Published in final edited form as:

Small. 2012 December 21; 8(24): 3839–3846. doi:10.1002/sml.201201367.

MULTI-RESERVOIR BIOADHESIVE MICRODEVICES FOR INDEPENDENT RATE-CONTROLLED DELIVERY OF MULTIPLE DRUGS

Hariharasudhan D. Chirra and Tejal A. Desai*

Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, CA 94158, U.S.A

Abstract

A variety of oral administrative systems such as enterically coated tablets, capsules, particles, and liposomes have been developed to improve oral bioavailability of drugs. However, they suffer from poor intestinal localization and therapeutic efficacy due to the various physiological conditions and high shear fluid flow. Fabrication of novel microdevices combined with the introduction of controlled release, improved adhesion, selective targeting, and tissue permeation may overcome these issues and potentially diminish the toxicity and high frequency of conventional oral administration. Herein, thin, asymmetric, poly(methyl methacrylate); PMMA microdevices were fabricated with multiple reservoirs using photolithography and reactive ion etching. They were loaded with different individual model drug in each reservoir. Enhanced bioadhesion of the microdevices was observed in the presence of a conjugated of targeting protein; tomato lectin to the PMMA surface. As compared to drug encompassing hydrogels, an increase in drug permeation across the caco-2 monolayer was noticed in the presence of a microdevice loaded with the same drug-hydrogel system. Also, the release of multiple drugs from their respective reservoirs was found to be independent from each other. The use of different hydrogel systems in each reservoir showed differences in the controlled release of the respective drugs over the same period of release. These results suggest that in the future, the microfabricated unidirectional multi-drug releasing devices will have an impact over the oral administration of a broad range of therapeutics.

Keywords

Oral drug delivery; microdevice; PMMA; bioadhesion; hydrogel

1. Introduction

Among the various conventional modes of drug administration, oral delivery of pharmaceuticals is a preferred route as it offers several advantages. It is less invasive, provides higher patient compliance, rapidly available, and has low cost of manufacturing. However, a unique set of intestinal barriers including the stomach's acidic environment, poor permeation of active therapeutics across the thick mucus and epithelial interface, an array of drug degrading intestinal enzymes, and limited retention time due to peristalsis and shear flow conditions limit the overall drug efficacy.^[1, 2] Although various oral delivery paradigms including enteric-coated capsules, liposomes, bioadhesive agents, and permeation enhancers were developed, many of these systems are administered with increased

*Corresponding Author. 1700 4th Street, Byers Hall 204, Box 2520, San Francisco, CA 94158, USA. Tel.: +1 415 514 4503; fax: +1 415 514 9656. tejal.desai@ucsf.edu.

frequency on an extended schedule, which is not practical for expensive and/or toxic drugs (chemotherapeutics).^[3–11] There are also instances of combination therapy, wherein multiple drugs are to be delivered at the same time for synergistic effects.^[12] Also the absence of targeting strategies for intestinal diseases such as, irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), and Crohn's disease results in an increased risk of side effects. To overcome these issues, oral drug delivery vehicles that require precise engineering and design control can be developed using microfabrication.

Although, techniques such as emulsification, droplet extrusion, solvent evaporation, or nanoprecipitation allow for the mass production of microparticles; the most common microfabricated vehicle, they usually tend to aggregate leading to polydispersity.^[13–15] Not only can polydispersity result in non uniform drug loading and release,^[16–18] but the symmetry of spherical particles can result in a loss of drug into the lumen caused by the omni-directional drug release at the mucus-particle interface.^[1] Therefore, we focus on the fabrication of reproducible, asymmetric, unidirectional, mucoadhesive microdevices for oral drug delivery.

