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Design of an apparatus incorporating a linear variable differential transformer for the measurement of type III bioadhesion to cervical tissue

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Summary

The design and initial evaluation of a bioadhesive strength testing apparatus are reported. Measurement of adhesive strength is mediated via a linear variable differential transformer (LVDT). The apparatus, constructed from Perspex, consists of a movable platform with a pedestal to which the bioadhesive formulation is attached. Tissue is secured to a second, upper pedestal linked to the LVDT via a sensor. During the test procedure the platform is moved downwards at a predetermined rate controlled by the motor drive logic board. As movement occurs to the upper pedestal this can be detected by the output from the LVDT which is recorded potentiometrically. The force generated is measured by a previously calibrated spring contained within the sensor housing. Different calibrated springs can be used to increase or decrease the sensitivity of the apparatus. Initial evaluation of the testing device was performed on a range of candidate bioadhesive film formulations. The effects on bioadhesion of varying the initial applied weight, mucus surface density, film thickness and film hydration were investigated. Excellent bioadhesion was achieved with the application of a dry sodium carboxymethylcellulose/sodium carbopol film to cervical tissue with a surface mucus layer.

Introduction

Adhesion can occur when two phases are maintained in immediate proximity by various categories of interfacial forces. When one or both of these phases are biological in nature then the adhesion is referred to as bioadhesion. Often, a distinctive feature of bioadhesion is that water is present in the adhesive mechanism. Three main categories of bioadhesion (types I–III) can be described based on experimental observations rather than any underlying mechanism (Park et al., 1987).

Type III bioadhesion is distinct from types I and II in that artificial adhesives are responsible for the adherence to biological substrates. A good example is the adherence of soluble polymers to various mucosal surfaces, a process often referred

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to as mucoadhesion. Type III bioadhesion is of primary interest with respect to the design of novel bioadhesive drug delivery systems.

Bioadhesive drug delivery devices have been developed for nasal, buccal, vaginal and ocular applications (Duchene et al., 1988). These sites are rich in a viscous secretion, mucus, which adheres to the adjacent epithelium, protecting it from mechanical, bacterial, viral and chemical attack. Mucus consists of water (up to 95% by weight), glycoproteins (0.5–5%), low proportions of lipids, mineral salts (1%) and 0.5–1% of free proteins (Duchene et al., 1988). The rheological properties of mucus, together with its adhesive and cohesive properties, are primarily attributable to the glycoprotein fraction.

The process of mucoadhesion occurs when a mucoadhesive material makes intimate contact with a mucosal surface and is retained by it for prolonged periods of time. Entanglement of mucus and polymer chains can then occur. Several models have been proposed to explain the process of mucoadhesion (Park et al., 1987) including mechanical, electrostatic, diffusion and adsorption theories. However, it is now clear that adhesion cannot easily be explained by any one simple model or theory. The theories proposed originate from observations on particular adhesive systems and have shortcomings when used to explain systems with a different configuration.

The evaluation of bioadhesive properties is fundamental to the development of novel bioadhesive delivery systems. Measurement of the mechanical properties of a bioadhesive material after interaction with a substrate is one of the most direct ways to quantify the bioadhesive performance. There are three types of test: tensile stress, shear and peel strength (Park and Park, 1990). Tensile stress loading involves the application of forces perpendicular to the plane. In shear testing, the force is applied parallel to the plane. Peel strength testing limits the stress to a fine line at the edge of the joint.

Most in vitro methods involve measurement of shear or tensile stress. Smart et al. (1984) described a method to study type II bioadhesion whereby a polymer-coated glass slide was withdrawn from a mucus solution. The force required to accomplish this was equated to mucoadhesion. A fluorescence probe technique was developed by Park and Robinson (1985) to determine bonding between epithelial cells and a test polymer. Other methods have involved measurement of the force required to detach polymeric materials from excised rabbit corneal endothelium. Mikos and Peppas (1986) described a method whereby a polymer particle was blown across a mucus-filled channel. The motion, recorded photographically, gave details regarding the adhesion process.

In the present study a new design for an adhesion tester for bioadhesive drug delivery systems is proposed. This design is based on the use of a linear variable differential transformer (LVDT). The apparatus has been used to evaluate the bioadhesive properties of prototype film drug delivery systems presently under development by us for the treatment of cervical intra-epithelial neoplasias (CIN I and II).

