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In vitro evaluation of mucoadhesive properties of chitosan and some other natural polymers

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Summary

A number of natural or partially modified polymers was screened for mucoadhesive properties by routinely measuring the force of detachment for swollen polymer films from pig intestinal mucosa in a saline medium. Surprisingly, hydroxypropyl- and carboxymethylcellulose showed almost no mucoadhesion, whereas the cationic polymer chitosan was fairly mucoadhesive in comparison to Polycarbophil as a reference substance. It is suggested that a strict difference be made between mucoadhesion of dry polymers on a wet tissue in air, and mucoadhesion of a swollen hydrogel in the presence of a third liquid phase. Cationic polymers should be further investigated with respect to possibly improved mucoadhesive properties in a neutral or slightly alkaline environment.

Introduction

Although polymers of the poly(acrylic acid) type are known for their excellent mucoadhesive properties, it would be unlikely if only this class of polymers were able to form mucoadhesive hydrogels. From a theoretical point of view, it can be predicted that polymers exist or at least could be synthesized with surface properties even more favourable for mucoadhesion than those of Polycarbophil gels (Lehr et al., 1991). Some years ago, Peppas and Burim (1985) analyzed the existing data and theories relevant to this topic. They came to the conclusion that a number of polymer characteristics are necessary for mucoadhesion which can be summarized as follows: (i) strong hydrogen-bonding groups (-OH, -COOH), (ii) strong anionic charges, (iii) high molecular weight, (iv) sufficient chain flexibility, (v) surface energy properties favouring spreading onto mucus. The latter three features still appear to be plausible in the light of more recent theories about mucoadhesion (Lehr et al., 1991). In contrast, negative charge and hydrogen-bonding capabilities are common to presently known mucoadhesives, but should not a priori be generalized. Instead, positively charged polymeric hydrogels could possibly develop additional molecular attraction forces by electrostatic interactions with negatively charged

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mucosal surfaces. In this context, the natural polyaminosaccharide chitosan appeared as an interesting candidate and was screened for mucoadhesive properties with respect to a possible oral application together with some other polymers.

Materials and Methods

If not otherwise indicated, the polymers tested were received from the Department of Polymer Chemistry, University of Ghent, Ghent, Belgium.

Cationic polymers

Chitosan is a deacetylated chitin (poly(*N*-deacetylglucosamine), obtained industrially by hydrolysing the aminoacetyl groups of chitin from crabs or shrimps in aqueous alkaline solutions. The structural formula of chitosan is shown in Fig. 1. Chitosan is insoluble in water but soluble in dilute aqueous acids (e.g. acetic acid). Technical applications are various, e.g. varnish for classical violins, water cleaner and contact lenses or cosmetic products such as hair sprays. Further general information about chitosan and its applications can be found in the literature (Zizakis, 1984).

Chitosan is commercially available from a number of suppliers in various grades of purity and average molecular weight. Chitosans tested in this study were gifts from Wella AD, Germany (Wella 'high viscosity' and 'low viscosity'; further specification not given), from Dainichiseika Co., Japan (Daichitosan H, MW 500 000–800 000, and Daichitosan VH, MW about 1 400 000, as indicated by the manufacturer), from Dr J. Knapczyk, Faculty of Pharmacy, University of Krakow, Poland (no further specification), and from Protan A/S, Drammen, Norway (Sea Cure 240, described as standard chitosan pharma grade, and Sea Cure 210+, described as chitosan glutamate). A further sample was purchased from Sigma, St Louis, U.S.A. (practical grade from crab shells).

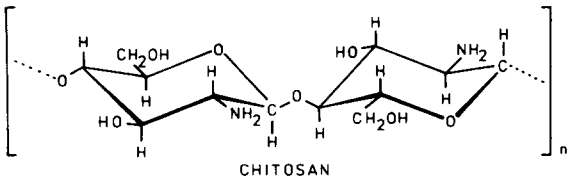

Fig. 1. Chemical structure of chitosan.

