The Correlation between Zeta Potential and Mucoadhesion Strength on Pig Vesical Mucosa

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The detachment forces of various polymers are frequently measured to determine their mucoadhesion strength. As the process of mucoadhesion is a consequence of interactions between the mucus layer on mucosa and mucoadhesive polymers, it is greatly dependent on mucus and polymer structure including their charge. It is also known that the glycosaminoglycan layer, which covers the urinary bladder mucosa surface, is highly negatively charged. Therefore, by measuring the zeta potential of polymer dispersions and mucosal homogenates an insight into electrostatic interactions during mucoadhesion can be obtained. In our experiments we chose three polymers, two anionic (polycarbophil, PC; sodium carboxymethyl cellulose, CMCNa) and one cationic (chitosan hydrochloride, CH), for which we expected different zeta potential values and different mucoadhesion strengths. The correlation between the zeta potential and the detachment force was determined. In addition to that, the zeta potential of the scraped surface layer of pig urinary bladders was measured to confirm its negative value. The mucoadhesion strength decreased in the following order: CH>CMCNa=PC. The zeta potentials for all three polymers and for porcine vesical mucosal homogenates were measured in Tyrode solution and two NaCl solutions with different ionic strengths. The lower values of the detachment force correlated well with the more negative zeta potential of the polymer, which might be a consequence of the greater repulsion between negative charges of polymers and glycosaminoglycans.

Key words zeta potential; mucoadhesion; urinary bladder mucosa; polycarbophil; sodium carboxymethyl cellulose; chitosan hydrochloride

The detachment forces between polymeric films or tablets and animal mucosae are frequently determined to evaluate the mucoadhesion strength of polymers. The most important interactions contributing to mucoadhesion are Van der Waals interactions and hydrogen bonds between mucoadhesive polymers and mucus. Mucus contains functional groups which are negatively charged and interact with the charged groups on polymers, thus influencing the mucoadhesion strength. The structure and composition of intestinal mucus is relatively well described in literature,[1] while there are very few data about the nature of the layer which covers vesical mucosa. The main components of intestinal mucus are glycoproteins. The oligosaccharide chains attached to protein core carry negative charge, due to sialic acid and sulfates. The layer on the surface of urinary bladder mucosa is composed of glycosaminoglycans (GAG), which carry a very high negative charge mostly due to sulfate groups attached to the GAG backbone. Hurst *et al.*[2,3] described the composition of the bovine and human GAG layers: heparan sulfate, dermatan sulfate and chondroitin sulfate are the most abundant GAG; they are bound to proteins and many of the free GAG molecules are only loosely adherent to the bladder mucosa surface. The presence of sulfated GAG in/on the urothelium was proved also by other researchers. Poggi *et al.*[4] used cold-cup biopsy from cadaveric human urinary bladder epithelium and found the sulfated GAG content ranging from 2.0×10^{-1} to 7.4×10^{-1} mmol/kg of dry, defatted bladder, while the amount of nonsulfated GAG in the same samples was approximately 10^3 times lower. Thus, it is obvious that the surface of the urinary bladder mucosa is highly negatively charged.

The GAG layer of the vesical mucosa is much thinner than the mucus layer of the intestines. According to Cornish[5] and Nickel[6] it is about 10—20 μm thick on an unfolded urothelium and many times thicker within the folds.

The zeta potential of a dispersion system is defined as the potential between the tightly bound surface liquid layer of a dispersed particle and the bulk phase of the solution. It is the measure of the net surface charge on the particles in a dispersion system. Therefore, the zeta potential can not only determine the electrostatic interactions between dispersed particles, but also serves as an important parameter in characterizing the properties of dispersion systems affected by this electrical phenomenon.[7] In cases where electrostatic interactions between a mucoadhesive polymer and GAG play a decisive role in mucoadhesion, zeta potential measurements of polymer dispersions could be used to predict the mucoadhesive properties of the polymer. In our previous work we showed that some polymers adhered more weakly to vesical than intestinal mucosa.[8] This effect was more pronounced for negatively charged polymers, which could at least partly be a consequence of a stronger repulsion on probably more negatively charged vesical mucosa. However, in another series of experiments[9] this type of correlation was not confirmed: with decreasing negative values of the zeta potential, a decreasing repulsion and an increasing mucoadhesion strength with negatively charged mucosa were expected, but the measurements showed a strong drop of the detachment force. In this case[9] the decreased zeta potential was a consequence of the presence of calcium ions, which, beside their impact on the zeta potential, have many other effects.

