Intestinal Patches for Oral Drug Delivery

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Purpose. Oral route offers an attractive mode of drug administration, although its applications are limited by poor stability of peptides and proteins in the gastrointestinal tract. In this article, we report a novel method based on intestinal patches for oral drug delivery. This method involves the use of millimeter size mucoadhesive patches that adhere to the intestinal wall and direct solute diffusion towards the wall similar to that observed in the case of a transdermal patch.

Methods. Intestinal patches were prepared by sandwiching a film of cross-linked bovine serum albumin microspheres between a film of ethyl cellulose and Carbopol/pectin. Delivery of three model drugs, sulforhodamine B, phenol red, and dextran was assessed in vitro using rat intestine.

Results. *In vitro* tests confirmed substantial unidirectional diffusion of model drugs from the patch across the intestinal wall. The presence of ethyl cellulose layer minimized release from the edges as well as from the back side of the patch into the intestinal lumen. *In vitro* experiments with rat intestine showed that patches were effective in delivering model drugs across the intestine. Trans-lumenal flux of model drugs from intestinal patches was about 100-fold higher compared to that from a solution due to localization of the solute near the intestinal wall and due to minimization of drug loss into the intestinal lumen.

Conclusions. Intestinal patches offer a novel approach for oral drug delivery.

KEY WORDS: intestinal delivery; mucoadhesive; patch; microspheres; *in-vitro.*

INTRODUCTION

Oral route has attractive advantages for drug delivery including ease of application and high patient compliance. However, for poorly absorbed molecules and enzyme-sensitive bioactive agents new strategies are required to achieve sufficient drug absorption into blood circulation. Several modifications of simple dosage systems including liposomes (1,2), microparticles (3,4), and nanoparticles (5–7) have been used as drug carriers to overcome poor drug bioavailibility. Particular attention has been paid to mucoadhesive micro/nanoparticles that adhere to intestine mucus and therefore prolong their migration time and extend drug release (8–10). However, several issues limit the applicability of these particle systems. Specifically: (i) drug release is not unidirectional, therefore certain fraction would get lost into the lumenal fluid and (ii) since the particle surface is exposed to the intestine fluid, bioactive agents encapsulated in these particles may not get sufficient protection from proteolytic degradation in the intestine.

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In this study, we describe a novel drug delivery system that offers several advantages over simple mucoadhesive particles. The proposed system attempts to mimic a transdermal patch that includes several layers that perform different tasks including adhesion, drug encapsulation, and protection from the surroundings. A schematic representation of the proposed system is shown in Fig. 1A. The proposed design consists of three layers; the first layer that consists of a mucoadhesive material enhances adhesion of the patch on the mucosal surface. The second layer comprises a monolayer of drug-loaded microspheres that are partially immersed inside the mucoadhesive layer. The third layer comprises a relatively impermeable membrane that encompasses the microspheres. We hypothesized that when the patches shown in Fig. 1A are introduced into the gastrointestinal tract, the mucoadhesive layer will stick to the lumenal wall. The drug may then be slowly released from the microspheres in a unidirectional way through the mucoadhesive layer into intestinal mucosa. The backing layer may minimize drug diffusion into the intestine and also minimize enzyme penetration into the patch. This method provides several significant advantages over conventional oral delivery systems, specifically the backing layer of the patch prevents drug leakage into the outer lumen and induces a unidirectional release of drug into the epithelial layer. This unidirectional release may result in increased local drug concentrations, which may enhance the absorption efficiency. Patches adhering to the lumenal wall by mucoadhesive layer should extend transit of drugs in the intestine, resulting in a sustained release behavior, and in case of bioactive agents such as peptides or proteins, protection of these agents by this patch system would reduce proteolysis. These millimeter size patches could be encapsulated in a capsule and delivered into the intestine. In this study, we describe fabrication and testing of these patches *in vitro*.

