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Enhanced Mucoadhesive Capacity of Novel Co-polymers for Oral Protein Delivery

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Abstract

Graft co-polymer networks have shown promise as devices for oral delivery of proteins. By increasing adhesion of these networks at the delivery site of the upper small intestine by utilizing small covalent chemical linkages caused by the addition of an aldehyde functional group we can make them more viable. These aldehydes bind covalently by way of a condensation reaction with the amines of the amino acids found in the glycoprotein network of the mucus layer of the small intestine to form imines. To investigate the effectiveness of this linkage the co-polymers are prepared in three different percentages of poly(ethylene glycol) (PEG) and aldehyde-modified PEG, and characterized through swelling, release and adhesion testing. The percentages of aldehyde-modified PEG used are 0.06, 0.6 and 3.3%. The swelling results indicate that the formulations with the aldehyde-modified PEG maintained the same pH sensitivity and transition around a pH of 5.8 as those formulations without the aldehyde moiety. Release results indicate that the release of insulin of the most promising 3.3% aldehyde formulation was successful with a release of about 80% after 3 h, which compares favorably with the similar release of the controls done in previous work. Adhesion testing was carried out through the use of a mechanical testing apparatus. Data have been gathered and plotted to give a detachment force (N) *versus* displacement (m) curve, of which the work of adhesion (μJ) was found by taking the area underneath the curve. Adhesion results indicate an increase to the already present adhesion of the co-polymers due to increased percentages of the aldehyde-modified PEG tethers where the 3.3% formulation showed an increase of 10–30 μJ over both control formulations.

Keywords

Drug delivery; adhesion; hydrogel; *in vitro*; aldehyde

1. Introduction

Administration of proteins has been an important unsolved problem of drug delivery. While scientists race to discover better treatments for the diseases that plague societies throughout the world, administration of many of these treatments poses problems that can be quite severe. Many who need specific proteins to help with their afflictions must take multiple injections daily. Many are unwilling to go through this ordeal and those who do still see other side-effects. Oral delivery of proteins, such as insulin for diabetics, would prove to be

convenient, improve patient compliance, and have better mimicry of dosage into the portal vein [1]. However, oral delivery has several obstacles that need to be overcome before it is a viable solution. These problems range from enzymes that digest the proteins to difficulties with the transport of large molecules across the mucosa and epithelial cell layer. These obstacles limit the overall drug bioavailability to ranges that are not commercially acceptable. However, contributions by pharmaceutical and biomaterials scientists have led to the development of novel carriers to be used as innovative drug-delivery systems. The performance of these systems was improved by synthesizing polymers that have desirable chemical, physical and biological properties, and favorable interactions with biological materials. These advancements have started to unlock ways around the problems that are encountered with oral drug delivery [2–6].

While there has been limited research in the area of delivery, there are still several separate courses of treatment that one suffering from diabetes mellitus (DM) might take. One such method is that of an insulin pump, which can better regulate the amounts of insulin throughout the day; this can be cumbersome and still requires subcutaneous penetration, however. There have been several other noninvasive forms investigated including administration through the transdermal tissue and nasal passages, but the results are inconsistent and require increased bioavailability before being considered as a viable option [7]. Another form of administration that has just been introduced to the market is an insulin inhaler [8]. However, there are limitations on who can use the inhaler. Those who smoke or have damaged lung tissue are discouraged from taking it as that can hinder uptake and decrease the already moderate bioavailability (10–20%) [8]. In other cases, research has been focused on developing a mucoadhesive delivery device for the oral administration of therapeutic agents to avoid intravenous therapy [9–11]. Some researchers are investigating the attachment of these mucoadhesive drug-delivery devices to the nasal, buccal, ocular, gastrointestinal, urethral, rectal and vaginal tissues in order to avoid any gastrointestinal degradation or first-pass hepatic metabolism [12]. Recently, the pharmaceutical community has become very interested in the development of new and improved mucoadhesive biomaterials with an objective of targeting specific parts of the body.

