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Report

A Simple Rheological Method for the *in Vitro* Assessment of Mucin-Polymer Bioadhesive Bond Strength

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A simple viscometric method was used to quantify mucin-polymer bioadhesive bond strength. Viscosities of 15% (w/v) porcine gastric mucin dispersions in 0.1 N HCl (pH 1) or 0.1 N acetate buffer (pH 5.5) were measured with a Brookfield viscometer in the absence (η_m) or presence (η_t) of selected neutral, anionic, and cationic polymers (0.1–2.5%, w/v). Viscosity components of bioadhesion (η_b) were calculated from the equation, $\eta_t = \eta_m + \eta_p + \eta_b$, where η_p is the viscosity of corresponding pure polymer solution as measured by an Ostwald viscometer. The forces of bioadhesion (F) were calculated from the equation, $F = \eta_b\sigma$, where σ is the rate of shear/sec. η_b's and F's for polyelectrolytes, e.g., polyacrylic acid, cationic gelatin, and chitosan were always higher in acetate buffer than in HCl. Validity of the technique and the effect of ionic charge, polymer conformation, and rate of shear on η_b and F are discussed, as is a comparison of this method to other methods for evaluating bioadhesive materials.

KEY WORDS: bioadhesion; mucin:polymer bioadhesive strength; viscosity; polyelectrolytes.

INTRODUCTION

The term bioadhesion describes a phenomenon in which synthetic or biological macromolecules and hydrocolloids are able to adhere to a biological tissue (1). The force of bioadhesion is the interfacial force which holds together the adhesive material and the biological tissue (2).

Determination of bioadhesive bond strength is important in the development of bioadhesive drug delivery systems as it can quantitatively compare different bioadhesive materials and allow for quality control testing. Numerous techniques for the measurement of bioadhesive bond strength (3–7) are based on adhesive bond-destruction methods, i.e., failure shear and/or tensile stress (8,9). Based on the excimer formation technique (10), Park and Robinson have developed another methodology utilizing fluorescent probes to evaluate the ability of water soluble polymers to adhere to conjunctival epithelial cells (11). These techniques are valuable as a comparative measure of bioadhesive performance, yet they cannot determine the absolute force of bioadhesion. Mikos and Peppas (12) have described a method based on fluid mechanics where absolute forces involved in static and dynamic adhesiveness of polymer particles to homogenized mucin can be calculated. However, this method requires specialized equipment. The present investigation describes a simple procedure to assess the absolute force of bioadhesion through monitoring viscometric changes in a system of porcine gastric mucin and selected polymers in solution.

THEORY

Porcine gastric mucin is a network of linear, flexible, and random coils of glycoprotein molecules (MW = 2×10^6) (2). The viscosity of a mucin colloidal dispersion is the net result of the resistance to flow exerted by individual chain segments, physical chain entanglement, and the noncovalent intermolecular interactions such as electrostatic, hydrogen, and hydrophobic bonding (2,13). These interactions are the identical forces involved in the process of mucin-polymer adhesion (1). Thus, forces in a mucin-bioadhesive system can be monitored by measurements of viscosity. Viscosity changes due to mucin interactions with small and large molecules are well reported (14–16). Rheological data for these reports were interpreted on a molecular basis involving physical and chemical interactions. For example, an increase in mucin viscosity in the presence of urea, serum albumin, or polylysine were thought to be due to an extension of mucin's three-dimensional configuration (15), macromolecular complexation (14,15), or mucin cross-linking (16), respectively. In fact, both physical and chemical bond energies in mucin-polymer interactions can be transformed into mechanical energy or work. This work causes changes in the shape or arrangement of macromolecules and is the basis for viscosity changes (13). Consequently, one may analyze the viscosity coefficient of a hydrophilic dispersion containing mucin and a bioadhesive polymer into its contributing components resulting in the empirical equation,

$$\eta_t = \eta_m + \eta_p + \eta_b \tag{1}$$

where η_t is the viscosity coefficient of the system, and η_m and η_p are the individual viscosity coefficients of mucin and the bioadhesive polymer, respectively. η_b is the viscosity

