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An in vitro mucosal model for prediction of the bioadhesion of alginate solutions to the oesophagus

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Abstract

This paper discusses the development of an in vitro model utilised to assess the adhesion of alginate solutions to porcine oesophageal tissue. The methodology involved the construction of retention apparatus onto which sections of tissue were mounted. Fluorescently labelled alginate solutions of known rheological profile were dispensed onto the tissue at a concentration of 2% w/v. A washing solution was applied at a specified rate to mimic saliva flow and the eluted material collected. Fluorimetric analysis allowed dose retention to be assessed as a function of time. The effect of the nature of the washing solution and the choice of alginate were investigated. It was found that after 30 min up to 20% of the applied alginate dose remained associated with the tissue, regardless of the alginate selected from the range examined. The nature of the washing medium did not have a significant effect on retention, irrespective of the inherent mucin concentration. Overall this study indicated that the technique presented offers a viable means of studying bioadhesion of liquids and also demonstrates that alginate solutions may have an application as bioadhesive agents for localisation within the oesophagus.

Keywords: Alginate; Bioadhesion; Mucoadhesion; Oesophagus

1. Introduction

Despite the extensive body of literature now available on drug targeting to specific regions within the gastrointestinal tract, there are comparatively few studies involving the delivery of drugs and/or therapeutic agents to the oesophagus. There are, however, a number of therapeutic areas whereby specific delivery and prolonged retention within the oesophagus is highly desirable (Dobrozsi et al., 1999). These include the treatment of upper gastro-oesophageal disorders including gastro-oesophageal reflux disease (GORD), heartburn, and dyspepsia. However, the transient

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nature of passage through this region of the body has rendered the development of suitable dosage forms difficult, as there is a need to balance the requirement for prolonged residence with the avoidance of oesophageal blockage.

In this investigation the potential of liquid alginates as bioadhesive agents has been examined, with a particular view to establishing the possibility of using relatively dilute solutions of this polysaccharide as coating agents. The concept of coating the oesophagus has received limited attention since early experiments involving sucralfate (Hardy et al., 1993). The rationale behind the development of such a coating lies in the longer term aim of providing a means to protect the oesophagus from chemical trauma or as a means of increasing drug residence time at the oesophageal surface without the concomitant blockage problems associated with the delivery of solid or semisolid dosage forms. Alginates are natural polysaccharides composed of two monomeric sugar units, α-L-guluronic acid (G) and β-D-mannuronic acid (M). The proportion and distribution of these monomers and their relative sequencing determines the chemical and physical properties of the alginate (Smidsrød and Draget 1996). These molecules exist as block copolymers containing segments of polyguluronate and polymannuronate sequences as well as fragments of an alternating character (Smidsrød and Draget, 1997). Alginates are currently utilised in the management of GORD, particularly in raft-forming systems. Such dosage forms generally comprise a mixture of sodium alginate with a bicarbonate salt that, on entering the acidic conditions of the stomach, generates carbon dioxide. The sodium alginate either precipitates out as alginic acid or, when calcium or aluminium antacids are incorporated, may form a crosslinked alginate gel raft. This raft floats on top of the gastric contents, its buoyancy aided by the bubbles of carbon dioxide released. A number of studies have been performed on the physicochemical aspects of raft formation (Washington et al., 1987) and this approach is now a well-established therapeutic strategy. Additionally, alginates have previously demonstrated bioadhesive potential (Chen and Cyr, 1970; Smart et al., 1984), hence it is logical to examine the possible extension of their use in GORD and other gastro-intestinal diseases, particularly in terms of the possibility of oesophageal adhesion. For the reasons given above, it is necessary to probe the bioadhesive properties of free-flowing alginate solutions for application to this target area. The use of free-flowing liquids as bioadhesives has only been investigated to a limited extent and established means of assessing solid and semi-solid bioadhesion such as tensile testing are clearly not appropriate in this situation. Previous studies of liquid bioadhesives include work that investigated the adhesive properties of polymeric solutions using a technique that assessed the difference in fluorescence of labelled cells before and after contact with the polymer in question (Park and Robinson, 1984). A second study involved the development of a 'continuous-flow adhesion cell' that not only measured the extent of adhesion but also assessed the duration of adhesion of polymeric solutions (LeRay et al., 1999). A parallel approach used for assessing microparticulate adhesion is that of Rao and Buri (1989) who developed a method to assess the retention of coated particles on rat stomach and jejunum tissue, termed the 'falling film' method. A known quantity of the particles was dispensed onto the tissue surface, the surface was washed to mimic physiological conditions and the washings were collected. The percentage of particles removed from the tissue substrate was then calculated by gravimetric analysis. This process allowed a retention profile of the dose adhering to the tissue to be deduced. Although this method was not used to assess the retention of liquid systems, it may be easily adapted for this use.

