changing COS contents in the hydrogel. bFGF in chemically-crosslinked COS/Pluronic hydrogel containing heparin showed sustained release profiles upon increasing the amount of COS.

References:

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