An Oral Delivery Device Based on Self-Folding Hydrogels

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Abstract

A self-folding miniature device has been developed to provide enhanced mucoadhesion, drug protection, and targeted unidirectional delivery. The main part of the device is a finger like bi-layered structure composed of two bonded layers. One is a pH-sensitive hydrogel based on crosslinked poly(methyacrylic acid) (PMAA) that swells significantly when in contact with body fluids, while the other is a non-swelling layer based on poly(hydroxyethyl methacrylate) (PHEMA). A mucoadhesive drug layer is attached on the bilayer. Thus, the self-folding device first attaches to the mucus and then curls into the mucus due to the different swelling of the bi-layered structure, leading to enhanced mucoadhesion. The non-swelling PHEMA layer can also serve as a diffusion barrier, minimizing any drug leakage in the intestine. The resulting unidirectional release provides improved drug transport through the mucosal epithelium. The functionality of this device is successfully demonstrated in vitro using a porcine small intestine.

1. Introduction

Many protein- and DNA-based drugs exhibit high sensitivity to the surrounding physiological conditions as a result of their delicate physicochemical characteristics and the susceptibility to degradation by proteolytic enzymes in biological fluids. They need to be properly protected during administration and their release needs to be precisely targeted and controlled. Typically, the intramuscular or intravenous injection is used for the administration of peptides and proteins. However, due to the undesirable nature of this method, such as pain, inconvenience and inconsistent pharmacokinetics, other routes have been considered. They include pulmonary, oral, nasal, buccal, rectal, ocular, vaginal, and transdermal delivery [1], among which oral administration is the most convenient and ideal route.

Although oral administration is a non-invasive route of drug delivery, peptides and proteins delivery through the gastrointestinal (GI) tract remains a highly challenging task because of their low bioavailability resulting from the pH fluctuation, proteolytic degradation, low transport efficiency, and short residence time. Enteric-coated systems have been commercially used for releasing drugs through oral administration [2]. The encapsulation of drugs within lipid vesicles also has the potential advantage of drug protection and high drug loading [3]. The inclusion of enhancers/promoters, protease inhibitors, and/or specific adhesion may help the diffusion of large molecules across the epithelial membrane. However, a major limitation is that these systems cannot fully protect the drugs and release them in a targeted area with a precisely controllable rate over a long period of time.

Mucoadhesive drug delivery systems (MDDSs) have attracted considerable interest because of their sustained drug release profile at the absorption site and increased drug bioavailability due to the intimate contact with the absorbing tissue. MDDSs typically present in

the form of symmetric micro- and nano-spheres or asymmetric patches. Mucoadhesion occurs through surface-to-surface contact. Micro-/nano-particles prepared by phase separation, microemulsion and spray drying have been successfully used as drug delivery carriers. [4-6]. These particles usually have polydisperse sizes and relatively simple structures. Additionally, the symmetric shape leads to drug release to all directions. Recently, several research groups have made efforts to design patch-like asymmetric delivery devices with functionalities such as drug protection and targeted unidirectional release [7-11]. However, the surface-to-surface adhesion for all these systems leads to the limited residence time due to the continuous shedding of surface mucus.

In this study, a novel particulate-like miniature device is developed based on the integration of a number of micro-manufacturing modules such as soft-lithography, micro-imprinting, and polymer self-folding. Approaches that are able to improve oral bioavailability, such as protective coating, mucoadhesive binding and mechanical grabbing are also applied in the device design.

2. Experimental

2.1. Materials

The pH-sensitive hydrogel was prepared from the monomer, methyacrylic acid (MAA, Sigma-Aldrich), and a crosslinking agent, tri(ethylene glycol) dimethacrylate (TEGDMA, Sigma-Aldrich). Hydroxyethyl methacrylate (HEMA, Sigma-Aldrich) crosslinked with diethylene glycol dimethacrylate (DEGDMA, Sigma-Aldrich) was used to prepare the non-swelling hydrogel. Both hydrogels contained 0.01mol of crosslinking agent/mol of monomer. A photoinitiator, 2,2-dimethoxy-2-phenylacetophenone (Irgacure 651, Aldrich), was used at 1 wt% of the monomer mixture. The free-radical photopolymerization of MAA/TEGDMA system was carried out in a water/ethanol mixture (1vs.1 ratio). The ratio of monomer to solvent during synthesis was 50:50 (w/w). The HEMA/DEGDMA system was polymerized in a water solution with a 40 wt.% solvent ratio. Poly(dimethylsiloxane) (PDMS) resin was purchased from Dow-Corning. A degradable Poly(-caprolactone) (PCL) were purchased from Sigma-Aldrich. Carbopol 934 was purchased from BF Goodrich (Cleveland, OH). All reagents, unless specified, are of analytical grade and were used without further purification. Two hydrophilic model drugs, acid orange 8 (AO8) and bovine serum albumin (BSA) were also purchased from Sigma-Aldrich. Fresh porcine small intestines were collected from The Ohio State University Lab Animal Resource.

