Micro- and Nanofabrication of Polymeric Devices and Films for Oral Drug Delivery Applications

By

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Micro- and Nanofabrication of Polymeric Devices and Films for Oral Drug Delivery Applications

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Abstract

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Oral drug delivery is the preferred route of administration due to its convenience to patients, low cost, and high patient compliance. However, the oral delivery of small molecules and protein therapeutics face physiological challenges that result in low drug solubility, poor absorption, and metabolic breakdown. This dissertation work describes the development of micro- and nanofabrication techniques to create drug delivery platforms that are designed to overcome these barriers. Micron-scale devices with planar, asymmetric geometries were fabricated using biocompatible materials with photolithography and inkjet printer techniques. These properties enhance device adhesion in the small intestine and are designed for unidirectional drug release toward epithelial tissue to release drug at high concentration and prolong drug exposure. Drug cargo comprising of either small molecules or peptides were efficiently loaded into microdevices with little waste using the inkjet printing approach and were capped with enteric or mucoadhesive polymers to control the rate of drug release in simulated intestinal environments. Finally, nanostructured surfaces were investigated as a means to enhance drug permeation across the intestinal epithelial barrier. Together, these results demonstrate how micro- and nanofabrication approaches can be used to overcome oral drug delivery challenges.
Dedicated to my family and Monica
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CHAPTER 1: INTRODUCTION
1.1 INTRODUCTION

Oral drug administration is the most preferred and common route of administration. As opposed to parenteral administration, the oral route typically causes neither tissue damage nor pain and requires less patient supervision, resulting in high patient compliance and decreased cost of care (1). Oral drug formulations may also provide advantages over intravenous drug formulations, which can involve injection of solubilizing excipients associated with toxicity and/or altered disposition of coadministered drugs (2-4). In addition to being the primary route for systemic drug administration, oral administration allows for localized drug treatment of gastrointestinal (GI) tissue. However, there is currently a lack of approaches to target diseased tissue (5, 6). Therefore, diseases of the GI tract are often treated through formulations designed for systemic administration, resulting in system-wide side effects (5, 7).

While oral administration is most preferred, approximately 50% of active pharmaceutical agents suffer from limited oral uptake (8, 9). The oral route is associated with issues with 1) drug degradation, 2) low drug solubility, and 3) low drug permeability, preventing uptake of intact drug into the bloodstream (10). Current approaches to improve drug uptake include permeation enhancers, excipients to enhance drug solubility or provide sustained drug release, micro- and nanoparticulate systems, drug conjugation and modification, enteric coating, metabolic and transporter protein inhibitors, and bioadhesive polymers and ligands, which have been reviewed in detail (6, 11-19). While these approaches allow for control over many properties of drug delivery systems, they do not typically provide precise geometric control, which can be used to facilitate interaction with the micro- and nanoscale features of GI tract physiology for increased adhesion and tissue permeability (20, 21).

Photolithography, soft lithography, and micro/nano-fabrication approaches can be used to fabricate oral drug delivery systems with precise control over feature geometry, symmetry, dimensions, material composition, and surface modification, allowing for design of microscale devices that specifically address physiological barriers of the GI tract. These fabrication technologies have also been reviewed in detail previously (6, 22-25). Application of these approaches to oral drug delivery has expanded to include biocompatible and bioadhesive polymers, asymmetric geometries, nanotopographical features, and materials that respond to environmental cues to improve drug uptake.

The goal of this dissertation is to combine existing oral drug delivery systems with the aforementioned novel technologies to address the challenges of drug loading and formulation, drug PK, tissue targeting, and drug permeation.

1.2 PHYSIOLOGICAL BARRIERS TO ORAL DRUG UPTAKE

1.2.1 Physiological considerations for oral drug delivery

The physiology of the human digestive tract presents significant barriers to oral drug delivery. Among these challenges are poor drug stability, low drug solubility, and mucosal barriers that limit the application of current oral dosing strategies to drugs that possess good bioavailability. In general, the digestive tract is composed of a series of compartments that work together using mechanical and chemical processes to help convert food into energy and nutrients for the body. Beginning with the oral cavity, multiple
accessory organs (i.e. teeth, salivary glands, tongue) break down food and secrete lubricants to help food pass into the pharynx, a tube that connects the mouth and the esophagus. Passing through the esophagus, the food reaches the stomach where the low pH and digestive enzymes help break down the food. The digested food moves to the small intestine for further digestion and absorption of nutrients and finally moves to the large intestine where water is absorbed and microflora further breaks down waste products prior to excretion.

The physiology throughout the digestive tract can differ significantly in the form of gastric mucosa, organization of the epithelium, and chemical microenvironment. Therefore, the physiological characteristics of the targeted region and all preceding regions must be considered when designing drug delivery platforms.