Incorporating mucoadhesive properties into a drug-delivery system has many significant advantages compared to traditional pharmaceutical dosage forms. The mucoadhesive properties can maintain the drug-delivery system in a specific location in the body, have a prolonged duration of contact with the tissue, and increase the treatment efficiency since the drug is locally maintained at the site of transport. This is important since localizing the drug at a targeted site of absorption and transporting the drug across the intestinal epithelial layer are two problems associated with the low bioavailability that often plagues oral protein delivery. Increased contact of the drug-delivery system with the mucosal absorptive membranes for an extended period could increase the absorption of the therapeutic agent, resulting in a higher drug bioavailability. This strategy is already used in many topical treatments, and improvement was successfully proved since the early stages of the mucoadhesion field [2–6].

Hydrogels with pH sensitivity make excellent candidates for the oral delivery route because of their ability to respond to their environment, they resemble natural living tissue, are biocompatible and provide the protection of proteins needed in harsh environments [13–17]. Hydrogels composed of high-molecular-weight poly(ethylene glycol) (PEG) grafted on poly(methacrylic acid) (PMAA), which will be designated P(MAA-g-EG), have been made and investigated. Briefly, the hydrogels swelling behavior occurs because of the formation of inter-polymer complexes between protonated pendant acid groups and the etheric groups on the graft chains. This inter-polymer complexation is a thermodynamically favorable event and is reversible [18, 19]. Acrylic-based polymers have been extensively used for

mucoadhesive applications because they exhibit high adhesive bond strengths in contact with tissues [20–22]. This increases residence time and drug bioavailability in most cases. Researchers believe that, in such systems, the free PEG chains that were grafted onto the gel act as mucoadhesive anchors, causing an increase in mucoadhesion and making these carriers very promising as systems for protein delivery [23].

There have been various attempts to improve the adhesive properties of polymers such as using linear PEG as an adhesion promoter [24], neutralizing ionic polymers [25], using a sustained hydration process for mucoadhesion [26] and developing polymer–adhesin conjugates [27, 28] to provide specific binding to the epithelia [29].

There have been numerous theories developed to describe the phenomenon of bioadhesion. There is no single theory that is widely accepted as the only mechanism for which bioadhesion occurs. This is especially true when describing our acrylic based hydrogel. A combination of theories has proved useful in describing the phenomena [30, 31]. The adsorption theory suggests that bioadhesion is due to secondary forces, such as van der Waals forces and hydrogen bonding. Figure 1 shows the diffusion theory, which describes the interpenetration of polymer chains in the interfacial region. All of the different theories of adhesion seem to play a role in the adhesive nature of the tethered hydrogel, but no analytical work has been able to define which has more of an impact [31–36].

Another way to increase adhesion is through creating stronger bonds between functional groups than those of secondary forces. Several different functional end groups have already been investigated for their adhesive and selective properties with more garnering increased interest. One such group is that of an aldehyde. The aldehyde functional group has promise for increasing mucoadhesion through the use of a Schiff base covalent reaction in which aldehyde groups undergo an acid-catalyzed condensation reaction under mild aqueous conditions with amines to form a covalent link, giving a permanent stabilized imine group. The linkage can also exist in a less permanent covalently linked intermediate state of a hemiaminal through alkylimino-de-oxo-bisubstitution. In the upper small intestine, the aldehyde functional group can be brought into contact with amines found on the glycoprotein network of the mucus lining the epithelial cells. This chemical linkage between the two should increase the mucoadhesive abilities of the hydrogel, yet still be discharged from the body when the mucus layer of the small intestine is renewed every 6 h [37–39]. These interactions should be prevented in the stomach because the tethers should be inside of the hydrogel in its complexed state.

Hydrogels used for oral delivery have met with limited success thus far. To move past the barriers that have been presented and discussed previously, new ideas and research must be considered. In this research, we propose the use of aldehydmodified PEG tethers for improved adhesion to the upper small intestine to address the barriers that should ultimately lead to meeting the ultimate goal of improved bioavailability.

