The effect of cross-linking on release of alkaline phosphatases of chitosan microspheres

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Introduction:

Chitosan, derived from chitin, 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] Cross-linking of hydrophilic polymers such as chitosans allows them to deliver a drug over extended periods of time. In this study, alkaline phosphatases (ALP) as a model protein was absorbed by chitosan microspheres and then cross-linked by genipin,[4] a natural cross-linking agent. The effect of genipin concentration on swelling ratio and the degree of cross-linking of chitosan microspheres and the elution of ALP from the chitosan microspheres were evaluated.

Methods:

Chitosan with 87.4% degree of deacetylation (DDA) was obtained from Vanson HaloSource (Redman, WA). ALP 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 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 dried. The chitosan microspheres with ALP were made by putting swollen chitosan microspheres (pre-swelled in DI water for 24 hours) into 1 mg/mL ALP solution for 24 hours and then rinsing with DI water and drying at room temperature. Microspheres without ALP absorbed were cross-linked as controls and to evaluate cross-linking concentrations on swelling. ALP absorbed cross-linked chitosan microspheres were obtained by putting dried ALP absorbed chitosan microspheres in 5 mM genipin solution for 2 hours and rinsing with DI water and drying. The swelling ratio of microspheres was determined by immersing the microspheres in a phosphate buffered saline (PBS) (pH=7.4) at room temperature for 24 hours with gentle shaking. Subsequently, the weight of the swollen microsphere was measured and swelling ratio was calculated. The degree of cross-linking 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 cross-linking. The elution of ALP was characterized by placing groups of 0.18 g chitosan microspheres with ALP (n=3) in 3 mL PBS at 37˚C with gentle shaking. At day 1, 2, 3, 7, 14, 21, 30, 37, 44, 51, 58 and 65, the PBS solution was changed and the amount of ALP in the eluate was determined by ALP assay (Sigma, St Louis, MO).

Results/Discussion:

Chitosan, containing hydroxyl and amino groups, is readily hydrated in water. Genipin cross-links chitosan by undertaking a ring-opening reaction.[4] As expected, cross-linking of plain chitosan microspheres with genipin decreased their swelling ratio with an increase of genipin concentration (Fig. 1). Also the degree of cross-linking increased with the genipin concentration (Fig. 2). The degree of cross-linking did not increase much after 1 mM genipin concentration. This was due to the outer layers of microspheres being cross-linked. This makes cross-linking of inner layers more difficult. ALP absorbed chitosan microspheres were cross-linked using 5 mM genipin solution for 2 hours in order to be cross-linked with less time to get high degree of cross-linking. It was found that the degree of cross-linking was 30.3% and swelling ratio was 100.3%. From Fig. 3, it was detected that ALP was released much less from cross-linked microspheres than that from the non-cross-linked microspheress over the entire 65 day test period. Some ALP may be lost during the process of cross-linking. After 30 days, ALP release was very small and hence accumulation levels did not increase substantially. The cross-linking can change the drug or protein delivery. The loading method of ALP is needed to be improved.

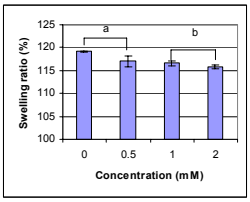

Fig.1 Swelling ratio of microspheres decreased with genipin concentration

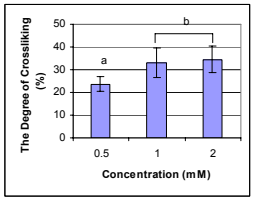

Fig.2 The degree of cross-linking increased with genipin concentration

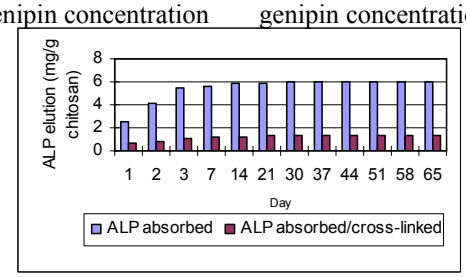

Fig. 3. The accumulated release profile of ALP from chitosan microsphers and cross-linked chitosan microspheres by genipin

Conclusions:

In this work, ALP absorbed chitosan microspheres cross-linked with genipin released ALP much less than that of non-cross-linked ones. The cross-linking of microspheres has effect on protein elution. These research data suggest that protein or drug release rates may be controlled by the degree of cross-linking and the swelling ratio of chitosan microspheres. The ALP loading method is needed to be improved.

References:

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