Interaction between Artificial Mucin Layer and Stimuli-Responsive Nanogel Particles for the Oral Peptide Delivery Observed in Simulated Intestinal Solutions by Using Colloid Probe AFM Method

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ABSTRACT

In order to characterize and control the adhesive behaviors of nanometer scaled stimuli-responsive gel particles designed for oral peptide delivery, their interaction with artificial mucin layer in the small intestinal solutions was determined by the colloid probe atomic force microscope method. The prepared nanometer scaled gel particles with a core-shell structure were designed to exhibit behaviors responsive to temperature and pH in solutions, consequently protect the incorporated peptide drug under harsh acidic conditions in the stomach, adhere and penetrate to the mucin layer in the small intestine, and thereafter release the drugs. Spherical agglomerates of the nanogel particles with several micron meters in diameter were prepared by the spray freeze drying method and adhered on the top of tip of commercial atomic force microscope. The interaction between the artificial mucin layer and nanogel surface determined by the colloid probe method depended on pH of the solution. Based on the possible transition of the surface-microstructure of nanogel particles following the pH change and the measured results from the colloid probe AFM method, the relationship between surface interaction and microstructure of nanogel was discussed.

Keywords: stimuli-responsive nanogel, oral peptide delivery, colloid probe AFM, mucin

1 INTRODUCTION

Significant advances in biotechnology and biochemistry have led to the discovery of a large number of bioactive molecules, such as peptides and proteins. However, bioavailability of peptides and proteins as therapeutic agents after oral administration is very low in general due to epithelial barriers of the gastrointestinal tract and gastrointestinal degradation by digestive enzymes. This requires a delivery system with specific abilities such as protecting the peptides from harsh manufacturing and physiological environment and enhancing the drug absorption at the preferable sites in the gastrointestinal tract.

One approach of achieving such specific functions is to use stimuli-responsive polymers. In our previous work, thermo- and pH-sensitive nanogel particles with core-shell structure (CSNPs) have been designed as a functional particulate device for oral peptide delivery [1]. The employed core and shell components for the CSNPs are poly(*N*-isopropylaclyramide) (p(NIPAAm)) hydrogel and poly(methacrylic acid) grafted with poly(ethylene glycol) monomethacrylate (p(MAA-*g*-EG)), respectively.

The proposed performances of the CSNPs thus designed are as follows: (1) Peptides can be loaded at such mild conditions as low temperature and neutral pH where both core and shell components swell; (2) Both components remain collapsed in the gastric fluid around body temperature, so that peptides can be protected from enzymatic degradation; (3) In the small intestine, the swollen p(MAA-*g*-EG) shell can adhere and penetrate to the mucin layer and peptide-transport enhancing effect in the mucosal cell lining, while the p(NIPAAm) core remains shrunken, allowing the peptide release in a prolonged manner. In order to adhere on the mucin layer and penetrate peptide transport, it needs to control and design the interaction between mucin and shell surface of CSNPs. It is expected that new testing system, colloid probe AFM method [2] is useful to characterize such surface interaction between colloidal particle and surface layer in solution.

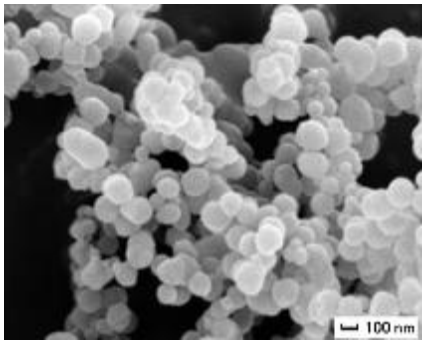
In this present paper, in order to characterize the above performance of CSNPs for the application of oral peptide delivery, the interaction between pH-sensitive nanogel particle and artificial mucin layer in the small intestinal solutions was determined by the colloid probe atomic force microscope method in aqueous solution with different pH.