1.2.2 Buccal Cavity

Delivery to the oral cavity is sometimes preferred over the conventional oral route because it avoids pre-systemic clearance by the liver and physiological challenges related to membrane permeability and absorption found in the gastrointestinal tract. Buccal mucosa includes a stratified squamous epithelium that comprises the upper and lower lips as well as the lining of the cheek and is richly vascularized. As such, buccal administration is an attractive target due to easy accessibility, mild pH microenvironment, and direct access to systemic circulation thereby avoiding the hepatic “first-pass” effect (26). However, drawbacks include the smaller surface area of membranes, about 170 cm$^2$, of which ~50 cm$^2$ of non-keratinized buccal tissue is available for drug absorption (27). In addition, saliva is constantly secreted in the oral cavity and can dilute and/or degrade labile drugs susceptible to enzymatic degradation. To bypass these physiological barriers, drug delivery platforms can be designed to prolong residence time with the mucosa, enhance drug permeation, and protect the drug from degradation.

1.2.3 Esophagus

The esophagus is a site not often utilized for drug delivery due to presence of a stratified, squamous epithelial layer that is known for its low permeability and very short residence time (28). For example, a 10 mL bolus has less than 16 s of residence time in the esophagus before reaching the stomach (29). Additionally, poor blood supply makes esophageal delivery less ideal for systemic delivery. Certain local diseases such as esophageal cancer, bacterial infection, and Barrett’s esophagus can be targeted specifically through the use of bioadhesives to improve local delivery (30). However, some solid dose formulations may become lodged in the esophagus, which is a concern. In this scenario, a high concentration of drug is delivered to a localized area, potentially causing damage to the tissue. As such, drug delivery to the esophagus must focus on devices that can improve device residence time while preventing permanent lodging of the device.

1.2.4 Stomach

The stomach is responsible for storing food temporarily and slowly releasing it into the small intestine. Within the stomach is a harsh microenvironment with a pH range of 1.0-2.5 that acts as a chemical factory for breaking down food and harmful pathogens (31).
To prevent self-digestion, the stomach possesses the extrinsic gastrointestinal barrier, a layer of mucus spanning 40-450 µm and bicarbonate ions secreted by surface epithelial cells (32, 33). Much of the mature epithelial cells have high turnover rate and die within a few days. Beneath this layer is the intrinsic barrier, which is composed of epithelial cells that seal the paracellular space with tight junctions. With a small surface area, there is little absorption that takes place making delivery difficult (34). Drug delivery systems that target diseases like peptic ulcer disease in the stomach must seek out methods to increase gastroretention time to avoid gastric emptying. This is primarily achieved by means of bioadhesive, swellable, expandable, or raft floating drug delivery systems.

1.2.5 Small intestine

Digested food from the stomach moves into the small intestine for further digestion and processing. The small intestine can be further divided into three structural parts: duodenum, jejunum, and ileum. The duodenum is a short structure that receives the gastric chyme from the stomach and secretes an alkaline solution of bicarbonate that neutralizes the stomach pH. The pH changes rapidly to about pH 6 in the duodenum and will rise to about 7.4 in the terminal ileum (35). The jejunum connects the duodenum to the ileum and possesses villi that increase its surface area for absorption of nutrients. The ileum is the final section that possesses similar physiological structure to the jejunum and absorbs remaining nutrients not absorbed by the jejunum.

Greater than 99% of the epithelial tissue is covered with enterocytes to form the villi, finger-like projections 0.5-1 mm in length (36). The role of villi is to use their apical projections, called microvilli, to increase surface area for greater absorption (37). The total surface area is approximately 400 m², although this varies with the individual, with an average measurable microvilli height and diameter of 0.67-1.36 µm and 0.08-0.15 µm, respectively (38). Covering the villi are follicle associated epithelium, M-cells, and mucus secreting goblet cells, which play a role in protecting the epithelium. The high surface area and numerous transport mechanisms make the small intestine a prime target for oral drug delivery (39).

However, there are multiple challenges that arise from its physiology. Drugs must survive the severe chemical microenvironment in the stomach prior to entering the small intestine. Once in the intestine, drugs are then exposed to numerous pancreatic enzymes and bile salts that can degrade drugs. In addition, the presence of a mucosal layer results in low drug bioavailability of most water-soluble macromolecules due to their low mucus permeability. Drugs must be able to cross the mucosal layer followed by a submucosal and areolar cell barrier where they interact with a plethora of transport pathways including transcellular transport, paracellular transport, or receptor/carrier-mediated processes to enter systemic circulation (Fig. 1-1) (13). Various microdevices and nanotechnology-based approaches propose to take advantage of the physiology of the small intestine (38, 40). The ability to control device dimensions in micro- and nanoscale allows targeting of specific villi and microvilli dimensions, leading to increased retention of the device, which may increase overall bioavailability (23). Furthermore, microfabrication can also incorporate permeation enhancers that promote absorption of therapeutics that have traditionally been difficult to use for oral drug delivery. This will be explored in further detail in later sections.
1.2.6 Colon