As already mentioned above the results from our previous work[8] indicate that positively charged polymers have higher affinity for urinary bladder than for intestinal mucosa. This article represents the continuation of the described work, as we want to further determine the relationship between the charge of a polymer and its bioadhesion properties. Three polymers, polycarbophil, chitosan hydrochloride and car-

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boxymethyl cellulose sodium were chosen. The decisive criteria for choosing the polymers were their high bioadhesion strength and different charge. Although, polymers that fulfilled these criteria differed in other physico-chemical properties great differences in zeta potential and thus the influence of zeta potential on bioadhesion strength are expected due to different charge of the polymers. It is not our intention to generalize the expected correlation, what we would like to show is that when choosing the bioadhesive polymers, their charge/zeta potential has to be considered as an important parameter in bioadhesion process.

MATERIALS AND METHODS

Materials The polymers used and their sources were as follows: chitosan hydrochloride (Protasan CL 213, Pronova, apparent viscosity of 1% solution in deionised water at 20 °C is 20—200 mPa·s), polycarbophil (Noveon AA1, Goodrich, molecular weight in billions) and sodium carboxymethyl cellulose (Fluka, viscosity of 2% solution in water at 25 °C is 400—1000 mPa·s). All the other solvents and substances used in this study were of analytical grade.

Tissue Preparation Urinary bladders from freshly slaughtered 7-month-old pigs of both sexes, weighing 90—110 kg, were obtained from a local slaughterhouse. The bladders were washed with aerated Tyrode solution and kept in Tyrode solution at 4 °C until use. Zeta potential measurements were performed within a few hours and detachment force measurements no later than within 24 h after the animals had been slaughtered. The mucosa of the urinary bladder corpus was used in all of the experiments.

Measurement of Detachment Forces The detachment force between the polymeric film and the animal mucosa was measured with a modified precision balance. Chitosan hydrochloride (CH), polycarbophil (PC) or sodium carboxymethyl cellulose (CMCNa) was dissolved in water or in methanol and films (1.25 mg of dry polymer per cm^2) were prepared on the glass plates. A suitable amount of Tyrode solution (10—19 μl/mg CH film, 2—13 μl/mg PC film and 10—19 μl/mg CMCNa film) was dispersed over the surface of the polymeric film and left for 2 h in a humid environment to hydrate, whereupon the glass plate was mounted on the upper clamp of the apparatus. The mucosal layer of a pig urinary bladder wall was separated from the underlying tissue and mounted on the lower support of the modified precision balance. After that the clamp with the tissue was slowly raised, a contact with the hydrated film was formed and an additional weight of 10 g was applied. The detachment force needed for the separation of the two surfaces was determined 2 min after the formation of the contact. The results are means of five to seven experiments.

Measurement of Zeta Potential Dispersions of the polymers with a concentration of 80 mg/l were prepared in Tyrode solution and in 0.5 and 0.1% w/v solutions of sodium chloride. After the dispersions were stirred overnight, their zeta potential was determined by Zetasizer 3000 (Malvern Instruments, GB). The polymer dispersions for the zeta potential measurements were prepared two to four times and each sample was measured 10 times.

For the zeta potential measurement of mucosal homogenates the luminal surface of urinary bladder mucosa was gently scraped with a plastic card. The obtained samples were homogenised using motorized Pelletle® tissue grinder (Kontes Glass co., Vineland, NJ, U.S.A.) and dispersed in Tyrode solution and in 0.5 and 0.1% w/v solutions of sodium chloride. The zeta potential was determined with the same instrument as described for the polymers. The impact of homogenisation time (1, 2, 5 min) and homogenate concentration in Tyrode solution (0.04—0.16% w/v) on the zeta potential was tested and no significant influences were found. The final measurements were performed on homogenates of five urinary bladders and for each diluted homogenate (0.08% w/v) the apparatus measured the zeta potential ten times.