2. Materials and Methods

2.1. Synthesis of Aldehyde-Modified PEG

PEG aldehyde was synthesized by a reaction outlined by Dr. Steven Ley [40]. Briefly, PEG monomethacrylate monohydroxyl (PEGMA) was dissolved with dichloromethane (DCM), 4 Å sieves and N-methyl morpholine N-oxide (NMO) (all from Sigma). The solution was purged with N₂ for 10 min and the catalyst, tetra-n-propylammonium per-ruthenate (TPAP, Sigma), was added. Following this step, the solution was purged with N₂ for an additional 10 min. The solution was stirred for 30 min until a change from dark green to black was observed, indicating the reaction was successful [40]. After mixing, DCM was added before

several salt washes were performed (potassium chloride, copper (II) sulfate, sodium sulfate) in a separatory funnel. The remaining organic layer was dried over several days to remove any remaining solvents. A Schiff's base assay composed of fuchsin sulfuric acid (Sigma) was used to detect aldehydes on a UV-Vis spectrometer. A standards curve was made using glutaraldehyde (Sigma) and the Schiff's base assay. Figure 2 shows the structure of aldehyde-modified PEGMA.

2.2. Hydrogel Synthesis

The methacrylic acid (MAA, Sigma) monomer was passed through a dehibit column to remove the inhibitor. The polymer films were prepared by free radical UV polymerization. The diluted monomer mixture was composed of MAA, and both PEGMA and aldehyde-modified PEGMA with a molecular weight of approx. 500. Irgacure® 184 (1-hydroxy-cyclohexyl-phenylketone, Ciba-Geigy), which was used as a photoinitiator, and tetraethylene glycol dimethacrylate (TEGDMA, Poly-sciences), which was used as a cross-linking agent, were also incorporated. Deionized water and ethanol were used as solvents. The monomer solution was made of MAA and EG units, in a ratio of 1:1, with three formulations containing aldehyde-modified PEGMA, in percentages of 0.06, 0.6 and 3.3%, incorporated with unmodified PEGMA, as well as solutions of P(MAA) with no PEG tethers and P(MAA-EG) with no aldehyde-modified PEGMA. TEGDMA was added as 1 mol% of the total monomer and Irgacure® 184 was added as 1 wt% of the total monomer.

The monomer solution was dissolved with a 50:50 (w/w) monomer/solvent ratio. The total solvent weight was composed of equal parts deionized water and ethanol. Once all components were added to the monomer mixture, the solution was sonicated until all materials were dissolved into solution. The mixture was purged with nitrogen for 20 min to remove oxygen, which acts as a free radical scavenger. The solution was pipetted between two glass slides (75 mm × 50 mm × 1 mm) separated by a 0.8 mm Teflon® spacer. The slides were placed under a UV light (Efes, Ultracure 100ss high pressure mercury lamp) and allowed to polymerize for 30 min.

After polymerization was complete, the thin films were washed with deionized water daily for 1–2 weeks to remove all unreacted components. After washing, the films were cut into 12-mm-diameter disks and dried under a vacuum (Heraeus VTR-5036) for 2 days at approx. 30°C. Fourier transform infrared spectroscopy (FT-IR) was used to determine if the Schiff's linkage was still taking place in the hydrogels with aldehyde-modified PEGMA.

2.3. Swelling Analysis

Dynamic swelling studies were performed using dimethylglutaric acid (DMGA, $I = 0.1$ M, Acros Organics) buffers. Ten buffers, with a pH range of 3.2 to 7.6, were prepared. The ionic strength of the buffers was controlled with 0.1 M NaCl (Sigma-Aldrich). The polymer disks were placed in 50 ml of the first DMGA buffer with a pH of 3.2 for 5 min at 37°C. Afterwards, the disks were taken out of the buffer, blotted to remove excess buffer, and weighed to determine the water uptake. The disks were then placed in the DMGA buffer of the next highest pH and the process was repeated up to the buffer with a pH of 7.6.

