Effect of Different Biological Membranes on In Vitro Bioadhesion Property

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ABSTRACT

The influence of biological membranes on in vitro bioadhesion property of Carbopol 934P (CP), Polyvinylpyrrolidone (PVP K90) and sodium carboxymethylcellulose (Cekol 10,000) was studied using a texture analyzer equipment. Polymer/biological membrane interaction was significantly influenced by the contact time used during the study. An increase in contact time also increased bioadhesion strength. An intermediate contact time of 180s was deemed to be the most suitable contact time. In addition, different polymers produced different values of bioadhesion strength. Moreover, polymer molecular weight is directly correlated with bioadhesion strength. Biological membrane, such as rabbit gastric mucosa, cow intestine, pig intestine or chicken pouch, is a less significant determinant in affecting the bioadhesion strength as compared to the type of polymer used. Chicken pouch, an easily available and uniform tissue, offers an attractive alternative to be employed as a model biological membrane in bioadhesion study.

Keywords: Bioadhesion, Carbopel, Polyvinylpyrrolidone, Chicken pouch, Sodium carboxymethylcellulose.

INTRODUCTION

In recent years, there is a great promise for utilization of bioadhesive polymers for local and systemic drug delivery formulations (1, 2). This is because these polymers can prolong the release of drug from dosage forms by localizing them at a specific site such as buccal tissue or other cavities (3, 4).

The determination of the mucosa adhesive bond strength is essential for the successful application of bioadhesive drug delivery systems. Various in vitro methods have been developed to study the bioadhesive strength of the preparations and different animal tissues were used as the model membranes. These included mouse peritoneum (5), rat intestine (6), rabbit stomach (7), bovine sublingual mucosa (1, 8), porcine buccal mucosa (9), chicken pouch (10, 11) and porcine gastric mucosa (12, 13). The pertinent questions raised concerning the use of animal tissues are the integrity of the tissues, the surface textures of the tissues, reproducibility of the results, and the ease of availability of the tissues, which could indirectly affect the studies.

So far, there has been very little work interrelating the various types of tissues in terms of bioadhesive strength. Smart (6) found a visibly non-uniform surface with pig oral mucosa. Moreover, Tobyn (12) found that different parts of the pig stomach gave different results due to their different surface characteristics.

The aim of this study was to investigate the suitability and consistency of easily available mucosal membranes (rabbit gastric mucosa, cow intestine, chicken pouch and pig intestine) as model for bioadhesive strength studies using three different hydrophilic polymers (polyacrylic acid, polyvinylpyrrolidone K90, and sodium carboxymethylcellulose).

MATERIALS AND METHODS:

Materials:

Polyacrylic acid, Carbopol 934P (CP) was purchased from B.F.Goodrich, Cleveland, OH, USA. Polyvinylpyrrolidone (PVP K90) was a gift from ISP Technologies Inc., Wayne, NJ, USA. Sodium carboxymethylcellulose (Cekol 10,000) was purchased from Metsa-Serla, Sweden. All other chemicals were of reagent grade and used as received.

Preparation of bioadhesive tablets:

CP, PVP K90 and Cekol 10,000 tablets of 13 mm diameter were prepared using IR hydraulic Press (Model P16, Beckman, UK). 200 mg of the polymeric materials were compressed at a pressure of 9 tonnes.
for 30s. Discs with a mean thickness of 1.57 ± 0.06 mm for CP, 1.45 ± 0.03 mm for PVP K90 and 2.05 ± 0.04 mm for Cekol 10,000 were obtained.

Bioadhesive strength measurements:

Tukey’s HSD test. Where appropriate, the results were also considered when p<0.05. Posterior analysis was carried out using multivariate tests. A statistically significant difference was obtained.