Materials and Methods

Chemicals

Hydroxypropylcellulose (Klucel MF), hydroxyethylcellulose (Natrosol 250 M-Pharm) and sodium carboxymethylcellulose (Blanose 7HF) were all obtained from Aqualon Ltd, Warrington, U.K. Carbopol 934P was purchased from B.F. Goodrich Co., Cleveland, OH. Sodium Carbopol was prepared according to the manufacturer's instructions (Carbopol technical manual GC-67). Methylcellulose (450 grade) was obtained from BDH Chemicals Ltd, Poole, U.K.

Tissue

Tissue used consisted of non-pathological samples of human uterine cervix dissected from the uterus at the cervical isthmus. At no stage was the cervix washed to remove mucus.

An incision was performed down to, and parallel with, the axis of the central cervical canal. This allowed the specimen to be opened from a conical to a planar configuration with the non-keratinized stratified squamous epithelium uppermost. It was then possible to cut cubes of tissue with a volume of approx. 0.5 cm^3. Each

cube of tissue was mounted on the adhesion testing apparatus using superglue (Loctite Superglue 3).

Construction of the bioadhesion tester

The tester was manufactured using 10 mm thickness, clear Perspex sheet. The overall design is shown in Fig. 1. The moveable carriage guide rails consisted of 3/8 inch stainless-steel rod. Linear bearings were obtained from Merco Ltd., Belfast, U.K. All electrical components were purchased from RS Supplies (Corby, U.K.). The tester was mechanized using a stepping linear actuator (RS No. 318–711) and this was controlled by a four-phase unipolar stepper motor drive board (RS No. 332-098).

The sensing apparatus incorporated a miniature d.c. energized LVDT (RS No. 646-476). Power supply to electrical components was by an

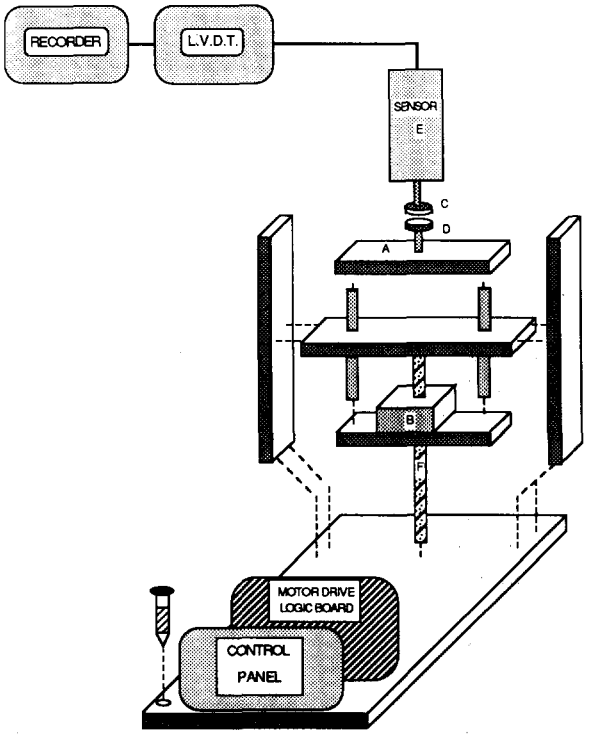

Fig. 1. Design of an apparatus for testing the bioadhesive strength of dosage forms. A, platform; B, stepping motor; C, upper pedestal (tissue mount); D, lower pedestal (bioadhesive mount); E, sensor housing; F, screw-threaded shaft.

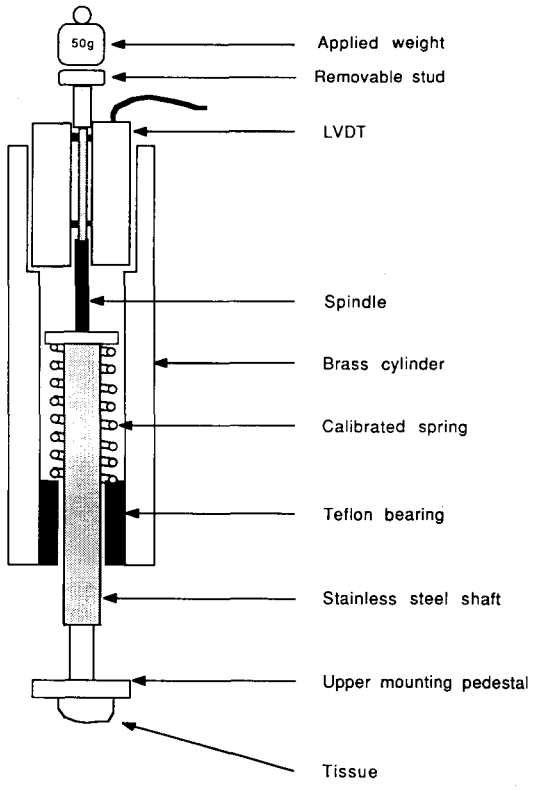

Fig. 2. Cross-section through sensor housing.