For equilibrium swelling, polymer disks were swollen in five different pH solutions for a span of 48 h at 37°C. One disk was placed in each of the five different DMGA buffers with a pH between 3.2 and 7.6. Afterward, the swollen disks were blotted to remove excess buffer and weighed in ambient air to determine the water uptake.

The following equation was used to determine the weight swelling ratio, Q :

$$Q = W_{\text{swollen}} / W_{\text{dry}}, \quad (1)$$

where W_{swollen} and W_{dry} refer to the weights of the co-polymer disk in swollen and dry states, respectively. These values were then used to plot Q vs swelling pH.

2.4. Insulin Loading and Release Analysis

Hydrogel films were dried, crushed and sieved with a mesh size of 45 μm . Images of particles were taken on a light microscope and processed using an image processing program. Particle size distribution was found to be 8.5 μm with a standard deviation of 5.8 μm . Although particles were not used in adhesion testing it is possible that the size may affect the overall adhesion with considerations of particle design with highest loading efficiency and greatest surface area determined in the future with an eye towards more downstream optimization. All glassware was treated with Sigmacote® (Sigma-Aldrich) to prevent insulin adsorption on the glass wall. Approximately 140 mg of crushed hydrogel particles were dispersed into 20 ml human insulin solution (500 $\mu\text{g}/\text{ml}$ in phosphate-buffered saline (PBS), pH 7.4) at 37°C and stirred for 4 h. Afterwards, 10 ml 0.1 M HCl (Sigma-Aldrich) was added to the solutions to collapse the hydrogel network. The particles were filtered and rinsed with 50 ml 0.1 M HCl and 50 ml DI-water to remove any surface bound protein. The loaded particles were then lyophilized.

The concentration of insulin in the loading and wash filtrates was determined by reverse-phase high-performance liquid chromatography (RP-HPLC). The amount of insulin loaded was determined based on the amount of insulin remaining in the loading filtrate to the initial insulin in the loading solution. Briefly, aliquots of 0.2 ml of the loading filtrate were taken using a 1 ml syringe with a 0.45 μm filter. The mobile phase for the RP-HPLC was composed of a mixture of mobile phase A (water with 0.1% TFA (v/v)) and mobile phase B (acetonitrile with 0.1% TFA (v/v)) on a Waters Symmetry300™ C18 column (5 μm , 3.9 $\times$ 150 mm). Samples of 20 μl were injected to a Waters 2695 separations module equipped with a 996 Photodiode Array detector at 1 ml/min. A 25–38% mobile phase B gradient was run for 10 min. Concentrations were determined based on the area under the curve compared to insulin standards with detection at 254 nm.

Release studies using the insulin-loaded polymer (ILP) were performed by adding 15 mg ILP to 30 ml PBS at pH 7.4 into a Distek Dissolution System 2100B. The solutions were stirred with 0.8 ml aliquots withdrawn using a syringe equipped with a 0.45 μm syringe filter at time intervals of 10, 30, 60, 90, 180, 360 and 1440 min. A constant release volume was maintained by addition of 0.8 ml fresh PBS. These studies were done in triplicate. The protein concentrations for the release time points were determined using a Micro BCA™ Protein Assay Kit (Thermo Scientific), measured by a microplate reader at 570 nm.

2.5. Adhesion Analysis

The adhesion of the polymers in contact with mucin was measured with a mechanical testing apparatus (Instron 4442, 50 N load cell). The hydrogels were swollen for 10 min in a PBS solution (pH 7.4) and fixed to the upper support with cyanoacrylate medical adhesive. Porcine mucin (Sigma-Aldrich, 1 ml of a 2 wt% solution) in DI water was deposited on the lower mount of the tensile tester. The 2 wt% was chosen to mimic the mucin percentage found in the small intestine. The two surfaces were brought into contact with an impingement force of approx. 0.05–0.1 N. The upper clamp was raised at a constant rate of 6 mm/min until complete detachment occurred, while the detachment force (N) vs displacement (m) was measured. The work of adhesion (μJ) was taken to be the area under the curve. This procedure was also performed in an aqueous environment with 10 ml PBS

buffer (pH 7.4) in ambient conditions, which is to say at room temperature and open to the atmosphere. To prevent dissolution in the PBS buffer, the mucin solution was made at 20 wt %. The added 10 ml PBS brought the overall solution back to 2 wt% of mucin solution.