In our previous work, we reported the development of a unidirectional drug releasing microfabricated device that was precisely engineered small enough to aid in better contact with the undulations of the intestinal wall, but large enough to avoid endocytosis.^[1, 19–22] We demonstrated that the unidirectional release of small molecules from the reservoir results in an increased local concentration of the drug in close proximity to the targeted epithelia. Also, these microdevices were designed to be flat and thin around 10 μm thick to minimize the shear stress experienced by the device side areas to the continuous intestinal liquid flow.^[1, 19, 23] These devices made up of an epoxy resin (SU-8) material had multiple drugs loaded in its single reservoir as layers of responsive hydrogels. As a result of this one drug per layer loading approach, the release of drug from the bottom most layer is dependent on the swelling/release properties of all the hydrogel layers – the drug encompassing bottom most layer, and the other top hydrogel layers acting as additional barriers. Similar is the case for the other drugs loaded in their respective middle hydrogel layers. Herein, we report the fabrication of a microdevice having multiple reservoirs using a more commonly approved biocompatible material -poly(methyl methacrylate) (PMMA) and the release of proteins from these devices. Other advantages of using PMMA include the potential functionalization of its methyl ester group and its extensive use as a resist in microelectromechanical systems (MEMS). The microdevices were fabricated using photolithography and reactive ion etching (RIE). The etching parameters of RIE can be easily modified to get reservoirs with varying depths that would prove advantageous for instances of loading expensive or low dosage drugs. Unlike the single reservoir system, the presence of multiple reservoirs enables one to load multiple drugs individually in different reservoirs, thereby enabling their release rates to be independent from each other. The hydrogel material properties were also modified by varying the crosslinking ratio or the amount of crosslinker; poly(ethylene glycol) dimethacrylate (PEGDMA) with respect to monomer monomethyl methacrylate (MMA).

Drug retention can be enhanced by improving the bioadhesive property of the microdevice. The increased planar surface area of the microdevice as compared to spherical microparticles/microspheres provides an enhanced bioadhesiveness to a flat surface, such as the gastrointestinal epithelial layer. Unlike microspheres, wherein surface functionalization results in particle agglomeration (via charge neutralization, hydrogen bonding, etc.), surface chemistry of microdevices can be uniformly or selectively modified with ease during on-wafer fabrication process. Our group has previously included bioadhesive targeting agents such as lectins that recognize and bind to intestinal mucosa.^[20, 24–26] Tao et al. demonstrated that after subsequent washing, microdevices functionalized with tomato lectin

bind to cell monolayer better than microspheres functionalized with lectin of same surface area.^[21] Ainslie et al. showed only a small fraction of the devices detach from the cell surface under flow, after initial binding.^[19] Herein, we demonstrate that our PMMA microdevices can also be lectin functionalized to provide enhanced bioadhesive properties. The use of microfabricated devices for oral drug delivery has the potential to improve efficacy of oral therapeutics for various disease treatments.

2. Results and Discussion

2.1. Fabrication of drug loaded microdevices

A series of photolithographic steps and reactive ion etching was used to fabricate 5600 PMMA microdevices per silicon wafer (figure 1.a.). Herein, circular shaped microdevices with three drug reservoirs were fabricated from PMMA with dimensions that would allow for *in vivo* transit through the mammalian gastrointestinal system (thickness of about ten microns, and the length of the maximum dimension being 200 μm). Though it is possible to fabricate a multitude of devices of varying dimensions and shapes, herein as shown in the SEM image (figure 1.b.), a circular 200 μm device with three 60 μm reservoirs was maintained as the prototype. Figure 1.c. shows the thickness profile of the device along the dotted line using a profilometer. The prototype device had a body thickness of about 7.5 μm , while the reservoirs were 5 μm deep. The thickness of the devices is chosen small enough to reduce the shear forces, per mass, experienced by the microdevice sides to flow conditions that can dislodge the device and disrupt therapeutic release and eventual permeation. The device body thickness limits the depth to which the reservoirs can be etched. In other words, the device thickness governs the volume of drug that can be loaded in a reservoir. By varying the spin speed of PMMA (1000 – 5000 rpm), the number of PMMA layers (1 – 3 layers), and baking (with or without a bake step in between layers), the thickness of the devices were varied from 3 – 12 μm . Any further layering had no significant effect on the thickness, while spin rates slower than 1400 rpm produced an edge bead of PMMA on the wafer that varied the aspect ratio significantly. The depth of the reservoirs (3.5 – 5 μm), and so the drug loading volume was easily adjusted by controlling the etch time or the ion flow rate (RIE power). This control over the drug volume is useful for instances of using expensive and toxic drugs for gastrointestinal (GI) delivery.

The drugs were loaded into the reservoirs as a drug encompassing hydrogel matrix using photolithography (figure 2.a.). Based on our prior work, the concentration of the photoinitiator DMPA was optimized to 6% and used to polymerize the entire of the reservoir volume.^[1] To confirm the stability of the drug-hydrogel matrix from staying in the reservoirs during flow conditions, the microdevice wafers were agitated (250 rpm) in PBS for three days. No significant loss or removal of the hydrogel from the reservoirs was observed ($98.6 \pm 0.9\%$ devices remained occupied with hydrogel). Figure 2.b. shows the fluorescent image obtained from single drug (Texas red-BSA) loaded microdevices. As observed, the drug uniformly filled all three reservoirs. From figure 2.c., it is observed that a series of spinning and UV exposure in the presence of respective individual reservoir masks leads to the filling of three different drugs to the three reservoirs with ease.

