Bioadhesive Drug Delivery Systems

Editors

Vincent Lenaerts, Ph.D.
Professor
Faculty of Pharmacy
University of Montreal
Montreal, Quebec, Canada

Robert Gurny, Ph.D.
Professor
School of Pharmacy
University of Geneva
Geneva, Switzerland

CRC Press, Inc.
Boca Raton, Florida

1990

Chapter 3

TEST METHODS OF BIOADHESION

Kinam Park and Haesun Park

TABLE OF CONTENTS

I. INTRODUCTION

Adhesion is defined as the state in which two surfaces are held together by interfacial forces.[1] Adhesion is referred to as bioadhesion, if one or both of the adherends are of a biological nature. Thus a *bioadhesive* is defined as a substance that is capable of interacting with biological materials and being retained on them or holding them together for extended periods of time.[2] Bioadhesion can occur by virtue of electrostatic and dipolar interactions, hydrogen bonding, or by the adsorption and interpenetration of macromolecules.[3] According to the definition, adhesives derived from biological objects (biological adhesives[4]) are not necessarily bioadhesives, if they are applied to nonbiological adherends.

There are a variety of bioadhesion phenomena and bioadhesives that satisfy the above definitions. For convenience, bioadhesion and bioadhesive are classified into three types based on phenomenological observation, rather than on the mechanisms of bioadhesion.[2] Type I bioadhesion is characterized by adhesion occurring between biological objects without involvement of artificial materials. Cell fusion[5] and cell aggregation[6] are good examples of Type I bioadhesion. Type II bioadhesion refers to adhesion of biological materials to artificial substrates. Type II bioadhesion can be represented by cell adhesion onto culture dishes[7,8] or barnacle adhesion to a variety of substances including metals, woods, and other synthetic materials.[9] Finally, Type III bioadhesion described adhesion of artificial substances to biological substrates, such as adhesion of polymers to skin or other soft tissues. All three types of bioadhesion are equally important. We will focus, however, on Type III bioadhesion, which is in accordance with the objectives of this book.

Type III bioadhesives have been used for quite a long time under different names. Many chemical adhesives have been used, instead of or in addition to sutures, for joining and sealing of tissues under the names of tissue adhesives,[10,11] clinical adhesives, biological glue,[12] or medical polymer adhesives.[13] A number of synthetic and natural polymers have been used as skin adhesives,[14] dental adhesives,[15] and mucoadhesives.[16]

The goal of the development of bioadhesives is to duplicate, mimic, or improve biological

adhesives, which are both durable where required and degradable where necessary, and nontoxic at all.[12] Developing a new bioadhesive and applying it for a particular use requires elucidation of the bioadhesion mechanisms. Understanding the bioadhesion mechanisms begins with evaluating bioadhesive performance of various candidate materials. Thus the evaluation of bioadhesive properties is fundamental for the development of new bioadhesives. This chapter describes currently known bioadhesion evaluation methods and parameters that are important to the testing and design of new bioadhesives.

II. EVALUATION OF BIOADHESIVE PROPERTIES

A. BIOADHESION EVALUATION METHODS

The first step in the selection of a bioadhesive for a particular application is to determine if its properties are suitable for the intended application. Testing is essential for the development, qualification, processing, and proper use of bioadhesives. Since there are a large number of bioadhesives in different physical forms and biological substrates of different nature, evaluation of bioadhesive properties is inherently complex and diverse. Bioadhesives, as well as nonbioadhesives, are usually tested as one component of a system of many parts. This means that a test of bioadhesives actually tests the properties of many components including the bioadhesive itself, the biological substrates, and other experimental conditions. Thus, evaluation and comparison of the properties of various bioadhesives can be obtained only if all the conditions of the test and the experimental procedures are kept constant. If the experiment is changed by varying a factor other than the bioadhesive, the test method can provide information about the contribution of the other component to the overall bioadhesive performance.[17]

It is not easy to extrapolate the behavior of a bioadhesive from a test to its performance in an actual *in vivo* application, since testing is generally made under a controlled environment that is far from the actual service condition. In addition, each test measures a particular aspect of bioadhesion and a particular property of a bioadhesive while the actual *in vivo* performance of a bioadhesive depends on various interdependent properties. It is extremely difficult to simulate the exact conditions that a bioadhesive may be subject to *in vivo*. Although it is expected that a certain test method will represent the actual *in vivo* performance of a bioadhesive better than others, it is not clear what parameter is most suitable for evaluating the *in vivo* bioadhesive performance. For this reason, various properties of a bioadhesive have to be measured, and the obtained parameters have to be compared with the actual *in vivo* performance of the bioadhesive.

B. STANDARD TEST METHODS

As described later in this chapter, a number of different test methods are used to measure the same property of a bioadhesive. There is a tendency for each investigator to use his or her own distinct test method. This is partly because there are no standard test methods specifically designed to measure a certain property of bioadhesives. The lack of standard test methods creates confusion among investigators, since data generated at different laboratories cannot be compared, and thus the real meaning of reported test values is not transferable. The standardization of test methods acceptable to everyone will improve the communication between researchers and allow them to speak a common language when comparing the test data and results.[18] Although there is a need for developing standard test methods designed for bioadhesives, fulfilling such a need will take time. Research on bioadhesives is still in its early stage, and more data will be necessary in order to have a consensus among investigators on standard test methods. Thus it appears that there is no alternative now other than trying various test methods to accumulate experimental data and improve our understanding of the bioadhesion phenomena. It is necessary, however, that investigators are aware of the need for establishing standard test methods.

C. QUANTIFICATION OF BIOADHESIVE PROPERTIES

The performance of a bioadhesive can be evaluated by various parameters, such as adhesion strength, adhesion number, or duration of adhesion. One is reminded that for measuring any given bioadhesive property, a small change in experimental variables, such as the initial loading pressure, the initial contact time, or the rate of removal of the adhesive, may result in significantly different values. Thus even the quantitative data may be considered to be subjective. In this regard, numerical values may have to be used on a comparative basis to establish adhesive properties measured by a certain specified procedure under specified conditions.

1. Adhesion Strength

Measuring mechanical properties of a bioadhesive may be the most direct way to quantify the bioadhesive performance. There are three basic types of stress that are most commonly used to measure the strength of adhesive joints. They are tensile, shear, and peel stress as shown in Figure 1. They are popular because they are relatively quick and simple to perform and enable poor candidates to be screened out.[19] In tensile loading, the forces are perpendicular to the plane of the joint. In shear loading, the stress is parallel to the plane of the joint. In both cases, the stress is distributed uniformly over the entire joint, and all of the adhesive is put to work at the same time.[20] In peel loading, the stress is limited to a very fine line at the edge of the joint.[20] Peel testing measures the ability to resist peeling forces, rather than mechanical properties of the adhesive.[17]

2. Adhesion Number

The measurement of adhesion strength using tensile, shear, or peel test will be very difficult, if a bioadhesive is in the form of small particles. In such a case, the adhesion number can be used to measure the adhesive properties. The adhesion number N_a is defined as the ratio between the number of particles N remaining after the application of a certain detachment force and the number of particles N_o originally present on the test surface.[21] The adhesion number is often expressed as a percentage.

$$N_a = (N/N_o)100 \quad (1)$$

The adhesive force can be evaluated from the adhesion number, since the detachment force is known, and it is numerically equal to but opposite in direction to the force of adhesion.[21] The use of an adhesion number, however, is good enough to evaluate and compare properties of various bioadhesive particles. Obviously, many variations of the adhesion number method can be used.

3. Durability

Probably the most important property of a bioadhesive is to maintain satisfactory performance in the actual service condition for a desired period of time. Thus the durability of a bioadhesive may be the ultimate parameter that should be used to compare various bioadhesives. Since the durability does not solely depend on the adhesive strength alone, other factors that affect it should be identified and examined. For structural adhesives, the durability of adhesive joints can be assessed by a number of methods, such as sustained load methods, the endurance limit method, cyclic stress testing, or fracture mechanics tests.[22] The durability of bioadhesives may be evaluated by changing experimental conditions for bioadhesive-tissue joints, such as changing pH, temperature, ionic strength, water content, etc. For example, Chen and Cyr[23] devised an apparatus that measured *in vitro* duration of adhesion of intraoral bandages (see Section III.A.1). The condition of the *in vitro* duration test was different in one significant respect from the actual condition in the oral cavity. Since the test system was completely submerged in water during *in vitro* testing, the excess water