2 EXPERIMENTAL PROCEDURE

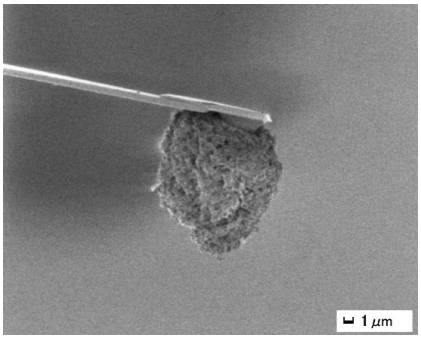
2.1 Preparation of Nanogel Colloid Probe and Mucin Substrate on Mica Surface

Nanogel particles were synthesized by a semi-continuous two-stage photo-initiated free radical dispersion polymerization technique. The detail preparation process had been reported in previous paper [1]. The core and shell

components for the CSNPs are poly(*N*-isopropyl-aclyramide) (p(NIPAAm)) hydrogel and poly(methacrylic acid) grafted with poly(ethylene glycol) monomethacrylate (p(MAA-*g*-EG)). Nanogel particles were dispersed into phosphate buffer aqueous solution with pH = 3.6 in order to shrink the shell of CSNPs. The solid fraction of this prepared suspension was 0.05 wt%. This dilute nanogel suspension was sprayed on the frozen copper plate and dried under vacuum for 24 hours. The freeze dried granule of nanogel with several micron meters in diameter was adhered on the top of a commercial tip for an atomic force microscope, AFM, by using a micro-manipulation system. The detail structure of a colloid probe preparation system was described in our previous papers [2, 3]. An example of nanogel granule and a colloidal probe prepared by the above system is shown in Fig. 1 (a) and (b), respectively.



(a) Freeze dried nanogel particle



(b) colloid probe of nanogel granule

Figure 1: FE-SEM observation of freeze dried nanogel granule and colloid probe.

Mucin substrate on mica was prepared by the following process. Firstly, silane coupling agent, 3-aminopropyl-triethoxy silane (Shietsu Chemical Co. Ltd., Japan, KBM-903) was added into aqueous solution with pH adjusted at 4.0, and agitated for 30 min for hydrolysis reaction of coupling agent. Small mica pieces were put into this solution and pH of aqueous solution was increased up to 12 in order to promote the condensation polymerization of coupling agent and mica surface. After slow shake of solution with mica pieces for 12 hours, mica pieces were picked up from solution and washed by ethanol to remove free coupling agent on the surface and obtained silane coupling treated mica (AP-mica).

Mucin particles derived from human stomach were dispersed in ultrapure water. Mucin suspension was filtered by a micro porous filter whose mean pore diameter was about 0.45 micrometers. Solid fraction of colloidal suspension of mucin was 0.5 wt%. Surface treated mica pieces by coupling agent was put into this mucin suspension and shaken for 24 hours. During shake of mica pieces in mucin suspension, mucin colloids were adsorbed on hydrophobic treated mica surface (M-AP-mica). The surface structure on mica was observed by an atomic force microscope, and the amount of silane coupling agent and mucin on mica was determined by a carbon, hydrogen and nitrogen analyzer.

2.2 Surface Interaction Measurement between Nanogel and Mucin Substrate

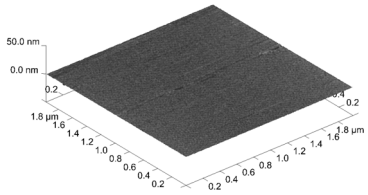
The interaction between a colloidal probe of nanogel particles and mica surface with each surface treatment, i.e., silane coupling and mucin adsorption, was measured by an atomic force microscope (PicoForce, Digital Instrument Co. Ltd.) in simulated intestinal solution prepared from phosphate buffer aqueous solution. In order to discuss the effect of pH on surface interaction between nanogel and mucin layer in stomach and small intestine, the pH of phosphate aqueous solution was adjusted at 3.6 for stomach and 5.3 and 6.8 for small intestine.

3 RESULTS AND DISCUSSION

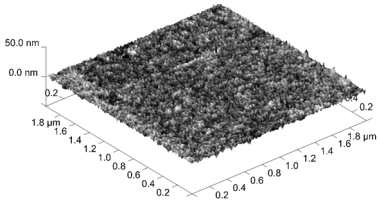
3.1 Surface Observation on Mica after Surface Modification

Surface structure change on mica after silane coupling and mucin treatment was observed by AFM and shown in Figure 2. The flat surface on mica was observed before surface treatment as shown in Figure 2(a). Surface roughness was increased by silane coupling treatment as shown in Figure 2(b). After mucin adsorption on mica surface, the smooth surface with local roughness was observed (Figure 2(c)). It seems that the mucin layer is formed on mica with silane coupling treatment.