Hystological Staining and Optical Microscopy Several pieces of tissue, with a surface of approximately 1 cm^2, were cut from the corpus of the isolated urinary bladders. From half of them mucus had been scraped off before cutting, the others had been left intact. All tissue samples were rapidly frozen in liquid nitrogen and the frozen tissue was sectioned into sections 10 μm thick perpendicularly to the luminal surface. After hemotoxylin-eosin staining of the sections their images were acquired using an Olympus BX 50 system microscope equipped with a Sony 3CCD colour video camera DXC-950P. The measurements of the urothelium thickness were done with the image-analysing software AnalySIS .

Statistical Evaluation The statistical evaluation was assessed using the two-sample *t*-test. Before applying this test, the *F*-test was used for evaluation of homoscedasticy. Depending on the results of this test, the *t*-test for equal or unequal variances was performed. Values of $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

Detachment forces were determined using polymeric films hydrated with certain amounts of Tyrode solution prior to the measurement. Different volumes of the solution were used for different polymeric films because they were able to accept different amounts of the liquid. The volume had to be large enough to spread over the polymer surface homogeneously, yet not so large to make the final polymer dispersion too fluid and drop off the glass plate. Measurements were performed within wide volume intervals, but only at 10 and 13 μl of Tyrode solution per mg of polymeric film were identical for all three polymers. Therefore, the statistical evaluation was performed only at these two volumes of Tyrode solution.

The results of detachment forces determination are shown in Fig. 1. As expected, the detachment force decreases with the increasing volume of Tyrode solution used for the hydration of polymeric films. It can also be seen that mucoadhesion strength decreases in the following order: CH> CMCNa=PC. It was proved that chitosan films had significantly higher detachment forces than the other two polymers at 10 and 13 μl of Tyrode solution used for hydration.

The zeta potentials of the polymers and mucosal homogenate dispersions in different media are shown in Table 1. For determining the zeta potential of mucosal homogenates, the luminal bladder surface was scraped off gently. Because GAG layer is so thin, it was impossible to scrape off only this layer, and thus the obtained homogenates contained also a few layers of surface mucosal cells (Fig. 2). However, as described earlier, sulfated GAG are present in

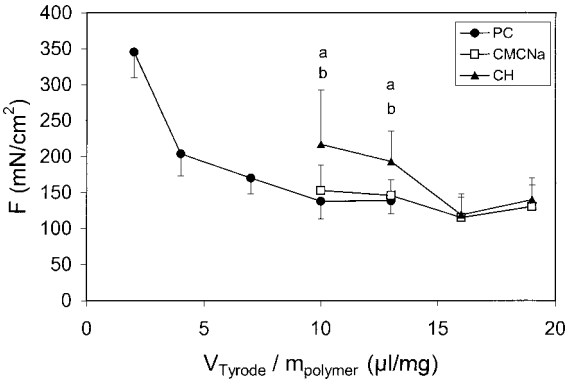

Fig. 1. Detachment Force (F) as a Function of Increasing Volume of Tyrode Solution (V) per mg of Polymeric Film Used for Hydration of Different Polymeric Films Weighing 1.25 mg/cm^2

Each point represents the mean±S.D. (n=5—7). The symbols a and b indicate the statistical significant difference between detachment force of CH and PC (a) or CH and CMCNa (b) at equal volume of Tyrode solution used for hydratation of polymeric films.

Table 1. Average Values and Standard Deviations of Zeta Potentials (ζ) of Polymers and Mucosal Homogenate in Tyrode Solution and in 0.5 and 0.1% w/v Solutions of Sodium Chloride

	ζ (mV)		
	Tyrode solution	0.5 % w/v NaCl	0.1 % w/v NaCl
PC	−30.59±0.37	−22.26±2.75	−25.21±0.81
CMCNa	−24.61±4.09	−9.39±4.29	−12.08±4.26
CH	−7.15±0.18	4.24±7.44	4.24±1.43
Mucosal homogenate	−20.4±2.4	−20.1±0.9	−26.2±1.8

Each point represents the mean±S.D. (n=2—4).

relatively high quantities on the surface of mucosa[2] and in the urothelium.[4] So we suppose that the zeta potential of the surface GAG layer also has a negative value, which might be close to the one determined in our study.