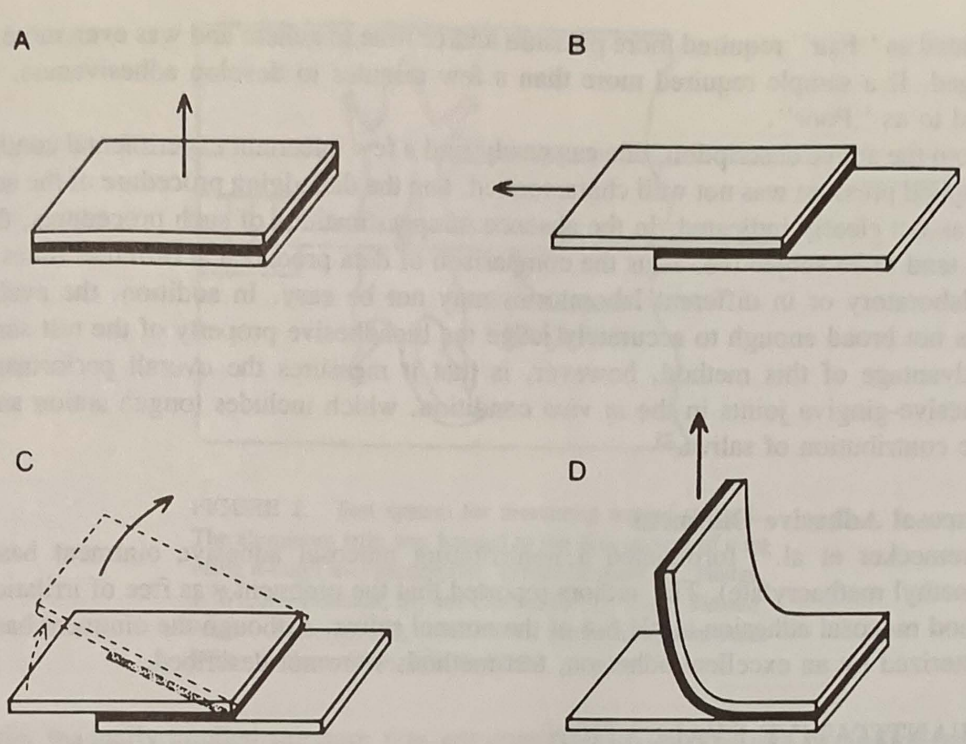

FIGURE 1. Testing of adhesive bonds by tensile (A), shear (B), and peel (C and D) tests. Peel tests with stiff detaching strip (C) are also known as cleavage tests[20] or bending tests.[23] Peel tests can be done at various peel angles. The white and black areas represent adherend and adhesive, respectively.

caused further and more rapid hydration of the hydrocolloid particles. As this additional water was absorbed, the adhesive bond was weakened due to the formation of a slippery mucilage. As a result, the *in vitro* test was considered to be an accelerated test for duration of adhesion in the actual service condition. The duration of adhesion for Orahesive® bandage ranged from 4 to 25 min in the *in vitro* tests, while the value ranged from 6 to 24 h under the actual clinical conditions.

III. *IN VIVO* AND *EX VIVO* EVALUATION OF BIOADHESION

This and the next two sections describe currently known *in vivo* and *in vitro* test methods. The test methods described here are neither the only test methods nor the standard test methods for particular bioadhesive-tissue systems. They can be modified for other applications. The test methods in this chapter are concerned only with Type III bioadhesion, i.e., adhesion of artificial substances to biological substrates such as gingiva, teeth, skin, or the gastrointestinal mucus layer.

A. QUALITATIVE EVALUATION

1. Intraoral Bandages

Chen and Cyr[23] designed a simple *in vivo* screening test for overall performance of intraoral bandages. Candidate hydrocolloid powders were mixed with polyisobutylene at a 6:4 ratio and pressed into disks about 1/16 in. thick. One side of the disk was covered with a thin polyethylene film of equal size. The exposed side was then pressed with a finger onto the anterior gingiva for 30 s. If the disk did not stick, pressure was continued for another 30 s. If a composition adhered to the gingiva under normal application pressure with the first 30 s and was difficult to dislodge, it was referred to as "Excellent". If a composition was easily dislodged, the performance was described as "Satisfactory". The composition

designated as "Fair" required more pressure and/or time to adhere and was even more easily dislodged. If a sample required more than a few minutes to develop adhesiveness, it was referred to as "Poor".

From the above description, one can easily find a few uncertain experimental conditions. The applied pressure was not well characterized, and the dislodging procedure of the adhered disk was not clearly indicated. In the absence of specifications of such procedures, the test results tend to be subjective. Thus the comparison of data produced at different times in the same laboratory or in different laboratories may not be easy. In addition, the evaluation scale is not broad enough to accurately judge the bioadhesive property of the test samples. The advantage of this method, however, is that it measures the overall performance of bioadhesive-gingiva joints in the *in vivo* condition, which includes tongue action and disruptive contribution of saliva.[23]

2. Mucosal Adhesive Ointment

Bremecker et al.[24] formulated a nonirritating mucosal adhesive ointment based on poly(methyl methacrylate). The authors reported that the ointment was free of irritation and had good mucosal adhesion at pH 6.4 of the normal saliva. Although the ointment base was characterized by an excellent adhesion, test methods were not described.

B. QUANTITATIVE EVALUATION

1. Tensile Testing

a. Tissue Adhesives

Leonard et al.[25] measured tissue bond strengths of various cyanoacrylate esters using rats. Midline incisions 3 cm in length, extending into the subcutaneous tissues, were made over the lumbar region of the anesthetized rats and the incision sealed with two drops of various alkyl cyanoacrylate monomers. The edges were held in apposition for up to 2 min until polymerization occurred depending on the nature of the monomers. The adhered wounds were pulled apart 1 h following wound closure as shown in Figure 2, and the adhesive strength was measured using a table model Instron testing machine at a cross-head rate (pulling rate) of 0.5 in./min.

Margules and Harris[26] used tension tests to compare the adhesive property of medical grade cyanoacrylate and acrylic adhesives. Test buttons were made of polymethylmethacrylate in a cylinder of 6.35 mm length and 6.35 mm diameter with a hole in the middle for pin placement. The skin was shaved and prepared for adhesion with benzalkonium chloride swabbing followed by air drying. The buttons with adhesives were applied to both wet and dry skin. The wet skin was prepared by swabbing the entire shaved area with normal saline. Test buttons were pressed to both wet and dry skin for 30 s. A strain rate of 2 cm/s was applied for each tension test, and the maximum yield strength was measured using a manually operated Chatillion force gauge.

b. Denture Adhesives

Ow and Bearn[27] developed a method to evaluate denture adhesives under controlled conditions *in vivo*. The adhesiveness was measured using a custom made pressure-sensitive device. Orahesive® was used as a model denture adhesive.

2. Peel Testing

a. Intraoral Bandages

Chen and Cyr[23] used a Chatillon gauge to quantitatively measure the force required to separate the bandage from oral mucous membrane or from the teeth. The authors concluded that the *in vivo* peel adhesion tests agreed reasonably well with those obtained in the *in vitro* lap-shear tests (Figure 1B, Section IV.C.1), although the curvature of the gingiva was

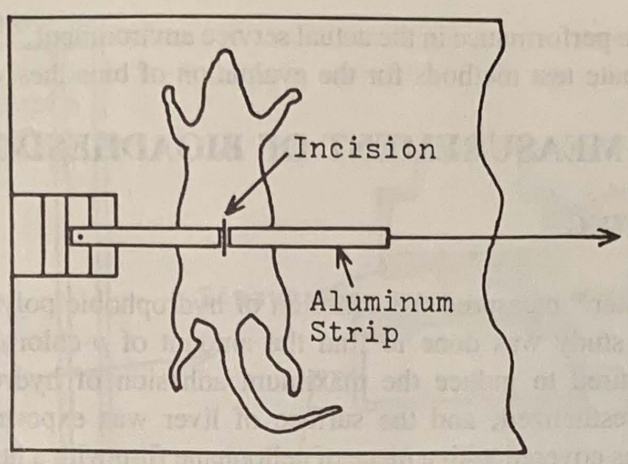

FIGURE 2. Test system for measuring wound strength. The aluminum strip was bonded to the skin surface of a rat using *n*-butyl α-cyanoacrylate. (From Leonard, F., Hodge, J. W., Jr., Houston, S., and Ousterhout, D. K., *J. Biomed. Mater. Res.*, 2, 173, 1968. With permission from John Wiley & Sons, Copyright © 1968.)

variable, manually applied pressure was not controllable, and the rate of pulling was also uncontrollable. The good agreement between results of *in vivo* and *in vitro* tests should be taken with caution, since the *in vitro* tests employed cellophane membranes instead of excised tissues. The issue of using artificial substrates in the evaluation of bioadhesives is briefly discussed in Section IV.C.

b. Prosthetic Skins

Skornik et al.[28] developed a quantitative technique to evaluate adherence of different types of prosthetic skins using a modified Keil tester. The rat's skin was excised from the dorsal surface and replaced with prosthetic material, which consisted of an adherent layer of nylon 66 velour and an outer barrier layer of polyurethane. Rats were selected at various days postapplication, anesthetized, and subjected to adhesion evaluation. The posterior end was freed from subcutaneous tissue and attached to the machine by means of a clamp. Pulling was maintained at a constant velocity (5 cm/s) in a posterior-to-anterior direction.

C. Indirect Evaluation

Lipatova described in his review[13] a method that was used to estimate the strength of adhesion joints under conditions approaching real ones. The effectiveness of closing the defected aortal wall or kidney wounds of animals by cyanoacrylate adhesives was investigated by measuring the maximum arterial pressure that broke the adhesion joints. The arterial pressure was raised by injecting adrenaline. The method achieved a maximum approximation to a real surgical situation and thus the results obtained from such study are of definite interest for clinical applications.

In the application of bioadhesives to controlled drug delivery systems, bioadhesives are used as platforms. Their major role is to secure dosage forms to a certain position of the body and thus to increase the overall drug absorption. As long as the desired bioavailability is achieved for a planned period of time using a particular bioadhesive, the actual adhesive strength of the bioadhesive to tissue surfaces may not be that important. Therefore, the bioadhesive performance may be inferred from measuring either the residence time at the target sites or bioavailability of drugs. For example, the gastric residence time or the gastric emptying pattern of bioadhesives can be noninvasively measured in a quantitative manner using gamma-scintigraphy.[29] Alternatively, pharmacokinetics can be used as a parameter

indicating bioadhesive performance in the actual service environment. These indirect methods are, in fact, the ultimate test methods for the evaluation of bioadhesives.