2.2. Conjugation of bioadhesive proteins to microdevices

The high surface area of the microdevice (23,000 μm^2) can be harnessed to facilitate multi-cell and multi-site attachment of the gastrointestinal mucosa to overcome issues associated with peristalsis and shear flow conditions experienced by current oral delivery systems. Tomato lectin is known to bind specifically to the N-acetylglucosamine moieties present on the epithelial cell lining of the intestinal wall, as modeled *in vitro* with caco-2 cells.^[27] Therefore, by introducing bioadhesive tomato lectin we can enhance the microdevice transit

time leading to increased drug retention, permeation, and eventual delivery. Tomato lectin was conjugated to the PMMA microdevice surface using two major steps: (a) the functionalization of PMMA to include amine groups via N-lithioethylene diamine aminolysis, and (b) the formation of amide bonds between the PMMA amines and the protein carboxylic acids using carbodiimide chemistry. The presence of amine functional groups and the ability of carbodiimide chemistry to bind the protein to PMMA surface were indirectly confirmed by probing the surface with fluorophore tagged tomato lectin. Figure 3 shows the fluorescent image of a microdevice that was initially tagged with FITC-tomato lectin and then used to introduce Texas red-BSA to the reservoirs. Since protein conjugation to the microdevices takes place for a time of 4 hr, it is done first prior to drug loading to avoid any drug loss associated with the swelling of hydrogel in the protein solution and eventual release of the drug. It is clear from figure 3 that the protein is mostly available on the surface of the PMMA microdevices and is readily available to recognize and bind with intestinal epithelia. The effect of using tomato lectin to introduce bioadhesive properties was confirmed using displacement studies (Table 1). Clearly the presence of tomato lectin on the surface of PMMA microdevices enhances the bioadhesive property of the microdevices. Although it may seem that the filling of reservoirs with drug-hydrogel matrix results in a reduction of overall bioadhesive property (59 %) as compared that of empty microdevices (71 %), this difference may just be from the number of asymmetric devices (conjugated to lectin on one side) that are not facing towards the caco-2 monolayer. It is also observed that a slight percentage of devices as such show binding to the caco-2 monolayer. This number can be increased by using a mucoadhesive material such as chitosan for fabricating the microdevice for enhanced oral drug delivery applications.^[28] The bioadhesive property of lectin coated microdevices may prove useful for the targeted treatment of various intestinal diseases such as IBD, IBS, and Crohn's disease.

2.3. Controlled *in vitro* drug release from microdevices

To measure the drug elution kinetics of the various microdevices, release of the different fluorophore tagged BSAs were monitored, *in vitro*, from hydrogel laden microdevices. BSA has a molecular weight of about 66 kDa ($14 \times 4 \times 4 \text{ nm}^3$) and is above the gastrointestinal limit of epithelial absorption (20 kDa).^[29] The volume of a single reservoir is approximately $1.4 \times 10^{-2} \text{ nL}$ and therefore a single drug loaded wafer (all three reservoirs loaded with same drug; figure 2.b.) or a multi-drug loaded wafer (different drug in different reservoir; figure 2.c.) holds approximately 85 ng of a single drug or 27 ng of each drug respectively. Similar drug loaded hydrogel boluses (hydrogel pellets with no microdevices) were polymerized as control samples and used for *in vitro* drug release studies. In the presence of a fluid, the hydrogel swells and allows the drug to diffuse out of the polymer matrix. The microdevices were added to the apical side of a caco-2 monolayer that possesses *in vivo*-like tight-junctions (1–3 nm) and drug concentration was measured in the basal side.^[30] Figure 4 shows the *in vitro* release profile of the single drug loaded wafers. Relative to the control (hydrogel bolus) sample, the microdevices show an enhanced permeation of drug across the caco-2 monolayer. This may be attributed to the fact that asymmetric microdevices release drug in a unidirectional way as compared to the hydrogel bolus to provide an increased concentration of drug across the device-cell interface. Similar results have been predicted by others, wherein the transport of high molecular weight proteins is attributed to the increased paracellular transport across the intestinal epithelium in the presence of a bioadhesive microparticle.^[31, 32] This increase in drug permeation caused by the presence of a microdevice is important in the context of being able to improve the oral bioavailability of large molecules.