There have also been a limited number of studies involving bioadhesion to oesophageal tissue. Dobrozsi et al. (1999) developed an everted rat oesophagus model to assess the retention of sucralfate suspensions on rat oesophageal tissue. This technique used a gravimetric assay to determine the extent of adhesion of liquid formulations to everted oesophageal tissue. Young and Smart (1998) developed a test that assessed mucoadhesion of given formulations to porcine oesophageal tissue; similar work was performed by Banning et

al. (1998). More recently magnetic resonance imaging was used to observe oesophageal retention of liquid and gel formulations in vivo (Potts et al., 2000).

In this study, a method is described for the investigation of adhesion of liquid polymeric solutions to oesophageal tissue. Particular attention was paid initially to optimisation of the experimental parameters associated with the technique and subsequently the adhesion of alginate solutions to oesophageal tissue was assessed. In addition, by utilising a range of alginates as well as washing media it was hoped that insights would be gained into the molecular characteristics and mechanisms associated with the adhesion process.

2. Materials and methods

2.1. Materials

A range of sodium alginates (LF120, SF120, H120L, SF200, LF10L, and LFR5/60) whose properties are listed in Table 1 were donated by FMC Biopolymer, Norway. The viscosity values of 2% w/v solutions at 37 °C were measured at a shear rate of 10/s using a TA Instruments (UK) AR1000N controlled stress rheometer. Fluoresceinamine (isomer I) supplied by Acros, UK; 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) from Lancaster, UK; acetone, phosphorus pentoxide and disodium fluorescein from Sigma, UK; dioxane from BDH, UK and sulphuric acid from M & B Laboratories, UK were used in the covalent labelling of the alginate. Chemicals used in the preparation of the artificial saliva included 'Lab-Lemco' powder, yeast extract and protease peptone supplied by Oxoid, UK; porcine gastric mucin (type II), sodium chloride, calcium chloride, potassium chloride and 40% urea supplied by Sigma, UK; sodium bicarbonate and sodium phosphate supplied by BDH, UK.

2.2. Methods

2.2.1. Preparation of fluorescently labelled alginate

Fluoresceinamine was covalently attached to the alginate chain according to a method adapted from previously published work (Blonk et al., 1995). The principle of the labelling reaction involves reacting the amine group of the dye with a small fraction of the carboxylic acid groups present in the alginic acid chain. Dioxane was present to help solubilise the dye. EDC acted as a dehydrating agent to remove water and thus enable the amide bond to form. An outline of the procedure is provided.

Alginic acid was prepared from sodium alginate (LF120) by acidification with sulphuric acid. The material produced was washed twice with both water and acetone then dried above phosphorus pentoxide at room temperature. Approximately 1.5 g of the dried and crushed alginic acid was added to 50 ml deionised water and stirred. One gram of EDC was then added to commence the dehydration reaction. Fifty milligrams of fluoresceinamine was dissolved in 30 ml 1,4-dioxane. This second solution was added slowly to the alginate/EDC suspension whilst stirring. Dissolving the dye in the dioxane aided dispersion of the fluorescent label to produce homogeneously labelled alginate solution. This solution was stirred overnight in a dark environment to prevent light

Table 1
Characteristic information on alginate samples used (data supplied by FMC Biopolymer except viscosity values)

Alginate	Molecular weight (kDa)	Viscosity of a 2% solution (Pa s)(shear rate = 10 s^{-1})	Fraction of G units
LF120	240	0.51	0.44
SF120	220	0.43	0.69
H120L	416	4.12	0.46
SF200	387	2.93	0.69
LF10L	75	0.02	0.45
LFR5/60	40	7.49×10^{-3}	0.64