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component due to bioadhesion and can be obtained by rearranging Eq. (1),

$$\eta_b = \eta_t - \eta_m - \eta_p \tag{2}$$

For Eqs. (1) and (2) to be valid, η_t, η_m, and η_p should be measured at the same concentration, temperature, time, and rate of shear. In the present study, the polymers solutions were Newtonian, and therefore, their viscosity is independent of time and the rate of shear. The force of bioadhesion, *F*, represents the additional intermolecular frictional force per unit area and was determined by (13)

$$F = \eta_b \sigma \tag{3}$$

where σ is the rate of shear per second. η_b was based on experimentally measured values in Eq. (2), and σ was obtained from the viscometer manual. Equation (3) is identical to the viscosity equation of Newtonian solutions but considers only the extra force due to bioadhesion. η_b is a direct estimate of the force of bioadhesion and, at a constant rate of shear, is useful for comparative investigations of bioadhesives. Since η_b may decrease with the increase in the applied rate of shear, σ, it was decided to use a high value of σ to eliminate weakly bioadhesive materials.

MATERIALS AND METHODS

Chemicals

Porcine gastric mucin (used as received without further purification), bovine serum albumin, dextran, heparin, and sodium acetate were purchased from Sigma Chemical Co. (St. Louis, MO). Polyacrylic acid and polybrene were obtained from Aldrich Chemical Co. (Milwaukee, WI). Polyethylene glycol was purchased from City Chemical Co. (New York, NY). Acid precursor porcine skin gelatin (297 bloom and 8.6 isoionic point) was obtained from Leiner and Sons. Chitosan, low-viscosity grade, was purchased from Proton Laboratories (Redmond, WA).

Equipment

Viscometric measurement for mucin/polymer dispersions were performed using a Brookfield viscometer, Model RTV, with a SC4-21 spindle and a small sample adaptor, R13, (Brookfield Engineering Laboratories, Stoughton, MD). An Ostwald viscometer (Fisher Scientific Co., Pittsburgh, PA) was used to estimate the viscosity coefficients of solutions of individual polymers. Most individual solutions possessed viscosity values close to that of the solvent and were below the accuracy limit of the Brookfield viscometer settings. Therefore, the Ostwald viscometer was used to perform rheological measurements of individual polymer solutions after recognizing their Newtonian character.

Experimental Procedure

Dried mucin was hydrated with 0.1 *N* hydrochloric acid or 0.1 *N* acetate buffer (pH 5.5) by gentle stirring for 3 hr at 25°C to yield a dispersion of 20% (w/v). For each polymer, two series of concentrations were prepared in either 0.1 *N* hydrochloric acid or 0.1 *N* acetate buffer (pH 5.5) except for chitosan. Chitosan was dissolved in 10% acetic acid to yield a 4% (w/v) stock solution. Chitosan solution was then diluted with 0.1 *N* hydrochloric acid or acetate buffer (pH 5.5) to give the concentrations of 0.10, 0.50, or 1.0% (w/v). Six-milliliter aliquots of mucin dispersions (20%, w/v) in 0.1 *N* hydrochloric acid or 0.1 *N* acetate buffer were mixed for 15 min with 2 ml of each polymer concentration in the corresponding solvent to give the concentrations of 0.10, 0.20, and 0.50 (w/v) for cationic gelatin, 0.10 and 0.50 (w/v) for polyaspartic acid and heparin, and 0.10, 0.50, and 2.50 (w/v) for all other polymers. The final concentration of mucin was 15% (w/v). Viscosities of mucin/polymer systems and that of mucin dispersions alone were measured at 25°C and a rate of shear of 93 $\sec^{-1}$. Viscometric measurements were performed after exactly 3 min of applying the shearing force. This time was chosen to allow the shear force to be homogeneously distributed throughout the sample. Mucin dispersions containing 1% (w/v) polyacrylic acid or chitosan were further examined at the shear rates of 4.75, 9.30, 18.60, 46.50, and 93.00 $\sec^{-1}$. The viscosity of all polymers (η_p) at their corresponding polymer concentrations in mucin-polymer systems was measured at 25°C using an Ostwald viscometer (11) assuming that the viscosity of distilled water is 0.890 cP at 25°C. The viscosity values from the Ostwald viscometer can be considered to be absolute viscosity coefficients under the assumption that the polymer solutions are Newtonian solutions, i.e., viscosity is independent of the applied shear stress. All polymers, except chitosan, have been considered to be Newtonian solutions at low concentrations similar to the concentrations used in this study (11). The viscosity coefficient of 1% chitosan solution in the presence of acetate buffer or hydrochloric acid were further determined with the Brookfield viscometer. Viscosity coefficients of chitosan in acetate buffer or hydrochloric acid showed no differences when measured by the Brookfield viscometer at a rate of shear of 93 $\sec^{-1}$ or when measured with the Ostwald viscometer. All viscometric experiments were repeated at least three times.