2.2. Device Design and Fabrication

The device mainly consists of three functional layers: a swelling layer, a non-swelling layer, and a mucoadhesive layer entrapped with drugs (shown in Figure 1A). The swelling bilayer was made of MAA crosslinked by TEGDMA and the non-swelling layer was HEMA crosslinked by DEGDMA. Soft-lithographic techniques were used to produce hydrogel bi-layered microstructures. The devices were fabricated following the procedures shown in Figure 2. A PDMS mold with a desirable surface pattern was made by casting a prepolymer and a curing agent at 10:1 weight ratio onto a complementary relief structure from the standard photolithographic process [12,13]. The HEMA monomer solution was brushed onto the PDMS mold with an applicator. The solution was trapped in the discrete wells due to discontinuous dewetting. After subject to UV radiation for 10 minutes, the MAA monomer solution was

brushed onto the cured PHEMA layer to prepare a bi-layered structure under another 15-minute UV radiation. A high light intensity and large dosage were applied to ensure high monomer conversion (around 99%). In order to further remove the residue monomer and unreacted initiator, distilled water was used to continually wash the structures in the wells covered by a strong flat membrane with 10 m isopore for 2 hours. To take out the bi-layered structures, the PDMS mold was placed on a sticky PHEMA film cured on a glass slide by briefly exposing the film to water vapor generated from a hot water bath. A solid weight (50g/cm^2) was set on the PDMS mold for 10 minutes. The mold was then removed with the bi-layered structures stuck to the PHEMA/glass slide. To make a mucoadhesive layer, Carbopol 934, PVA and the model drug were mixed to form homogeneous solution in distilled water (1:1:1, 10wt.%), which was then brushed onto the PDMS mold with proper fixtures. Water was allowed to evaporate and a mucoadhesive layer was formed. To transfer the mucoadhesive layer, the PDMS mold was placed onto the bi-layered structures. A solid weight (around 500g/cm^2) was set on the PDMS mold for 10 minutes. Eventually, the mucoadhesive layer adhered to the bi-layered structures due to the compressing and the drug-layer sticking after it was totally dried out. By using this simple approach, we can make both micro- and millimeter sized devices (240 μm – 4 mm). The typical dimensions of device were shown in Figures 1(A) and (B). When the device is conveyed into the small intestine, it may directly target onto the small intestine surface due to the Carbopol mucoadhesion. Then the bi-layered structures may fold into the mucosa in a 'grabbing' manner, resulting in better drug protection and enhanced mucoadhesion (Figure 1C).