The effect of using multi-reservoir devices loaded with different individual drug in each reservoir as compared to our previously used single reservoir systems loaded with layers of

different drugs was also studied (figure 5). In the case of the single reservoir system loaded with multiple drugs loaded in layers of hydrogels, the release of the different drugs depended on the swelling kinetics of the overlaying hydrogel layers.^[1] This dependency on the swelling of other hydrogel layers acts as additional barriers for the different drugs to release from the microdevices. But it is observed from figure 5 that unlike the layered single reservoir systems, the release of all three model fluorophore BSAs from the three reservoir prototype device is independent from each other. This independent release behavior proves useful for combination therapies, wherein multiple drugs are to be delivered at the same time at the same place. All three drugs show linear release up to three hours, after which steady state is reached, which is consistent with the amount of drug loaded in each microdevice per wafer.

In addition to the molecular weight and amount of drug loaded, the properties of the drug encompassing polymer matrix can also be modified to control the release kinetics. The polymer can be chosen specifically to release the drug via degradation or in response to external stimuli (pH, temperature, etc).^[32–42] Here, we modified the swelling property of the hydrogel matrix in each reservoir to provide different release kinetics by modifying the crosslinking ratio of the hydrogel. Increasing crosslinking ratio of PEGDMA from 15 % for Texas red-BSA loaded reservoir to 30 % for FITC-BSA reservoir to 45 % for DNP-BSA reservoir was used for this study. It is observed from figure 6 that Texas red-BSA released faster than FITC-BSA that released faster than DNP-BSA. In other words, the controlled release of different drugs is dependent on the crosslinking ratio of the hydrogel system. This is due to the fact that lower crosslinking ratio (15 %) results in the formation of a less tighter/loose mesh network leading to an increased diffusion of the drug, while higher crosslinking ratio (45 %) results in the formation of a highly tighter mesh network leading to a decreased diffusion of the drug. A similar effect can also be obtained with the use of different molecular weight (length of the chain) monomers or crosslinkers.^[43] The use of different polymer systems of varying release and degradation kinetics in each reservoir enables the use of microdevices for timed release of different drugs for effective therapy^[44, 45] Timed release of drugs from microdevices may enable more effect delivery of therapeutics to different regions of the gut as the device transits through the intestinal tract.

3. Conclusion

The development of oral drug delivery platforms for administering drugs in a safe and effective manner across the gastrointestinal tract is of much importance. Unlike symmetric spherical microparticles that have issues of polydispersity, non-uniform drug loading and release, and drug loss into the lumen, the use of microfabricated asymmetric microdevices could overcome some of these limitations. This study has demonstrated the successful fabrication of multi-reservoir PMMA microdevices using photolithography and reactive ion etching. The flat, thin device feature enables to maximize the contact area between the microdevice and the intestinal epithelium. Reactive ion etching proved useful for varying the well geometry in order to modulate the amount of drug loaded in a microdevice, while the presence of multiple reservoirs enabled the loading of multiple model drugs in the same microdevice. Bioadhesive tomato lectin was selectively conjugated to the reservoir containing side of the PMMA microdevices using aminolysis and carbodiimide chemistry, leading to better adhesion between the device and epithelium. Unlike the layered multi-drug loaded single reservoir microdevices, the release of different drugs from individually loaded multi-reservoir devices was independent from each other. This independent controlled release property of multi-reservoir microdevices was harnessed to modify the release kinetics of the different drugs by using different hydrogel systems. Tunable microdevices may allow for enhanced efficacy in oral drug delivery and targeting.

4. Materials and methods

4.1. Fabrication of PMMA microdevices

4.1.1. Materials for microdevice fabrication—All chemicals were purchased from Sigma Aldrich and used as received, unless noted otherwise. Concentrated sulfuric acid, hydrogen peroxide (30%), acetone, methanol, and isopropanol were used for standard RCA pre-cleaning of the wafers. The device material poly(methyl methacrylate); (PMMA) of molecular mass 950,000 suspended in anisole (11%), Shipley 1818 positive photoresist, microposit 351 developer, and 1112A photoresist remover were purchased from Microchem. Positive masks for fabricating the device body (200 μm circles) and its reservoirs (three 60 μm circles inside the 200 μm bigger body circle) were obtained from CAD art services (Badon, OR). The three 60 μm circles were placed on the corners of an equilateral triangle equidistant from the center of the 200 μm circle.