3. Results

3.1. Analysis of Chemical Structure

FT-IR spectroscopy was performed to investigate the functionality of the aldehyde-modified PEGMA after polymerization to the pH-sensitive hydrogel. Spectra were taken of the Schiff's reagent, a dry sample of the 3.3% aldehyde-modified PEGMA formulation and a 3.3% aldehyde-modified PEGMA formulation soaked in Schiff's reagent and washed in DI water to remove excess unreacted Schiff's reagent as seen in Fig. 3a and 3b.

Figure 3a shows a broad peak between 3000 and 3500 cm^{-1} for the Schiff's reagent spectra. This broad peak distinguishes the amine and aromatic groups found in the fuchsin sulfurous acid of the Schiff's reagent, which is noticeably absent from the dried 3.3% sample but is evident in the soaked and washed 3.3% sample.

In the washed 3.3% sample (Fig. 3b) there are two distinct peaks at 1175 and 1300 cm^{-1} that are absent in the dried 3.3% sample. These two peaks are shifted due to the steric hindrance with such large molecules but indicate the presence of sulfurous acid. Sulfurous acid is a component of the Schiff's reagent, which is absent from the initial hydrogel sample.

3.2. Hydrogel Swelling

Dynamic and equilibrium swelling studies were performed to investigate the pH sensitivity and water uptake of these samples with the incorporation of the aldehyde-modified PEGMA in comparison to two control samples of PMAA and P(MAA-EG). The dynamic water uptake for each formulation is shown in Fig. 4a and 4b. All formulations maintain pH sensitivity with a transition around a pH of 5.8. All formulations have similar swelling ratios with high points of 1.5 and low points of 1.0 with one exception. The PMAA formulation has a maximum of 3.3 and a minimum of 1.4 due to the lack of increased hydrogen bonding brought by the PEG tethers.

The equilibrium water uptake for each formulation is shown in Fig. 5. All formulations maintain pH sensitivity with a transition around pH 5.7. All formulations have similar swelling ratios with a maximum of 6.8 and minimum of 1.4, with one exception. The PMAA formulation has a maximum of 13.9 and a minimum of 2.8 due to the lack of increased hydrogen bonding brought by the PEG tethers. The aldehyde-modified PEGMA formulations for both dynamic and equilibrium swelling show continued pH sensitivity, as seen with the control formulations.

3.3. Insulin Loading and Release Analysis

Insulin load and release studies were performed to determine how incorporation of the aldehyde-modified PEGMA tethers would affect release characteristics. To determine any detrimental effect by the new aldehyde tethers, load and release studies were performed on the highest aldehyde-modified PEGMA tethered formulation (3.3%). As discussed previously, RP-HPLC was used to determine that the amount of insulin loaded was at 93%. The fractional insulin released is shown in Fig. 6. The graph shows a minimum of 0.19 after 10 min at the first time point and then an exponential increase to 0.73 at the last time point of 3 h.

3.4. Adhesion Analysis

Adhesion studies were performed to investigate the effectiveness of the chemical Schiff's linkage in aiding mucoadhesion when compared to the interpenetration and hydrodynamic forces seen in the control formulations. Figure 7a and 7b shows typical graphs of the force of detachment (N) vs displacement (m) curves. The two graphs are of the lowest and highest order results retrieved from testing, respectively. This can be seen in the overall increase in the magnitude of the maximum force on the y-axis with Fig. 7a peaking at 0.09 N and Fig. 7b peaking at 0.35 N. Figure 7a shows the curves obtained in the tensile experiment present three different regions, indicated with the letters A, B and C. From point A to B, the disk is completely in contact with the mucin solution and the graph peaks at a maximum force as the two surfaces begin to detach. A large force is necessary for starting the detachment process. In the second portion of the curve, from point B to C, the graph begins decaying as the mucin solution detaches and moves to the exterior portion of the disk. The graph begins to tail off, corresponding to the only contact with the disk, being through a taut string of mucin. Point C indicates the break point, where the two surfaces completely detach causing the graph to return to zero, ending the experiment. The work of adhesion (μJ) was calculated to be the area under the curve.