Dispersions for zeta potential measurements were first prepared in Tyrode solution, which was also used in the measurements of detachment forces between polymeric films and urinary bladder mucosa. But the concentrations of polymers differed a lot in the two experiments (detachment force: approximately 10% w/v; zeta potential: 0.008% w/v) and this produced additional intra- and inter-molecular interactions during mucoadhesion measurements. It was, however, impossible to measure zeta potentials in concentrations as high as those used for mucoadhesion, so we decided to perform measuring zeta potential under conditions, which would reflect the properties of the polymer only and would be influenced by environmental factors as little as possible. This was not assured when measuring zeta potential in Tyrode solution, because it is influenced by its buffer capacity and ionic strength. As our method of determining the zeta potential did not allow us to use dispersions of the polymers in bidistilled water, measurements of the zeta potential in NaCl solutions with different concentrations and no buffer properties were performed as well. Dispersions of the polymers and urinary GAG were prepared in 0.5 and 0.1% w/v solutions of NaCl. The results show that in Tyrode solution as well as in both solutions of NaCl, CH, which is a cationic polymer, has the least negative zeta potential value or even a positive one, and PC has the most negative one. We assume that the same relation of zeta potentials for the three polymers would also have been obtained if dispersions had been prepared in bidistilled water. Therefore, it is expected that during mucoadhesion electrostatic interactions between urinary GAG and these three polymers are in a relation similar to that of the zeta potentials obtained in this experiment. So, if electrostatic interactions prevail, the mucoadhesion strength between these polymers and negatively charged urinary GAG is expected to be: CH>CMCNa=PC. This was experimentally confirmed (Fig. 1) and the correlation between detachment force and zeta potential is shown in Fig. 3.

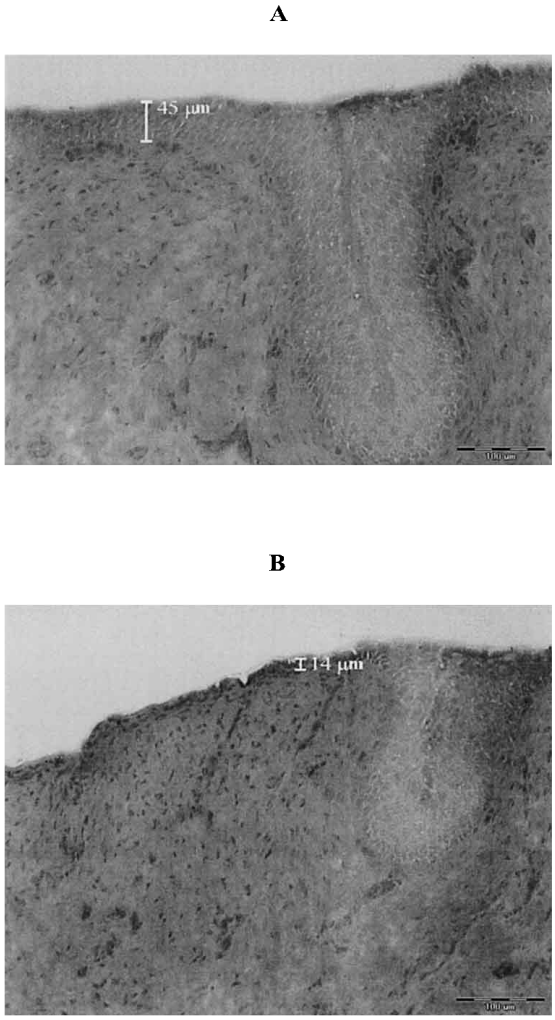

Fig. 2. Intact (A) and Scraped (B) Urinary Bladder Mucosa Stained by Eosin-Hematoxylin

The thickness of the urothelium is marked.