Dimethylaminoethyl (DEAE)-dextran prepared from dextran of an average MW of 500 000 was purchased from Sigma, St Louis, U.S.A.

Aminodextran (batch code T70) was synthesized at the laboratory of one of the authors (E.H. Schacht). Molecular weight was indicated to be 70 000 with a degree of substitution of 10%.

Non-ionic polymers

Hydroxypropylcellulose (Klucel HF NF) was received as a kind gift from Aqualon (Wilmington, U.S.A.). This type was the highest viscosity grade available (MW 1 150 000).

Scleroglucan: glucans are homopolysaccharides consisting of glucose subunits, such as dextran, starch or cellulose. They vary in water solubility and the type of glycosidic bonds (e.g. $1 \rightarrow 3$, $1 \rightarrow 4$, $1 \rightarrow 6$).

Hydroxyethyl starch is known to form gels with water at concentrations between 1 and 5%.

Anionic polymers

Pectin consists primarily of poly(galacturonic acid), partially esterified in the form of its methyl ester, which determines its water solubility. It is known to gel in the presence of sugar, acid and Ca^{2+}, but hydrolyzes in alkali.

Xanthan gum is a polysaccharide produced by fermentation of glucose by *Xanthomonas campestris*. It consists of glucose, mannose, potassium glucuronate, acetate and pyruvate. It forms gels which are said to be insensitive to changes in temperature, pH, and salts. The batch tested was a sample of Keltrol T (Kelco Co., San Diego, U.S.A.).

Carboxymethylcellulose (CMC) was used in different viscosity grades. A high- and a low-viscosity grade (1500 ± 400, and 30–40 mPa s for 1% solution, respectively) were received from BDH (Poole, U.K.); a medium-viscosity grade batch (500 mPa s) was obtained from Fluka (Buchs, Switzerland). This polymer was tested as

it has been reported to be an excellent mucoadhesive (Smart et al., 1984).

Polycarbophil is a high molecular weight poly(acrylic acid) copolymer, loosely cross-linked with divinyl glycol. On account of its known excellent mucoadhesive properties, this polymer served as reference. Carbopol EX-55 resin was a gift from BF Goodrich (Cleveland, U.S.A.).

Usually, polymers were dissolved in water in a concentration of 1% (m/v). Chitosans were dissolved in dilute acetic acid (1%, v/v) with the exception of chitosan glutamate (Sea Cure 210 +, Protan) which can be dissolved in water. Polycarbophil was dispersed in methanol. Cover glasses (24 × 24 mm) were coated by dispensing 300 μl of the polymer solutions on their surface and drying in air. Polymer content of the resulting film was about 1 mg/cm^2.

TABLE 1

Survey of mucoadhesive properties of various polymers

Polymers	Force of detachment (mN/cm^2)
Cationic polymers	
Chitosan (Wella 'low viscosity')	3.9 (1.2)
Chitosan (Wella 'high viscosity')	6.7 (0.7)
Chitosan (Dr Knapczyk)	5.7 (1.1)
Daichitosan H	8.0 (5.7)
Daichitosan VH	9.5 (2.4)
Sea Cure 240	4.1 (2.9)
Sea Cure 210+	9.5 (2.5)
Chitosan (Sigma)	6.6 (3.0)
Polycarbophil/Daichitosan VH blend	11.9 (2.5)
DEAE-dextran	0
Aminodextran	0
Non-ionic polymers	
Scleroglucan	2.8 (2.8)
HE-starch	0.6 (0.8)
HPC	0
Anionic polymers	
CMC (low viscosity)	1.8 (1.1)
CMC (medium viscosity)	0.3 (0.3)
CMC (high viscosity)	1.3 (1.0)
Pectin	0
Xanthan gum	0
Polycarbophil	17.6 (3.6)

Indicated is the mean (SD) force of detachment of 2 to 6 measurements.