Digesting food moves from the small intestine and into the large intestine, or colon, which is the final site of the digestive system before excretion. Compared to other oral drug delivery sites, the colon is host to lower digestive enzyme activity, higher pH compared to the stomach and small intestine, and a long residence time of up to 5 days (41, 42). Furthermore, the gut microflora can carry out chemical reactions that may metabolize a number of different drugs. Delivery to the colon is important for treatment of bowel diseases including Crohn’s disease, ulcerative colitis, and colorectal cancer. Specific targeting of these diseases would benefit from local delivery by using less drug and can reduce side effects that arise from systemic delivery. As the colon is the most distal target, device design must ensure that drug release and absorption do not occur in the stomach or small intestine while protecting the drug against degradation in the variable microenvironments encountered. However, a major drawback to targeting the colon is the inherent variability of the patient’s pH levels, gastric emptying times, and differing microflora (43, 44). As a result, the unique microenvironment found in each patient leads to variable drug release and poor bioavailability, which makes it challenging to deliver consistent, therapeutic doses. Therefore, drug delivery vehicles must take these physiological challenges into consideration.

1.2.7 Concluding remarks for physiology

There are a number of physiological barriers that must be considered in the design of new devices to transport and protect drugs with subsequent delivery to targeted tissue. Therefore, it is important to design systems capable of taking advantage of biology by means of increased adhesion to promote retention time, efficient drug loading to deliver a significant dose within the therapeutic window, and enhanced permeation at target sites.

1.3 MICRO/NANOFABRICATION TECHNOLOGIES

1.3.1 Advantages of micro/nanofabrication technologies

Micro/nanofabrication approaches provide precise control over device geometry, surface modification, material composition, symmetry, and size, all of which can be used to design drug delivery systems for specific interactions with GI tract tissue (47, 48). By designing particles or devices within the right size regime, it is possible to target delivery to the intestinal villi, which possesses micron-sized folds and pits (49). In addition to local delivery, microfabricated devices can be further enhanced with ways to control release kinetics and/or delivery on demand (50). Tailored release kinetics would be a dramatic improvement over bolus dose delivery in which there is very little control over drug transport. Finally, microfabrication technologies are capable of scale-up with high product reproducibility, key features that will be important in the eventual translation to human use (47).

1.3.2 Micro/nanofabrication techniques

There are numerous technologies already available that can be used to fabricate a micron-scale particle or device with well-controlled geometric parameters. The most
widely-established method is photolithography. The general schematic of the process is shown in Fig. 1-2. This process involves the deposition of a photoresist polymer onto a base substrate, typically silicon. The polymer is selectively etched with UV radiation to form the desired pattern after the development process. The remaining polymer material serves as a protective layer during chemical or reactive ion etching processes which remove the underlying substrate. Finally, the protective polymer is stripped away and only the substrate is left behind. Higher resolution techniques involve the use of electron-beam or ion-beam lithography. A more comprehensive description of the approach can be found in the article by Altissimo (51).

Hard patterned substrates, such as silicon, are used in a process called soft lithography (52, 53). This process serves to create a ‘negative’, or inverse replica, of the master mold pattern. Typically, a soft elastomer polymer known as poly(dimethyl siloxane) (PDMS) is cured on the master mold and peeled away to reveal the replica pattern on its surface. Other photo-curable polymers may be used such as polyurethane acrylates (54). Replicating from the ‘negative’ mold recreates the original pattern. Soft lithography possesses great utility due to the fact that the original master mold and subsequent molds can be used multiple times.

There exist other fabrication techniques that can be used to fabricate micron-sized drug carriers and are discussed in the following reviews (23, 55).

1.3.3 Micro/nanofabrication materials

Minimizing cell toxicity is an important consideration in the fabrication of oral drug carriers. Therefore, the material that is used to fabricate the particle or device should be non-toxic to cells. There are two main categories of materials used for micro/nanofabrication: (i) traditional microfabrication materials and (ii) biocompatible materials.

Traditional microfabrication materials used in the semiconductor industry comprise of silicon-based materials and polymeric photoresists. Silicon and silicon-based materials, such as silicon dioxide, are typically used as a microelectromechanical system (MEMS). In a comprehensive analysis of silicon, silicon dioxide, and silicon nitride, Voskerician et al. implanted these materials into rats and assessed their biocompatibility over several weeks (56). Inflammatory exudate concentrations were similar to control animals, indicating that the materials were tolerated. In addition to silicon, researchers have investigated an epoxy-based polymer known as SU-8, a common photoresist used in microfabrication. Multiple groups have used this material as a delivery vehicle and even an implantable agent (57, 58). Nemani et al. showed that SU-8 implanted in mice had minimal inflammation and low levels of immune system activation. Given that the time scale for drug delivery is orders of magnitude shorter compared to implantation, any potential toxicity is expected to be further minimized. However, SU-8 is currently not FDA-approved as a bulk material and more extensive studies would be required to assess its potential as a drug carrier.

