생체접착성 Alginate-WGA 마이크로입자의 점막 전달 시스템으로서의 연구

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Bioadhesive Alginate-WGA Microparticles for Mucosal Delivery Systems

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Introduction

The main effect of bioadhesion is to improve the therapeutic efficiency of various active ingredients by increasing their residence time at their optimal activity or resorption. Lectins are proteins which bind specifically to sugars, and therefore agglutinate cells and polysaccharides and glycoconjugates. New systems for specific bioadhesion have been proposed recently. One approach consists in the conjugation of lectins with colloidal polymeric particles which may constitute either a drug carrier for therapeutical applications (drug resorption or local activity) or a vaccine adjuvent [Irache et al., 1994].

Spray drying method has been widely used for the preparation of microparticles due to the following advantages: It is a well-established technology, involves readily available equipment, and is able to produce large amounts of microparticles. It is an expeditious, single-step process, and the resultant microparticles have a narrow size distribution.

Recently, a biosensor based on surface plasmon resonance (SPR) has been used to analyze interactions of glycoproteins, glycopeptides, and oligosaccharides with lectins in real-time without fluorescence or radioisotope labeling [Zeng et al., 1998].

In this study, lectin(WGA) was conjugated on alginate and alginate-WGA microparticles were prepared by the spray drying method. The activity of microparticles with gastric pig mucin (GPM) was studied in vitro. In addition, the activity of WGA conjugated alginate with GPM was studied by surface plasmon resonance (SPR).

Experimental

Materials

Lectin from triticum vulgaris (wheat germ, WGA), mucin (Type Ⅱ: crude from porcine stomach) Triton X-100 were purchased from Sigma. N-hydroxysuccinimide, 11-mercaptoundecanoic acid(MUA), alginic acid sodium salt, calcium chloride dehydrate, 1,1'-carbonylimidazole (CDI) were purchased from Aldrich.

Conjugation of WGA to alginate

Alginate powder was treated with CDI in anhydrous DMSO (alginate is not soluble in DMSO) for 48 hours

at room temperature. The activated alginate was washed with excess DMSO to remove unreacted CDI and 0.2M borate buffer, pH 9.5 containing WGA was added to the powder. The ligand coupling step was allowed to continue for 24hours at room temperature. Unbound lectin was removed from the swollen powder by careful washing with borate buffer [Mathiowitz et al., 2001]. The product was analyzed by NMR spectroscopy.

Preparation of alginate and alginate-WGA microparticles

2% w/v of sodium alginate (Na-A) or alginate-WGA were spray dried by a Buchi B-191 Mini Spray Dryer. The resultant microparticles were dispersed in 0.1M CaCl2 solution with vigorous stirring at 2000rpm at room temperature for 10min. The cross-linked microparticles were washed with water, centrifuged at 7000rpm and vacuum dried. The size and shape of the microparticles were examined by Scanning Electron Microscopy(SEM) [Coppi et al., 2001].

In vitro studies with gastric pig mucin (GPM)

GPM was chosen as a model to determine the in vitro activity of alginate and alginate-WGA microparticles towards the sugar residues of a glycoprotein. The activities of alginate and alginate-WGA microparticles were determined by mixing 1 ml of the GPM suspension in PBS (1mg/ml) with 1ml of the suspension of the alginate and alginate-WGA microparticles in PBS. After incubation, the samples were centrifuged for 30min at 7000rpm and the remaining free GPM in the supernatants was measured by UV spectroscopy. The amount of interacted GPM was calculated as the difference between references (without alginate and alginate-WGA microparticles) and the supernatants of the samples, since interacted GPM was sedimented together with the microparticles [Irache et al., 1994].

SPR experiments

For SPR measurements, Spreeta^{TM} Evaluation Module (SPR-EVM-BT) Surface Plasmon Resonance Biosensor (Nomadics, Inc. OK, USA) was used (figure 1). MUA monolayers were formed on gold films by pumping 1mM ethanolic solution through the flow cell of the SPR sensor for 30min at a flow rate of 40μℓ/min at room temperature [Woodbury et al., 1998;].