RESULTS AND DISCUSSION:

Effect of contact time on bioadhesion:

Wong (14) reported that probe speeds of 0.5 to 1 mm s⁻¹ and contact forces of 0.5 to 1 N did not cause any significant effect on bioadhesion, at a contact time of 180s, however, a variation in contact time significantly affected the bioadhesion property. In this study, both the contact force and the separating probe speed were maintained at 0.5 N and 1mm s⁻¹, respectively, whereas the contact time was varied from 60, 120, 180, 300 to 600 s. This was to investigate the influence of contact time on bioadhesion.

Figures 1 and 2 show the effect of contact time on the peak detachment force and work of adhesion, respectively. It can be inferred from the two figures that, at all the five contact times employed, both the peak detachment force and work of adhesion were increased with an increase in the contact time. This is in good agreement with the results obtained by Tobyn (12) and Wong (14). Contact time is an important factor in bioadhesion, since a sufficient contact time ensures adequate hydration and swelling of the polymer, enhances interpenetration of moisture and formation of non-covalent interaction, which in turn, promotes mucoadhesion.

Multivariate test showed that peak detachment force and work of adhesion at different contact times were significantly different (p<0.05) for CP, PVP K90 and Cekol 10,000. Further analysis by Tukey’s HSD test indicated that the five contact times resulted in four subsets for the peak detachment force but three subsets for the work of adhesion, for all the three polymers. In general, short and long contact times also gave rise to low and high peak detachment force and work of adhesion, while intermediate contact times resulted in subsets of intermediate peak detachment force and work of adhesion. Contact time of 180s consistently produced bioadhesion strength belonging to the intermediate subset, reflecting reproducibility of results obtained from this timing. As a result, 180s was selected as the contact time in the second part of the study.

The final ranking (in descending order) for the three polymers in terms of peak detachment force and work of adhesion was found to be: CP 934P > PVP K90 > Cekol 10,000. Pearson correlation coefficients showed that the peak detachment force and work of adhesion were directly correlated with the molecular weight of the polymer for all the four biological membranes used (Table 1).

The molecular weights of CP, PVP K90 and Cekol 10,000 are around 3,000,000, 1,300,000 and 400,000, respectively, where CP exhibited the highest molecular weight, followed by PVP K90, and lastly Cekol 10,000. This result suggested that the molecular weight of the polymer used was a factor affecting the bioadhesion strength of the polymers. Polymers with higher molecular weight also resulted higher bioadhesion strength. This finding is in agreement with Huntsberger’s (15). He reported that adhesive strength increased as the molecular weight of adhesive polymer increased. Tobyn (13) also found that the molecular weight of the polyacrylic acid used had crucially influenced the observed work of detachment between the polymer and pig gastric tissue. They explained that the increase in molecular weight of polymer would lead to an increase in the internal cohesion of the molecules, which augmented the mucoadhesion.
Figure 1: The influence of contact time on peak detachment force for CP, PVP K90 and Cekol 10,000 at a probe withdrawal speed of 1 mm/s and a contact force of 0.5 N (n=10) mean ±S.D.

Figure 2: The influence of contact time on work of adhesion for CP, PVP K90 and Cekol 10,000 at a probe withdrawal speed of 1 mm/s and a contact force of 0.5 N (n=10) mean ±S.D.

Figure 3: The influence of biological membrane on peak detachment force for CP, PVP K90 and Cekol 10,000 at a contact time of 180s, a probe withdrawal speed of 1 mm/s and a contact force of 0.5 N (n=10) mean ±S.D.

Figure 4: The influence of biological membrane on work of adhesion for CP, PVP K90 and Cekol 10,000 at a contact time of 180s, a probe withdrawal speed of 1 mm/s and a contact force of 0.5 N (n=10) mean ±S.D.

Table 1: Pearson’s correlation coefficients for peak detachment force and work of adhesion as a function of polymer molecular weight using various biological membranes.

Among the three polymers studied, CP produced the highest bioadhesion mean values. This was attributed to its good adhesion property through strong entanglement and penetration with mucin chains, due to the presence of a large number of carboxylic acid groups, which provide the ability to form hydrogen bonds. Moreover, CP also swells readily in water, thus offering a large adhesive surface for maximum contact (16).