MATERIALS AND METHODS

Formulation

To fabricate patches shown in Fig. 1A, cross-linked bovine serum albumin (BSA, Sigma Chemicals, St. Louis, MO) microspheres were first prepared. BSA microspheres were prepared by dispersing 0.5 ml 25% (w/v) BSA solution in 20 ml mineral oil (Fisher Scientific, Pittsburgh, PA, USA) at a speed of 1500 rpm. Microspheres were loaded with three different model drugs, sulforhodamine B (MW 558.7 Da, Molecular Probe, Eugene,OR, USA), phenol red (MW 376.4 Da, Acros Organics, NJ, USA), and FITC-dextran (MW 70,000 Da, Sigma Chemicals, St. Louis, MO, USA) in separate batches. In each case, an aqueous solution of these solutes (2.4% for sulforhodamine B, 2.0% for phenol red, and 2.5% for FITC-dextran, all w/v) was added to BSA solution prior to dispersing it in mineral oil. 100 μl of an aqueous solution of glutaraldehyde (25% v/v, Sigma Chemicals, St. Louis, MO) was added to the W/O emulsion and BSA was allowed to cross-link for 2 h (11). Cross-linked BSA microspheres were washed first in petroleum ether, then in ethyl ether, and finally in acetone. This procedure produced uniform microspheres of size in the range of 10–30 μm.

To make a mucoadhesive layer, Carbopol 934 (BF Goodrich Co. Cleveland, OH, USA) and pectin (Sigma Chemi-

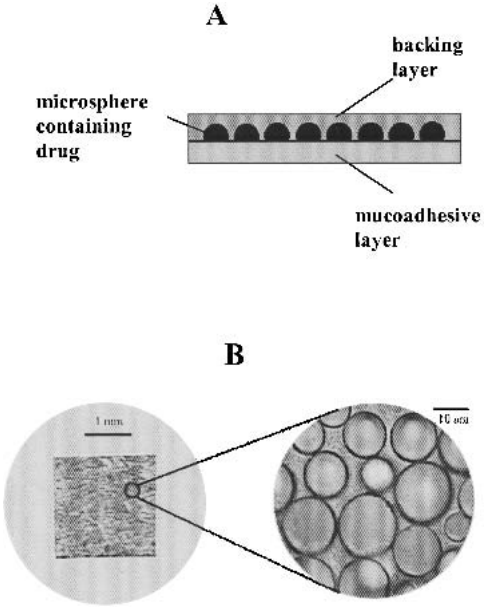

Fig. 1. (A) Schematic representation of the patch design. The patch consists of a film of a mucoadhesive polymer. A monolayer of cross-linked bovine serum albumin (BSA) microspheres (10–30 μm) is dispersed on the mucoadhesive film. The drug to be delivered is encapsulated in the microspheres. The microsphere monolayer is covered by a film of poorly permeable polymer. (B) Intestinal patches (4 mm^2) prepared using method described in this paper. Right figure indicates the microstructure of the patch.

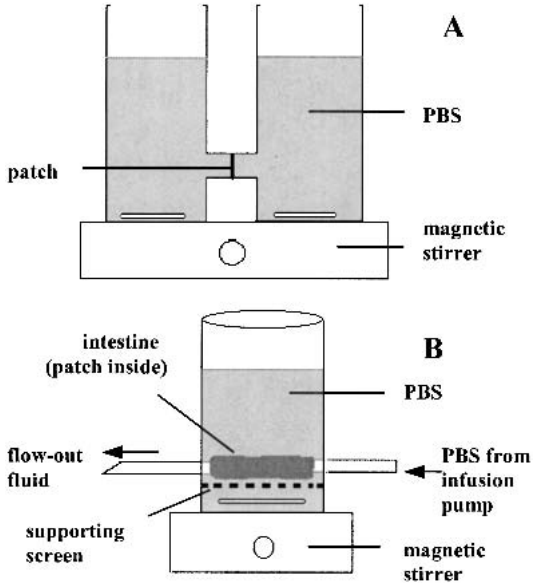

Fig. 2. (A) Schematic representation of the diffusion cell used to measure release of model drugs from the patch. (B) Schematic representation of flow-through set-up for measurement of transport across the intestinal wall.

cals, St. Louis, MO, USA) mixture solution (1:1, 10% w/v total) was cast evenly on a teflon plate by a spatula. Water was allowed to evaporate and a mucoadhesive film (about 5 μm in thickness) was formed. Microspheres were then spread uniformly on this mucoadhesive layer. To uniformly spread microspheres, a suspension of microspheres was dispersed on a partially dried mucoadhesive layer. A cover slide was used to press the microspheres against the mucoadhesive layer. This procedure usually resulted in the formation of a monolayer of microspheres. The spacing between the microspheres was controlled simply by controlling the number of microspheres added per unit area of mucoadhesive film. The microspheres adhered to the mucoadhesive layer after the mucoadhesive layer was totally dried out. The ethylcellulose (EC) solution in acetone (20 mg/ml) was placed onto the monolayer. Since the bottom part of the microspheres was adhered to mucosdhesive layer, EC did not completely cover microspheres.