IV. *IN VITRO* MEASUREMENT OF BIOADHESIVE STRENGTH

A. TENSILE TESTING

1. Tissue Adhesives

Wang and Forrester[30] measured the adhesion of hydrophobic polymers to parenchymal tissue of rats. Their study was done to find the amount of *p*-chlorobenzoyl chloride (an acylating agent) required to induce the maximum adhesion of hydrophobic polymers to tissue. Rats were anesthetized, and the surface of liver was exposed through a midline incision. The liver was covered with a piece of cellophane film with a hole (1 cm in diameter) in the center. A thin layer of the rubber or silicone adhesive solution containing various amounts of the acylating agent was applied with a spatula over the exposed portion of the liver. A regular laboratory cork (size 00) was also coated with a thin layer of the polymer adhesive and pressed gently to the previously coated area of the liver surface. After 30 min, a portion of the organ was excised, and placed immediately on the platform of a tension tester. The cork was pulled at a rate of 1.3 mm/s and the force for detachment was measured using a Chattillon motorized tension tester.

Matsumoto[10] devised a tension test that provided the evaluation of both speed of bonding and bond strength of various cyanoacrylate adhesives. Fresh samples of beef lung and muscle tissue were used as model adherends. They were cut into $^1/_2$ in. diameter cylindrical plugs and wiped free of excess water and blood. The cyanoacrylate adhesive was applied, and the tissue samples were pressed together for a few seconds. At regular intervals, the tensile strength of the bonds was measured.

2. Gastric Adhesives

Park and Robinson[31,32] measured the force required to separate a polymer specimen from freshly excised rabbit stomach tissue using a modified automatic surface tensiometer (Fisher Autotensiomat®). The isolated stomach was opened with scissors, washed with saline solution to remove its contents, and kept at 4°C saline solution until use. A section of the tissue was cut from the fundus and secured, mucosal side out, onto a tissue mounting device prepared in the laboratory using rubber stoppers and aluminum serum bottle seals with a hole (10 mm) in the center (Figure 3). Another section of the stomach was secured on the modified plunger of a 20 ml plastic syringe and suspended from the balance beam of the tensiometer. Test bioadhesives (acrylic hydrogels) were synthesized in a sheet form, cut into disks using a cutting mold, and placed over the upper tissue in the test solution. The contact between the disk and the lower tissue was maintained for 1 min with the initial weight of 1.8 g. After a predetermined time, usually 1 min, the force to separate two tissues was measured by lowering the bottom tissue mounting device at a constant rate of 0.2 in/s until the polymer layer became detached from the mucus layer.

When tissues are not available in large quantity, bioadhesive hydrogels can be secured onto other substrates. We have found that nitrocellulose membrane is an excellent substrate for securing acrylic hydrogels. Figure 4 shows interaction of cross-linked poly(acrylic acid), which was secured on the nitrocellulose membrane, with excised gastric tissue of rabbits in the arificial gastric juice. As the bioadhesive disk was separated from the tissue surface after contact for 1 min as described above, the mucus layer was pulled away from the tissue surface with the bioadhesive disk. Eventually, the breakup occurred in the mucus layer close to the disk instead of the interface between the disk and the mucus layer. A visual as well as microscopic inspection of the disk at the end of the experiment showed the mucus residues remaining on the disk.

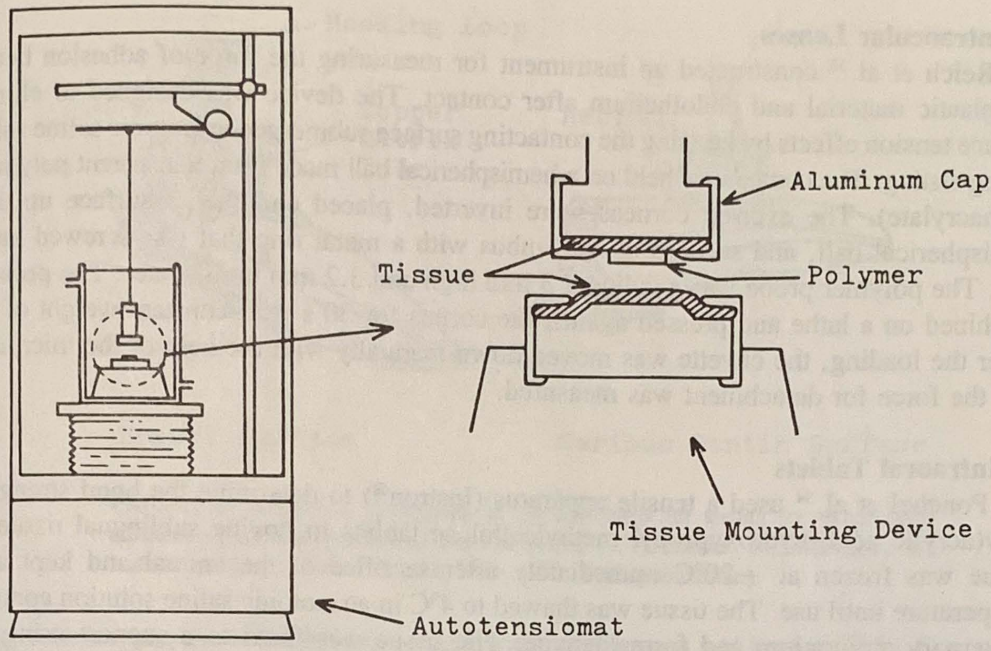

FIGURE 3. Test system for measuring the adhesive strength between acrylic hydrogels and the gastric tissues. Test hydrogel is placed between two tissue layers.

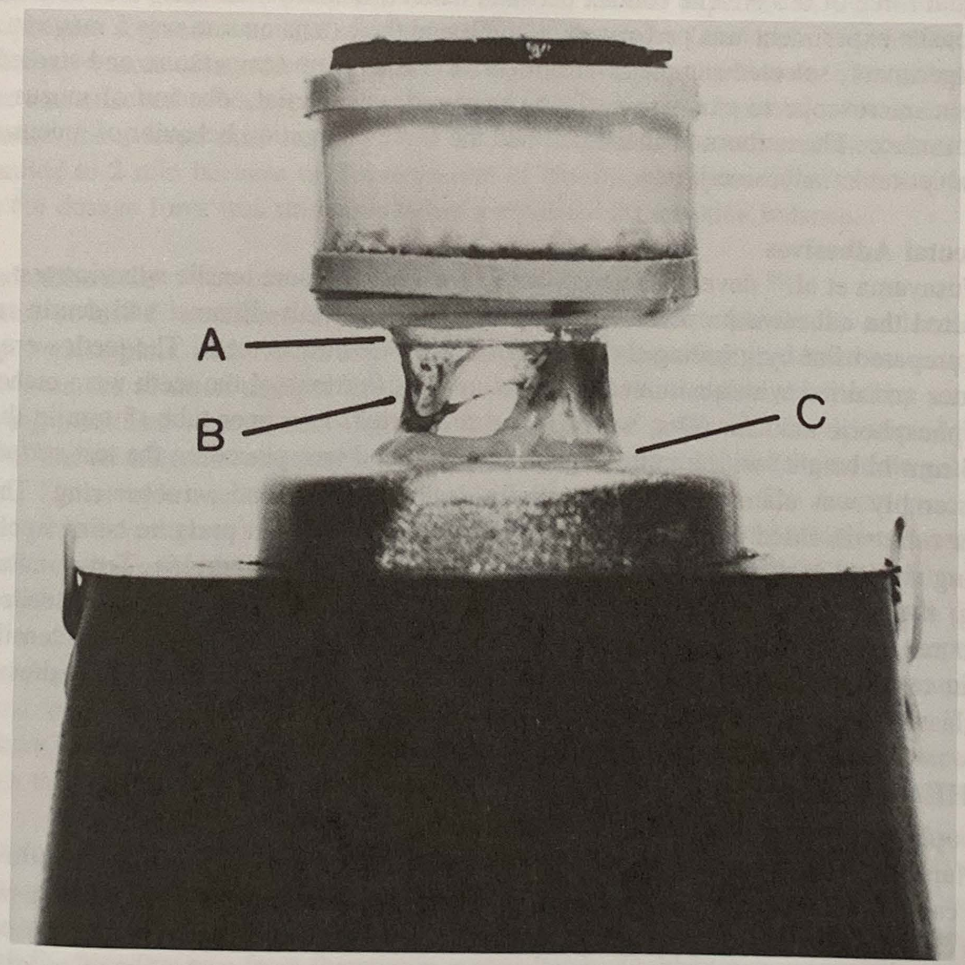

FIGURE 4. Measurement of adhesive strength between cross-linked poly(acrylic acid) (A) and the rabbit gastric tissue (C) using the system shown in Figure 3. The bioadhesive disk (A) was secured on the nitrocellulose membrane. The mucus residues are clearly seen at the bottom of the disk after the cohesion failure occurred in the mucus layer (B).

3. Intraocular Lenses

Reich et al.[33] constructed an instrument for measuring the force of adhesion between the plastic material and endothelium after contact. The device was designed to eliminate surface tension effects by keeping the contacting surface submerged in isotonic saline solution during testing. The cornea was held on a hemispherical ball made from transparent poly(methyl methacrylate). The excised corneas were inverted, placed endothelial surface up on the hemispherical ball, and secured at the limbus with a metal ring that was screwed into the ball. The polymer probe was a cylinder 3 mm high and 3.2 mm in diameter. The probe was machined on a lathe and pressed against the cornea for 30 s with constant weight of 16 g. After the loading, the cuvette was moved down manually with the help of the micrometer, and the force for detachment was measured.

