The effect of crosslinking of chitosan microspheres with genipin on protein release

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Statement of Purpose:

Chitosan, one of the most abundant polysaccharides found in nature, has been widely researched for biomedical applications.[1,2] In drug delivery applications, chitosan has been used as a vehicle for drug, protein and gene delivery.[3] Crosslinking of hydrophilic polymers such as chitosans allows them to deliver a drug over extended periods of time. In this study, immuno bovine albumin fraction V (IBAFV) as a model protein was mixed with chitosan to make microspheres and then crosslinked by genipin,[4] a natural crosslinking agent. The effect of crosslinking time on swelling ratio, the degree of crosslinking and the elution of IBAFV from the microspheres was evaluated.

Methods:

Chitosan with 87.4% degree of deacetylation (DDA) was obtained from Vanson HaloSource (Redman, WA). IBAFV was bought from MP Biomedical Inc. (Aurora, OH). Genipin was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other reagents were reagent grade. The plain chitosan microspheres were made using a solution of 3.5% chitosan in 2% acetic acid dripped via a syringe pump into NaOH and methanol solution. Microspheres were rinsed with deionized water to pH<8 and then crosslikend in 0.5 mM genipin solution at room temperature (rt) for 4, 8 and 16 hrs. The chitosan microspheres with IBAFV were made in the same way using a solution of 3.5% chitosan and 1% IBAFV in 2% acetic acid. The swelling ratio of plain microspheres was determined by immersing the microspheres in a phosphate buffered saline (PBS) (pH=7.4) at rt for 24 hrs with gentle shaking. Subsequently, the weight of the swollen microsphere was measured and swelling ratio was calculated. The degree of crosslinking of the plain chitosan microspheres was determined by ninhydrin assay.[4] The assay determines the percentage of free amino groups remaining in the chitosan microspheres after crosslinking. The elution of IBAFV was characterized by placing groups of 0.2g chitosan microspheres with IBAFV (n=3) in 2.5mL PBS at 37˚C with gentle shaking. At day 1, 2, 3, 7, 17, 24 and 31, the PBS solution was changed and the amount of protein in the eluate was determined by bicinchoninic acid (BCA) assay (Pierce, Rockford, IL).

Results / Discussion:

Chitosan, containing hydroxyl and amino groups, is readily hydrated in water. It was found that genipin may undertake a ring-opening reaction to form an intermediate aldehyde group due to the nucleophillic attack by the amino groups in chitosan. As expected, crosslinking of plain chitosan microsphere with genipin decreased its swelling ratio with the increase of crosslinking time (Fig. 1). Also the degree of crosslinking of plain chitosan microspheres increased with the crosslinking time (Fig. 2). The degree of crosslinking did not increase much after 8 hrs. This may be due to the outer layers of microspheres being crosslinked. This makes crosslinking of inner layers more difficult. From Fig. 3, it was detected that IBAFV was released less from crosslinked microspheres than that from the non-crosslinked microspheress over the entire 31 day test period. The amount of the released IBAFV decreased with the increase of the density of crosslinking during the experimental period. The crosslinking can extend the drug or protein delivery over longer period.

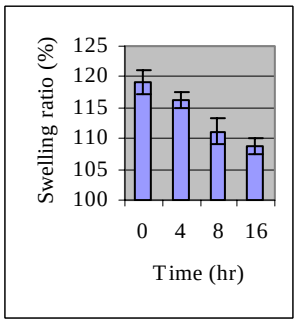

Fig.1 Swelling ratio of microspheres increased with genipin crosslinking time

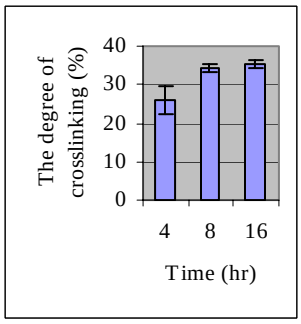

Fig.2 The degree of crosslinking increased with time up to 8 hrs

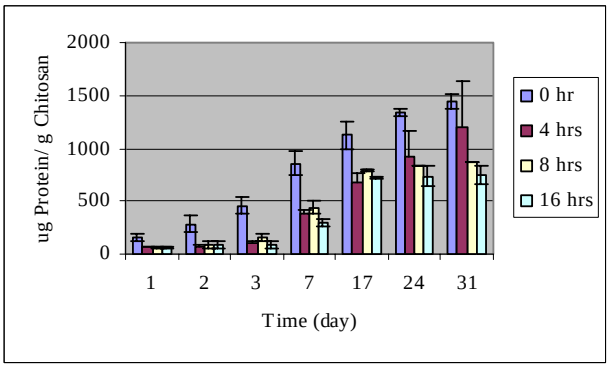

Fig.3 The accumulated release profile of IBAFV from chitosan microspheres after crosslinking with genipin.

Conclusions:

In this work, chitosan microspheres crosslinked with genipin released IBAFV more slowly than non-crosslinked microspheres. This slower release rate may be beneficial to the long term drug or protein delivery. These research data suggest that protein or drug release rates may be controlled by the degree of crosslinking and the swelling ratio of chitosan microsphere.

References:

1. Martino AD, Sittinger M, Risbud MV. Biomaterials. 2005;26:5983-5990.
2. Khor E, Lim LY. Biomaterials. 2003;24:2339-2349.
3. Sinha VR, Singla AK, Wadhawan S et al. Inter J Pharm. 2004;274:1-33.
4. Mi FL, Tan YC, Liang HC et al. J Biomater Sci Polymer Edn. 2001;12:835-850.