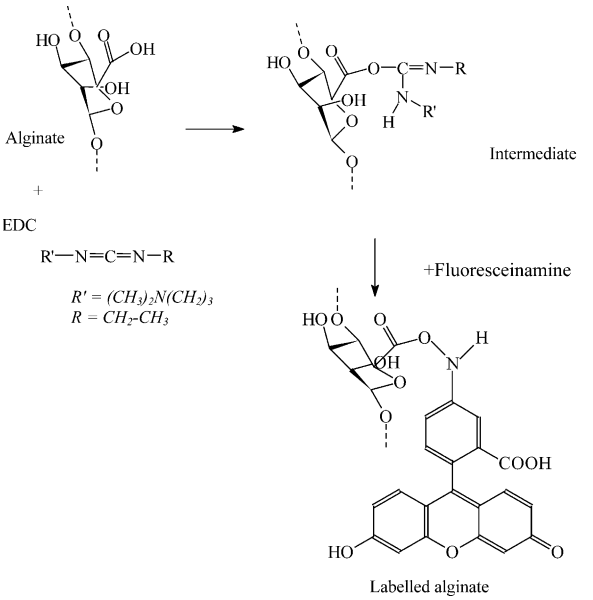

Fig. 1. Schematic representation of the covalent labelling of alginate using fluoresceinamine.

degradation of the fluorescent component. The reaction solution was then neutralised slowly to pH 7 using concentrated sodium hydroxide solution and the pH monitored throughout this process (Hanna Instruments 8520 pH meter; calibrated at both pH 4 and at pH 7). The neutral solution was then exhaustively dialysed against deionised water to remove any free dye or other small molecular weight impurities. Once the dialysis was complete the labelled sodium alginate solution was freeze dried (Edwards Micro Modulyo with an Edwards RV3 pump) and stored in an airtight container at 0–5 °C. Fig. 1 shows a schematic representation of the labelling process. The excitation and emission absorption frequencies of an aqueous solution of the material produced were found to be 489 and 515 nm respectively. The viscosity of a 2% w/v solution of the labelled alginate was found to be 0.75 ($\pm$0.03) Pa s at a shear rate of 10/s and 37 °C compared to 0.51 ($\pm$0.02) Pa s for the unlabelled 2% w/v LF120. The difference in viscosity observed was unexpected. It was thought that the labelling process may degrade the alginate chain resulting in a reduced viscosity of the labelled material. Presently an exact explanation for the enhanced viscosity observed is not available. One suggestion is that the chemical alteration of the polymeric chain leads to enhanced network arrangement due to the formation of additional cross-links. However, for future experiments it was assumed that the labelling process did not lead to the alginate displaying atypical behaviour in the adhesion experiments performed.

Gel permeation chromatography was performed to assess the purity of the resulting labelled alginate. A single peak was observed indicating the presence of only one species rather than a separation of free dye and labelled alginate. If efficient attachment is assumed, the proportion of substituted uronic acid units is estimated as 0.5% from the quantities used. Alternative fluorescent solutions were prepared by addition of sodium alginate to solutions of disodium fluorescein (0.001% w/v). The maximum excitation and emission absorption wavelengths of these solutions were found to be 490 and 510 nm respectively.

2.2.2. Retention model

Porcine oesophageal tissue was obtained from freshly slaughtered animals. The outer muscle layers were removed exposing the inner epithelial tube. Inner tissue sections were flash frozen and stored at -20 °C until required. Tissue was allowed to thaw overnight in a refrigerator then equilibrate to room temperature prior to use. This method of preparation has been shown to retain the histological integrity of the tissue (Levine et al., 1987).

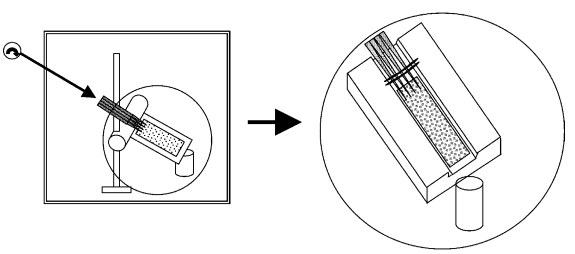

Fig. 2. Schematic representation of the retention model apparatus used.