Figure 8 shows the work of adhesion (μJ) for PMAA, P(MAA-EG) and all three weight percentage ratios of aldehyde-modified PEGMA (0.06, 0.6 and 3.3%) in ambient surroundings with 2 wt% mucin solution and PBS buffered bath with 20 wt% mucin solution. Figure 8 shows a minimum of 10.7 μJ for the PMAA formulation in ambient surroundings and a maximum of 48.1 μJ for the 3.3% aldehyde-modified PEGMA formulation in PBS buffered bath surroundings with noticeable increase of mucoadhesion through all formulations between ambient and buffered bath testing and a noticeable increase between control formulations of PMAA and P(MAA-EG) and the 3.3% aldehyde-modified PEGMA formulation.

4. Discussion

4.1. Analysis of Chemical Structure

As stated previously, the objective of this work was to determine if the aldehyde groups are still functional after undergoing the polymerization process to the hydrogels. ATR-FT-IR was used on dry and wet samples and compared against each other, as well as the spectrum of the Schiff's base assay to determine functionality and binding to amines of the fuchsin sulfurous acid in the Schiff's base assay.

In Fig. 3a the broad peak between 3000 and 3500 cm^{-1} for the Schiff's reagent spectra indicates the amine and aromatic groups found in the fuchsin sulfurous acid of the Schiff's reagent. This peak is absent from the dried 3.3% sample but evident in the soaked and washed 3.3% sample. This indicates that the aldehyde bound to the Schiff's reagent since there are no aromatics or amines present in the initial hydrogel sample.

In Fig. 3b, the two distinct peaks at 1175 and 1300 cm^{-1} found in the washed 3.3% sample but absent in the dried 3.3% sample are shifted due to the steric hindrance with such large molecules and indicates the presence of sulfurous acid. These double peaks indicate that the aldehyde is bound to the Schiff's reagent since there is no sulfurous acid present in the initial hydrogel sample. The aldehyde-modified PEGMA polymerized to the hydrogel and maintained functionality throughout the polymerization process.

This study allows us to confirm that the aldehyde-modified PEG tethers are still functional. This indicates that they survived the harsh conditions of the polymerization process and remain active to be utilized in the adhesion to the gastrointestinal tract.

4.2. pH Sensitivity and Overall Water Uptake

The objective of this work was to indicate that incorporation of the new aldehydemodified PEG tethers has no negative effect on the swelling or pH sensitivity of the hydrogels.

It is important to understand the phenomenon of complexation through hydrogen bonding. Inter-polymer complexation forms between electron deficient moieties and moieties containing regions of high electron density. This inter-polymer complexation is a thermodynamically favorable event and is reversible. In acidic surroundings, the co-polymer is in a collapsed state because of the hydrogen bonding between the ether group of the PEG chain and the carboxylic group of the PMAA [18, 19]. The pH must be sufficiently below the pK_a to allow for sufficient protonation of the carboxylic acids group. As the pH values reach the pK_a value of MAA (4.8), the carboxylic acids are ionized. This ionization causes the dissociation of the hydrogen bonds, resulting in a repulsive interaction between the PEG chains and the MAA molecules. This phenomenon is commonly known as decomplexation.

While there is hydrogen bonding between the PMAA and the PEG chain, the bonds can be disrupted. It has been shown that the methyl group of PMAA acts as an electron receiver and stabilizes the carboxylic acid group. The increase in electron receiving due to the methyl group causes an increase in the stability of the carboxylic acid's hydrogen bonding, further stabilizing the complexation [41].