4. Intraoral Tablets

Ponchel et al.[34] used a tensile apparatus (Instron®) to determine the bond strengths of poly(acrylic acid)-hydroxypropyl methylcellulose tablets to bovine sublingual tissue. The tissue was frozen at −20°C immediately after sacrifice of the animal and kept at that temperature until use. The tissue was thawed to 4°C in an isotonic saline solution containing quaternary ammonium and formaldehyde. The tissue was fixed to a support using a cyanoacrylate adhesive. In a bioadhesion test, 15 μl of water was uniformly placed on the exposed side of the tablet, and the two surfaces were brought immediately in contact with an initial force of 0.5 N. The contact between tablet and mucus was maintained for 10 min. The tensile experiment was performed at 26°C, and the extension rate was 5 mm/min. After the experiment, selected samples were dried for 3 d at room temperature and studied in an electron microscope to examine surface changes due to partial adhesion of mucus on the tablet surface. The authors further examined the force-elongation behavior of mucus-mucus and tablet-tablet adhesive joints.

5. Dental Adhesives

Fusayama et al.[35] developed an apparatus for a nonpressure tensile adhesion test, which evaluated the adhesive properties of the restorative materials. Enamel and dentin surfaces were prepared flat by grinding the facial surface of the human teeth. The teeth were stored in water and dried with air immediately before use. Portions of the teeth were etched with 40% phosphoric acid for 60 s, washed, and then dried. A copper tube (5 mm in diameter and 4 mm in height) with a knife edge at its lower end was placed on the test surface, and the assembly was clamped with a specially designed frame and a rubber ring. Then the copper tube was filled with restorative resin with no substantial pressure being applied. A hooking wire loop was inserted into the resin before setting (Figure 5). Ten minutes after filling, the specimens were immersed in water at 37°C and stored for an extended time period ranging from 1 week to 3 months before they were subjected to the tensile test. Similar tension tests were used to evaluate adhesive properties of periodontal dressings,[36] and glass ionomer cement to dentin and enamel.[37,38]

B. SHEAR TESTING

1. Esophageal Adhesives

Marvola et al.[39] developed a method to study the tendency of dosage forms to adhere to the esophageal wall. The study was initiated by the fact that adherence of the drug products to the esophageal wall resulted in high local drug concentrations, which in turn caused drug-induced esophageal ulceration or stricture in humans. Thus their goal was to find less sticky dosage forms, instead of higher strength bioadhesive dosage forms.

The esophagi were removed from pigs and kept in Tyrode solution at 4°C until use.

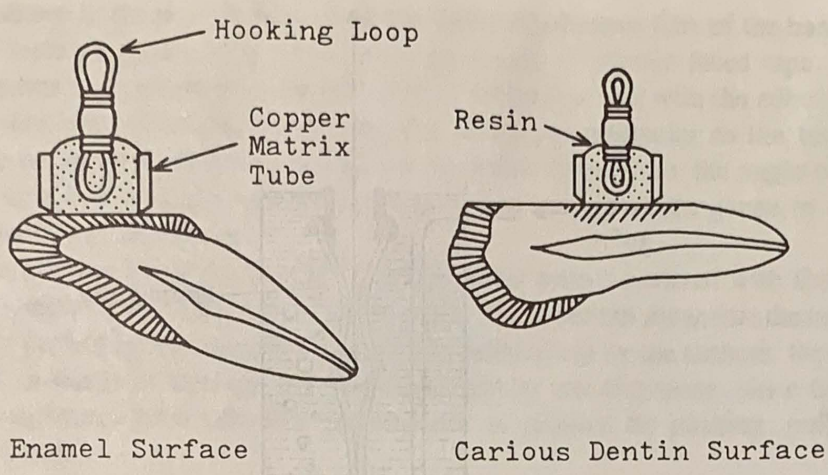

FIGURE 5. Schematic description of the specimens for testing the nonpressure adhesion of restorative resin. (From Fusayama, T., Nakamura, M., Kurosaki, N., and Iwaku, M., *J. Dent Res.*, 58, 1364, 1979. With permission.)

During experiments, the solution was aerated with pure oxygen and kept at 37°C. Segments 6 to 7 cm long were cut from the esophagus and mounted in an organ bath as shown in Figure 6. The lower end of the esophageal segment was tied off, and the upper end was attached around a glass tube (diameter of 15 mm). In another experiment, the lower end of the esophageal segment was also attached around a glass tube (diameter of 8 mm) to wash the mucosa. A hole (diameter of 1 mm) was drilled in the dosage forms to be tested. The product was attached to a copper wire (diameter of 0.25 mm) and placed in the esophageal preparation for a fixed time ranging from 10 s to 3 min. In most cases, the contact time was limited to 2 min because of disintegration of the dosage forms. The force required to detach the dosage form was measured using a modified prescription balance.

2. Mucoadhesives

Smart et al.[16,40] measured interaction of soluble polymers with mucus molecules by coating a glass plate with water-soluble polymers and measuring the force to remove it through a mucus solution using a tensiometer. Crude mucus samples obtained by scraping guinea pig intestines were mixed with the same volume of water and slowly stirred for 24 h at 4°C. The mixture was then centrifuged at 32,500g for 30 min, and the middle gel layer retained. The sample was stored frozen in 1 ml aliquots until use. The frozen mucin gel was thawed in a 5 ml glass vial placed in the water bath at 20°C. The vial was then transferred to a platform that could be mechanically lowered at a rate of 1 mm/min. Glass plates were coated with test polymer materials by dipping into a 1% polymer solution and oven drying at 60°C to constant weight. The polymer-coated glass plates, 11 mm wide, were suspended from a microforce balance. The platform was then raised until the plate was completely immersed in the mucus gel. The plate was left in contact with the mucin gel for 7 min, after which the platform was lowered at a rate of 1 mm/min. The maximum force necessary to detach the plate from the gel was recorded.

3. Gastric Adhesives

Leung and Robinson[41] measured the bioadhesive properties of copolymers of acrylic acid and methyl methacrylate using the tensiometer described in Section IV.A.2. The force to detach the bioadhesives from the gastric mucus layer of rabbits was measured by pulling the upper tissue in the direction parallel with the lower tissue.

4. Dental Adhesives

Jedrychowski et al.[42] compared the adhesive effects of various adhesion promoters

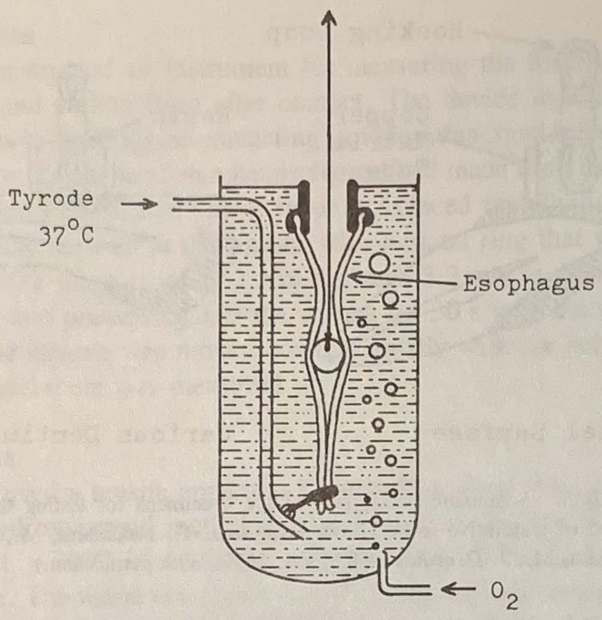

FIGURE 6. Measurement system for the force necessary to detach oral dosage form from esophagus. (From Marvola, M., Vahervuo, K., Sothmann, A., Marttila, E., and Rajaniemi, M., *J. Pharm Sci.*, 71, 975, 1982. Reproduced with permission of the copyright owner, the American Pharmaceutical Association.)

utilizing acid-etched human permanent teeth. Extracted, human, caries-free permanent mandibular incisors were embedded in acrylic bases, and surfaces of the teeth were cleaned with a pumice slurry. The washed and dried teeth were etched with 50% phosphoric acid, rinsed, and dried with compressed oil-free air. The tooth was covered with a template with three holes of 0.01 in. diameter. The template insured that each resin sample tested would have the same resin-enamel contact area. The adhesion promoters were applied with a cotton pellet immediately before the testing resin was mixed and applied to the treatment area. The resin was applied by tamping it into a 0.125 in. diameter plastic tube. The tube was placed over the treatment area, and the resin was condensed against the enamel surface with a wooden dowel. The sample was stored in 100% humidity for 24 h at 37°C. The bond strengths were determined using an Instron® universal testing machine at a cross-head speed of 0.05 in./min. The wire loop was placed around the tube to measure a pure shear force. The loop was pulled in the direction parallel to the enamel surface.

C. *IN VITRO* BIOADHESION TESTS USING NONBIOLOGICAL SUBSTRATES

It is natural to expect that excised biological tissues are used for *in vitro* bioadhesion experiments. However, if a large number of candidate bioadhesives are to be screened, obtaining enough tissues by sacrificing many animals may be difficult and may not be economical. In such cases, nonbiological substrates can be used in place of tissues. According to the definition, then, the tests are not bioadhesion tests anymore. The only way to justify the use of nonbiological substrates for *in vitro* bioadhesion tests is to find the correlation between test results obtained using biological tissues and nonbiological substrates.

1. Intraoral Bandages

Chen and Cyr[23] used lap-shear and bending test methods (Figures 1B and 1C) to determine the adhesive property of intraoral bandages. These methods provided a quantitative expression of wet adhesive strength by measuring the force necessary to separate a sample bandage from a wet dialyzing cellophane representing gingiva. The preparation of the sample bandages

was described above in Section III.A.1. Both the thin polyethylene film of the bandage and the cellophane were cemented to a plastic slide by means of double-faced tape. The lap-shear measurements were made with the direction of the pull in line with the adhesive layer. Bending resistance was measured by placing the slides perpendicular to the base of the platform. In the bending resistance experiments, the value depends on the angle of pull. In both tests, the adhesion strength was measured either by using a strain gauge or manually increasing the force for separation.