Fig. 2 illustrates the apparatus used to determine the retention of an alginate dose on oesophageal tissue. A Perspex® mounting block was manufactured with dimensions 100 mm length by 60 mm width and 15 mm deep. A groove was cut into this block in a central position; the dimensions of the groove were 100 mm long by 12 mm wide and 5 mm deep. The mounting block was permanently attached to a clamp that was able to rotate through approximately 120°. The clamp was attached to a stand and placed within a temperature and humidity controlled environment, an adapted Gallenkamp industrial humidity cabinet (model BR185H). This apparatus allowed the temperature and humidity to be controlled and maintained via a dry and wet bulb and appropriate differences were set using hygrometric tables. The cabinet had sealed glove access to enable procedures to be performed during the experiment. The washing medium delivered to the tissue was supplied via a peristaltic pump (Watson Marlow model 202). The flow was split into four channels to provide an even distribution of the media over the entire tissue section. The material washed from the surface of the tissue was collected into glass vials. The vial was changed at 3-min intervals to enable a profile of the material eluted at stages throughout the experimental procedure to be obtained.

A 60 mm by 12 mm longitudinal section of oesophageal tissue was cut and placed into the groove cut on the mounting block. The exterior of the tissue section was inherently tacky from the removal of the muscular layer and thus no permanent fixative was required. It was noted that there was no motion of the tissue section for the duration of each experiment. The tissue section was discarded if residual surface debris was evident. The tissue section was not washed as this process may affect the surface properties and hence the adhesive interaction. A measured dose of labelled alginate was dispensed evenly over the tissue surface. The section was retained horizontally for a set period after which it was inclined to a designated angle and washed at an appropriate rate with the washing medium. The experimental procedure was observed to ensure that there was no significant disruption to either the uniformity of flow of the media or the alginate moiety. Fluorimetric analysis of the eluted material using a fluorescence spectrophotometer (Perkin Elmer PE-204) allowed the amount of alginate removed from the tissue to be calculated according to a previously recorded calibration curve. This technique allowed both the extent and duration of adhesion to be quantified. In the first instance, the effect of the angle of the tissue inclination, the duration for which the alginate was in intimate contact with the epithelium prior to washing (termed the equilibration period) and the rate of flow of the washing medium were investigated. These experiments were performed under standardised conditions of a set dose size of 1 ml and an alginate concentration (LF120) of 2% w/v, labelled with disodium fluorescein. Equilibration times of 60 s were used prior to washing unless otherwise stated. Deionised water was used as the washing medium and was set to flow over the tissue surface at a rate of 1 ml/min. The temperature was set to 37 °C and the humidity was maintained at a level greater than 90% RH. Parameters investigated included alginate retention onto biological and also inert substrates and a comparison of the retention profile of the covalently attached label and the labelling with a simple disodium fluorescein solution. These parameters were then fixed for subsequent investigations as indicated.

Four washing media were used during the study; deionised water, two artificial saliva formulations (Table 2) and human saliva collected from a healthy volunteer at least 1 h after eating or drinking. Human saliva samples were collected as required and used within 1 h of collection.

3. Results

3.1. Basic experimental parameters

Prior to examining the effects of both the nature of the adherent material and the washing media, preliminary investigations were performed into the basic experimental parameters to be used. All results are quoted as the mean of the percentage of the original dose that remained adhered

Table 2
Formulae of artificial salivas used during the study

Artificial saliva I		Artificial saliva II	
Chemical	Amount (g/l)	Chemical	Amount (g/l)
'Lab-Lemco' powder	1.0	$NaHCO_3$	0.42
Yeast extract	2.0	–	–
Proteose peptone	5.0	$NaH_2PO_4 \cdot H_2O$	0.91
Porcine gastric mucin	2.5	Porcine gastric mucin	2.7
NaCl	0.35	NaCl	0.43
$CaCl_2$	0.2	$CaCl_2$	0.22
KCl	0.2	KCl	1.49
40% urea	12.5	–	–