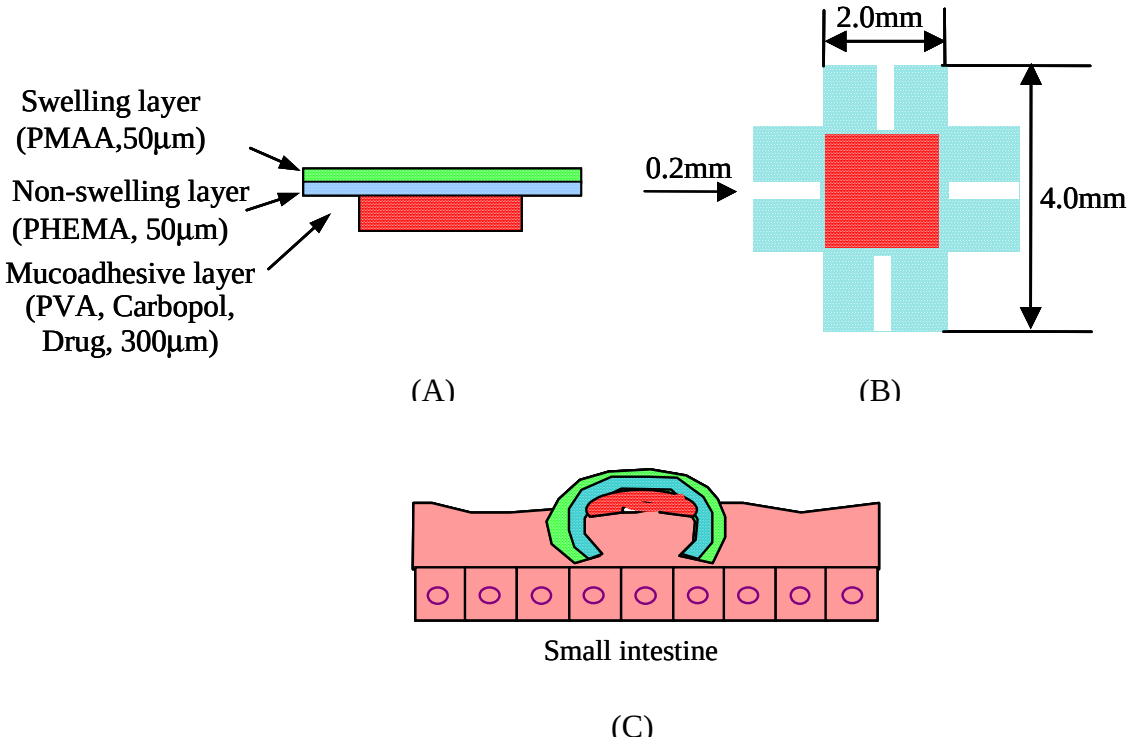

Figure 1 Schematic of the 3-layer device from (A) side view and (B) top view, and (C) folding on the small intestine surface.

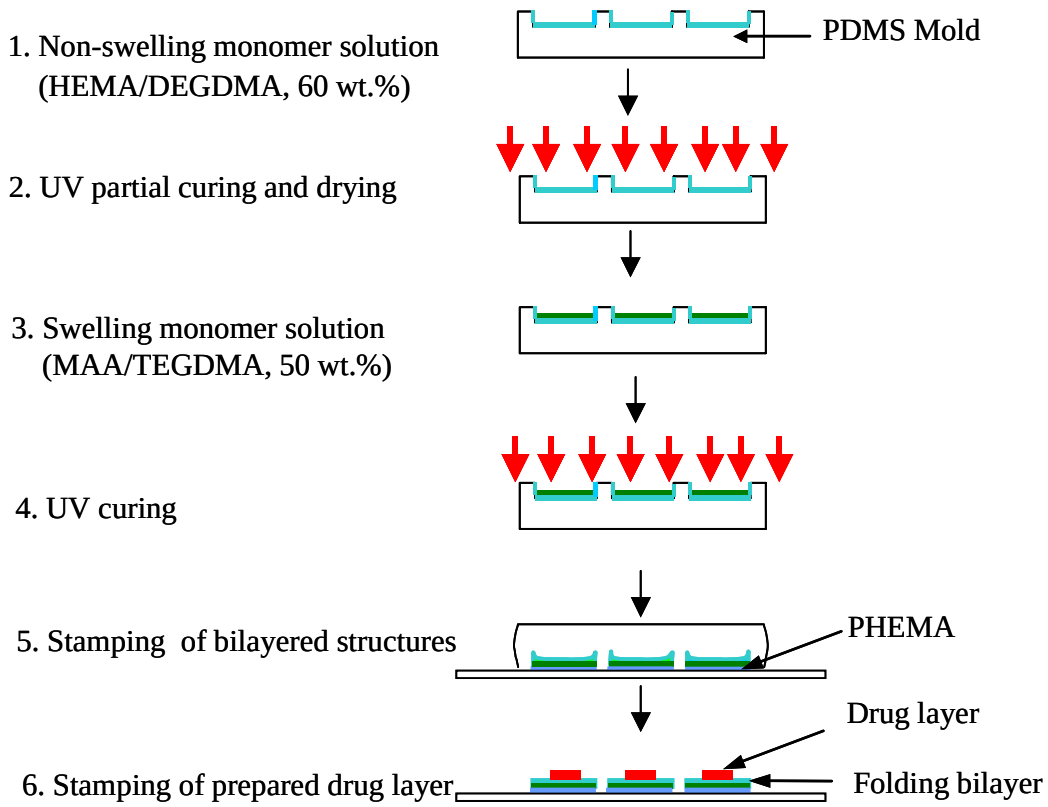

Figure 2 Fabrication procedure of the miniature devices.