The film was allowed to dry and was cut into squares or circles (~2–4 mm^2) (Fig. 1B).

Characterization of the Patch System

Release of model drug (Sulforhodamine B) from patches was measured *in vitro* into phosphate buffered saline (PBS, pH 6.8, 0.01 M). To distinguish drug release from the mucoadhesive side and the backing side of the patch, the patches were placed in a custom-designed diffusion cell (Fig. 2A). The cell comprised two chambers placed side-by-side with an opening provided between the chambers of about 12.56 mm^2. A patch (16 mm^2) was placed between the two chambers and each chamber was filled with 5 ml PBS. Vacuum grease was used to avoid leakage of PBS. Amount of model drug released from either side of the patch into the solution was quantified at 565 nm using a spectrophotometer (UV-1601 Shimadzu Corporation).

To assess drug release from the edge of the patch, the mucoadhesive and backing side of the patch was sandwiched between two layers of aluminum sheet. The aluminum was adhered to the patch surface by cyanoacrylate polymer (Sigma Chemicals, St. Louis, MO, USA). With this adhesive, aluminum sheet can form a tight seal on the flat surface of the patch. Because aluminum possesses low permeability, if any release of drug is observed, it should have originated from the sides of the patches. Amount of model drug (Sulforhodamine B) leaked from the sides of the patches was quantified at 565 nm using the same spectrophotometer.

In Vitro Absorption Test

To investigate whether our novel mucoadhesive patch system would have any enhancing effect on drug transport across intestine, trans-lumenal delivery of three model drugs, sulforhodamine B, phenol red, and FITC-dextran was measured. The experiments were performed in a custom-built *in vitro* perfusion device (Fig. 2B). Briefly, Rats (Sprague Dawley, 8–20 weeks) were sacrificed and intestine was removed. All animal procedures were performed using institutionally approved protocols. Intestine was cleaned by rinsing with 40 ml PBS, then it was cut into small pieces (~3–4 cm) and stored at −70°C until used. We have tested the permeability of intestine before or after freezing at −70°C. The freezing process didn't cause a significant difference in the intestine permeability. For convenience, we used frozen small intestine in our *in vitro* experiments. Before the experiment, patches were placed in the thawed intestine prior to mounting the intestine in the transport cell. For this purpose, the intestine was flipped inside out and the patch was placed on the intestine. The intestine was flipped back again and was mounted on two tubes (4.2 mm outside diameter). The two tubes (see Fig. 2B)

are removable. To mount the intestine on both tubes, we first took out one tube and mounted the intestine on it. The other tube was inserted through the chamber wall and then the intestine was mounted on it. The tubing was then put back into its original position. The space between the tubing and the chamber wall was sealed by vacuum grease.

The intestine was immersed in 10 ml PBS and was infused with PBS at a flow rate of 0.05 ml/min. This perfusion system mimics *in vivo* intestine fluid movement. Perfusion fluid exiting the intestine was collected every 10 minutes (sulforhodamine B, phenol red) or 30 minutes (FITC-dextran). Spectrophotometry (565 nm for sulforhodamine B, 560 nm for phenol red, and 480 nm for FITC-dextran) was performed to assess the amount of drug released from the patch but retained in the intestine. Concentration of model drugs in the receiver fluid was also assessed every 10 or 30 minutes to determine the amount of drug transported across the intestinal wall. Control experiments were performed by injecting the same total amount of drug into the lumen.