after 30 min plus or minus the standard deviation where $P > 0.05$ and $n = 6$. Initially the effect of the angle of inclination was studied; physiological conditions dictate that the human oesophagus is orientated vertically when standing and horizontally when reclining. Early studies indicated that in order to ensure even flow of the washing medium over the tissue a shallow angle was required; hence it was necessary to establish a set of conditions. No significant difference in the retention of an alginate dose was observed when the angle of inclination was altered within the range of 45–75° to the vertical ($P > 0.05$, $n = 6$) at any of the time points investigated. An angle of 60° was selected for future studies as this angle gave the most reproducible results. Equilibration periods of 60 and 300 s were compared. Again, no statistically significant effect was noted in either the extent or duration of alginate adhesion at the time points investigated ($P > 0.05$, $n = 6$). An equilibration period of 60 s was utilised in further experiments as this was thought to have greater physiological relevance. A one-way analysis of variance (ANOVA) test showed that none of the parameters investigated showed significant differences compared to those associated with the inherent variability of the system. A further experiment was performed to ascertain that the choice of marker and substrate were appropriate. In particular, it was possible that the disodium fluorescein itself was adhering to the tissue irrespective of the incorporation into the alginate solution; hence comparative studies were performed using an equivalent concentration aqueous solution of the marker. Fig. 3 shows retention data at three time points and indicates that an aqueous solution of disodium fluorescein was not retained to any measurable extent, whereas the alginate systems incorporating disodium fluorescein demonstrated an adhesive interaction, with approximately 20% of the original dose retained after 30 min. Similarly it was necessary to establish whether the labelling technique used (covalent attachment or simple mixing) had an influence on retention. The retention of the covalently labelled alginate was compared to the retention of alginate associated with disodium fluorescein in an aqueous mixed system. The results can be seen in Fig. 4. The overall retention profiles are similar for the alginate solutions labelled via the two different methods. However, large differences in the extent of adhesion are

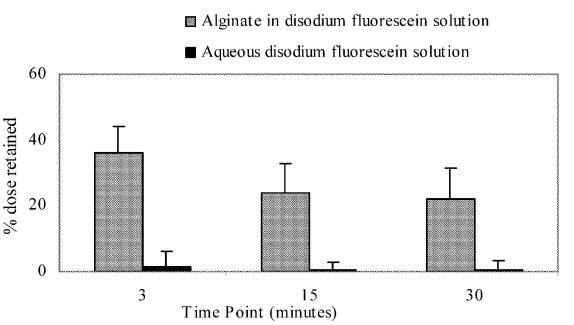

Fig. 3. Retention data for disodium fluorescein in 2% w/v sodium alginate LF120 and in water. (Mean values are shown where $n = 6$ and the error bars represent standard deviation).

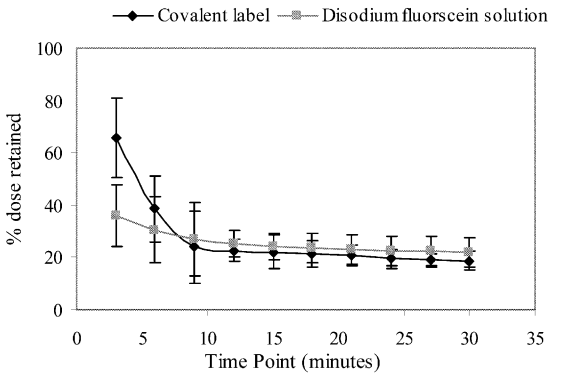

Fig. 4. Time profiles for 2% w/v LF120 covalently labelled alginate and sodium alginate associated with disodium fluorescein. (Mean values are shown where $n = 6$ and the error bars represent standard deviation).

noted in the initial stages, these are statistically significant only at the 3 min time point. Likely explanations for this phenomenon include the viscosity difference between the two applied solutions and also the diffusion of the water-soluble disodium fluorescein from the alginate moiety into the washing media. This anomaly was considered to be acceptable as the adhesion in the early stages is affected by many parameters and it is believed that the adhesion in the latter stages may be more useful in providing an insight into the nature of the adhesive interaction. The alginate associated with disodium fluorescein was used in further studies as the preparation of this solution was a more rapid and reproducible procedure. It is, however of use (and significance in the current studies in terms of future work) to note that fluorescently labelled alginates may be prepared. It was also necessary to establish whether the observed adhesion effect was general or whether there was evidence of specificity for biological tissues. A comparative study using cellulose acetate membrane in place of oesophageal tissue indicated that the retention was significantly lower for the inert polymeric material compared to the ex vivo tissue (Fig. 5).