Altai stabilized power supply (13.8 V-3 A) and output was recorded on a Linseis chart recorder.

Construction and calibration of the sensor

The sensor was constructed as shown in Fig. 2. With no load on the sensor it remained in resting equilibrium and the LVDT output was set as the sensor baseline on the chart recorder. Incremental addition of weights unto the pedestal compressed the internal spring, pulling the spindle out of the LVDT and thus causing a voltage change. This displacement signal was recorded, giving a measure of the tensile stress detected. Thus, the compressibility of the spring and its ease of compression determined the sensitivity and weight range over which the sensor can operate in a linear fashion.

Bioadhesive formulations

Stable, clear, viscous (depending on grade) aqueous gels were prepared either from a single

polymer (3% w/w) or from a mixture of two polymers (1.5% w/w of each component, with the exception of sodium Carbopol which was used at a concentration of 0.15% w/w). Traces of incorporated air were removed by overnight refrigeration. Candidate gels were poured over Teflon-coated metal plates and allowed to dry overnight in a cold air stream, after which they could be peeled off, cut and conveniently stored as a flexible film.

Method of testing

Tissue was securely mounted on the upper pedestal, attached to the sensor. The lower pedestal held the bioadhesive test film secured by double-sided adhesive tape.

The sensor body was secured on an adapted retort stand that was moveable along the vertical axis by means of a manually operated screw thread drive. The sensor and tissue were lowered as a complete unit unto the stationary film mounting pedestal. Adjustments were made as necessary to maintain intimate contact and a zero baseline output on the recorder. After applying a fixed mass for a predetermined period of time the tester was actuated and the lower pedestal moved away from the tissue at a constant speed of 17 mm min^{-1}.

Evaluation of bioadhesive properties

Effect of initial application weight and duration of application An initial force was applied to samples of a candidate film (sodium carboxymethylcellulose/ sodium Carbopol) via weights placed on top of the sensor housing. The film was adhered to cervical tissue, and the resultant initial force allowed to act through the sensor housing for one minute prior to testing. The applied weight was varied from 7.9 to 50.6 g. A further experiment involved the use of a single weight applied for various initial time periods ranging from 5 to 120 s.

Effect of film hydration Samples from a sodium carboxymethylcellulose/ sodium Carbopol film (thickness 0.063 ± 0.004 mm; mean $\pm$ S.D.; $n = 5$) were hydrated by soaking in water for various times prior to measuring their adhesion to cervical tissue.

Effect of film thickness Films (sodium carboxymethylcellulose/ sodium Carbopol) of varying thicknesses were cast using different gel dilutions. The effect on bioadhesion of varying the film thickness was determined.

Effect of mucus surface density Cervical tissue samples were graded visually with respect to the density of surface mucus. This can vary widely according to the condition of individual tissue samples. Mucus surface density was altered either by (a) air-drying, (b) swabbing to allow only a light surface dampness, (c) dabbing the tissue with cotton wool swabs to remove excess mucus, leaving a medium surface thickness of mucus still present, or (d) saturating the tissue surface with mucus, if necessary by transferring to the cut tissue sample mucus from adjacent areas. The bioadhesion of a hydroxypropylcellulose film was determined with respect to cervical tissue samples treated by each of these methods.

Formulation effects The bioadhesive properties with respect to cervical tissue were evaluated for a range of candidate film formulations. In each case an initial weight of 57 g was applied for 1 min prior to the commencement of each test.

Results and Discussion

Many techniques are available for measuring bioadhesion (Park and Park, 1990). Each is different in its conception and has limited areas of application. These adhesion testers are usually adaptations of existing laboratory apparatus such as tensiometers, microbalances and motor-driven pulley systems incorporating spring balances as measuring devices. The lack of uniformity in bioadhesion testing technology prompted the design and development of the device described in this study.