RESULTS AND DISCUSSION

Intestinal Patches as a Novel Oral Drug Delivery System

The method described in this article for oral drug delivery is inspired by the design of a transdermal patch. A schematic of the proposed oral patch is shown in Fig. 1A. The proposed patches offer several advantages over standard oral tablets, sustained release formulations, and mucoadhesive microspheres. Specifically, the patches offer high surface area per unit mass of the patch, thereby increasing their adhesion on the intestinal wall. This feature offers particular advantages over microspheres, which allow limited contact area. Adhesion of patches on the wall should also localize the drug near the wall thereby offering increased concentration gradient for its transport. The protective layer of the patch also offers two advantages. First, this layer minimizes drug loss into the intestine, thereby forcing the drug to diffuse towards the intestinal wall. Furthermore, this layer also minimizes enzyme penetration into the patch, thereby offering protection for sensitive drugs. These patches (a few mm^2 in size) could potentially be incorporated into a capsule that releases the patches in the intestine. The patches would subsequently adhere to the intestinal wall and deliver the drug into the wall. In this study, we show the feasibility of such patch design.

The proposed patch design could in principle be achieved by sandwiching a layer of drug between a layer of a mucoadhesive polymer and a poorly permeable polymer. Because the area of the flat face of the patch is much larger (mm^2) than that of the edge (μm^2), one would expect that the drug would be primarily released from the flat surface. Furthermore, since the mucoadhesive layer can be designed to be more permeable than the backing layer, the drug should be primarily released from the mucoadhesive side. To assess this hypothesis, we prepared a sandwich of sulforhodamine between two layers of EC (~5 μm thick). Diffusion coefficients of low-molecular weight solutes in EC have been previously reported in the literature. Specifically, Siepmann et al reported diffusion coefficient of a small solute (theophilline, MW ~200 Da) in EC of about 1.0×10^{-10} cm^2/s (12). Based on this diffusion coefficient, we calculated from Fick's first law that it should take approximately 2 hours for 99% sulforhodamine to release from an EC-sulforhodamine-EC sandwich (4 mm^2). However our experimental data showed that 99% sulforhodamine is released in less than 10 minutes. This rather unexpected result was attributed to water penetration into the patch from the edges. Specifically, penetration of water into the patch destabilizes its structure, thereby causing near complete release in less than 10 minutes. Accordingly, minimization of drug leakage or water penetration from the edge is of utmost importance in designing these patches.

A simple solution to leakage from edges would be to coat individual patches with a poorly permeable material. However, this strategy requires fabrication of individual patches, making it practically challenging. Accordingly, we designed a novel strategy to minimize drug leakage from sides. In this strategy, a monolayer of drug-loaded microspheres is spread on the mucoadhesive film. The monolayer is then covered by a solution of EC, which penetrates between the microspheres and creates a low-permeability continuous phase in which drug-loaded microspheres are dispersed. When the patch is cut into smaller pieces, the breakpoint usually occurs between microspheres, thereby making it difficult for the drug to leak from edges. We tested whether this strategy indeed reduces lateral drug leakage. For this purpose, a patch of sulforhodamine BSA microspheres dispersed in EC (~30 μm thick) was sandwiched between two layers of aluminum sheet. The aluminum was adhered to the patch surface by cyanoacrylate. With this adhesive, the aluminum sheet can form a tight seal on the flat surface of the patch. If we did see any drug leakage, it should have originated from the edges rather than from the flat surface. Figure 3 shows release of sulforhodamine B from this system with (closed circles) or without (open squares) EC protection. Release of sulforhodamine B from EC-protected microspheres was statistically different ($p < 0.05$) than that without EC protection. In the absence of EC protection, more than 70% sulforhodamine was released within 10 minutes. However, addition of EC substantially reduced the release to less than 10% in 60 minutes. The protective effect of EC may be further increased by optimizing of microsphere separation and EC concentration.

We next assessed whether the backing EC layer indeed offers protection for drug release. In other words, we assessed whether drug release from the mucoadhesive side of the patch is indeed faster than that from the backing side. For this purpose, a patch was prepared as shown in Fig. 1. The patch

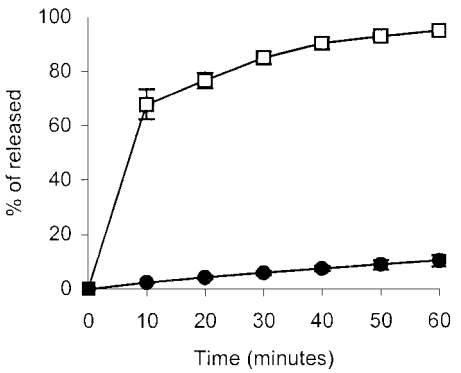

Fig. 3. Release of sulforhodamine B from the patch with (●) and without (□) ethyl cellulose protection. Error bars = SD, n = 3. Drug released from the edge was substantially reduced by the protection of ethyl cellulose.