All compositions in both the dynamic and equilibrium study showed pH sensitivity at pH 5.8 and 5.7, respectively. There was also an increase in water uptake as pH increased, indicating that the addition of the aldehyde moiety does not affect the bonds that cause complexation and decomplexation. Of note is the drop between the weight swelling ratio of the PMAA formulation, which has maximums of 3.3 and 13.9 for dynamic and equilibrium, respectively, and all compositions with tethers, which have maximums of 1.5 and 6.8 for dynamic and equilibrium, respectively. This was to be expected with fewer ether group sites due to the lack of PEG chains, thus decreasing the hydrogen bonding and the carrier's ability to keep fluid out.

This study allows us to confirm that the new tethers do not affect the desired properties of the pH sensitive hydrogels. Thus, we expect that the new carriers could maintain the same characteristics through the oral delivery process.

4.3. Insulin Loading and Release Analysis

The objective of this study was to analyze the effects of any interference the incorporation of the aldehyde-modified PEGMA tethers on the insulin release. This has implications because of the success of prior work with pH-sensitive hydrogels [42].

Briefly, Fig. 6 shows the fractional insulin release with an exponential increase of up to 0.73 fractional release after 3 h, indicating that release of insulin was successful.

This study allows us to confirm that insulin release is still possible with the new tethers of the pH-sensitive hydrogel. It is expected that these systems could maintain significant insulin release with potentially more adhesion.

4.4. Adhesion Analysis

The objective of this study was to determine any increase in adhesion in dry and wet conditions that the addition of the aldehyde-modified PEG tethers have on the hydrogels.

During the mucoadhesive experiments, the hydrogel disks were previously swollen in a buffer solution of pH 7.4 for 10 min. The first step necessary for the formation of a

mucoadhesive bond between a polymer and the mucus is the wetting and swelling of the polymer. This step is crucial since the amount of water imbibed by the polymer at the time of adhesion will affect the interaction of the system with the mucus at the interface. Ten minutes of exposure to a buffer solution was sufficient to swell the system to conditions similar to those in the upper small intestine. This value simulates the physiological situation of a hydrogel traveling through the GI tract to the upper small intestine, where it is deswollen under the conditions described above, to the target site of adhesion, where it starts swelling and decomplexing.

Different PEG-tethered designs were studied but all with a constant molecular weight. The objective was to improve the mucoadhesive performance of a cross-linked P(MAA) hydrogel, a well-known mucoadhesive polymer, by decorating it with PEG tethers that have different ratios of the adhesive moiety. The PEG tethers in this research were decorated with an aldehyde functional group. The basic P(MAA-EG) hydrogel utilizes many secondary chemical interactions. These secondary chemical interactions include ionic bonds, van der Waals interactions and hydrogen bonding, with hydrogen bonding probably being the most important secondary interaction in mucoadhesion. Some functional groups that form hydrogen bonds are hydroxyls and carboxyls, which are both present in the basic P(MAA-g-EG) system. However, while these types of forces are weak, numerous interaction sites led to strong mucoadhesion [43]. Upon entering the high-pH conditions of the upper small intestine, hydrogen bonds are formed between the hydrogel and the mucus. There are some ionic interactions but not a significant amount. These secondary forces hold long enough for swelling and, as a result, interdiffusion will take place. This happens because the PEG chains are not interacting with the P(MAA) backbone at the higher pH conditions. Consequently, the PEG chains are able to diffuse through the mucus and enhance mucoadhesion. We did not test the effect of increased tether length by increasing the molecular weight of the PEG as we wanted to show the ability of the aldehyde moiety to increase adhesion alone. It is possible that increase tether length could increase adhesion by increased interpenetration or the opposite could occur as longer tethers could fold into themselves causing less adhesion. Future work in a separate study would need to be performed to determine which is more likely. The aldehyde moiety will complement these interactions with the addition of another possibly stronger chemical reaction with that of a Schiff's base covalent linkage. This occurs when the aldehyde forms a chemical linkage with an amine functional group similar to those found on the N-terminus of the glycoproteins that help compose the mucus layer that coats the small intestine.