RESULTS

Tables I and II show viscosity coefficients of all tested polymers as well as their corresponding bioadhesion components and forces of bioadhesion at pH 1 and pH 5.5, respectively.

Neutral Polymers. At pH 1, the force of bioadhesion of polyethylene glycol, bovine serum albumin, and dextran increased as the polymer concentration increased. At 0.1% (w/v), polyethylene glycol had a force of 365 dyne/cm^2, which increased to 1328 dyne/cm^2 at a concentration of 2.5% (w/v) of the polymer. Parallel results were observed for dextran. Bovine serum albumin had a sharper increase in the force of bioadhesion from 141 dyne/cm^2 at 0.1% (w/v) to 6383 dyne/cm^2 at a concentration of 2.5% (w/v). At pH 5.5, concentrations of 0.1 and 0.5% (w/v) for polyethylene glycol and 0.1% (w/v) for dextran caused no viscometric changes in the mucin dispersion, i.e., the components of bioadhesion and the force of bioadhesion equaled zero. However, the force of bioadhesion started to increase at polymer concentrations of 2.5% (w/v), being 1899 dyne/cm^2 for polyethylene glycol, 2769 dyne/cm^2 for dextran, and 1918 dyne/cm^2 for bovine serum albumin. No major changes in bioadhesion

Table I. Polymer Viscosity (η_p), the Component of Bioadhesion (η_b), and the Force of Bioadhesion (F) in 0.1 N Hydrochloric Acid

Polymer	MW	Polymer concentration (w/v, %)	η_p (cps)[a]	η_b (cps)[a]	F (dyne/cm^2)[a]
Polyethylene glycol	4,000	0.10	0.89 ± 0.00	3.92 ± 1.08	365 ± 100
		0.50	0.93 ± 0.00	6.28 ± 1.07	584 ± 100
		2.50	1.04 ± 0.00	14.28 ± 1.08	1328 ± 100
Bovine serum albumin	70,000	0.10	0.89 ± 0.00	1.52 ± 1.71	141 ± 159
		0.50	0.91 ± 0.01	15.92 ± 1.11	1481 ± 102
		2.50	1.11 ± 0.03	68.63 ± 2.43	6383 ± 226
Dextran	71,500	0.10	0.89 ± 0.00	1.51 ± 1.08	140 ± 100
		0.50	0.97 ± 0.09	6.24 ± 1.17	580 ± 109
		2.50	1.32 ± 0.03	18.73 ± 1.10	1742 ± 102
Polybrene	4,500	0.10	0.89 ± 0.00	0.00	0
		0.50	0.89 ± 0.00	0.00	0
		2.50	1.04 ± 0.00	7.98 ± 1.08	742 ± 100
Cationic gelatin	193,000	0.10	0.93 ± 0.00	3.88 ± 1.08	361 ± 100
		0.20	1.01 ± 0.00	7.88 ± 1.08	733 ± 100
		0.50	1.69 ± 0.01	41.51 ± 1.09	3860 ± 101
Chitosan	652,000	0.10	1.43 ± 0.01	18.90 ± 1.16	1758 ± 108
		0.50	4.68 ± 0.09	54.32 ± 1.17	5052 ± 109
		1.00	16.07 ± 0.47	73.27 ± 2.12	6814 ± 197
Polyaspartic acid	35,000	0.10	0.89 ± 0.00	0.00	0
		0.50	0.89 ± 0.00	0.00	0
Heparin	48,500	0.10	0.89 ± 0.00	0.00	0
		0.50	0.89 ± 0.00	0.00	0
Polyacrylic acid	90,000	0.10	0.89 ± 0.00	0.00	0
		0.50	0.98 ± 0.00	11.49 ± 2.40	1069 ± 223
		2.50	1.92 ± 0.02	61.76 ± 2.62	5744 ± 243

[a] Values reported as mean ± SD ($n = 3$).