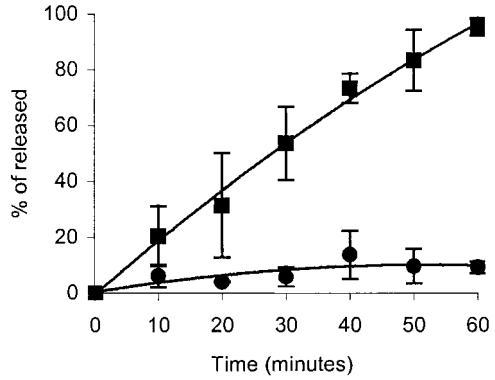

Fig. 4. Release of sulforhodamine B from the mucoadhesive (■) and backing side (●) of the patch in 60 minutes.The amount of drug released from mucoadhesive side was significantly higher ($p < 0.05$). Error bars = SD, n = 3.

was mounted in the diffusion cell and the amount of drug released from both sides was measured. Figure 4 compares the amount of sulforhodamine released from the mucoadhesive side (closed squares) with that from the backing side (closed circles). At any instance, the amount of drug released from the mucoadhesive side is significantly higher ($p < 0.05$) compared to that from the backing side. The Figure shows that more than 95% of the drug is released from the mucoadhesive side. To further confirm this observation, the mucoadhesive side of this patch was placed on the mucosal side of the rat intestine *in vitro*. The back of this patch was covered by another intestine mucosal layer. After immersing in PBS for 10 minutes, the intestine pieces were removed and observed for sulforhodamine B penetration (Fig. 5). Significantly more sulforhodamine B was found on the patch's mucoadhesive side (Fig. 5A) compared to that on the backing side (Fig. 5B). These images also confirmed that the release of drugs from the patch is indeed unidirectional.

In Vitro Tests

We next assessed whether intestinal patches can enhance transport of three model drugs, sulforhodamine B (easily absorbed molecule, MW 558.7 Da), phenol red (poorly absorbed molecule, MW 376.4 Da), and FITC-dextran (poorly absorbed large molecule, MW 70,000 Da). Patches (8 mm^2) loaded with these model drugs were prepared and placed in the intestine as shown in Fig. 2B. Patches remained attached to the intestinal wall even in the presence of a constant fluid flow (0.05 ml/min). Fig. 6A–6C, respectively, show translumenal delivery of sulforhodamine B, phenol red, and FITC-dextran from the patches (closed squares). The Figures also show the transport of corresponding model drugs from the solution (closed circles). In the case of sulforhodamine (Fig. 6A), about 30% of sulforhodamine loaded in the patches was delivered across the intestine in 60 minutes. On the other hand, only ~10% of sulforhodamine in the solution appeared across the intestine. For a phenol red patch, the transport fraction is about 45% compared to 10% in solution form (Fig.

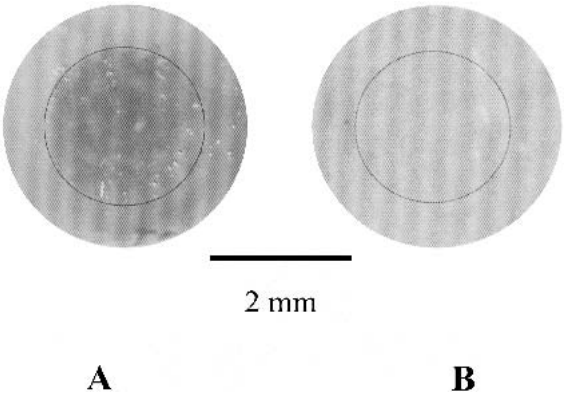

Fig. 5. Visualization of sulforhordamine B diffused into intestinal mucosa from the patch's mucoadhesive layer (A) or backing layer (B). Significantly more sulforhodamine B was observed on the patch's mucoadhesive side (dark stain within the circle).