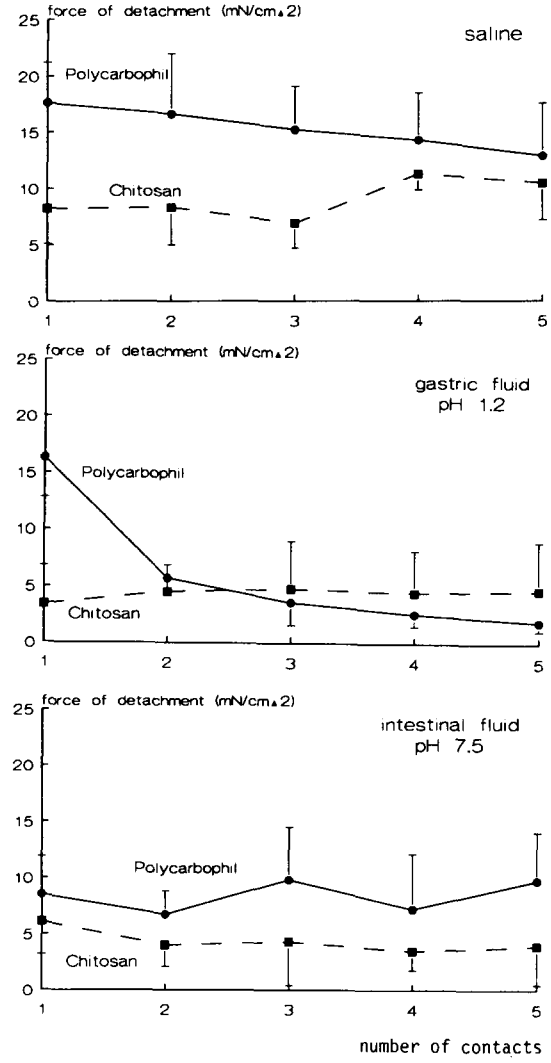

Fig. 2. Repeated adhesion of, respectively, Polycarbophil (Carbopol EX-55) and a high molecular weight chitosan (Daichitosan H) in various physiologically relevant fluids (N = 3.5).

The force of detachment for polymer-coated cover glasses from pig intestinal mucosa in various test fluids (37°C) was measured as previously described (Lehr et al., 1990). The test fluids used were isotonic saline (0.9% NaCl in demineralized water), artificial gastric fluid (pH 1.2) and artificial intestinal fluid (pH 7.5). The latter two media were prepared according to the corresponding USP monographs, but without adding any enzymes. Dry films were swollen in the test medium

for 5 min and then brought into contact with the tissue under very slight pressure (~ 10 mN) and kept in this position for an additional 1 min. A vertically acting force was slowly increased until the polymer became detached. Polymer and tissue samples were used only once per measurement. To study repeated adhesion, this procedure was repeated several times, retaining the same polymer and mucosa specimen.

Wash-off tests were performed as follows: pieces of pig intestinal tissue (about 2 × 2 cm) were mounted onto object glasses (3 × 1 inch) with cyanoacrylate glue. The edges of the tissue were additionally secured with Parafilm®. Six glasses were connected with a suitable support using rubber rings. About 100 microspheres (315–400 μm) of poly(2-hydroxyethyl methacrylate) (PHEMA), coated with the mucoadhesive polymer under investigation (Lehr et al., 1990), were spread onto each wet, rinsed tissue specimen and counted. Immediately thereafter, the support was hung onto the arm of a USP tablet-disintegration tester, permitting a slow, regular up and down movement (~ 30 min^{-1}) in a test fluid kept at 37°C. At given intervals, the motor was stopped and the number of beads still adhering onto the tissue was counted.

Results

For an initial screening, the force of detachment for polymer films from pig intestinal mucosa was measured in saline at 37°C. A survey of the results is given in Table 1.