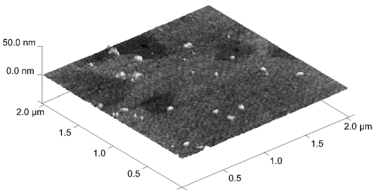
In order to confirm the surface adsorption of mucin on mica, the amount of reacted carbon and nitrogen on mica after each surface treatment is shown in Figure 3. Carbon and nitrogen content on mica was increased by each surface treatment, silane coupling and mucin adsorption. Since both observation and measurement were carried out after surface washing by ethanol, mucin colloids were adsorbed and strongly fixed on mica surface.



(a) Original mica surface



(b) After silane coupling treatment (AP-mica)



(c) After mucin adsorption (M-AP-mica)

Figure 2: Surface observation on mica without and with surface treatment by AFM.

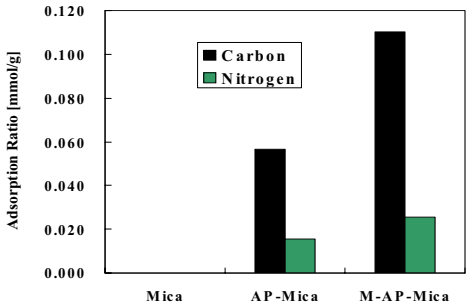


Figure 3: Amount of carbon and nitrogen on mica after each surface treatment.

3.2 Influence of Surface Treatment on Interaction between Nanogel and Substrate

Figure 4 shows the effect of surface treatment on mica on force curve between nanogel colloid probe and mica. The force curve in solution without surface treatment is shown in this figure. The pH of solution was adjusted at 6.8. Before surface treatment on mica, van der Waals type and electrostatic attractive force were only observed. If silane coupling treatment was carried out on mica, the attractive force was much larger than that of original mica. On the contrary, after adsorption of mucin on mica, the long range repulsive force was observed. Since nanogel and mucin have a negative charge at pH = 6.8, this repulsive force is genelated by the over rap of electrostatic double layer on mucin and nanogel.

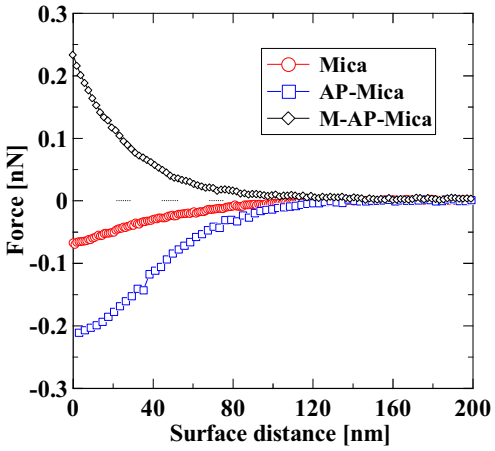


Figure 4: Force curve between nanogel colloid probe and mica substrate with and without surface treatment (Trace process).

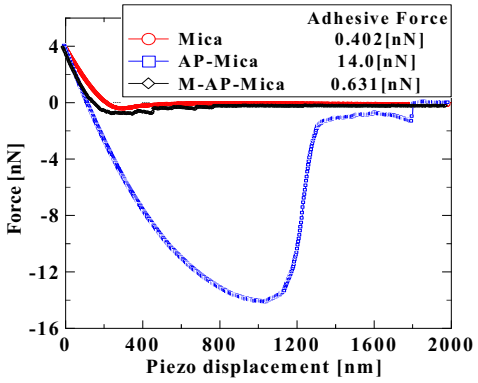


Figure 5: Force curve between nanogel particle and surface treated mica in PB aqueous solution with pH=6.8 (Retrace process).

In order to discuss the long range attractive force between nanogel and AP-mica surface, force curve during retrace process between nanogel colloid probe and substrate is shown in Figure 5. Since the long range attractive force between nanogel and AP-mica was observed, it is estimated

that the coupling agent is not reacting with the mica surface in the monolayer, multi-layer of coupling agent formed on mica and produced the long range and nonlinear attractive force by the bridge formation between hydrocarbon chain of coupling agent and nanogel surface. On the contrary, original mica and mocin adsorbed mica (M-AP-mica) were not formed such long range attractive force between nano-gel particle. By the surface adsorption of mucin, the surface was changed ridge structure as shown in Figure 2 (c) and the long range attractive force was disappeared.