2.3. Swelling Studies

To prepare hydrogel samples for swelling test, a monomer solution was transferred to a glove box where it was kept under a nitrogen atmosphere. Nitrogen was bubbled through the solution for 20 minutes. Then the mixture was pipetted between two glass slides separated by a Teflon spacer. The thickness of the spacers was 0.3mm. The setup was then placed under a UV light for photopolymerization at 2.0 mw/cm^2. The cured hydrogels were then rinsed in double deionized water for 5 days to remove unreacted monomer, initiator and sol fraction. Subsequently, the monomer-free disks were cut into samples with a 5.0 mm diameter for swelling test.

Swelling tests were performed at various pH values ranging from 3.0 to 7.0 to characterize the hydrogel behavior in the GI tract. The buffer solutions with different pH values were prepared by mixing the citric acid with appropriate amounts of sodium phosphate solution. Sodium chloride was used to adjust the ionic strength of all solutions to I=0.1M, which is the near-physiological condition. For the swelling test, the dried hydrogel samples were weighed and placed in the buffer solution at room temperature (25°C). The hydrogels were taken out of the solution at pre-selected time intervals. After the extra water on the surface was removed by laboratory tissue, the weight of the wet hydrogels was measured. The weight-swelling ratio was calculated by the weight of the swollen sample to the weight of the dried

sample. Self-folding of the hydrogel bilayers was observed and recorded in a buffer solution and on the porcine small intestine. All animal procedures were performed based on the institutional protocols.

2.4. Mucoadhesion Measurement

The detachment between the device and a segment of porcine small intestine was measured in a flow trough and a microbalance. First, a sacrificed small intestine was longitudinally cut into small pieces (2cm $\times$ 3cm), sliced lengthwise to spread flat, exposing the lumen side, bonded on the trough bottom by super glue, then washed with 50 ml phosphate buffer saline (PBS) solution. Before the pump drove the buffer solution through the trough, the sample was gently dropped on the intestinal surface. The buffer solution with a high viscosity was prepared by mixing 0.2wt% Xanthan Gum (CP Kelco, Wilmington, DE) in a pH=6.5 buffer for a solution viscosity of 87.9 cp. By controlling the flow rate, the residence time of samples on the intestinal surface was determined through the microscope observation.

To prevent the acidic degradation in the stomach and maintain the device geometry, this device could be loaded in an enteric capsule. In vitro perfusion experiments were carried out to determine the device adhesion in the small intestine. Briefly, a 15cm of the isolated segment was placed horizontally on a bench top and was connected to a tubing so that the lumen could be filled with a pH=6.5 PBS buffer at a volumetric flow rate of 1.0 ml/min. A capsule containing three devices was placed in the intestine. After 20 minutes, a longitudinal incision was made in the intestine to observe the device attachment. The experimental temperature was maintained near 37°C.

2.5. Delivery Performance

To evaluate whether the self-folded device has any improved effect on drug protection and transport, targeted unidirectional release was conducted for trans- epithelium delivery of two model drugs in a side-by-side diffusion chamber. Having rinsed with PBS buffers, the jejunum part of the intestine was cut into a disc shape of 2.2 cm in diameter and placed on a support between the two chambers (the effective diffusion area was 2.83 cm^2). Before the experiment, the prepared device (the dimension was shown in Figures 1A and B) was placed onto the jejunum surface in the donor chamber. Subsequently, 8 ml of pH=6.5 buffer solution was simultaneously injected into both the donor chamber and the receptor chamber at room temperature (25°C). The setup was subjected to constant shaking at 180 rpm. At predetermined time intervals, 0.15 ml buffer solution was taken from the receptor chamber for concentration test. To maintain a constant volume, 0.15 ml fresh PBS buffer was added after each sample was withdrawn.

AO8 release was measured by monitoring its absorbance at 490 nm using a microplate reader (GS Spectra MAX250). The concentration of AO8 in the buffer solution was obtained from a calibration curve, and the amount of AO8 release at time t (M_t) was calculated from accumulating the total AO8 release up to that time. The fractional drug release, $M_t/M_{0,}$ could then be calculated. Here M_0 is the amount of initially loaded AO8. For the BSA release experiment, 0.1 ml samples were taken and replaced by fresh buffer. After accounting for dilution caused by previous measurements, protein concentrations were measured with a Bio-Rad protein assay using the microplate assay protocol. The color change of the dye in

response to the concentration change was monitored by measuring the absorbance at 595 nm on the same microplate.

