MUCOADHESION OF POLYMER-COATED LIPOSOMES TO RAT INTESTINE *IN VITRO*

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Multilamellar liposomes consisting of dipalmitoyl phosphatidylcholine (DPPC) and dicetyl phosphate (DCP) in a molar ratio of 8:2 (DPPC:DCP=8:2) were coated with three different types of polymers: chitosan, polyvinyl alcohol having a long alkyl chain, and poly(acrylic acid) bearing cholesterols. The existence of polymer layers on the liposome surface was confirmed by measuring the zeta potential of the liposomal particles. The mucoadhesive function of the polymer-coated liposomes was evaluated *in vitro* using rat intestine. A particle counting method using the Coulter counter was adopted to evaluate the adhesive % of liposomes. Chitosan coated liposomes showed the highest adhesive % among the polymer-coated liposomes tested. No adhesive % was observed for the non-coated liposomes. The adhesion of chitosan-coated liposomes to the intestine wall was confirmed by fluorescence microscopy using pyren loaded liposomes.

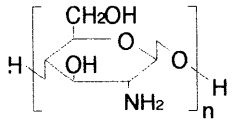
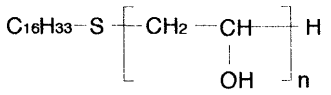
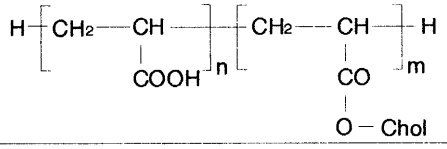
KEYWORDS liposome; mucoadhesion; chitosan; coating; polyvinyl alcohol; poly(acrylic acid)

Mucoadhesive dosage forms have received substantial attention as a novel drug delivery system to improve the bioavailability of drugs by prolonging the residence time and controlling drug release characteristics. The most extensively studied dosage forms having a mucoadhesive function are tablets for oral or buccal administration. The devices are completed by formulating mucoadhesive polymers such as hydroxypropylmethycellulose and Carbopol, and the adhesive properties have been tested with several methods.[1] A multi-unit bioadhesive system has also been prepared by coating microspheres of poly-hydroxyethyl-methacrylate with mucoadhesive polymers in laboratory scale equipment.[2] Recently, Pimienta et al.[3] demonstrated the bioadhesive properties of isohexylcyanoacrylate nanocapsules coated with poloxamers and poloxamine on rat ileal segments *in vitro* using a labeled compound.

The present paper reports that polymer coated liposomes function as a multi-unit bioadhesive device. The mucoadhesive property of the liposomes coated with three different polymers were tested with a rat intestine tube *in vitro* based on a particle counting method using the Coulter counter(Coulter Multisizer II, COULTER).

Liposomes were prepared with 24mg of L-α-dipalmitoylphosphatidylcholine (DPPC, Nippon Oil and Fats Co.) and 2.24mg of dicetyl phosphate(DCP, Sigma) by a thin film hydration method. A phosphate buffer solution (1ml, pH 7.4) was used in hydration. The polymers used for liposome coating are chitosan (CS, Katakurachikkarin Co.), polyvinyl alcohol having a long alkyl chain (PVA-R, Kuraray Co.) and poly(acrylic acid) bearing cholesterols (PAA-Chol), which was prepared with poly(acrylic acid) (Sigma).[4] The properties of polymers are summarized in Table I. PVA-R and PAA-Chol dissolved in a phosphate buffer solution (pH7.4), and CS dissolved in an acetate buffer solution (pH4.4) were used for coating of the liposomes. Polymer coating was carried out by mixing the resultant liposomal suspension (0.2ml) with the polymer solution (-1.5%, 0.2ml), followed by incubation.

Table I. Properties of Polymers Used for Liposome Coating

Polymer	Structure	Note
Chitosan		Degree of deacetylation = 85% Molecular weight = ca. 150,000
PVA-R		Degree of polymerization = 480
PAA-Chol		Molecular weight of PAA = 250,000

The size of liposomes measured with Cis-1(Galai Inc.) was 5-6 μm before and after polymer coating, which suggested that no aggregation of liposomal particles occurred during the polymer coating process. The change in surface properties of liposome particles was detected by measuring the zeta potential of the particles with a zeta meter (Laser Zee Meter 501, Penken, Inc.). The values of zeta potential of the polymer coated liposomes were found to be changed with the increasing concentration of polymer solution used in coating (Fig. 1). The change in zeta potential value of PAA-Chol coated liposomes was mainly attributed to the electric charge of the polymers fixed on the surface of the liposomes. Consideration of an ion complex formation of CS with DCP on the surface of liposomes in the coating process could explain the decrease of zeta potential of CS coated liposomes. In the case of the non-charged polymer, PVA-R, the formation of a polymer layer on the surface of liposomes could lead to moving the sheared plane to the solution side, which could then be responsible for the change of the zeta potential. These results confirmed the existence of a fixed polymer layer on the surface of liposome particles.

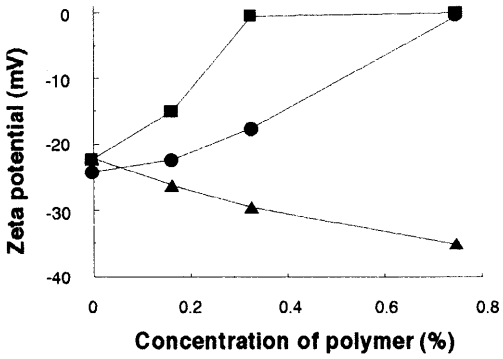


Fig. 1. Zeta Potential of Liposomes Coated with Various Polymers at pH7.4
(●) : Chitosan, (■) : PVA-R, (▲) : PAA-Chol; Lipid composition : DPPC:DCP=8:2.