4.1.2. Microfabrication process—Photolithography and reactive ion etching were used to create $200 \times 8 \mu\text{m}$ cylindrical PMMA microdevices with three $60 \times 5 \mu\text{m}$ cylindrical reservoirs over 3-inch silicon wafers. Each wafer was cleaned in piranha solution (3:1:: $\text{H}_2\text{SO}_4\text{:H}_2\text{O}_2$) for 20 min, and rinsed with deionized water thrice. Wafers were then rinsed with acetone, methanol, isopropanol, and baked (100 $^\circ\text{C}$; 2 min) to remove all impurities. Figure 1.a. shows the scheme of steps involved in the microfabrication process. The wafers were spin coated twice with PMMA (1400 rpm, 30 s) using a Headway Research PW101 spinner (Garland) to get the microdevice body layer. Baking was done before and after the second coat (110 $^\circ\text{C}$, 1 min) on a vented hot plate to remove solvents from the PMMA layer. After cooling, the wafers were spin coated with positive photoresist (5000 rpm, 30 s) and pre-baked (110 $^\circ\text{C}$, 1 min). The cooled wafers were then exposed to UV light (405 nm; 16 mW/cm²; 20 s) of a mercury lamp using a Karl Suss MJB3 mask aligner holding the positive photomask that defines the 200 μm microdevice body. The photoresist was developed (75 s) in a solution of 351 microposit developer to DI water (1:3 vol%). The wafers were then rinsed in a DI water cascade, blown dry with nitrogen, and post-baked (110 $^\circ\text{C}$, 1 min). The exposed PMMA was dry etched away using a Surface Technology Systems PE1000 AC Plasma Source Reactive ion etcher (RIE; PETS Inc.; 20% oxygen flow; 30 mTorr pressure; 455 W) for 8 minutes. After etching, any residual photoresist was removed using a 1112A photoresist remover (1 min), followed by water, isopropanol rinse, and blown dry with nitrogen.

Once the device body was defined, a second photolithography step was performed to define the microdevice reservoirs. The wafers were spin coated with positive photoresist (5000 rpm, 30 s), pre-baked (110 $^\circ\text{C}$, 1 min), and again exposed to UV light using the mask aligner through the second photomask designed to define the three 60 μm microdevice reservoirs. The reservoirs were aligned to the microdevice body using front side alignment techniques on the same mask aligner. Following exposure, the wafers were developed as before using the 351 developer-DI water mixture, rinsed in a DI cascade, nitrogen dried, and post-baked. The unmasked reservoir defining areas were reactive ion etched as before for 8 minutes. The depth of the reservoirs can be controlled by the etching time, but for this work, 5 μm deep reservoirs were obtained. Any residual resist was removed by using 1112A resist remover solution. Characterization of the microdevice dimensions was done using an Ambios Technology XP-2 Stylus Profiler (0.05 mm/s; stylus force of 0.8 mg), while a Novel X my-SEM (Lafayette, CA) scanning electron microscope was used to visualize the microdevices.

4.2. PMMA-protein binding chemistry

4.2.1. Surface aminolysis—The bioadhesive property to the PMMA microdevices is provided by binding targeting proteins to their surface. Amine groups were introduced to the

PMMA microdevices using N-lithioethylenediamine. Briefly, N-lithioethylenediamine was synthesized by purging ethylenediamine (19.8 mL) with nitrogen (30 min). Butyllithium (400 μ l) in cyclohexane (2 M) was then added to ethylenediamine and the reaction was allowed to proceed under nitrogen atmosphere for 3 hours under constant stirring. The PMMA microdevice containing wafers were surface modified to include amines only on the sides containing the reservoirs. The wafers were rinsed in DI water, blown dry with nitrogen, and placed on a petri dish that was supplied with nitrogen. After nitrogen purging (2 min), N-lithioethylenediamine (500 μ l) was added to the wafers and evenly applied to coat all microdevices (3 min). The wafers were taken out and immersed in DI water to stop aminolysis and eventual release of pH responsive PMMA microdevices from the wafer. After gentle washing in DI water, the wafers were blown dry with nitrogen.

4.2.2. Surface immobilization of protein—The amines were conjugated to model protein tomato lectin (FITC-labeled) using 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC; Invitrogen) and N-Hydroxysuccinimide (NHS; Invitrogen). Briefly, model protein (600 μ l of 1 mg/mL) in MES buffer (pH 5.5), EDC (13 μ l of 100 mM) and NHS (13 μ l of 200 mM) were added and allowed to react (20 min). Once, the carboxylic acid groups of the proteins were modified into an activated EDC-NHS ester, the reaction was stopped by adding β -mercaptoethanol (0.8 μ l of 14 M). After a min, the pH of the protein mixture was raised to 7.4 by adding sodium bicarbonate and immediately applied to the amine functionalized PMMA microdevice wafers. The binding of the amine groups of PMMA with the modified carboxylic acid groups of the protein was allowed to take place for 4 hr, after which, the wafers were extensively rinsed with DI water to remove any non-covalently bound protein.