Bioadhesion evaluation of different biological membrane:

The bioadhesive properties of tablets containing CP, PVP K90 and Cekol 10,000 using different biological membranes are shown in Figures 3 and 4. Four different types of animal membranes were
utilized in this study. They were rabbit gastric mucosa, cow intestine, chicken pouch and pig intestine.

It was interesting to notice that for PVP K90 and Cekol 10,000, there was no statistically significant difference in the peak detachment force for the four different membranes used. Tukey’s HSD test indicated that the membranes of pig, cow, chicken and rabbit all belonged to one homogeneous subset, whereas for CP, membranes of cow, chicken and rabbit belonged to one homogeneous subset, while pig membrane was in another subset. The effect of animal membranes on the work of adhesion were generally divided into two subsets for all the polymers studied, where membranes of cow and pig were mostly in one subset and membranes of rabbit and chicken in another subset. These results suggested that the type of polymers used in the study, played a more important role in determining the bioadhesion properties, than the type of animal membranes employed.

Hoogstraatea (17, 18) has reported that different animal species have different epithelial tissues. For example, the buccal mucosa of rabbits and hamsters are quite different from that of human because rodents use their cheeks for the storage of food, and therefore the tissue is keratinized due to constant mechanical friction. Shojaei (19) suggested that most commonly utilized laboratory rodents have keratinized oral epithelium at all regions of their oral mucosa, and are, therefore, not completely simulating the human mucosa. Although a relatively flat and uniform surface may be obtained with intestinal tissue e.g. cow and pig intestinal tissues, a different site of the intestine may have different surface characteristics (20). Moreover, their use may involve sacrificing a lot of animals, since in bioadhesion study, each mucosa can only be used once for each measurement. The pig oral mucosa is thought to be the most suitable model for studies of buccal conditions because there are essentially very little difference between human and pig buccal mucosa in terms of lipid content, composition, membrane morphology and permeability barrier function (21). In addition, other animals, such as dog and monkey, have also been employed in bioadhesion studies Hoogstraatea (17, 18).

Nevertheless, in this study it was found that there was little or no significant effect on the bioadhesion properties from the various types of biological membranes employed. Rabbit gastric mucosa, cow intestine and pig intestine did not show significant advantage over chicken pouch. Chicken pouch, an easily available biological membrane, has a non-keratinized, uniform surface morphology. The integrity of the membrane was well maintained throughout the study. In addition, sacrificing a small number of animals has already provided a sufficient amount of uniform membrane tissue for the study.

New technologies are exploring the more advanced drug delivery systems, such as bioadhesive dosage forms, to offer a better way of targeting drug release. Further investigations may be necessary to provide better understanding of the in vivo and/or in vitro performance of the bioadhesive oral preparations. The further investigations such as; In vitro bioadhesion test using real human buccal tissue and non-biological substrate

It is natural to expect that excised biological tissues be used for in vitro bioadhesion experiments. However, if a large number of potential bioadhesive materials are to be screened, obtaining enough biological membrane by sacrificing many animals may be difficult and non-economical. In such cases, non-biological substrate can be used instead of biological tissues. One way of justifying the use of non-biological substrate for in vitro bioadhesion test is to investigate the correlation between test results obtained using animal biological tissues, real human buccal tissue and non-biological substrate membranes.

CONCLUSIONS:

Polymer/biological tissue interaction could be critically influenced by contact time during in vitro bioadhesion study. In the present study, it was found 180s, is a good contact time to be utilized. In addition, different polymers produce different values of bioadhesion strength. Moreover, polymer molecular weight is directly correlated with bioadhesion strength. Biological membrane, such as rabbit gastric mucosa, cow intestine, pig intestine or chicken pouch, is a less significant determinant in affecting the bioadhesion strength as compared to the type of polymer used. Chicken pouch, an easily available and uniform tissue, offers an attractive alternative to be employed as a model biological membrane in bioadhesion study.

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