A washing medium was utilised to mimic saliva flow within the oesophagus, as this process may be expected to be a major factor in the removal of any adherent material. It was considered necessary to study the effect of flow rate in the first instance, using deionised water as the medium. A number of references are available that quote the flow rate of human saliva. For example, two separate studies report unstimulated human saliva flow to be 0.33 and 0.39 ml/min respectively (Wang et al., 1998; Wolff and Kleinberg, 1998). A maximum flow rate of 10 ml/min of stimulated saliva has also been reported (Driezen, 1970). Flow rates of 0.4 and 1.0 ml/min were investigated. Again, the results did not differ significantly ($P > 0.05$, $n = 6$) and a rate of 1.0 ml/min was used for subsequent studies.

3.2. Investigations into the effect of washing media and alginate chemistry

A range of apposite washing media were investigated; natural saliva, two distinctly different artificial saliva formulae and deionised water. In addition to the issue of flow rate discussed above, the choice of relevant media is also important. The difficulties associated with the use of natural saliva in experimental procedures including the considerable inherent variability have been fully discussed previously (Dawes, 1974; Rudney, 1995). Artificial salivas provide a means by which this variability may be reduced. Many different artificial saliva formulations have been reported in the literature to reflect most aspects of natural saliva behaviour (Vissink et al., 1983; Levine et al., 1987). The mucin concentration within the

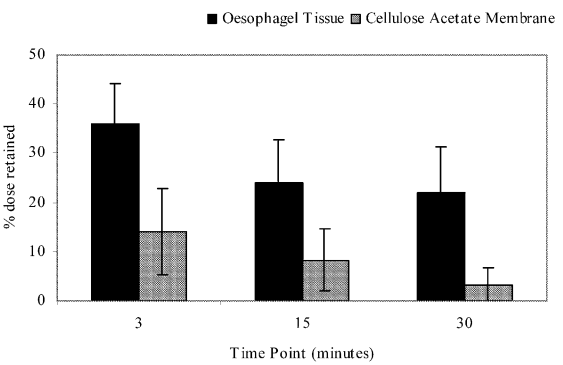

Fig. 5. Comparison of retention profiles for 2% w/v sodium alginate LF120 on oesophageal tissue and cellulose acetate film. (Mean values are shown where $n = 6$ and the error bars represent standard deviation).

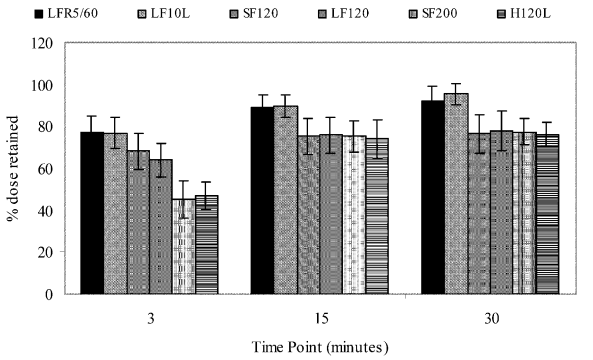

Fig. 6. Retention data for a range of 2% w/v solutions of sodium alginate, associated with disodium fluorescein. (Mean values are shown where $n = 6$ and the error bars represent standard deviation).

formulation used was also considered in this study as mucin may play an important role in the adhesion process (Mortazavi et al., 1992). Two artificial salivas were selected; the first (I) was based on a formulation used widely within the dental field and has a similar rheological profile to natural saliva (Embleton et al., 1998). The second (II) was developed in order to mimic the ionic environment within the mouth (Lentner, 1981). Table 2 compares the formulations of these artificial salivas. No significant differences in the retention of the alginate dose were observed using the four different washing media ($P > 0.05$, $n = 6$).

The retention profiles of the range of alginates are shown in Fig. 6. The retention of the different alginates was similar over the time points shown, although statistically significant lower retention of alginates LF10L and LFR5/60 was observed at all three time points highlighted ($P < 0.05$, $n = 6$). Alginates H120L and SF200 were retained to a significantly greater extent than the remaining alginate range at the 3 min time point although this retention was not significantly different at either 15 or 30 min.

Two further studies were performed to probe the nature of the interaction between oesophageal tissue and alginate; the size and concentration of the dose applied. LF120 was utilised as the standard alginate as the properties are mid range with respect to molecular weight and G fraction. The retention of 1 and 2 ml doses of 2% w/v alginate were compared at 30 min. It was observed that at 30 min the percentage of the 2 ml dose retained ($7.7 \pm 6.9\%$) was approximately half that of the 1 ml dose ($21.9 \pm 9.5\%$). This data may be transformed to provide the volume of alginate retained on the tissue surface at 30 min. No statistically significant difference was found in the volume of alginate retained for the two doses applied.