Table II. Polymer Viscosity (η_p), the Component of Bioadhesion (η_b), and the Force of Bioadhesion (F) in 0.1 N Acetate Buffer, pH 5.5

Polymer	MW	Polymer concentration (w/v)	η_p (cps)[a]	η_b (cps)[a]	F (dyne/cm^2)[a]
Polyethylene glycol	4,000	0.10	0.93 ± 0.00	0.00	0
		0.50	0.94 ± 0.00	0.00	0
		2.50	1.22 ± 0.00	20.42 ± 1.71	1899 ± 159
Bovine serum albumin	70,000	0.10	0.93 ± 0.00	1.47 ± 1.71	137 ± 159
		0.50	0.93 ± 0.00	13.48 ± 1.71	1254 ± 159
		2.50	1.02 ± 0.00	20.62 ± 1.71	1918 ± 159
Dextran	71,500	0.10	1.01 ± 0.01	0.00	0
		0.50	1.06 ± 0.01	8.54 ± 1.71	794 ± 159
		2.50	1.50 ± 0.00	29.77 ± 4.10	2769 ± 381
Polybrene	4,500	0.10	0.93 ± 0.00	0.00	0
		0.50	0.93 ± 0.00	0.00	0
		2.50	1.10 ± 0.00	19.24 ± 1.71	1789 ± 159
Cationic gelatin	193,000	0.10	1.04 ± 0.00	13.42 ± 1.71	1248 ± 159
		0.25	1.17 ± 0.00	31.95 ± 1.71	2971 ± 159
		0.50	1.05 ± 0.00	65.06 ± 1.71	6051 ± 159
Chitosan	652,000	0.10	1.37 ± 0.02	21.30 ± 3.00	1981 ± 279
		0.50	3.35 ± 0.02	70.63 ± 1.54	6569 ± 140
		1.00	10.45 ± 0.44	137.56 ± 2.86	12793 ± 266
Polyaspartic acid	35,000	0.10	0.93 ± 0.00	0.00	0
		0.50	0.93 ± 0.00	0.00	0
Heparin	48,500	0.10	0.93 ± 0.00	0.00	0
		0.50	0.93 ± 0.00	0.00	0
Polyacrylic acid	90,000	0.10	1.11 ± 0.00	12.11 ± 2.72	1126 ± 253
		0.50	2.76 ± 0.00	15.03 ± 2.03	1398 ± 189
		2.50	6.00 ± 0.02	111.17 ± 8.20	10339 ± 763

[a] Values reported as the mean ± SD ($n = 3$).

components or forces were observed with pH change except for 2.5% bovine serum albumin.

Polyanions. Neither heparin nor polyaspartic acid demonstrated measurable bioadhesion components or forces at either pH 1 or pH 5.5 at the tested concentrations. Polyacrylic acid possessed concentration-dependent increases in the bioadhesion component. Viscometric changes caused by polyacrylic acid at pH 1 were less than those at pH 5.5.

Polycations. Polybrene showed no viscometric changes at concentrations of 0.1 and 0.5% (w/v) at pH values of 1 or 5.5. At a 2.5% (w/v) polybrene concentration and a pH 1, the viscosity increased to give a force of bioadhesion of 742 and 1789 dyne/cm^2 at pH 5.5. Both cationic gelatin and chitosan caused large increases in the viscosity of mucin-polymer dispersions. These increases were proportional to the polycation concentration. The increase in the force of bioadhesion in acetate buffer was markedly higher than corresponding values in hydrochloric acid.

The relationships between the component of bioadhesion and polymer concentration of chitosan and polyacrylic acid are illustrated in Fig. 1. Chitosan showed higher component of bioadhesion values than polyacrylic acid at the same concentrations; both polymers showed higher components in the presence of acetate buffer (pH 5.5) than in hydrochloric acid (pH 1). The effect of the rate of shear on the component of bioadhesion is shown in Fig. 2. The component of bioadhesion for chitosan decreases with an increase in the rate of shear changes in either medium. Changing the rate of shear did not markedly change the component of bioadhesion for polyacrylic acid.