In the design of the various operating components of the tester several factors were considered. Firstly, the device had to embody a system for interchanging the pedestals used to mount the bioadhesive film or gel, thereby allowing tensile stress, shear strength and peeling tests to be undertaken. Secondly, a rigid, motor-driven carriage was needed to give a powerful, yet highly

controlled, rate of advance, sufficient to generate the forces needed to cause failure of the adhesive bond. An integral sensor capable of yielding a readily captured signal was required. The sensor had to support tissue samples and have sufficient sensitivity over a wide testing range. Many bioadhesion tests require application of a mass for a fixed period and so an accessible method of allowing this was necessary. Finally, the apparatus had to be light, easily portable and inexpensive to manufacture.

The initial conception was to manufacture the structure using stainless steel. However, it soon became apparent that weight would become excessive and machining the components lengthy and expensive. Therefore, a new design using light, yet strong, Perspex was conceived. This material is easily cut, machined and reasonably inexpensive. It can be polished to produce an aesthetically acceptable device. To mechanize the latter a stepping actuator was selected. This can produce extremely accurate movements using a series of pulses. The pulse is triggered using a logic drive board. This drives the central mechanism of the motor through a fixed angle of rotation. The rate of delivery of these pulses, which ultimately determines the speed of the motor, can be varied electronically to give a large range of constant advance rates. The torque produced is relatively high with a starting force of 120 N and therefore the use of gearing is not required.

The motor was fixed onto a square carriage that was free to move vertically through two linear bearings. The former was designed to pull a stationary threaded shaft through its centre and thus drive the carriage up or down. The lower mounting pedestal could then be secured to the upper cross-member of the carriage together with other interchangeable mounts if so required.

The sensor was based on an LVDT that produces a d.c. output which varies linearly with respect to the vertical displacement of its central spindle. This spindle was welded to a stainless-steel rod that acts to compress a spring as a load is applied through it. The greater the load, the larger the distance the spring is compressed and thus the further out of the LVDT the spindle is pulled. The spring can be manufactured to fit the internal dimensions of the sensor and wound with wire of sufficient stiffness to give the necessary testing range of forces in which the bioadhesive strength is expected to lie. Displacement (y) was linearly related to the applied weight (x) over a range of 50–320 g ($y = 0.390x - 0.107$; $R^2 = 1.000$) for the stiffer spring and 10–100 g using a lighter spring ($y = 1.338x - 6.667$; $R^2 = 0.998$).

As can be seen in Fig. 2 the spindle in the LVDT was accessible from above. This was used as a convenient method of applying fixed masses during the process of contact between the tissue and film. A metal stud similar in shape to the lower mounting pedestal could be placed into the LVDT, acting down through the central axis of the sensor and ultimately through the tissue and unto the film. This weight was easily applied and removed after fixed time periods. The sensor could be orientated to act in both the vertical and horizontal positions with an outer casing of sufficient strength to allow rigid clamping unto the retort stand.

A mucus-covered tissue, human cervix, was cut and mounted as described. All the bioadhesive formulations tested bonded to the tissue with ease. The bioadhesion of candidate polymer films was tested by measuring the tensile force required to rupture their bonding to this tissue. Upon activation of the device and the pulling apart of the film/tissue interface, the sensor produced a typical hard copy on the recorder (20 mm min^{-1}) as shown in Fig. 3. Quite distinct regions are apparent which profile the events during the process of detachment. Initially, the sensor sits at equilibrium (the baseline position) until the tissue is brought into contact with the film. This produces a slight negative dip as this contact pushes the spindle up a minute distance, thus confirming intimate contact. As the weight is applied, the tissue compresses slightly giving a positive displacement as the spindle is drawn out again. When the weight is removed the tissue relaxes and with adjustments in the sensor height it is possible to realign the output to the baseline once more. The bioadhesive bond mechanically links the motor directly to the spring and once activated it does work to compress the spring. As the spring shortens it opposes this process with a

proportionally increasing force until a point is reached at which this force equals the force of bioadhesion. After the rupture of the bond mucus was clearly seen residing on the partially hydrated film surface. Adhesion was often so aggressive that, after bond rupture, epithelial cells were ripped off the tissue surface. This was confirmed by applying histological stain (methylene blue) prior to testing and observing, after separation, areas on the tissue surface with plaques of coloured material removed. Stretching in the tissue sample deviates the line initially from linearity but this returns and peaks at the point of detachment. The height of the peak from the baseline is converted into units of force using the calibration curve.