The data show a trend of increasing adhesion from no tethers in the case of PMAA to those with tethers, as well as an increase in adhesion with an increase in the amount of aldehyde-modified PEGMA. This is the case for both ambient and buffered bath conditions, although the trend is not seen with the buffered bath conditions until reaching the higher percentages of aldehyde-modified PEGMA. This indicated that interpenetration forces cause an initial increase in adhesion from PMAA to P(MAA-EG) but that the addition of the added chemical bonding through the Schiff's linkage causes a greater increase in the adhesion and adhesion will only increase with more aldehyde-modified PEGMA due to the availability of more bonding sites.

Also of note is the overall increase in the force of adhesion between the ambient and PBS buffered bath solutions, especially for the PMAA formulation. This occurs due to the added swelling that takes place within the water bath. As stated previously, the PMAA formulation has a larger water uptake than that of any other formulation. This is due to the lack of the hydrogen bonding that takes place between PEG and PMAA in all tethered formulations. These hydrogen bonds cause for tighter junctions and smaller uptake of fluid and, thus, swelling. Since PMAA does not have these interactions, it has more uptake of fluid and

more swelling, which is still taking place in the water bath during the mucoadhesion experiments. The increased swelling allows for more of a wicking action to take place between the hydrogel and the mucin substrate, causing an increase in adhesion.

This study allowed us to determine the increased adhesion the aldehyde-modified PEG tethers provide for. With increased adhesion over other formulations, these new materials could potentially improve drug delivery due to increased residence time in the upper GI tract.

5. Conclusions

The present work was performed to determine the effectiveness of using chemical linkages by utilizing a reactive functional group, in this case an aldehyde moiety, to increase the limited adhesion of the hydrogel. The better adherence to the small intestine would lead to increased residence time, release at the delivery site, and would presumably affect the bioavailability of the protein. Hydrogels with and without PEG tethers and with varying percentages of aldehyde-modified PEG tethers were synthesized and characterized to determine any improved adhesive capacity without affecting the overall characteristics of the oral delivery device. Swelling studies showed that the aldehyde-functionalized hydrogels retained their pH sensitivity at pH 5.5. Release studies showed the aldehyde-functionalized hydrogels maintained the fractional insulin release seen in previous work with non-functionalized hydrogels. Widely accepted *in vitro* tensile testing showed improved adhesive capacity with increased chemical linkages with the aldehyde moiety in both dry and wet conditions. These findings indicate the potential these functionalized hydrogels have for improved oral drug delivery.

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Figure 1.

Upon contact, the interface between two polymer systems disappears and chain interpenetration or diffusion occurs. (a) Systems separated, (b) the two systems being brought into surface contact and (c) the interpenetration of the two systems into the other.

Figure 2.
Structure of aldehyde-modified PEGMA.

Figure 3.

FT-IR spectra of (top to bottom) P(MAA-EG) hydrogel with 3.3% aldehyde-modified PEGMA in the dry state, Schiff's reagent for aldehydes and P(MAA-EG) hydrogel with 3.3% aldehyde-modified PEGMA after being in Schiff's reagent (a) and washed in DI water (b). This figure is published in colour in the online edition of this journal, which can be accessed via <http://www.brill.nl/jbs>

Figure 4.

Dynamic swelling results to gauge pH sensitivity. (a) Dynamic swelling of all formulations and (b) close up of four formulations. This figure is published in colour in the online edition of this journal, which can be accessed via <http://www.brill.nl/jbs>

Figure 5.

Equilibrium swelling of all formulations to gauge pH sensitivity. This figure is published in colour in the online edition of this journal, which can be accessed via <http://www.brill.nl/jbs>

Figure 6.
Fractional insulin release of 3.3% aldehyde-modified PEGMA hydrogel formulation.

Figure 7. Force of detachment vs displacement from the Instron test with highest and lowest order results. (a) The lowest order force curve of a P(MAA) disk, (b) the highest order force curve of a 3.3% aldehyde disk.

Figure 8.

Work of adhesion (μJ) from Instron testing with ambient and buffered bath data for all formulations. This figure is published in colour in the online edition of this journal, which can be accessed via <http://www.brill.nl/jbs>