4.3. Drug loading of microdevices

Single or multiple drugs are loaded to the microdevice reservoirs using photolithography. Briefly, hydrogel-drug prepolymer solutions were prepared by mixing crosslinker poly(ethylene glycol) dimethacrylate (PEGDMA; 750 mol wt; 2 mL) with photoinitiator dimethyl acetophenone (DMPA; 300 μ l of 60 mg/mL) in monomer monomethyl methacrylate (MMA), and model fluorophore-drug in PBS (200 μ l of 3 mg/mL). The model fluorophore-drug was dissolved in PBS via sonication prior to mixing with the crosslinker-monomer solution. The different fluorophore-drugs used were fluorescently labeled bovine serum albumins (BSA) – fluorescein isothiocyanate-BSA (FITC-BSA; ex: 494 nm; em: 520 nm), Texas red-BSA (ex: 596 nm; em: 615 nm), and 2,4-dinitrophenylated-BSA (DNP-BSA; ex: 360 nm; em: 385 nm). Upon mixing all ingredients for hydrogel prepolymer solution, the mixture was sonicated (30 min) to ensure equal distribution of initiator and drug.

For single drug loaded microdevices, the prefabricated wafers were spin coated (3000 rpm, 30 s) with the respective single drug prepolymer solution (300 μ L) and exposed to UV light (90 s) using the mask aligner (figure 2.a.). The photomask used for single drug loading in all three reservoirs is a negative photomask designed to allow light to pass through all three 60 μ m reservoirs for photopolymerization of the prepolymer solution into a drug encompassing hydrogel matrix. Development was done using DI water (30 s) and blown dry using nitrogen. For loading of multiple drugs individually in their respective reservoirs, a series of spin coating, alignment, exposure, development, and drying was done using three different negative masks, each allowing light to pass through only one of the reservoirs for photopolymerization (figure 2.a.). Also, a similar multi-drug loaded wafer was obtained by varying the crosslinking ratio of the prepolymer solution. The crosslinking ratios (PEGDMA:MMA) were 15:85, 30:70, and 45:55 for Texas red-BSA, FITC-BSA, and DNP-

BSA respectively. Fluorescent microscopy of the protein conjugated and drug loaded devices was done using an Olympus BX60 microscope (Mellville, NY).

4.4. In vitro drug permeation studies

The drug loaded microdevices were released within 2 min from the wafers using potassium hydroxide (KOH; 8 M) solution that was preheated (40 °C). The released microdevices being less dense than water was ultra centrifuged (30 kDa Amicon ultra centrifugal filters) and washed with PBS twice for release and permeation studies. Human colorectal adenocarcinoma epithelial cells (caco-2 ATCC) were grown to confluency (transepithelial electrical resistance plateau at 900–1000 Ω) on 50% collagen-ethanol (Type 1, Becton Dickinson, Franklin Lakes, NJ) treated 24-well Transwell® inserts. The caco-2 cells were maintained in Modified Eagle's Media (MEM) with fetal bovine serum (Invitrogen; 20%), L-glutamine (2 mM), sodium bicarbonate (1.5 g/L), glucose (2.5 g/L), HEPES buffer (10 mM), sodium pyruvate (1.0 mM), and penicillin/streptomycin (1 mg/mL) for 5 days or more prior to seeding. The microdevice solution containing about one third of devices from a single wafer (~1850 devices; 500 μ L) was added to the Transwell® insert. Discrete time (20 min) samples were taken from both the upper and lower chamber of the Transwell® for the different drug loaded systems and observed for fluorescence using a Packard Fluorocount Microplate Fluorometer (Meriden, CT). All errors are calculated as standard deviations from their mean values (triplicates were done).

4.5. Bioadhesive displacement studies

Caco-2 monolayers were grown to confluency on 6 well plates under standard conditions. About 100 microdevices per sample (with and without lectin and/or hydrogel; triplicates were done) were incubated on the monolayer surface using PBS (30 min; 37 °C). The wells were then visualized using the microscope. The wells were then displaced five times in a controlled vertical fashion with PBS. The initial view field that was imaged before displacement was imaged again and the microdevices were traced in a MS Word grid. Devices that were within 80% of their initial area were considered still stationary and bioadhesive. Those microdevices that were outside the initial 80% area were considered to be displaced.