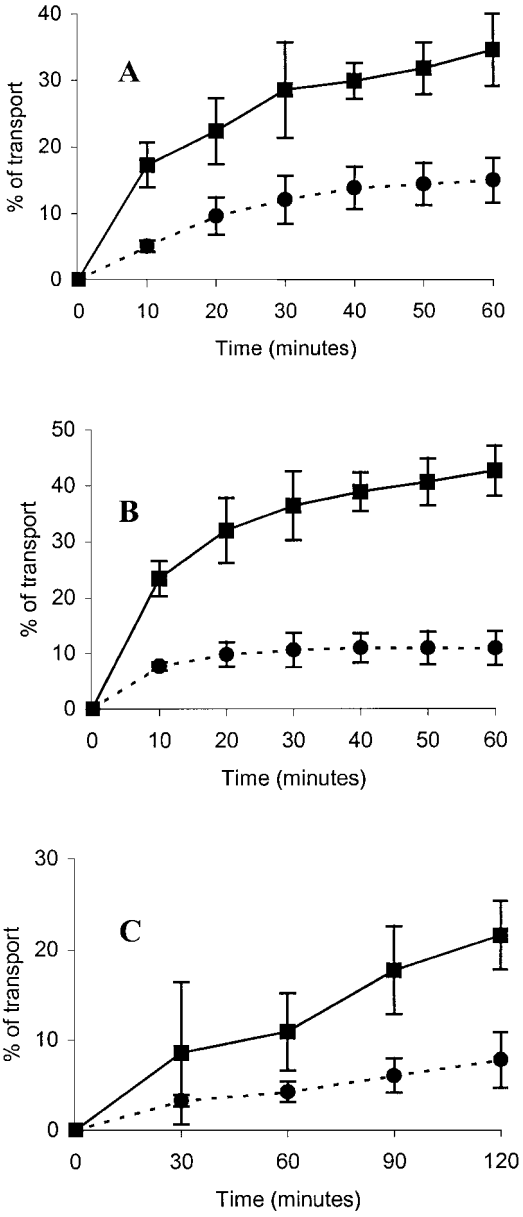

Fig. 6. A–C respectively shows release of three model drugs, sulforhodamine B, phenol red, and FITC-dextran from the patch (■) and from the solution (●) across the intestinal wall. Significant enhancement of transport was observed in three model drugs in this patch system ($p < 0.05$). Error bars = SD, n = 3–4.

6B). More than 20% of dextran was delivered across the intestine in the patch system within 120 min, while less than 10% of the dextran was delivered in the solution form (Fig. 6C). Significant enhancement of transport was observed in three model drugs in the patch system ($p < 0.05$).

Note that the area of the intestine available for transport is significantly different in the case of the patch and the solution. Specifically, the area available for sulforhodamine transport from the solution is about 471 mm^2 (30 mm long intestine and diameter of 5 mm). On the other hand, the patch possessed an area of 8 mm^2. Hence it should be interesting to compare solute delivered per unit available area for various cases shown in Fig. 6. This normalization is based on the fact that drug is delivered only underneath the patch (Fig. 5A–B). After normalizing the same data in Fig. 6 by the intestinal area available for transport, the sulforhodamine flux (amount delivered per unit area per unit amount loaded in the patch) from patches is more than 150-times higher compared to that from a solution. Similar enhancements were also obtained for poorly absorbed small molecules (phenol red) and poorly absorbed large molecules (FITC-dextran). This enhancement is attributed to two factors: (i) localization of the drug close to the intestinal wall, thereby providing a high concentration gradient for delivery; and (ii) maintaining unidirectional diffusion towards the wall.

CONCLUSIONS

This study proposes a novel intestinal patch system for oral drug delivery. Data show that this patch system significantly enhanced transport of three model drugs (sulforhodamine B, phenol red and dextran-70) across rat intestine during *in vitro* perfusion experiment. Drug leakage from the patch edge was substantially reduced by introducing microsphere structure in the patch matrix. Model drugs were primarily released unidirectionally from the mucoadhesive side of the patch. It is suggested that this patch system may be especially advantageous for delivery of peptides and proteins that are sensitive to enzymatic degradation. Further studies should focus on assessing the protective effect of these patches against the intestinal enzymes and *in vivo* tests of the patches.

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