The retention of the 2, 3 and 5% w/v doses were statistically similar at 30 min as 21.9 ± 9.5, 19.8 ± 10.2 and $19.3 \pm 9.7\%$ respectively, of each dose was retained on the tissue surface. However, after 3 min statistically significant differences were observed in the retention of the doses; 36.1 ± 8.1, 61.6 ± 9.8 and $87.2 \pm 12.8\%$ of the 2, 3 and 5% w/v doses were retained respectively.

4. Discussion

The basic studies performed established some of the fundamental experimental parameters associated with this technique and indicated that factors such as the angle of inclination and the washing medium flow rate did not have statistically significant effects on alginate retention over the range of conditions studied. Although differences were observed in the early retention of the alginate dose according to the labelling method selected, simple mixed solutions were used for future studies as the more relevant adhesion data collected at the later time points (30 min) was not affected by the choice of label investigated. The poor retention of alginate to cellulose acetate membrane indicated that the adhesive process is dependent on the nature of the substrate and is not simply a function of the viscosity or 'stickiness' of the applied solution. It may also be a measure of the surface properties of the tissue including local roughness and the presence of mucin. It is also worth noting that during these preliminary studies approximately 20% of the applied alginate dose was retained after 30 min. While it would be misleading at this stage to claim that this technique correlates with the in vivo situation the figure of 20% is nevertheless encouraging, as there is a clear implication that therapeutically significant retention is feasible.

The studies involving the washing medium and the choice of alginate showed a relatively low dependence on either factor. Our initial expectation was that a significant factor in the adhesion process would be the presence of swallowed mucin on oesophageal tissue. While the samples used were not entirely representative of the in vivo situation one might reasonably expect there to be some effect when a mucin-containing washing medium was applied. If mucoadhesion rather than bioadhesion is the dominant factor in the adhesive process the mucin present in the washing medium may interact with the alginate layer and increase removal of the alginate entity. The absence of any discernible effect tends to suggest that the interaction with mucus may not be the dominant factor in oesophageal adhesion.

The use of a range of alginates allowed the relationship between molecular properties and adhesion to be investigated. There was some evidence of a greater retention of the high molecular weight materials in the initial stages, with no discernible relationship being apparent with regard to the M:G ratio. This observation lead to the proposal of a theory of a double adherent layer. The double layer is comprised of a lower layer of alginate that comes into direct contact with the oesophageal tissue surface and an upper alginate layer that resides on top of the lower adhered alginate layer. The retention of the upper alginate layer is dependant upon the cohesive forces that exist within the applied alginate solution. It is well documented that high molecular weight polymers exhibit stronger cohesive forces thus this theory may explain the relatively poor retention of the low molecular weight alginates investigated. This theory may also be linked to a previous observation made that low molecular weight polymers demonstrate poor bioadhesive potential (Chen and Cyr, 1970). The retention of the lower layer is dependant upon the adhesion forces between the alginate component and the upper oesophageal tissue surface. This lower adherent layer may be comprised of only a certain volume of alginate applied that is able to make direct contact with the tissue surface. Evidence for this theory is provided by the results of the study that investigated the effect of the size and concentration on dose retention. It was noted that a finite volume of alginate was retained at the 30 min time point regardless of the size or concentration of the applied dose. This theory also highlights the importance of assessing the duration of adhesion as well as the initial profile as it demonstrates the limitations of in vitro models. The data collected from this apparatus in the initial stages provides information on the adhesive and cohesive forces involved in the bioadhesive process whereas the data collected at later time points provides data more specifically about the adhesive interface.

5. Conclusions

A novel method has been developed to allow investigation into the adhesion of liquid formulations to oesophageal tissue, with a particular view to establishing the potential of alginate solutions as liquid bioadhesive systems. A range of experimental factors have been investigated in order to optimise the experimental protocol and to identify parameters that may influence the adhesion process. The development of the double adherent layer theory has provided insights into the nature of the adhesive interface. It has been demonstrated that alginates can be retained on porcine oesophageal tissue in potentially therapeutically significant quantities for prolonged periods of time, indicating that this approach shows promise either as a means of protecting the oesophagus or as a means of delivering drugs to this tissue.

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