Acknowledgments

This work was supported by National Institute of Health grant EB011664 and Z Cube Zambon Research Venture.

References

1. Ainslie KM, Desai TA. Lab Chip. 2008; 8:1864. [PubMed: 18941687]
2. Eaimtrakarn S, Itoh Y, Kishimoto JI, Yoshikawa Y, Shibata N, Takada K. Int J Pharm. 2001; 224:61. [PubMed: 11472815]
3. Okumu, FW.; Cleland, JL. Implants and injectables. In: Rathbone, MJ.; Hadgraft, J.; Roberts, MS., editors. Modified release drug delivery technology. Marcel Dekker; New York: 2003. p. 633
4. Saffran M, Kumar GS, Neekers DC, Pena J, Jones RH, Field JB. Biochem Soc Trans. 1990; 18:752. [PubMed: 2083667]
5. Iwanaga K, Ono S, Narioka K, Kakemi M, Morimoto K, Yamashita S, Namba Y, Oku N. J Pharm Sci. 1999; 88:248. [PubMed: 9950646]
6. Ponchel P, Irache JM. Adv Drug Deliv Rev. 1998; 34:191. [PubMed: 10837678]
7. Arango MA, Ponchel G, Orecchioni AM, Renedo MJ, Duchene D, Irache JM. Eur J Pharm Sci. 2000; 11:333. [PubMed: 11033077]
8. Lehr CM. J Control Release. 2000; 65:19. [PubMed: 10699266]
9. Fasano A, Uzzau S. J Clin Invest. 1997; 99:1158. [PubMed: 9077522]

10. Fasano A. Trends Biotechnol. 1998; 16:152. [PubMed: 9586237]
11. Schwarz UI, Gramatte T, Krappweis J, Oertel R, Kirch W. Int J Clin Pharmacol Ther. 2000; 38:161. [PubMed: 10783825]
12. White NJ. Antimicrob Agents Chemother. 1997; 41:1413. [PubMed: 9210658]
13. Bugarski B, Li QL, Goosen MFA, Poncelet D, Neufeld RJ, Vunjakg A. AIChE J. 1994; 40:1026.
14. Wolters GHJ, Fritschy WM, Gerrits D, Vanschilfagaarde R. J Appl Biomater. 1992; 3:281. [PubMed: 10147997]
15. Leach DT, Simpson WT, Val TN, Yu ZS, Lim KT, Park EJ, Williams RO, Johnston KP. AAPS Pharm Sci Tech. 2005; 6:E605.
16. Lai SK, Hida K, Man ST, Chen C, Machamer C, Schroer TA, Hanes J. Biomaterials. 2007; 28:2876. [PubMed: 17363053]
17. Rejman J, Oberle V, Zuhorn IS, Hoekstra D. Biochem J. 2004; 377:159. [PubMed: 14505488]
18. Randall CL, Leong TG, Bassik N, Gracias DH. Adv Drug Deliv Rev. 2007; 59:1547. [PubMed: 17919768]
19. Ainslie KM, Lowe RD, Beaudette TT, Petty L, Bachelder EM, Desai TA. Small. 2009; 5:2857. [PubMed: 19787677]
20. Tao SL, Lubeley MW, Desai TA. J Control Release. 2003; 88:215. [PubMed: 12628329]
21. Tao SL, Desai TA. J Control Release. 2005; 109:127. [PubMed: 16274828]
22. Tao SL, Popat KP, Desai TA. Nat Protocols. 2006; 1:3153.
23. Ahmed A, Bonner C, Desai TA. J Control Release. 2002; 81:291. [PubMed: 12044568]
24. Wood KM, Stone GM, Peppas NA. Biomacromol. 2008; 9:1293.
25. Wood KM, Stone GM, Peppas NA. J Control Rel. 2006; 116:e66.
26. Arbos P, Arangoa MA, Campanero MA, Irache JM. Int J Pharm. 2002; 242:129. [PubMed: 12176235]
27. Rocca J, Shah K. Drug Delivery Technology. 2004;4.
28. Lehr CM, Bouswarta JA, Schacht EH, Junginger HE. Int J Pharm. 1992; 78:43.
29. Goldie, R. Immunopharmacology of epithelial barriers. Page, C., editor. Elsevier; 1994.
30. Kitchens K, Kolhatkar R, Swaan P, Eddington N, Ghandehari H. Pharm Res. 2006; 33:2818. [PubMed: 17094034]
31. Uskokovic V, Lee PP, Walsh LA, Fischer KE, Desai TA. Biomaterials. 2012; 33:1663. [PubMed: 22116000]
32. Fischer KE, Aleman BJ, Tao SL, Daniels RH, Li EM, Bunger MD, Nagaraj G, Singh P, Zettl A, Desai TA. Nano Lett. 2009; 9:716. [PubMed: 19199759]
33. Hilt JZ, Peppas NA. Int J Pharm. 2005; 306:15. [PubMed: 16253449]
34. Al Malyan M, Becchi C, Boncinelli S, Ashammakhi N. Minerva Anesthesiol. 2007; 73:173. [PubMed: 17159768]
35. Saylor DM, Kim CS, Patwardhan DV, Warren JA. Acta Biomater. 2007; 3:851.
36. Angelos S, Yang YW, Patel K, Stoddart JF, Zink JJ. Angew Chem Int Ed. 2008; 47:2222.
37. Kwon IC, Bae YH, Kim SW. Nature. 1991; 354:291. [PubMed: 1956379]
38. Schwendeman SF, Amidon GL, Levy RJ. Macromol. 1993; 26:2264.
39. Edelman ER, Kost J, Bobeck H, Langer R. J Biomed Mater Res. 1985; 19:67. [PubMed: 4077873]
40. Kost J, Leong K, Langer R. Proc Natl Acad Sci. 1989; 86:7663. [PubMed: 2813349]
41. Ghodsian FF, Brown L, Mathiowitz E, Brandenburg D, Langer R. Proc Natl Acad Sci. 1998; 85:2403.
42. Gutowska A, Bark JS, Kwon IC, Bae YH, Cha Y, Kim SW. J Control Release. 1997; 48:141.
43. Chirra HD, Hilt JZ. Langmuir. 2010; 26:11249. [PubMed: 20486693]
44. Grayson ACR, Choi IS, Tyler BM, Wang PP, Brem H, Cima MJ, Langer R. Nat Mater. 2003; 2:767. [PubMed: 14619935]
45. Grayson ACR, Cima MJ, Langer R. J Biomed Mater Res Part A. 2004; 69A:502.