The results are in accordance with Takeuchi *et al.*,[10] who showed that positively charged liposomes had a higher adhesion than negatively charged ones due to the negatively charged mucin layer of the intestine. Beside that He *et al.*[11] evaluated the adsorption of mucin type III and I-S on chitosan microspheres at various conditions. The mucin adsorption was proportional to the absolute values of the positive zeta potential of chitosan microspheres and negative zeta potential of mucin. Good correlation between both parameters was explained by the fact that the interactions between mucin and chitosan microspheres are dominated by electrostatic attractions. On the other hand, Tur *et al.*[12] reported that a more

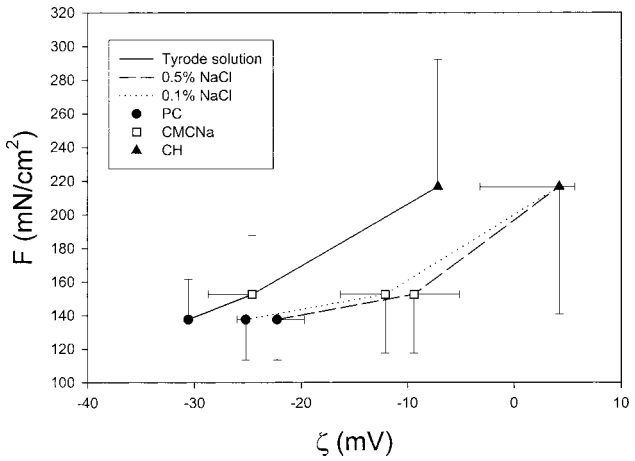

Fig. 3. Relationship between Detachment Force (F) and Zeta Potential (ζ)

Dispersions for zeta potential measurements were prepared in Tyrode solution and in 0.5 and 0.1% w/v solutions of sodium chloride, and the obtained zeta potentials (mean±S.D.; n=2—4) in all three solutions are presented as a function of the detachment force (mean±S.D.; n=5—7) determined by using 10 μl of Tyrode solution per mg of polymeric film for its hydration.

negative zeta potential due to the increased ionization of cross-linked polyacrylic and polymethacrylic acid resulted in an increased detachment force between the polymers and rabbit stomach tissue. This more negative zeta potential of the polymers ensured a better uncoiling of the polymers to interpenetrate with mucin molecules.

In sum, the detachment forces for three different polymers, two anionic (polycarbophil, carboxymethyl cellulose) and one cationic (chitosan hydrochloride), and the zeta potentials of polymeric dispersions were determined. The zeta potentials for all three polymers and for porcine vesical mucosal homogenates had negative values in all solutions, with the exception of CH dispersions in NaCl solutions. The lower values of the detachment force correlated well with a more negative zeta potential of the polymer, which might be a consequence of the greater repulsion between negative charges of the polymers and GAG. Beside zeta potential, other properties of polymers, such as their chemical nature and structure, contribute to their mucoadhesion strength, but the results obtained in this study additionally confirmed a possible involvement of electrostatic interactions in mucoadhesion.

REFERENCES

1) Allen A., *Br. Med. Bull.*, **34**, 28—33 (1978).
2) Hurst R. E., Zebrowski R., *J. Urol.*, **152**, 1641—1644 (1994).
3) Hurst R. E., Rhodes S. W., Adamson P. B., Parsons C. L., Roy J. B., *J. Urol.*, **138**, 433—437 (1987).
4) Poggi M. M., Johnstone P. A. S., Conner R. J., *Urol. Oncol.*, **5**, 234—237 (2000).
5) Cornish J., Nickel J. C., Vanderwee M., Costerton J. W., *Urol. Res.*, **18**, 263—266 (1990).
6) Nickel J. C., Emerson L., Cornish J., *J. Urol.*, **149**, 716—718 (1993).
7) Li L. C., Tian Y., "Encyclopedia of Pharmaceutical Technology," Vol. 16, eds. by Swarbrick J., Boylan J. C., Marcel Dekker, New York, 1997, pp. 429—458.
8) Bogataj M., Mrhar A., Korošec L., *Int. J. Pharmaceut.*, **177**, 211—220 (1999).
9) Kerec M., Bogataj M., Mugerle B., Gašperlin M., Mrhar A., *Int. J. Pharmaceut.*, **241**, 135—143 (2002).
10) Takeuchi H., Yamamoto H., Kawashima Y., *Adv. Drug Deliv. Rev.*, **47**, 39—54 (2001).
11) He P., Davis S. S., Illum L., *Int. J. Pharmaceut.*, **166**, 75—88 (1998).
12) Tur K. M., Ch'ng H. S., *Int. J. Pharmaceut.*, **160**, 61—74 (1998).