The authors observed that the results of the lap-shear tests correlated with those of the *in vivo* screening tests (Section III.A.1) in many cases. This does not mean that the cellophane membrane is the perfect replacement for gingiva. As pointed out by the authors, the adhesive performance of an intraoral bandage may not be judged by one test alone, since the *in vivo* qualitative test measures total adhesive performance as affected by pushing, pulling, and angular lifting forces.

2. Gastric Adhesives

The bioadhesive property of cross-linked poly(acrylic acid) was measured using excised gastric mucus layer of rabbits as described in Section IV.A.2. As shown in Figure 4, the cross-linked poly(acrylic acid) disk was secured onto the nitrocellulose membrane. In the course of the experiments measuring bioadhesive properties of various polymers, it was observed that the cross-linked polyacrylamide did not interact with the nitrocellulose membrane as tenaciously as the cross-linked poly(acrylic acid).[43] It was further observed that the adhesive properties of the copolymers of acrylic acid and acrylamide to the nitrocellulose membrane were dependent on the contents of acrylic acid. The trend of interaction between the copolymers and the nitrocellulose membrane was the same as that between the copolymers and the gastric mucus layer,[32] although the exact adhesive strengths were different. Thus it may be said that the nitrocellulose membrane can be used instead of the gastric mucus layer to comparatively examine the bioadhesive strengths of acrylic hydrogels, at least copolymers of acrylic acid and acrylamide.

V. OTHER *IN VITRO* TEST METHODS OF BIOADHESION

A. ADHESION WEIGHT METHOD

Smart and Kellaway[40] developed a test system where suspensions of ion exchange resin particles flowed over the inner mucosal surface of a section of guinea pig intestine, and the weight of the adherent particles determined. Although the method was of limited value due to poor data reproducibility resulting from fairly rapid degeneration and biological variation of the tissue, it was possible for them to determine the effect of particle size and charge on the adhesion after 5 min contact with everted intestine. A sieve size fraction (63 to 178 μm) showed a significant increase in the weight of the intestine. No weight changes were observed with smaller sieve size fractions. This is a variation of the adhesion number method described in Section II.C.2.

B. FLUORESCENT PROBE METHOD

Park and Robinson[44] studied polymer interaction with the conjunctival epithelial cell membrane using fluorescent probes. The study was done in an attempt to understand structural requirements for bioadhesion in order to design improved bioadhesive polymers for oral use. The membrane lipid bilayer and membrane proteins were labeled with pyrene and fluorescein isothiocyanate, respectively. The cells were then mixed with candidate bioadhesives and the changes in fluorescence spectra were monitored. The fluorescence spectrum of pyrene in the cell membrane showed two distinct bands known as monomer and excimer

bands. It was known that the ratio of excimer/monomer was dependent on the viscosity of the environment.[45] Thus the idea was to detect the change in membrane viscosity by measuring the excimer/monomer ratio. The fundamental assumption of this approach was that the change in membrane viscosity was directly related to the adhesive strength of the test polymer. Polymer binding to membrane proteins was examined using fluorescence depolarization.

This technique is useful to quantitatively compare interactions of various soluble polymers with the cell membrane. It, however, fails to measure the interaction of water-insoluble polymers to the cell membrane due to the interference of the insoluble polymers to fluorescence spectra. In addition, the size of the cell must be measured using an electrical particle counter to accurately interpret the data.

C. FLOW CHANNEL METHOD

Mikos and Peppas[46] have developed a flow channel method that utilizes a thin channel made of glass and filled with 2% (w/w) aqueous solution of bovine submaxillary mucin, thermostated at 37°C (Figure 7). Air was used as a model fluid (A in Figure 7) to test the adhesive characteristics of polymers for nasal application. A gas cylinder containing air was used for the gas flow. The gas stream was passed through a humidifier, where it was saturated at 37°C. The flow cell (C), consisting of two parallel plexiglass plates 15 cm long and 4 cm wide, was constructed with a jacket (E) connected to a water bath (F) and outside insulating fiberglass (G). The temperature of the cell was measured using a thermometer (L). A particle of a bioadhesive polymer (size in the range of 10 and 200 μm) was placed on the mucin gel (D), which had a depth of 0.5 cm through the top of the flow cell (H). Both the static and the dynamic bioadhesive behavior of the particle were determined by taking pictures of the motion of the particle at frequent intervals using a camera (I) and a light source (J). The velocity of the particle was measured using a permanently attached micrometer (K). The flow rate of the air was increased slowly and at a constant rate using a regulating valve with gauge (B), and the time corresponding to the particle detachment was recorded. As pointed out by the authors, this experiment can be done using freshly excised mucus layer or gastrointestinal tissue.

The bioadhesive force is equal to the hydrodynamic force necessary for the particle detachment, assuming no cohesion failure occurs. In the simplest case of a rigid particle interacting to a rigid surface, the hydrodynamic force (F) exerted on the sphere is

$$F = 6\pi fuvR \tag{2}$$

where $f = 1.7009$, u is the air viscosity, R is the particle radius, and v is a characteristic velocity.[47]

D. FALLING LIQUID FILM METHOD

Teng and Ho[48] developed a technique that used excised intestinal segment and microsize particles (Figure 8). Polymer-coated latex particles were prepared by adding a known volume of 1% polymer solution to the cleaned latex particles (5×10^8 particles/ml) in water and stirring at least for 2 h. To further prepare the polymer coated particles in the buffer solutions varying in ionic strength, an aliquot of the polymer coated particles in water was directly transferred into a beaker containing the desired buffer solution to give about 5×10^6 particles/ml. The suspension was subsequently sonicated for 30 s and used 15 min later. The small intestine obtained from Sprague-Dawley rats was cut into segments of desired length, and the lumen was cleaned with normal saline. The intestinal segment was cut lengthwise with surgical scissors and immediately spread out on the flute prepared by cutting open the Tygon tubing (1 in. internal diameter). The Tygon flute was supported by a platform

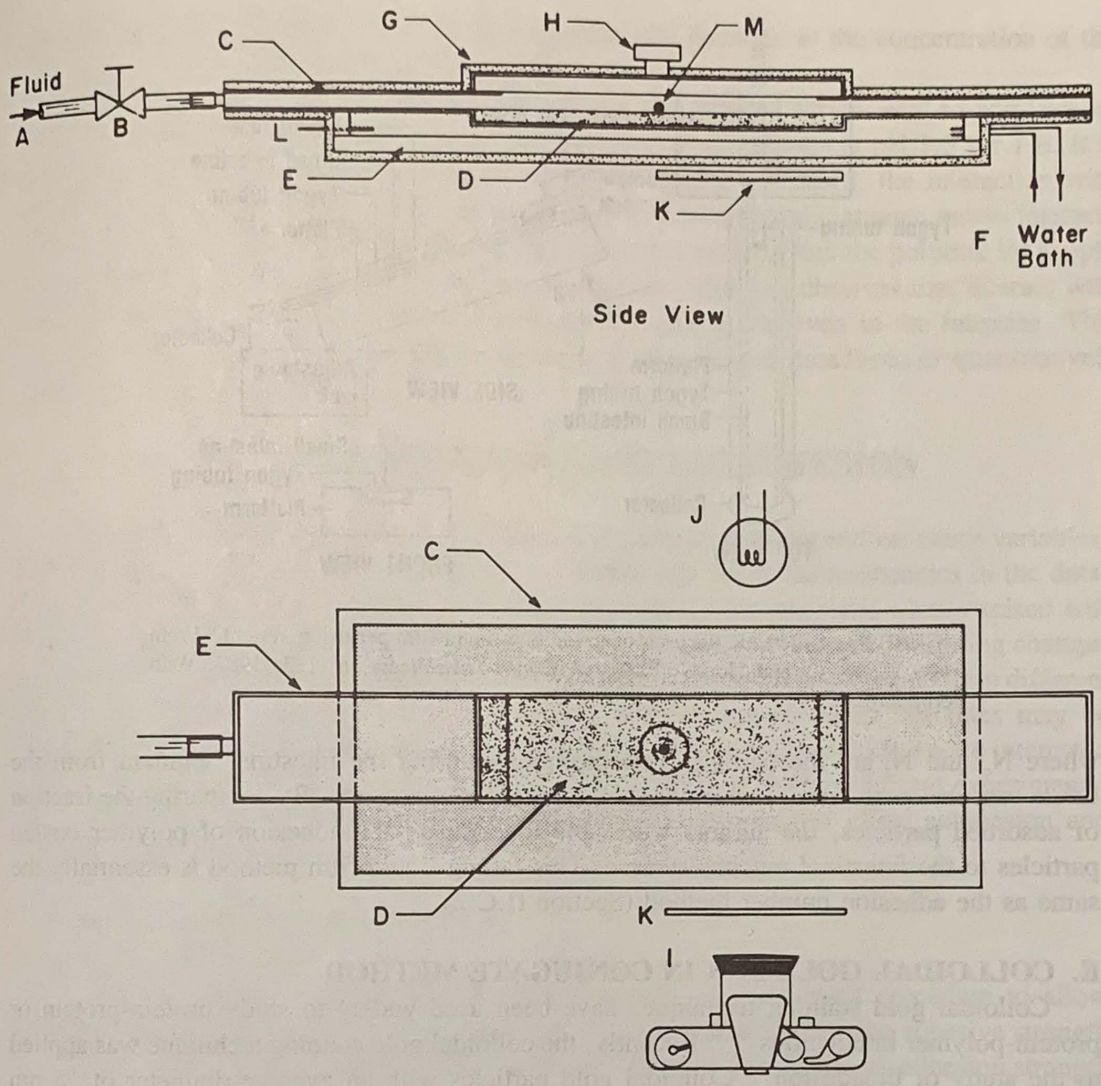

FIGURE 7. Flow channel device simulating the bioadhesive behavior of polymer particles in contact with mucus. See text for details. (From Mikos, A. G. and Peppas, N. A., *Proc. Int. Symp. Controlled Release Bioact. Mater.*, 13, 97, 1986. With permission.)