3. Results and Discussion

3.1. Swelling Studies

The pH-sensitive hydrogel, PMAA has been studied extensively as a promising candidate for oral delivery of peptide and protein drugs through the gastrointestinal tract because of its unique swelling property. Figure 3 exhibits the dynamic swelling behavior of the hydrogels in different buffer solutions. As can be seen, the dried hydrogels swelled at all pH conditions due to the adsorption of water into the porous structure. In the high pH buffers, PMAA hydrogels swelled rapidly and achieved a much higher weight-swelling ratio. This was because ionization of the carboxyl groups (the pendent group of MAA) occurred as the solution become less acidic, resulting in dissociation of the hydrogen bonds between the carboxylic acid groups of MAA and the oxygen of the ether groups of TEGDMA. The dissociation of hydrogen bonds, combined with the electrostatic repulsion force, caused the hydrogel network to swell quickly and greatly under an osmotic pressure. Below a pH of 6.5, the swelling ratio drastically decreased to a small value. This implied that the hydrogel was in a relatively collapsed state. On the other hand, PHEMA is a neutral hydrogel, which has no ionizable groups on its side chain. With a change of pH values, this material exhibited very little swelling in buffer solutions. In addition, since the solvent content in HEMA monomer solution (40 wt.%) was less than that in MAA solution (50 wt.%), PHEMA hydrogels had more compact structures than PMAA gels with the same crosslinking ratio. Although DEGDMA has a shorter chain than TEGDMA, its contribution could be neglected when considering the low amounts of crosslinker.

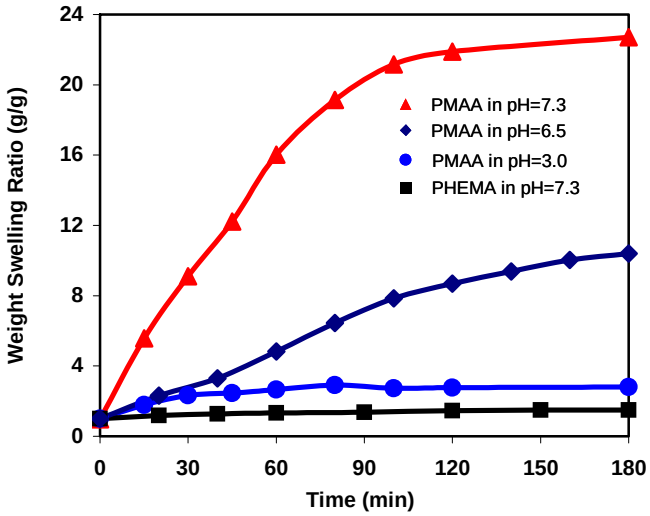

Figure 3 Dynamic swelling behavior of PMAA and PHEMA hydrogels.

3.2. Mucoadhesion Measurement

The layered shape of the device maximizes its contact area with the intestinal wall, while the thin side areas minimize its exposure to the liquid flow through the intestine.

Additionally, since the bilayers curl into the mucus in the mode of “grabbing”, it is expected to provide more resistance to mucus shedding than conventional mucoadhesion. Thus, the residence time can be significantly increased due to the combination of the “grabbing” adhesion of the folding bilayers and the conventional adhesion of the mucoadhesive layer. This enhanced performance was demonstrated in the flow test. In the experiment, to minimize the influence of other factors than the bi-layered folding, much attention was paid to the preparation and the sample loading. At the same height, samples with similar dimensions were randomly dropped on the intestinal surface using tweezers without external force and the flow rate was gradually adjusted from 4.0 to 5.5 ml/s. Figure 4(A) summarizes the number of bound samples remaining on the mucus surface as a function of the flow time. For each case, the initial bound samples were equal. In three minutes, the bound sample for PHEMA was zero. That indicated there was no the remaining PHEMA on the small intestine. For the PCL patches (the drug layer adhered on PCL layer) and the folded devices, all initial samples still stayed on the mucosal surface at 60 minutes. In order to distinguish the more adhesive system, a higher flow rate (5.5 ml/s) was used in the measurement. According to the Figure 4(B), the average residence time for the PCL patch was around 72 minutes. The folded devices showed the longest average residence time, around 103 minutes.