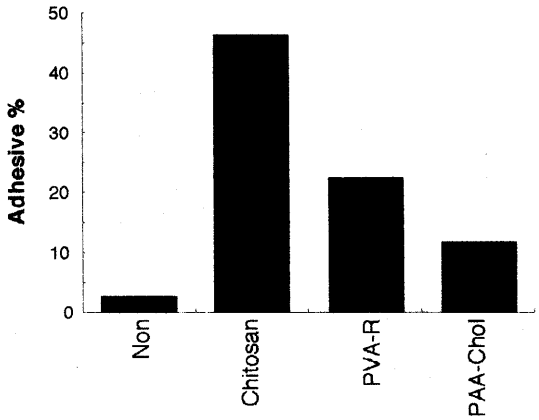


Fig. 2. Adhesive % of Liposomes Coated with Various Polymers to the Intestinal Tube of Rat

To evaluate the mucoadhesive function of the polymer-coated liposomes *in vitro*, a particle counting method using the Coulter counter was used after confirming a linear relationship between the liposomal concentration (μm lipid/ml) and the number of liposomes measured. The mucoadhesive test was carried out using an intestine tube (15cm) isolated from the Wistar rats. After washing the intestine tube with a saline solution, the tube was filled with the liposomal suspensions diluted by 100 times with a phosphate buffer solution (pH7.4), and then sealed with closers. The tubes were incubated in a saline solution at

37°C for 1 h and the number of liposomal particles was measured both before and after incubation. As the particle size distribution of liposomes was observed to be retained after incubation, the mucoadhesive % was presented by the following equation.

$$\text{Adhesive \%} = (\text{No} - \text{Ns})/\text{No} \times 100$$

where No and Ns are the number of liposomes before and after incubation, respectively.

As shown in Fig. 2, CS coated liposomes showed the highest adhesive % among the three different polymer-coated liposomes. The mucoadhesive function was thought to be offered by the polymer layer fixed on the surface of liposomes, because non-coated liposomes showed no adhesive %. An interpenetration between the polymeric and the mucous networks is considered to be responsible for the adhesion. The adhesive % of CS-coated liposomes depended on the CS concentration used for coating (Fig. 3). Combining the results shown in Figs. 1 and 3, it could be concluded that the more effective coating lead to the higher adhesive %.

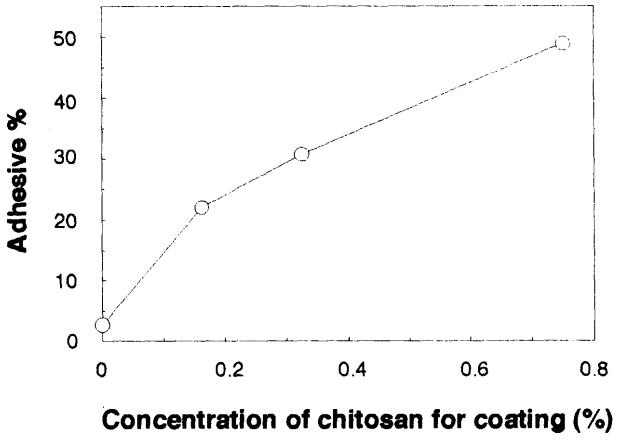


Fig. 3. Effect of Concentration of Chitosan for Coating on Adhesive % of Chitosan Coated Liposomes to the Intestinal Tube of Rat
Lipid composition : DPPC:DCP=8:2.

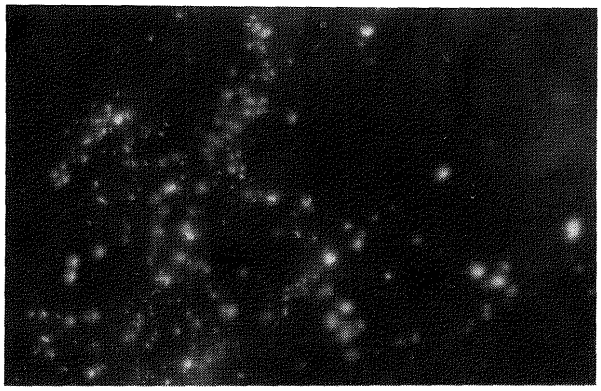


Fig. 4. Fluorescence Microscopic Photograph of Pyren Loaded Liposomes Adhered to the Rat Intestine Wall

The same adhesion test was conducted using pyren loaded CS coated or non-coated liposomes to confirm the mucoadhesion of CS coated liposomes optically with fluorescence microscopy. After the adhesion test, the intestine tube was washed with an excess amount of saline solution and the inside wall of the intestine was observed with a fluorescence microscope (AXIOPHOT, Karl Zeiss). A large number of liposomes was observed on the picture when the CS coated liposomes were applied in the adhesion test, while no liposomal particles were found in the case of non-coated liposomes (Fig. 4).

This report suggests that polymer-coated liposomes are a promising device as a multi-unit mucoadhesive system. Liposomes can encapsulate both hydrophilic and hydrophobic drugs, including peptide drugs. These results presented here encourage us to evaluate the effectiveness of polymer-coated liposomes as an oral drug delivery system *in vivo*.

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