composed of a plastic foam board. The angle of inclination was adjusted by a laboratory jack to 78°. The prepared intestinal segment mounted on the Tygon flute was perfused using a perfusion pump and a sample syringe for 10 min with a buffer to remove any loosely held mucus. With the aid of the pump, a liquid film of the buffer solution was established on the intestinal segment. In the next 2 min, samples of the elluent solution were collected and used as a control solution for the particle counting. They observed a constant sloughing of an extraneous substance, presumably mucus, with time. To test the adhesion of polymer-coated particles to the intestinal surface by the perfusion of the particle suspension, 0.5 ml samples of the elluent particle solution were collected, and the number of particles remaining in the sample was counted using an electronic particle counter (Coulter). The fraction of particles adsorbed on the mucous layer (F_a) was measured using the following equation.

$$F_a = 1 - N_f/N_o \tag{3}$$

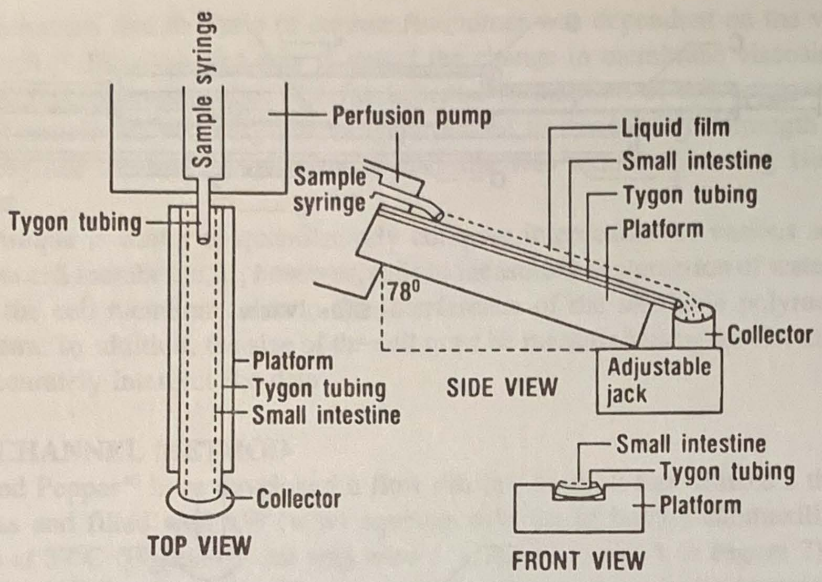

FIGURE 8. Schematic diagram of the falling liquid film perfusion system. (From Teng, C. L. C. and Ho, N. F. L., *J. Controlled Release*, 6, 133, 1987. With permission.)

where N_o and N_l are the particle concentrations entering the intestinal segment from the dilute suspension reservoir and leaving the segment, respectively. By comparing the fraction of adsorbed particles, the authors were able to compare the adhesion of polymer coated particles to the intestinal mucous surface. This falling liquid film method is essentially the same as the adhesion number method (Section II.C.2).

E. COLLOIDAL GOLD-MUCIN CONJUGATE METHOD

Colloidal gold staining techniques have been used widely to study protein-protein or protein-polymer interactions.[49,50] Recently, the colloidal gold staining technique was applied to the study of bioadesion.[51] Colloidal gold particles with an average diameter of 18 nm were prepared by boiling $HAuCl_4$ in the presence of trisodium citrate.[50,52,53] The formation of the monodisperse colloidal gold particles was indicated by a color change from dark blue to red. The concentration of colloidal gold particles can be easily calculated by measuring the absorbance at 525 nm. The absorbance of 1.0 at 525 nm corresponds to 8.5×10^{11} particles per milliliter.[50] The colloidal solution was cooled and centrifuged. The colloidal gold particles were resuspended in the buffer solution of the desired pH. The buffer concentration was always less than 5 m*M*. To this solution was added bovine submaxillary mucin solution dialyzed against deionized distilled water. Mucin molecules adsorbed onto the gold particles and stabilized them. The total amount of the mucin necessay to stabilize the colloidal gold particles depends on the pH. We varied pH and the total amount of added mucin, and found that the conjugates were very stable if 0.2 ml of mucin (at least 0.112 mg/ml) was added to a 2 ml of the colloidal gold solution (7.7×10^{11} particles per milliliter or absorbance of 0.9 at 525 nm) at pH 1.3. The colloidal gold and mucin were gently mixed using a rotary mixer for 15 min at 10 rpm. The mixture was then centrifuged at 20,000g for 45 min to remove unadsorbed mucin molecules. The sedimented mucin-gold conjugates were resuspended in the desired buffer solution. Once conjugates are formed, they are very stable under all conditions so that the pH and ionic strength of the solution can be varied. The advantage of using the mucin-gold conjugates is that the red color is formed on the bioadhesive hydrogels as the hydrogels interact with the conjugates. Thus the adhesive interaction between them can be easily quantified either by measuring the intensity of the

red color on the hydrogel surface or by measuring the decrease in the concentration of the conjugates from the absorbance change at 525 nm.

Figure 9 shows various hydrogels differing in the ratio of acrylic acid to acrylamide. Those hydrogels were allowed to react with mucin-gold conjugates at pH 1.3 for 1 h. It is clear from the figure that as the content of acrylic acid is increased, the interaction with mucin is also increased. Using this technique, we have also found that once mucin interacts with poly(acrylic acid) at pH 1.3, the mucin is not dissociated from the polymer by simply changing pH to 7. This suggests that the poly(acrylic acid) bioadhesives that interact with mucin molecules in the stomach will maintain the interaction even in the intestine. This technique can be used to screen a large number of potential bioadhesives quantitatively under a variety of conditions.

VI. FACTORS AFFECTING BIOADHESION

The results of any bioadhesion experiment are expected to depend on many variables. The lack of proper control of experimental variables will cause inconsistencies in the data. Some of the variables are extremely difficult to control. For example, when excised soft tissues are used for *in vitro* tests, it is not clear how much the tissues are undergoing changes under the experimental time period. It is also not clear whether tissues obtained from different animals are in the same condition. The effects of such uncontrollable variables may be minimized by using a large number of tissue samples. The factors in which we are interested here are those that can be controlled, but are often ignored in the bioadhesion experiments. Voyutskii discussed in detail in his excellent book the factors that affect autohesion and adhesion of nonbioadhesives.[54]

A. EXPERIMENTAL CONDITIONS

1. Initial Contact Time

In the evaluation of the bioadhesive performance, it is a normal procedure to allow bioadhesives and tissue surfaces to contact for a certain time period before adhesive strength is measured. The optimum initial contact time that results in the maximum adhesion strength depends on many variables, such as the nature of bioadhesives and tissues, the initial pressure, or water content.

In case of cyanoacrylate adhesives, the initial contact has to be maintained at least until polymerization occurs. Thus the optimum initial contact time depends on the nature of the monomer, since the polymerization time is different for different monomers[25,55] (Section III.B.1.a). If bioadhesive tablets are used[34,39] (Sections IV.A.4 and IV.B.1), the initial contact time as well as the amount of water applied onto the tablets will determine the bioadhesive strength by controlling the dissolution of the tablets. When dry or partially swollen hydrogels are used, the gels will continue swelling as long as the contact is maintained with the wet tissues. Continuous swelling of the bioadhesive hydrogels may have a profound effect on the overall bioadhesion. Even hydrogels swollen to equilibrium may have different bioadhesive properties depending on the initial contact time. As the contact time is increased, the interaction and mutual entanglement between bioadhesive polymer chains and the tissue glycoprotein molecules may be substantially increased.[31,32] When lens materials were in contact with the endothelial cell layer, the period of the initial loading (16 g) was limited to 30 s, since much longer times always completely damaged the endothelial cells (Section IV.A.3).[33] Obviously the contact time can be extended if lower pressure is applied or vice versa. For practical reasons, it is preferable to adjust other variables so that the optimum contact time is less than a minute.