With conventional pressure-sensitive adhesives an initial pressure must be applied for a given time in order to form the adhesive bond. An investigation was made of this effect with respect to the adhesion of a model film to cervical tissue. Weights were applied onto a removable stud (Fig. 2) for a period of 1 min immediately prior to testing. The weight acted through the sensor housing directly on the film-mucus bond. The resulting effect on bioadhesion is given in Table 1. Table 2 shows the influence on bioadhesion of varying the application time of a constant applied weight. Clearly, there was no discernible relationship between either the applied weight and the resulting force of adhesion (in N), or between the duration for which the weight was applied and the subsequent bioadhesive force. The initial applied weight given in Table 1 is the weight of the removable stud (7.9 g), and this value is incorporated into all other weight values used. The lack of importance of initial pressure in forming the bond between the film and the cervical surface clearly differentiates this form of adhesion from conventional pressure-sensitive adhesion.

TABLE 1

Effect of initial application weight on the bioadhesion of a sodium carboxymethylcellulose / sodium Carbopol film (1.5: 0.15% w / w in the gel state) to cervical tissue

Initial application weight (g)	Force of bioadhesion (N) (± S.D.) ($n = 4$)
7.9	1.763 ± 0.501
14.6	1.640 ± 0.171
20.6	2.297 ± 0.280
30.6	1.619 ± 0.483
50.6	1.860 ± 0.530

TABLE 2

Effect of varying the duration of an initial applied weight (20.6 g) on the bioadhesion of a sodium carboxymethylcellulose / sodium Carbopol film (1.5:0.15% w / w in the gel state) to cervical tissue

Application time (s)	Force of bioadhesion (N) (± S.D.) ($n = 4$)
5	1.648 ± 0.799
15	2.201 ± 0.370
30	1.521 ± 0.896
60	1.962 ± 0.703
120	1.751 ± 0.882

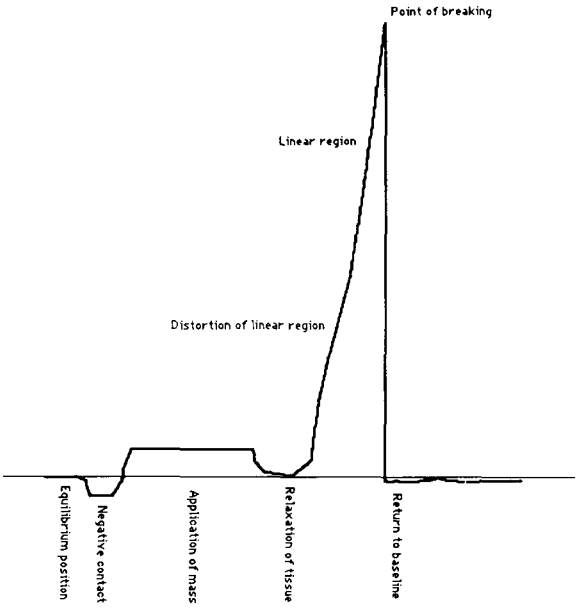

Fig. 3. Annotated output from sensor.

Film hydration was found to be of particular importance in the bioadhesion process (Table 3). Clearly, the largest bioadhesive force was obtained when a dry film was applied to the cervical tissue surface and for this reason all other studies

TABLE 3

Effect of initial hydration time on the bioadhesion of a sodium carboxymethylcellulose / sodium Carbopol film (1.5:0.15% w / w in the gel state) to cervical tissue

Film hydration time (s)	Force of bioadhesion (N) (±S.D.) ($n = 4$)
0	1.822±0.561
5	0.363±0.044
15	0.290±0.049
30	0.235±0.021
60	no adhesion

A weight of 57 g was applied initially for 1 min immediately prior to testing.

reported in this paper were performed by applying films in their dry state to the cervical surface.

Robert et al. (1988) suggested that hydration was significant because, when incomplete, adhesive properties were greatly increased. Chen and Cyr (1970) stated that an optimum water concentration was needed to sufficiently hydrate the hydrocolloid matrix, allowing maximum adhesion to develop. If hydration was excessive then adhesion was lost as a slippy mucilage formed. Dried hydrogels will adhere aggressively to moist tissue. They function by hydration at the tissue surface, with the film swelling rapidly and penetrating into surface depressions.