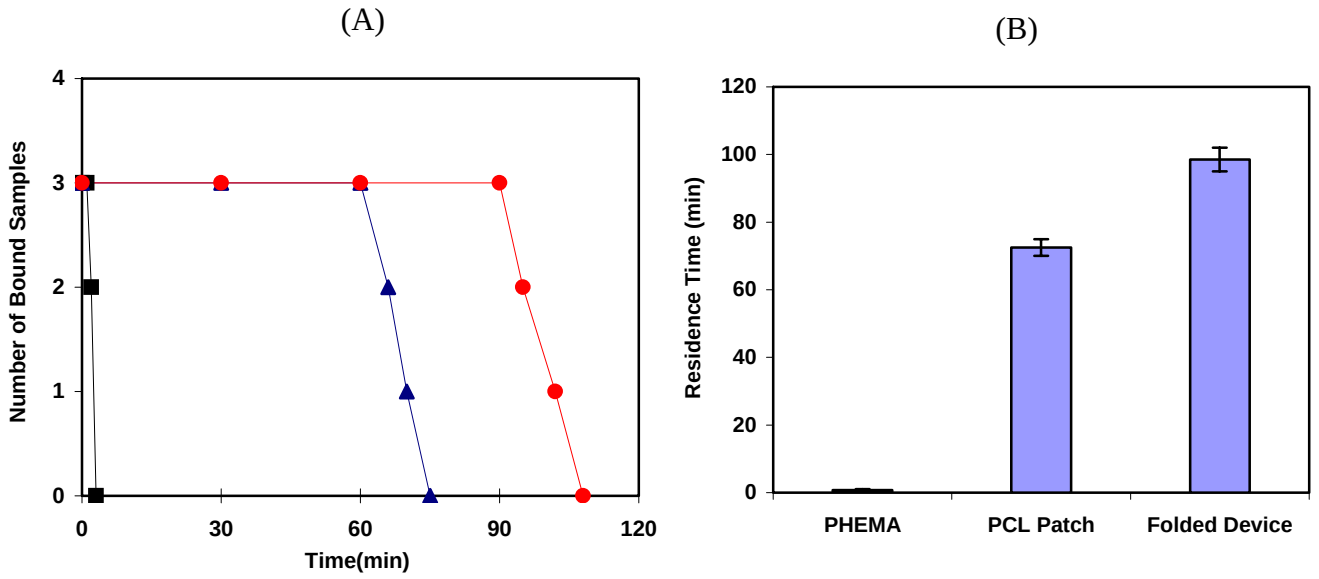

Figure 4 (A) Number of bound samples and (B) residence time for different samples attached to intestinal mucus in the flow test.

To visually demonstrate the folding behavior and enhanced mucoadhesion, a folding device tinted with blue dyes was placed on the mucus surface and a digital camcorder was used to record its folding process from the side view. Figure 5(A) shows the recorded folding behavior of a bi-layered device with each layer having a thickness of 10 μm. In the beginning, the device adhered on the mucus surface. Around 2 minutes, the bi-layered structure started to fold into the mucus and at 4 minutes the structure completed the folding. The temperature is a very important factor influencing the response time of the bilayers, as well as residence time of the folded device. The typical body temperature, 37°C was used for the response studies. The swelling ratio of PMAA in pH=6.5 buffer was increased to 11.01 from 10.39 and the response

time was improved to 2 minutes as a result of temperature increasing. The residence time of the folded device also increased due to the increased extent of folding.

Snapshots shown in Figure 5(B) describe the device attachment in the flow test. As a control, a PCL patch was also placed on the mucosal surface. At the beginning, both devices attached onto the surface tightly in the flow field. After 65 minutes, the PCL patch started to detach from the surface. Around 70 minutes, the patch was completely washed away from the mucosal surface. Due to the combined effect of mucoadhesion and self-folding, the folded device could stay on the mucus for a longer time. It started to detach at 82 minutes and was finally washed away at approximately 108 minutes. The detachment was due to the mucus shedding, not the unfolding of the bi-layered structure.