2. Initial Pressure

It is a common experience that the manner of applying pressure to contact adhesives

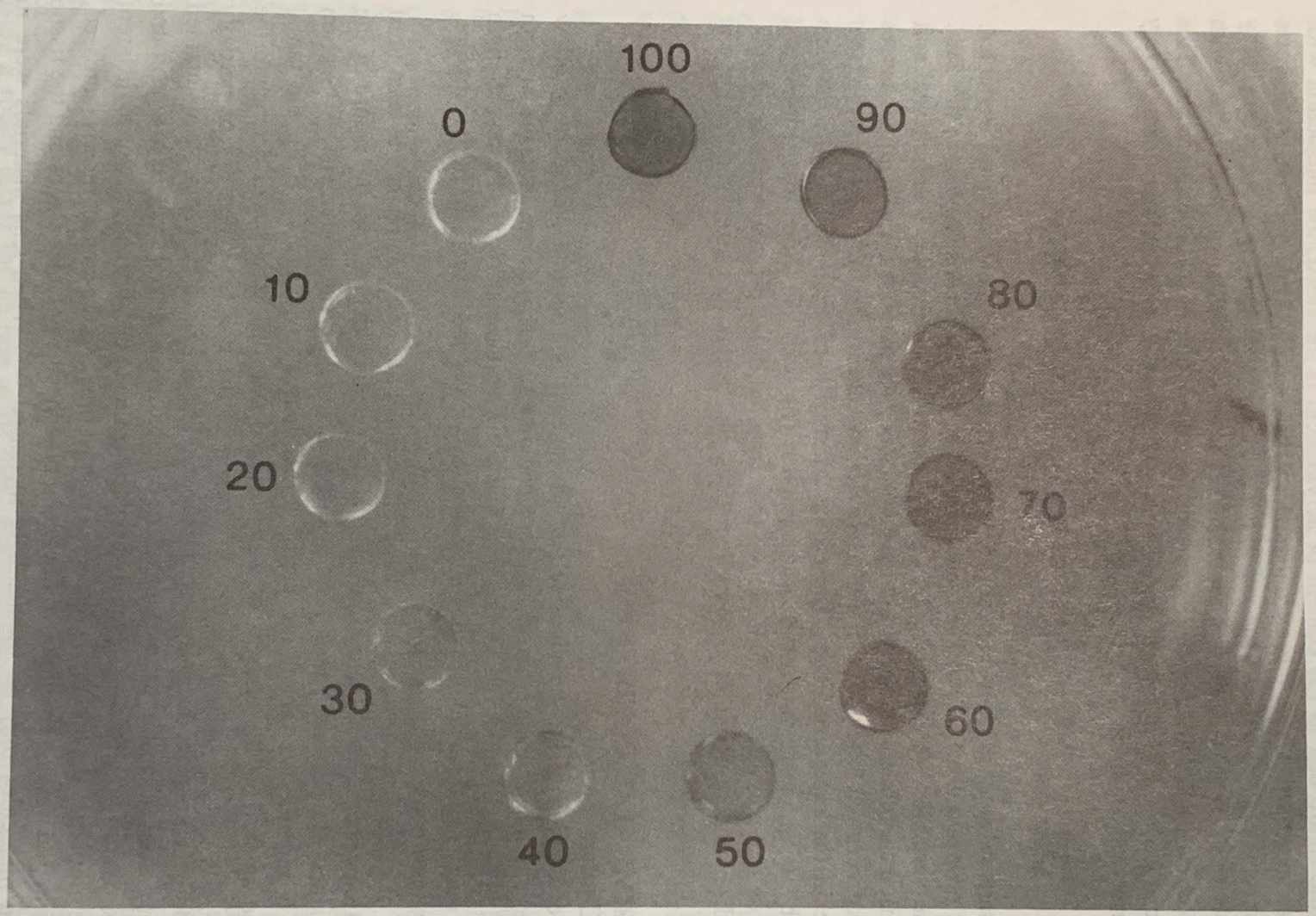

FIGURE 9. Interaction of colloidal gold-mucin conjugates with various hydrogels at pH 1.3. The hydrogels in disk shape are cross-linked copolymers of acrylic acid and acrylamide. The numbers describe percentages of acrylic acid in the copolymers. The colloidal gold-mucin conjugates begin interaction with the polymer disk of 50% acrylic acid. The interaction is indicated by the appearance of red color of the conjugates.

and tissues affects the measurement and results in inconsistent values.[31,35] Park and Robinson[31] examined the effect of the pressure applied to contact the gastric tissue layer and various hydrogels. They found that the adhesion strength was increased linearly as the applied force was increased up to a certain level. In the early stage of their research, however, the authors did not take such effect into account in measuring the adhesion strength. Thus the effect of pH on the bioadhesion of polycarbophil, which was measured in the absence of such consideration,[56] had to be corrected later.[31] Noticing the linear relationship between the tensile strength and the applied pressure, they were able to extrapolate back to the zero applied pressure and determine the intrinsic bioadhesiveness. One surprising, but very reasonable, result was that poly(hydroxyethyl methacrylate) had a negative adhesion with the gastric mucus layer. In other words, they did not adhere in the absence of the applied pressure. This kind of information is essential in the design of oral dosage forms, since the external pressure cannot be applied to them in the gastrointestinal tract (GIT). On the other hand, external pressure can be easily applied to the intraoral or skin bioadhesives.

3. Speed of Testing

The speed of testing is the relative rate of motion of test fixtures during the test.[18] In most cases, the speed of testing is maintained at a constant rate using a motor-driven device. The rate can be maintained manually also. The testing speed is generally chosen empirically to observe the largest difference between different bioadhesives. The speed of testing described in Sections III and IV ranges from 1 mm/min[16] to 5 cm/s.[28] It is necessary to fully examine the effects of the testing speed on the bioadhesives strength. According to Voyutskii,[54] the work of adhesion increases with the increase of the peeling rate of the adhesive from the substrate.

4. Temperature

In most bioadhesion experiments, the temperature may be fixed at either room temperature or 37°C. The study on the effect of temperature, however, is important in understanding the behavior of adhesives. As pointed out by Voyutskii,[54] there are two different effects of temperature on the adhesion strength. The effect of temperature at which the adhesive bond is formed should be distinguished from the effect of temperature at which the force for detachment is measured. In reality, it may be the case that the bioadhesive is applied at room temperature and detached at 37°C. The drastic changes in temperature at which adhesion force is measured may be useful in evaluating the durability of bioadhesives.

B. BIOLOGICAL FACTORS

1. Treatment of Tissues

The adhesion of cyanoacrylate and acrylate adhesives to skin depends on the content of water on the skin surface[26] (Section III.B.1.a). The acrylic adhesives do not adhere measurably to wet skin while the cyanoacrylates do not adhere to dry skin.[26] The bioadhesive properties of some polymers, such as polyurethane prepolymer or silicone elastomer,[30,57] depend on the hydrophobicity of the tissue surface. Thus it appears that the priming of the tissue surface with water or hydrophobic agents, whether intentional or not, has significant influence in the evaluation of bioadhesives. The adhesion of acrylic hydrogels to the gastric mucus layer is known to depend in part on the flexibility of mucin molecules.[31] Park and Robinson[31] observed that treating the gastric mucus layer with *N*-acetylcysteine increased the adhesion strength up to 40%, presumably due to the increased flexibility of the mucin molecules. On the other hand, treating with glutaraldehyde resulted in a 30% decrease in the adhesion strength.

2. Mucin Turnover

The natural turnover of the tissue surface should be considered for the *in vivo* application

of bioadhesives. For example, mucoadhesives adherent to the gastric mucus layer may be removed from the surface regardless of their bioadhesive strength by the normal mucin turnover, which may be shorter than the desired time period for drug delivery. In such case, the difference between various mucoadhesives may not be observed. In addition, the natural mucin turnover in the GIT will result in a substantial amount of soluble mucin molecules. Teng and Ho[48] noticed that surface fouling by mucus was unavoidable despite the efforts made to wash out loosely bound mucus from the intestinal wall. Studies using mucus-coated particles showed the presence of an interfacial barrier between the particles and the intestinal mucus surface. Thus, in practice, mucus contamination of polymer coated particles could lead to unfavorable conditions for bioadhesion, even though the particle surfaces have been originally designed to promote adhesion to the intestinal mucus wall.[48] The exact turnover rate of the mucus layer remains to be determined. When strong gastric mucoadhesives such as poly(acrylic acid) are used, it is common to observe the cohesion failure at the mucus layer as shown in Figure 4. The rupture did not occur at the interface between polymer and mucus but rather inside the mucus.[34] Thus, once a bioadhesive is separated from the mucus layer, there is little chance that the contaminated bioadhesive will readhere to the mucus layer.

VII. SUMMARY

The studies on the Type III bioadhesion and bioadhesives are not new. Only recently, however, was the concept of using bioadhesives to controlled drug delivery established. Research on the evaluation of bioadhesives is still in its early stage. Poor understanding of biological tissue surfaces has resulted in the lack of universal test methods, which in turn results in poor understanding of bioadhesion mechanisms. Although a number of test methods are already available as described in this chapter, they are by no means perfect test methods. We need to continue modifying old test methods and developing new ones as they may be necessary.

REFERENCES

1. *Annual Book of ASTM Standards,* Vol. 15.06, Sect. 15, American Society for Testing and Materials, Philadelphia, PA, 1984.
2. **Park, K., Cooper, S. L., and Robinson, J. R.,** Bioadhesive hydrogels, in *Hydrogels in Medicine and Pharmacy,* Vol. 3, Peppas, N. A., Ed., CRC Press, Boca Raton, FL, 1987, chap. 8.
3. **Eirich, F. R.,** Bioadhesion as an interphase phenomenon, in *Biocompatibility of Implant Materials,* Williams, D., Ed., Sector Publishing, London, 1976, chap. 19.
4. **Wake, W. C.,** *Adhesion and the Formulation of Adhesives,* Applied Science, London, 1976, chap. 13.
5. **Sowers, A. E., Ed.,** *Cell Fusion,* Plenum Press, New York, 1987.
6. **Edwards, J. G.,** The biochemistry of cell-adhesion, *Prog. Surf. Sci.,* 13, 125, 1983.
7. **Grinnell, F.,** Cellular adhesiveness and extracellular substrata, *Int. Rev. Cytol.,* 53, 65, 1978.
8. **Taylor, A. C.,** Adhesion of cells to surfaces, in *Adhesion in Biological Systems,* Manly, R. S., Ed., Academic Press, New York, 1970, chap. 3.
9. **Hillman, R. E. and Nace, P. F.,** Histochemistry of barnacle cyprid adhesive formation, in *Adhesion in Biological Systems,* Manly, R. S., Ed., Academic Press, New York, 1970, chap. 6.
10. **Matsumoto, T.,** *Tissue Adhesives in Surgery,* Medical Examination, Flushing, NY, 1972, 195.
11. **Chu, C. C.,** Survey of clinically important wound closure biomaterials, in *Biocompatible Polymers, Metals, and Composites,* Szycher, M., Ed., Technomic Publishing, Lancaster, 1983, chap. 22.
12. **Gross, L. and Hoffman, R.,** Medical and biological adhesives, in *Handbook of Adhesives,* 2nd ed., Skeist, I., Ed.,Van Nostrand Reinhold, New York, 1977, 818.
13. **Lipatova, T. E.,** Medical polymer adhesives, *Adv. Polym. Sci.,* 79, 65, 1986.
14. **Cleary, G. W.,** Transdermal controlled release systems, in *Medical Applications of Controlled Release,* Vol. 1, Langer, R. S. and Wise, D. L., Eds., CRC Press, Boca Raton, FL, 1984, chap. 7.