From Table 4 it is apparent that the bioadhesive force is directly related to film thickness, increasing with an increase in the thickness of the

TABLE 4

Effect of film thickness on the bioadhesion of a sodium carboxymethylcellulose / sodium Carbopol film (1.5:0.15% w / w in the gel state) to cervical tissue

Film thickness (mm) (±S.D.) ($n = 5$)	Force of bioadhesion (N) (±S.D.) ($n = 5$)
0.076±0	1.806±0.477
0.059±0.006	1.750±0.634
0.040±0	0.683±0.113
0.030±0.008	0.519±0.096
0.019±0.002	0.379±0.131
0.015±0.005	0.300±0.112
0.010±0	0.198±0.038

A weight of 57 g was applied initially for 1 min immediately prior to testing.

TABLE 5

Effect of the surface density of mucus on the bioadhesion of a hydroxypropylcellulose film (3% w / w in the gel state) to cervical tissue

Level of surface mucus	Force of bioadhesion (N) (±S.D.) ($n = 4$)
Air-dried tissue	0
Damp tissue (light mucus coat)	0.176±0.035
Dabbed tissue (medium mucus coat)	0.243±0.044
Tissue surface saturated with mucus	0.112±0.025

A weight of 57 g was applied initially for 1 min immediately prior to testing.

model film formulation used for the study. For this reason it is necessary to control the film thickness within reasonable limits when other parameters are to be studied in respect of their effect on bioadhesion.

In the particular case of cervical tissue, the surface density of mucus varies with the stage of the oestrus cycle. Since it was impossible to control this in respect of the available tissue samples, the level of mucus on the cervical surface was artificially altered either by drying in air, swabbing to remove the majority of mucus, dabbing to remove only excess mucus, or saturating the tissue surface with mucus (Table 5). There was no resulting bioadhesion when dry films were applied to dry tissue samples, clearly showing the importance of film hydration since hydrated films do adhere strongly to dry tissue.

Excess mucus was found to reduce the bioadhesive force. The extent to which mucus is involved in the bonding process to the hydrated film is not clear, but the magnitude of the force required to rupture the adhesive bond is certainly larger than that normally associated with mucoadhesion, suggesting that the primary bonding may be between the tissue surface itself and the hydrated film.

A range of candidate film formulations of controlled thickness were investigated for their bioadhesive properties (Table 6). The tissue mucus level was controlled as far as reasonably practial in these experiments to give a medium thick-

TABLE 6

Effect of film formulation on the bioadhesion to cervical tissue

Formulation	Force of bioadhesion (N) (±S.D.) ($n = 4$)
HPC/HEC	0.550 ± 0.094
HPC/MC	0.638 ± 0.126
HPC/NaCP	0.297 ± 0.071
HPC	0.287 ± 0.077
HEC/MC	0.753 ± 0.109
HEC/NaCMC	1.101 ± 0.096
HEC/NaCP	0.301 ± 0.082
HEC	0.287 ± 0.077
NaCMC/MC	0.904 ± 0.138
NaCMC/NaCP	1.404 ± 0.068
NaCMC	1.492 ± 0.130

A weight of 57 g was applied initially for 1 min immediately prior to testing. All films contained either 3% w/w of single polymer or 1.5% w/w of each polymer (two component films) in the gel state, with the exception of sodium Carbopol (0.15% w/w). HPC, hydroxypropylcellulose; HEC, hydroxyethylcellulose; MC, methylcellulose; NaCP, sodium Carbopol; NaCMC, sodium carboxymethylcellulose.

ness of surface mucus. There was a wide variation in bioadhesive properties to cervical tissue as determined by the adhesive force. The greatest bioadhesion was observed with films containing sodium carboxymethylcellulose. However, it is also necessary to consider the mechanical properties of the film in addition to bioadhesion. For this reason, although single-component sodium carboxymethylcellulose films had the highest force of adhesion, a two-component film with sodium Carbopol was preferred for its greater tensile strength and flexibility. These films had excellent bioadhesion to cervical tissue.

The LVDT bioadhesive tester described in this study was able to differentiate clearly between the adhesion produced by the various polymeric film formulations, and could further be used to investigate the effect on bioadhesive force of a range of variable parameters. The results obtained suggested that dry film application will give extremely strong bonding to cervical tissue. The LVDT apparatus was simple and rapid to operate, and gave reproducible results in hard copy format.

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