With the exception of Polycarbophil and some of the chitosan samples, none of the other polymers showed appreciable mucoadhesive properties under the conditions of this routine test. Macroscopically, the viscosities of the solutions prepared from the different chitosan samples could be observed to correlate with the measured mucoadhesive performance, indicating that a higher molecular weight would indeed favour mucoadhesion. An exception was the sample of chitosan glutamate (Sea Cure 210 +, Protan) which formed an only slightly viscous solution but nonetheless scored well in the adhesion test. As one of the most promising candidates, Daichitosan H was selected for further evaluation. The sample of Daichitosan VH was received later upon special request and could not be studied in more detail.

The force of detachment after repeated adhesion of the same polymer and tissue specimen in different physiologically relevant media is shown

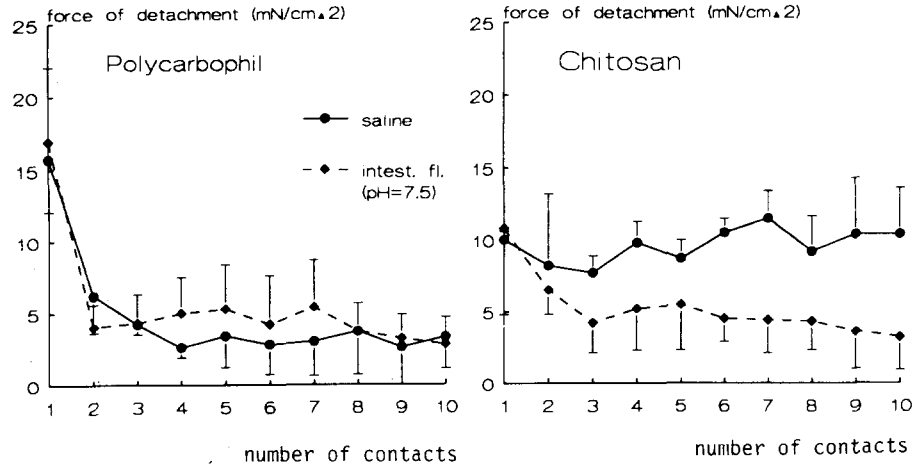

Fig. 3. Repeated adhesion of Polycarbophil and Chitosan after immediate contact of the dry polymer films.

in Fig. 2. The films were swollen for 5 min prior to initial contact in the respective media. For comparison, values previously measured with Polycarbophil are also displayed in the same graphs. As was the case for Polycarbophil, chitosan yielded the highest force of detachment in the non-buffered saline medium. Mucoadhesive performance in simulated gastric (pH 1.2) or intestinal (pH 7.5) fluids was significantly lower, but yielded about the same values in both cases. This behaviour is different from that of Polycarbophil, which was clearly less mucoadhesive in the intestinal fluid than in gastric fluid. This difference was even more pronounced after repeated adhesion without prehydration of the polymer films (Fig. 3).

Polycarbophil showed a marked drop in mucoadhesion after the initial contact in both saline and intestinal fluid (gastric fluid was not studied), whereas such a decrease could not be observed for chitosan. In the saline medium, chitosan appeared to be better suited for repeated adhesion, since it did not become inactivated after the first contact. However, in none of these experiments did the force of detachment for chitosan films exceed that for Polycarbophil, at least not after the initial contact. Only in intestinal fluid, where Polycarbophil showed the weakest mucoadhesion, did the two Polymers yield comparable values.

After air-suspension coating of PHEMA microspheres, the durability of mucoadhesion was determined by performing wash-off tests from pig intestinal mucosa in the same three test fluids (Fig. 4). As was previously found for Polycarbophil (in preparation), mucoadhesion of chitosan-coated beads resisted permanent hydrodynamic shear for more than 18 h in saline, but became slowly detached from the tissue in intestinal fluid. In gastric fluid, however, chitosan-coated beads were washed off very rapidly, whereas Polycarbophil coated beads in the same medium resisted for more than 18 h.