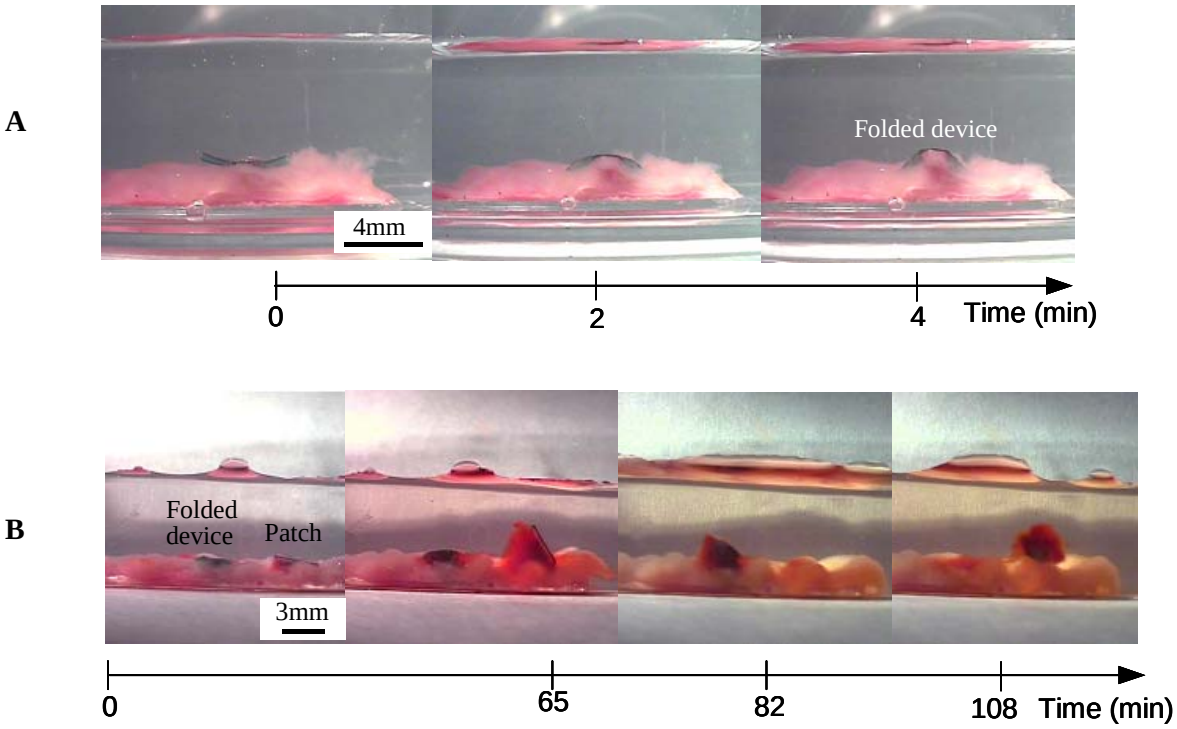

Figure 5 Dynamic processes for (A) folding behavior and (B) enhanced mucoadhesion. Buffer pH=6.5 and 25°C.

3.3. Delivery Performance

A side-by-side diffusion chamber was used for drug release studies. When the device was attached to the intestinal surface, the drug concentration change in the donor chamber indicated the leakage in the small intestine. Figure 6 compares AO8 leakage of delivery systems with different protection layers. Due to good mucoadhesion, a simple PMAA layer could adhere to the mucus surface tightly and the leakage was very low in the beginning. After 60 minutes, the high swelling of PMAA hydrogel, however, led to a very large permeability resulting in severe drug leakage through the protection layer. For the PCL layer, the drug could gradually leak into the donor chamber from the edge of the patch. For the bi-layered structure, since the PHEMA protection layer has a lower permeability than the PMAA layer, it served as a barrier to provide protection from drug leakage. Furthermore, the folded structure prevented

the leakage from the edges. Consequently, the total leakage from the folded device was very low, less than 30% of loaded drugs after 2 hours.

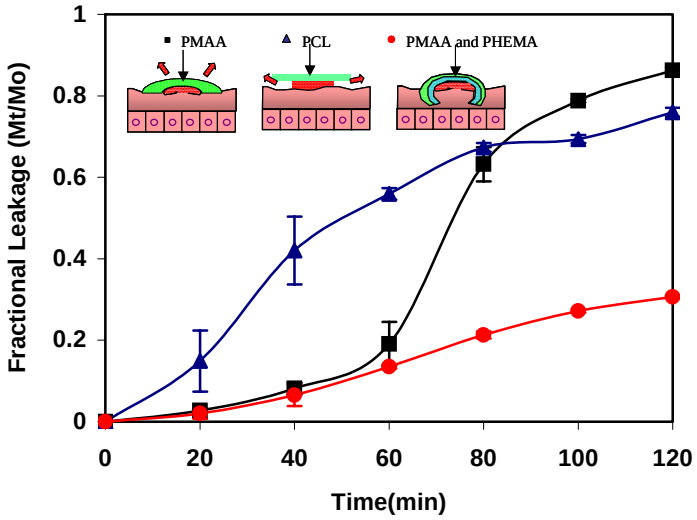

Figure 6 The fractional leakage of AO8 from the drug reservoir with different protection layers (thickness=20 μm) at pH=6.5 and 25°C. Error bar = SD, n = 3.

For in vitro drug transport across the mucosal epithelium, we separated the mucosal membrane from the serosal compartment of the small intestine. The isolated mucosal membrane was loaded in the side-by-side diffusion chamber for the diffusion measurement. The drug concentration in the receptor chamber indicates the transferred drugs.