DISCUSSION

Bioadhesive drug delivery devices have been proposed and formulated to be localized onto a biological surface. A bioadhesive force is required between the drug device and the biological surface successfully to retain the device and retard the natural clearance processes. Efforts to characterize the force of bioadhesion resulted in various hypotheses on the structural requirements for bioadhesion of polymer-mucin systems (1,2). General agreement exists on the characteristics of molecular weight, hydrogen bond formation ability, and molecular dimensions and configurations. However, the importance of electrostatic charge is apparently ambiguous. Polycations (e.g., polylysine and polybrene) and strong polyanions (e.g., heparin or polyglutamic acid) did not exhibit considerable adhesion to conjunctival epithelial cells as tested by the fluorescent probe technique (11). Polyacrylic acid polyanion showed maximum adhesion by the same test (11). On the other hand, cationic methacrylamide copolymers showed a higher affinity to rabbit intestinal rings compared to unmodified polymers (17). According to a tensile stress test (18), loosely cross-linked polyacrylic acid showed more adhesion power to excised rabbit stomach at pH 2 (i.e., in its unionized form, pK_a = 4.7), and adhesion diminishes as the pH reaches neutrality.

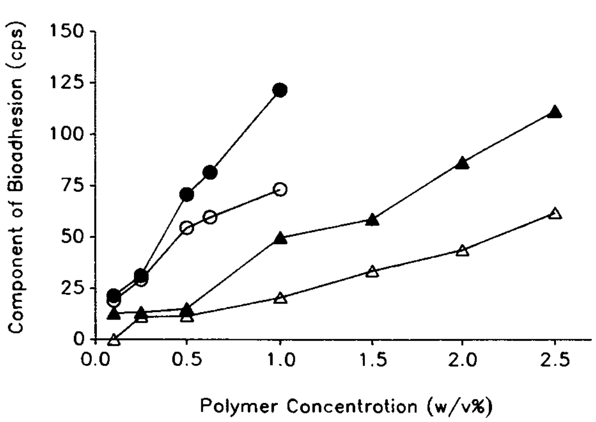

Fig. 1. Effect of polymer concentration (w/v, %) on the component of bioadhesion (cps). (●) Chitosan in the presence of acetate buffer or (○) in hydrochloric acid and (▲) polyacrylic acid in the presence of acetate buffer or (△) in hydrochloric acid.

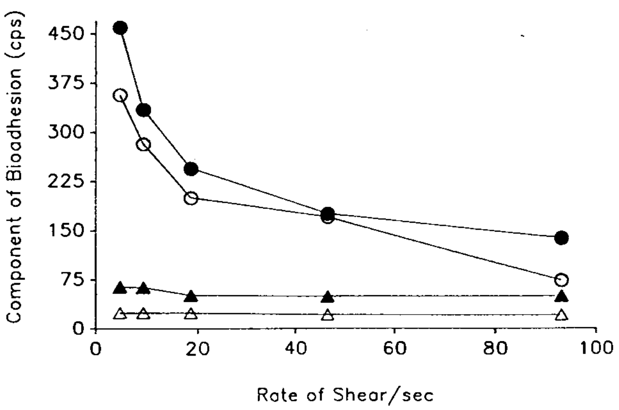

Fig. 2. Effect of the rate of shear (sec^{-1}) on the component of bioadhesion (cps). One percent (w/v) chitosan (●) or polyacrylic acid (▲) in the presence of 0.1 *N* acetate buffer or in 0.1 *N* HCl (open symbols).