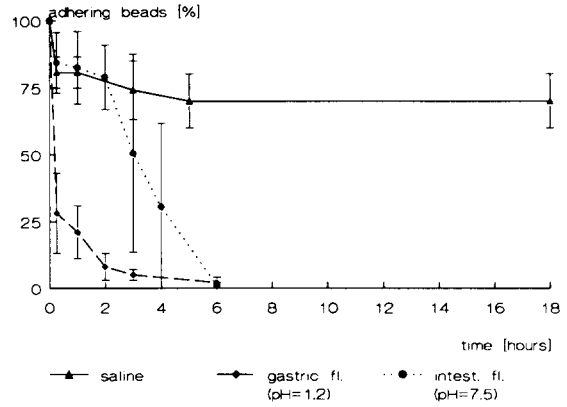

Fig. 4. Wash-off test of chitosan-coated microspheres from pig intestinal mucosa ($N = 3$).

Discussion

As Table 1 shows, mucoadhesive polymers are relatively rare. Furthermore, the results illustrate the need for a clear definition of this term. Hydroxypropylcellulose has frequently been called a mucoadhesive, however, it showed no adhesion at all in this study. The same was observed for all grades of carboxymethylcellulose, for which the highest scores – even higher than for the poly(acrylic acid) derivative Carbomer – were reported earlier by Smart et al. (1984). The same polymer films which did not adhere in saline, however, showed extremely strong adhesion to mucosal tissue in air, appreciably exceeding the range of the test instrument. It is likely that a number of so-called mucoadhesive polymers adhere only under conditions where the amount of interstitial liquid is limited. The mechanism of adhesion in this case is probably that of capillary attraction between a dry, water-absorbing polymer and a wet, mucosal surface. This should not be confused with the peculiar interaction between two hydrogels (polymer and mucus) in equilibrium with a third liquid phase. It is suggested that the term mucoadhesion be used only for the latter phenomenon as it can be observed in a wet and not just a humid environment.

Although not yet competitive in comparison with Polycarbophil, the 'true' mucoadhesive properties of chitosan are interesting to note. To the authors' knowledge this is the first time that this has been reported in the literature. At least, the data might provide some useful information for the further development of even better mu-

coadhesives. The weak, short-lasting mucoadhesion of chitosan in an artificial gastric fluid can be explained by the solubility of the pure polymer in acidic solutions. This could possibly be overcome by chemical cross-linking or blending with other, non-soluble polymers or drugs. In agreement with recent findings reported by Park et al. (1989), however, cationic polymers are likely to be superior mucoadhesives especially in a neutral or slightly alkaline medium as would be desirable for adhesion in the small or large intestines. It was observed that chitosan underwent minimal swelling in artificial intestinal fluid. This is explained by the poor water solubility of the free base, but could possibly be improved by substituting the free amino groups with short alkyl chains in order to increase the pK_a and hence the ionization of these groups at higher pH, which will increase swelling and possibly also mucoadhesion.

Miyazaki et al. (1988) reported prolonged absorption of indomethacin in rabbits after oral administration of granules prepared from a 1:2 mixture of drug and chitosan. Without providing experimental evidence, they explained their results as being due to prolonged gastric retention of the presumably floating granules, but did not discuss possible bioadhesive properties of their formulation. Takahashi et al. (1990) studied the formation of polyion complexes between chitosan and sodium polyacrylate, which could also be interesting as mucoadhesives. Mixing of an aqueous solution of chitosan (Daichitosan VH) and a methanolic dispersion of Polycarbophil in a mass ratio of 1:1 resulted in a highly viscous gel with mucoadhesive properties intermediate between those of the parent polymers (see Table 1).

Conclusion

From this in vitro study, Polycarbophil appeared to be the best performing mucoadhesive in comparison to all other polymers tested. Nonetheless, the possibility of developing new, and possibly even better, mucoadhesives on the basis of cationic high molecular weight polymers such as chitosan should be investigated in the future.

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