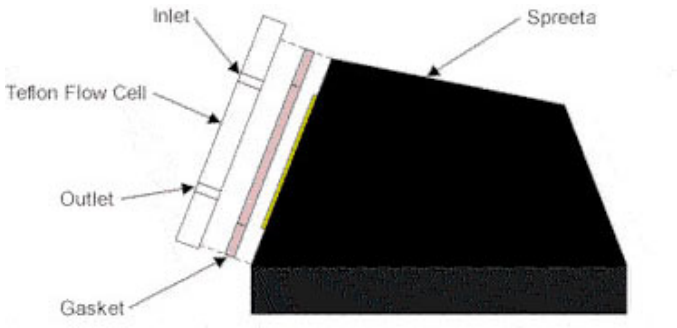
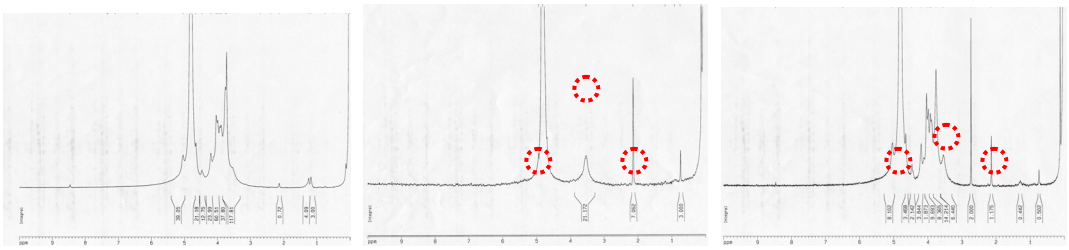


Figure 1. Spreeta sensor with flow cell

Results and Discussion

The NMR spectra of alginate, WGA and alginate-WGA are shown in figure 1. The characteristic peak of WGA appears in 0.74, 2.1, 3.5 ppm which are also present in alginate-WGA NMR peaks and verifies that WGA

was conjugated on alginate.

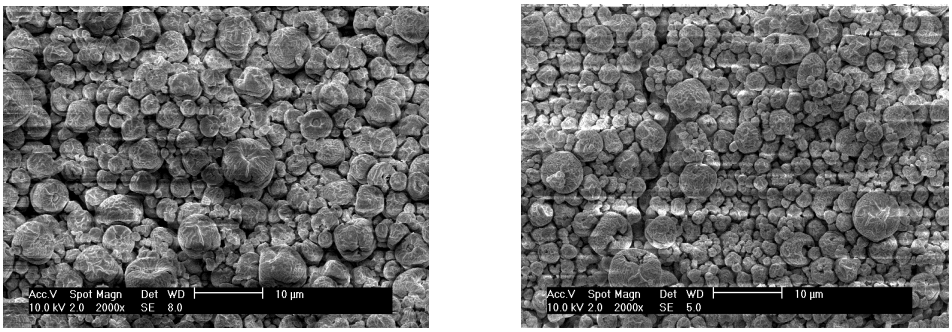


(a)alginate (b)WGA (c)alginate-WGA

Figure 1. The NMR spectra of alginate, WGA and alginate-WGA

(a) alginate, (b)WGA, (c)alginate-WGA

Figure 2 shows the SEM photograph of alginate and alginate-WGA microparticles. The microparticles are spherical and less than 10㎛, therefore suitable to be taken up by the Peyer's patches.



(a) alginate microparticles (b) alginate-WGA microparticles

Figure 2. The SEM photographs of alginate and alginate-WGA microparticles.

The amount of interacted GPM with alginate and alginate-WGA microparticles are shown in figure 3. The alginate-WGA microparticles interacted with GPM, whereas alginate microparticles didn't interact with GPM very much. This result can be attributed to the interaction between GPM and WGA in the alginate-WGA microparticles.