Figure 1.

(a) Schematic representation of the process of fabricating PMMA microdevices, (b) a scanning electron microscopic image of the fabricated microdevice prototype (200 μm circular device with three 60 μm circular reservoirs), and (c) the dimensions of the microdevice, as measured using a profilometer (dotted line). The scale bar represents 100 μm .

Figure 2.

(a) Schematic process overview for fabricating single or multi-drug loaded microdevices using photolithography, (b) a fluorescent micrograph showing the presence of a single model drug (Texas red-BSA) loaded in all three reservoirs of the same microdevice, and (c) a fluorescent micrograph composite of a multi-drug (Texas red-BSA; red, FITC-BSA; green, DNP-BSA; blue) loaded microdevice as individual drug in separate reservoirs. The white circle highlights the microdevice area.

Figure 3. A fluorescent micrograph composite confirming the conjugation of model fluorophore (FITC)-lectin to the surface of PMMA microdevice (green) and showing the loading of model drug (DNP-BSA; blue).

Figure 4.

The enhanced permeation of different single drug loaded microdevices as compared to their respective drug loaded hydrogel bolus (control; without devices) through a caco-2 epithelial monolayer on collagen treated Transwells[®]. The concentration was normalized with respect to total drug loaded in each microdevice wafer (N=3).

Figure 5. The independent permeation of different model drugs from their respective reservoirs of the same microdevice across the caco-2 epithelial monolayer on collagen treated Transwells[®] (N=3).

Figure 6.

Controlled release and permeation of different model drugs loaded into their respective reservoirs of the same device using different crosslinking ratio/amounts of crosslinker (PEGDMA). Increasing or decreasing the amount of PEG resulted in a slower or faster release of similar molecular weight drug respectively. This proves useful for timed release therapy of various intestinal diseases (N=3).

Table 1

Displacement studies, wherein microdevices were incubated with and without caco-2 cell monolayer in 6 well plates. The wells were displaced five times in a vertical fashion and device location was observed by comparing the before and after micrographs.

Caco-2 monolayer	Microdevice	Drug-hydrogel	Tomato lectin	Binding [%]
–	+	+	+	0 ± 0
+	+	+	–	2 ± 1
+	+	–	+	71 ± 8
+	+	+	+	59 ± 6