15. **Brauer, G. M. and Huget, E. F.,** Dental adhesives, in *The Chemistry of Biosurfaces,* Vol. 2, Hair, M. L., Ed., Marcel Dekker, New York, 1972, chap. 16.
16. **Smart, J. D., Kellaway, I. W., and Worthington, H. E. C.,** An in-vitro investigation of mucosa-adhesive materials for use in controlled drug delivery, *J. Pharm. Pharmacol.*, 36, 295, 1984.
17. **Portelli, G. B.,** Testing, analysis, and design of structural adhesive joints, in *Structural Adhesives,* Hartshorn, S. R., Ed., Plenum Press, New York, 1986, 407.
18. **Shah, V.,** *Handbook of Plastics Testing Technology,* Wiley-Interscience, New York, 1984, 1.
19. **Hartshorn, S. R.,** Introduction, in *Structural Adhesives,* Hartshorn, S. R., Ed., Plenum Press, New York, 1986, 1.
20. **Been, J. L.,** Bonding, in *Encyclopedia of Polymer Science and Technology,* Vol. 1, Interscience, New York, 1964, 503.
21. **Zimon, A.D.,** *Adhesion of Dust and Powder,* 2nd ed., Consultant Bureau, New York, 1982, chap. 3.
22. **Hartshorn, S. R.,** The durability of structural adhesive jonts, in *Structural Adhesives,* Hartshorn, S. R., Ed., Plenum Press, New York, 1986, 347.
23. **Chen, J. L. and Cyr, G. N.,** Compositions producing adhesion through hydration, in *Adhesion in Biological Systems,* Manly, R. S., Ed., Academic Press, New York, 1970, chap. 10.
24. **Bremecker, K. D., Strempel, H., and Klein, G.,** Novel concept for a mucosal adhesive ointment, *J. Pharm. Sci.*, 73, 548, 1984.
25. **Leonard, F., Hodge, J. W., Jr., Houston, S., and Ousterhout, D. K.,** Alpha-cyanoacrylate adhesive bond strengths with proteinaceous and nonproteinaceous substrates, *J. Biomed. Mater. Res.*, 2, 173, 1968.
26. **Margules, G. S. and Harris, D. L.,** Adhesives for intact skin: a preliminary investigation, *Med. Biol. Eng. Comput.*, 18, 549, 1980.
27. **Ow, R. K. K. and Bearn, E. M.,** A method of studying the effect of adhesives on denture retention, *J. Prosthet. Dent.*, 50, 332, 1983.
28. **Skornik, W. A., Dressler, D. P., and Richard, J. K.,** Adherence of prosthetic skin, *J. Biomed. Mater. Res.*, 2, 447, 1968.
29. **Theodorakis, M. C., Digenis, G. A., Beihn, R. M., Shambhu, M. B., and DeLand, F. H.,** Rate and pattern of gastric emptying in humans using ^{99m}Tc-labeled triethylenetetraamine-polystyrene resin, *J. Pharm. Sci.*, 69, 568, 1980.
30. **Wang, P. Y. and Forrester, D. H.,** Conditions for the induced adhesion of hydrophobic polymers to soft tissue, *Trans. Am. Soc. Artif. Int. Organs,* 20, 504, 1974.
31. **Park, H. and Robinson, J. R.,** Physico-chemical properties of water insoluble polymers important to mucin/epithelial adhesion, *J. Controlled Release,* 2, 47, 1985.
32. **Park, H. and Robinson, J. R.,** Mechanisms of mucoadhesion of poly(carboxylic acid) hydrogels, *Pharm. Res.*, 4, 457, 1987.
33. **Reich, S., Levy, M., Meshorer, A., Blumental, M., Yalon, M., Sheets, J. W., and Goldberg, E. P.,** Intraocular-lens-endothelial interface: adhesive force measurement, *J. Biomed. Mater. Res.*, 18, 737, 1984.
34. **Ponchel, G., Touchard, F., Duchene, D., and Peppas, N. A.,** Bioahesive analysis of controlled release systems. I. Fracture and interpenetration analysis in poly(acrylic acid)-containing systems, *J. Controlled Release,* 5, 129, 1987.
35. **Fusayama, T., Nakamura, M., Kurosaki, N., and Iwaku, M.,** Non-pressure adhesion of a new adhesive restorative resin, *J. Dent. Res.*, 58, 1364, 1979.
36. **Haugen, E., Espevik, S., and Mjor, I. A.,** Adhesive properties of periodontal dressings — an in vitro study, *J. Periodontal Res.*, 14, 487, 1979.
37. **Powis, D. R., Folleras, T., Merson, S. A., and Wilson, A. D.,** Improved adhesion of a glass ionomer cement to dentin and enamel, *J. Dent. Res.*, 61, 1416, 1982.
38. **Goldman, M., DeVitre, R., and Pier, M.,** Effect of the dentin smeared layer on tensile strength of cemented posts, *J. Prosthet. Dent.*, 52, 485, 1984.
39. **Marvola, M., Vahervuo, K., Sothmann, A., Marttila, E., and Rajaniemi, M.,** Development of a method for study of the tendency of drug products to adhere to the esophagus, *J. Pharm. Sci.*, 71, 975, 1982.
40. **Smart, J. D. and Kellaway, I. W.,** In vitro techniques for measuring mucoadhesion, *J. Pharm. Pharmacol.*, 34, 70P, 1982.
41. **Leung, S. H. S. and Robinson, J. R.,** The contribution of anionic polymer structural features to mucoadhesion, *J. Controlled Release,* 5, 223, 1988.
42. **Jedrychowski, J. R., Caputo, A. A., and Foliart, R.,** Effects of adhesion promoters on resin-enamel retention, *J. Dent. Res.*, 58, 1371, 1979.
43. **Park, K. and Park, H.,** unpublished data, 1987.
44. **Park, K. and Robinson, J. R.,** Bioadhesive polymers as platforms for oral-controlled drug delivery: method to study bioadhesion, *Int. J. Pharm.*, 19, 107, 1984.

45. **Dembo, M., Glushko, V., Aberlin, M. E., and Sonenberg, M.,** A method for measuring membrane microviscosity using pyrene excimer formation. Application to human erythrocyte ghosts, *Biochim. Biophys. Acta,* 552, 201, 1979.
46. **Mikos, A. G. and Peppas, N. A.,** Comparison of experimental technique for the measurement of the bioadhesive forces of polymeric materials with soft tissues, *Proc. Int. Symp. Controlled Release Bioact. Mater.,* 13, 97, 1986.
47. **O'Neill, M. E.,** A sphere in contact with a plane wall in a slow linear shear flow, *Chem. Eng. Sci.,* 23, 1293, 1968.
48. **Teng, C. L. C. and Ho, N. F. L.,** Mechanistic studies in the simultaneous flow and adsorption of polymer-coated latex particles on intestinal mucus. I. Methods and physical model development, *J. Controlled Release,* 6, 133, 1987.
49. **Goodman, S. L., Hodges, G. M., and Livingstone, D. C.,** A review of the colloidal gold marker system, *Scanning Electron Microsc.,* 1980/II, 133, 1980.
50. **Park, K., Simmons, S. R., and Albrecht, R. M.,** Surface characterization of biomaterials by immunogold staining-quantitative analysis, *Scan. Microsc.* 1, 339, 1987.
51. **Park, K. and Park, H.,** A new approach to study mucoadhesion: colloidal gold staining, *J. Int. Pharm.,* in press.
52. **Frens, G.,** Controlled nucleation for the regulation of the particle size in monodisperse gold suspensions, *Nature London Phys. Sci.,* 241, 20, 1973.
53. **Horisberger, M.,** Evaluation of colloidal gold as a cytochemical marker for transmission and scanning electron microscopy, *Biol. Cellulaire,* 36, 253, 1979.
54. **Voyutskii, S. S.,** *Autohesion and adhesion of high polymers,* Interscience, New York 1963, chap. 6.
55. **Leonard, F.,** Hemostatic applications of alpha cyanoacrylates: bonding mechanism and physiological degradation of bonds, in *Adhesion in Biological Systems,* Manly, R. S., Ed., Academic Press, New York, 1970, chap. 11.
56. **Ch'ng, H. S., Park, H., Kelly, P., and Robinson, J. R.,** Bioadhesive polymers as platforms for oral controlled drug delivery. II. Synthesis and evaluation of some swelling, water-insoluble bioadhesive polymers, *J. Pharm. Sci.,* 74, 399, 1985.
57. **Wang, P. Y.,** Adhesion mechanism for polyurethane prepolymers bonding biological tissue, in *Biomedical Applications of Polymers,* Gregor, H. P., Ed., Plenum Press, New York, 1975, 111.