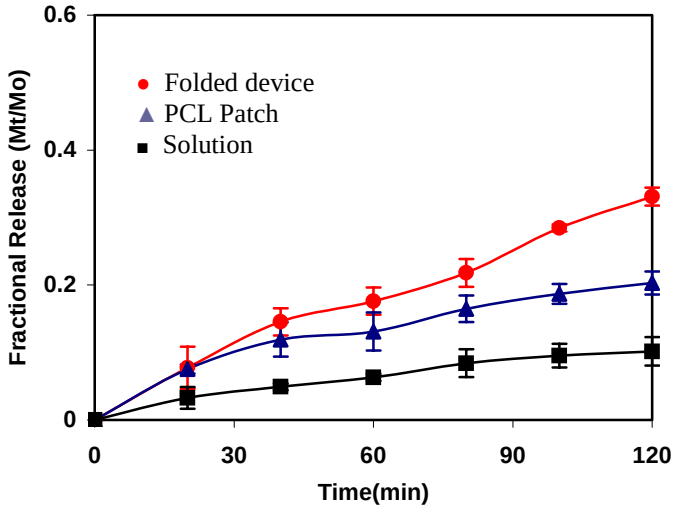

Figure 7 AO8 transport from different systems across the mucosal epithelium at pH=6.5 and 25°C. Error bar = SD, n = 3.

Figure 7 compares the AO8 transport from different systems across the mucosal epithelium. The squares indicate the homogeneous solution loaded into the donor chamber.

The triangles and the circles are for the PCL patch system and the folded device, respectively. All three systems had an equal amount of loaded drug. The figure shows that only about 12% AO8 in the solution was delivered through the mucosal epithelium in 120 minutes, while 20% AO8 loaded in the patch system could transfer across the intestinal membrane. The self-folded device showed the highest drug transport fraction (33%) due to its localized high drug concentration.

To compare the release behavior of drugs with different sizes, BSA was also used as a model drug. In the experiment, the BSA loading concentration was about 3 times higher than that of AO8 in order to provide easy detection by UV spectroscopy. Figure 8 shows the BSA transport profile from a folded device and the homogeneous solution at room temperature. As can be seen, the self-folded device exhibited an improved BSA transport fraction. Compared with Figure 7, the transport of large molecules across the mucosal epithelium was much more difficult than small molecules.

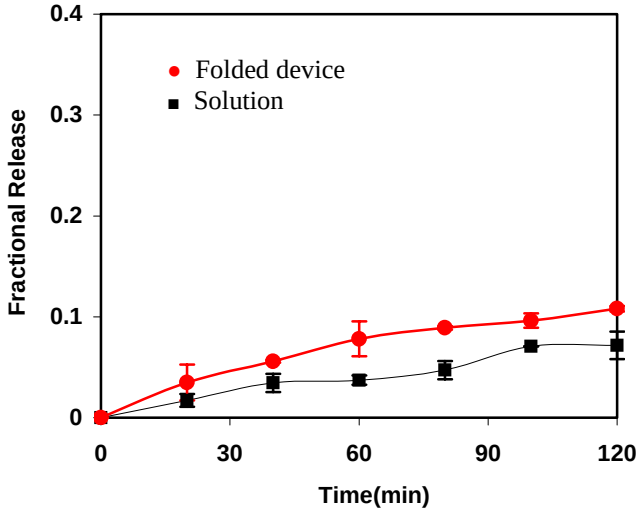

Figure 8 BSA transport from different systems across the mucosal epithelium at pH=6.5 and 25°C. Error bar = SD, n = 3.

4. Conclusion

In summary, a self-folding miniature hydrogel device has been developed based on the integration of a number of micro-manufacturing modules. They demonstrated multi-functionalities such as enhanced mucoadhesion, lower drug leakage, and improved unidirectional delivery. The enhanced mucoadhesion due to self-folding increased the residence time at the target site, and led to improved drug transport. The PHEMA layer served as a diffusion barrier to provide good drug protection and prevented the drug leakage.

This novel delivery device design can be of great benefit for the advancement of oral administration of proteins and DNAs. Since the mucus layer covers many tissues at other specific sites, this device may be applied for ocular, buccal, vaginal and rectal administrations as well. The polymer self-folding phenomena at the microscale can also be applied as a probe for bio/chemical sensing, carriers in cell-based bioreactors, and tissue clamping.

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