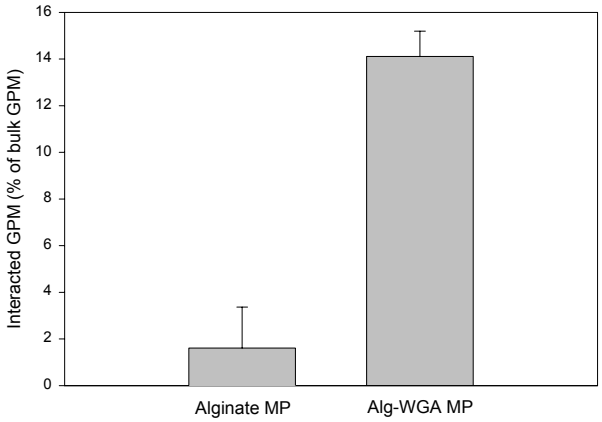


Figure 3. GPM binding to alginate and alginate-WGA microparticles.

Figure 4 shows the change in refractive index over time during the binding of MUA on gold surface of SPR

sensor. The refractive index doesn't change very much during the washing of sensor surface after the MUA attachment. It can be concluded that the MUA was bound to the gold surface of sensor. The SPR curves that the carboxyl group of MUA is activated and GPM is attached to this activated MUA, the alginate-WGA is bound to GPM will be studied in near future.

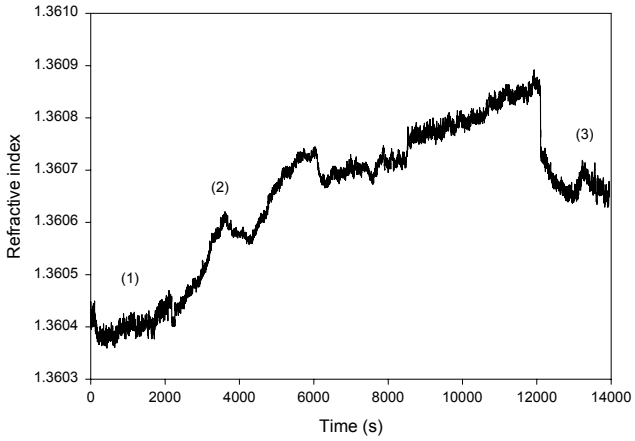


Figure 4. Binding of MUA on gold surface of SPR sensor: (1) flow of ethanolic solution as a baseline, (2) flow of 1mM MUA ethanolic solution, (3) flow of ethanolic solution for washing.

References

Coppi, G., Iannuccelli, V., Leo, E., Bernabei, M. T. and Cameroni, R., "Chitosan-Alginate Microparticles as a Protein Carrier", Drug Development and Industrial Pharmacy, **27**, 393 (2001).

Irache, J. M., Durrer, C., Duchene, D. and Ponchel, G., "Preparation and characterization of lectin-latex conjugates for specific bioadhesion", *Biomaterials*, **15**, 899 (1994).

Irache, J. M., Durrer, C., Duchene, D. and Ponchel, G., "In vitro study of lectin-latex conjugates for specific bioadhesion", *Journal of Controlled Release*, **31**, 181 (1994).

Mathiowitz et al., "Bioadhesive microspheres and their use as drug delivery and imaging systems", US patent 6,217,908 B1 (2001).

Woodbury, R. G., Wendin, C., Clendenning, J., Melendez, J., Elkind, J., Bartholomew, D., Brown, S. and Furlong, C. E., "Construction of biosensors using a gold-binding polypeptide and a miniature integrated surface plasmon resonance sensor", Biosensors & Bioelectronics, **13**, 1117 (1998).

Zeng, X., Murata, T., Kawagishi, H., Usui, T. and Kobayashi, K., "Analysis of specific interactions of synthetic glycopolypeptides carrying N-acetyllactosamine and related compounds with lectins", *Carbohydrate Research*, **312**, 209 (1998).

Acknowledgement

This research was funded by Center for Ultramicrochemical Process Systems sponsored by KOSEF and partially by Brain Korea 21 Project.