3.3 Effect of pH on Surface Interaction between Nanogel and Mucin

Figures 6 and 7 show the effect of pH on force curve during trace and retrace process between nanogel and mucin layer. Since the p(MAA-*g*-EG) shell swelled and had a negative charge in neutral pH condition, the long range electro-steric repulsive force appeared between mucin and nanogel surfaces. At low pH condition, since shell shrank and formed ridge structure, the repulsive force and affecting distance was reduced.

Attractive force was strongly depended on pH as shown in Figure 7. Since the shell formed a ridge structure at low pH (pH = 3.6, simulated in stomach), the observed maximum adhesion force was high, however, the nanogel and mucin was separated, instantaneously. Once each surface separated, no interaction was observed. On the contrary, since shell swelled in neutral pH condition (pH = 6.8, simulated in small intestine), the long range attractive and non linier interaction was observed. This long distance attractive force is useful to capture nanogel particles at the mucin layer in small intestine. The design of shell molecular structure was success in the first step to penetrate to peptide into small intestine.

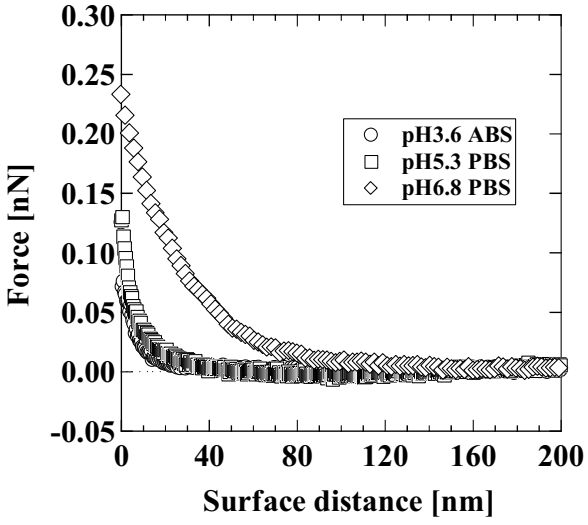


Figure 6: Effect of pH in solution on force curve between nanogel and mucin (Trace process).

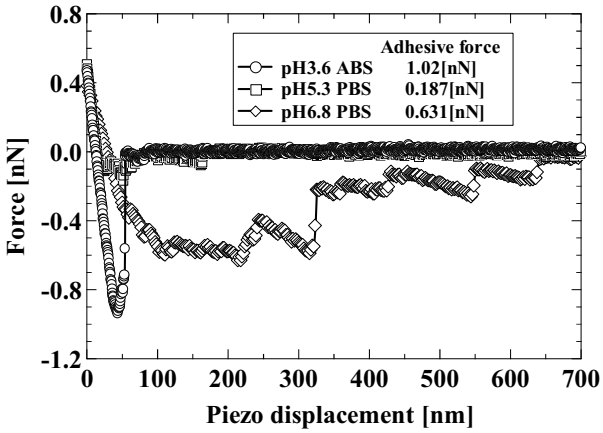


Figure 7: Effect of pH in solution on force curve between nanogel and mucin, retrace process.

4 CONCLUSION

By establishing the preparation methods of nanogel colloid probe and mucin layer on mica, the change of pH stimuli-responsive shell was evaluated by colloid probe AFM method. Since the p(MAA-*g*-EG) shell swelled and generated long range attractive interaction with mucin layer in neutral pH condition, it is possible to adhere on the mucin layer in small intestine and penetrate peptide by using this CSNPs.

REFERENCES

[1] H. Ichikawa, Y. Yamasaki and Y. Fukumori, "Novel composite nanoparticles of temperature-sensitive hydrogels with pH-sensitive polymeric layer for oral drug delivery", In New Trends in Polymers for Oral and Parental Administration, Editions De Sante, Paris, pp. 257-260 2001.

[2] W. A. Ducker, T. J. Senden and R. M. Pashley, "Measurement of forces in liquids using a force microscope," Langmuir, 8, 1831-36, 1992.

[3] H. Kamiya, S. Matsui and T. Kakui, "Analysis of action mechanism of anionic polymer dispersant with different molecular structure in dense silicon nitride suspension by using colloidal probe AFM," Ceramics Trans., vol. 152, 83-92, 2004.

[4] M. Tsukada, R. Irie, Y. Yonemochi, R. Noda, H. Kamiya, W. Watanabe and E. I. Kauppinen, "Adhesion force measurement of a DPI size pharmaceutical particle by colloid probe atomic force microscopy", Powder Technology, 142, 262-269, 2004.