In this investigation, cationic gelatin and chitosan showed high interactions with mucin in both hydrochloric acid and acetate buffer. At a pH value of 1, mucin carboxylic acid groups (from terminal sialic acids) will be unionized (pK_a = 2.6), and therefore, the force of interaction is not solely due to ionic attractions. Both gelatin and chitosan are rich in hydroxyl and amino groups and capable of hydrogen bond formation. Chitosan is a linear molecule and can more easily interpenetrate a mucin random coil than gelatin which is a branched macromolecule. Moreover, chitosan in this study had a higher molecular weight than gelatin and increases the probability for chitosan-mucin interfacial interactions. At higher pH values (4.7 for chitosan and 5.5 for gelatin) the sialic acid was in the anion form, hence ionic attraction along with other forces resulted in more bioadhesive forces. Polybrene is a quaternary ammonium compound (i.e., its cationic nature is pH independent), and in 0.1 *N* hydrochloric acid (pH 1) minimum electrostatic interactions are expected between the unionized carboxylic acid terminal groups of mucin and the permanent positive charge of polybrene. However, other bonds such as polar, nonpolar, or physical bonds may exist. The low bioadhesion component of polybrene may be due to its low molecular weight along with its large side chains, which could sterically inhibit formation of the above mentioned bonds especially those due to physical entanglement. The slight but significant increase in the force of bioadhesion to polybrene at pH 5.5 is clearly due to electrostatic attractions created as the mucin terminal carboxylic groups became ionized.

Polyanions of relatively small molecular weights such as heparin and polyaspartic acid did not change the viscosities of mucin dispersions at pH 1 or 5.5. Zero values of the force of bioadhesion of heparin and polyaspartic acid are consistent with the results of the fluorescent probe method by Park and Robinson (11). Polyacrylic acid, on the other hand, demonstrated high bioadhesion forces at both pH 1 and pH 5.5. Interestingly, the interaction of polyacrylic acid with mucin was stronger at pH 5.5 than at pH 1. These results are compatible with the results obtained using the fluorescent probe technique (11) yet are in disagreement with the results generated by the tensile stress method (18).

The limitation of bond destruction methods (3–7) is the result of the nonuniform stress distribution along the bond line caused by the inherent differences in the substrate (i.e., mucin) moduli and the adhesive polymer (8). Therefore, it cannot be assumed that the breakdown of a gastric tissue-polymer-gastric tissue system occurs only at the polymer-gastric tissue interface. System breakdown may occur inside the polymer layer if the polymer-polymer cohesive forces are less than the polymer-mucin adhesive forces.

Although electrostatic repulsion exists between anionic groups of polyacrylate and the mucin terminal carboxylate groups at pH 5.5, the high force of bioadhesion of polyacrylic acid can be explained on the basis of molecular shape dynamics of polyelectrolytes described by Katchalsky (19). Polyacrylic acid molecules exist in a random coil form when unionized. This contracted form reflects the numerous intramolecular hydrogen bonds between the unionized carboxylic groups. Ionization of such a molecule results in diminishing the intramolecular hydrogen bonds and generates a stretched cylindrical shape, which is then more able to penetrate a mucin network than the coil form. Interaction between two anionic macromolecules is further supported by the fact that the viscosity of polyacrylic acid in 0.1 *N* acetate buffer is higher than in 0.1 *N* hydrochloric acid. The high viscosity of the ionized polyacrylic acid molecules (η_p) indicates that the repulsion due to similar negative charges is less than the other intermolecular bonds, which are able to hold together repelling molecules.

For neutral polymers such as dextran and bovine serum albumin, molecular branching reduced polymer-mucin interactions to almost the same degree as a smaller linear polymer such as polyethylene glycol 4000. A very steep increase in the bioadhesive force was observed for bovine serum albumin bioadhesion component at 2.5% (w/v) (pH 1) and may represent a concentration threshold requirement prior to molecular rearrangement for maximum complex formation.

Porcine gastric mucin was used as a model mucin (15). The behavior of other mucins, such as submaxillary, ocular, or gastric mucin from different sources, in the presented method remains to be determined. However, since all mucins appear to share general physical, structural, and rheological properties (2,15), it is believed that porcine gastric mucin is a satisfactory model for primary evaluation of bioadhesive candidates.

In conclusion, data generated by the viscometric assessment method of bioadhesion are in agreement with an already established fluorescent probe technique. The viscometric method is, however, a simpler alternative since it utilizes the tissue surface component involved in the process of bioadhesion rather than the whole cell. The viscometric method also introduced the parameter, η_b, which is an empirical determinant of the absolute force of bioadhesion. η_b could be a compound parameter representing various physicochemical properties of the bioadhesive polymer, e.g., molecular weight and configuration and electric charge. Further investigations may lead to predictive mathematical models that incorporate the above properties. The ease of the technique may be of practical importance in evaluating potential bioadhesive materials for pharmaceutical dosage forms.

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