In addition, microdevice fabrication have shifted towards the use of already well-established biocompatible materials, which comprise of relatively non-toxic polymers. One such polymer is poly(methyl methacrylate) (PMMA), which is an FDA-approved material commonly used in bone cement and contact lenses (59). It is already widely utilized as a photoresist in electron-beam lithography and has been used extensively to fabricate planar, low-aspect ratio microdevice bodies (60-62). Other biocompatible
polymers include poly(lactic-co-glycolic) acid (PLGA), poly(ethylene glycol) (PEG), polycaprolactone (PCL), chitosan, and gelatin. These materials are either derived from existing natural materials (i.e. chitosan and gelatin) or synthetically made (i.e. PLGA and PEG) and possess a positive track record of use in animals and humans to demonstrate their biocompatibility (63-66). Beyond using these materials as drug carriers, these polymers can be used for promoting adhesion, regulating drug release, and/or enhancing drug permeation and will be mentioned in detail in later sections.

1.3.4 Rationale for planar, asymmetric drug-releasing devices

Spherical particles release drug omni-directionally, which is inefficient and leads to drug waste. More recent designs have used cylinders with flat surfaces that can increase surface area contact with the GI wall, promoting improved adhesion, while possessing a drug reservoir that releases in one direction (67-69). This unidirectional drug release, if facing intestinal enterocytes, can enhance local drug concentration and improve bioavailability compared to omni-directional particles or bolus dose. By combining targeting moieties or a ‘rough’ nanostructured surface, it is possible to increase the probability in which the devices will face the enterocytes (70, 71). Finally, devices can be fabricated with a very low-aspect ratio body. This promotes geometry-mediated adhesion while decreasing the shear force per mass to improve residence time of the microdevices (69). This will be further discussed in the following section.

1.4 STRATEGIES TO IMPROVE MICRO/NANOFABRICATED ORAL DRUG DELIVERY ADHESION

1.4.1 Rationale for promoting adhesion

One particular interaction that is advantageous for oral drug delivery is bioadhesion, as adhesion to GI tract enhances drug uptake by (i) prolonging device residence time and drug exposure and (ii) allowing for release of drug in high concentrations proximally to epithelial tissue for enhanced permeation effects (20, 21). Drug delivery platforms can be fabricated to adhere to the lining of the GI tract via geometric, mechanical, biochemical, nanotopographical and/or motion-based approaches (72, 73).

1.4.2 Geometry-mediated adhesion

Microscale drug delivery systems are capable of enhanced adhesion over macroscale drug delivery systems as a result of their high surface-area-to-mass ratio and ability to become entrapped within the microscale villi (6). Geometry-based approaches can further promote device adhesion by utilizing a flat or planar device shape that is typical of microdevices for oral drug delivery (21, 60, 69, 71, 74-76). As shown in Fig. 1-3, a planar geometry promotes adhesion by (i) increasing the contact area available for interaction with the epithelial lining of the GI tract and (ii) decreasing the force exerted on the devices from the fluid flow in the GI tract (20, 21). Furthermore, microdevices can be fabricated asymmetrically with a drug reservoir on only one side of the device, providing unidirectional drug release to create a steep concentration gradient to increase drug permeation. Tao et al. investigated the effect of device geometry on adhesion by incubating planar, asymmetric devices with dimensions of 150 × 150 × 5 µm over a
monolayer of Caco-2 intestinal epithelial cells and exposing the devices to multiple washing steps (74). After washing, 68% of the planar microdevices remained adhered while 17% of PMMA microspheres of similar surface area remained adhered. When loaded with the model drug fluorescein and added to a Caco-2 monolayer under flow conditions, these devices increased permeation of drug 10-fold over that of a bolus dose (75). Furthermore, Chirra et al. demonstrated the effect of planar device geometry on adhesion in vivo (69). When PMMA microdevices 200 µm in diameter and 8 µm in thickness were administered to mice, they showed 27% retention in the proximal small intestine after 2 hours while PMMA microspheres of similar surface area demonstrated 12% retention. When loaded with drug, the planar PMMA microdevices provided a four-fold increase in oral bioavailability of acyclovir, a Biopharmaceutics Classification System (BCS) class III poorly permeable drug, relative to that of a bolus dose.

1.4.3 Biochemical surface modifications to enhance adhesion

In addition to providing geometry-mediated enhancement in bioadhesion, micro and nanofabricated oral drug delivery platforms can be surface modified with bioadhesive compounds to promote adhesion. Microdevices are typically fabricated on a silicon wafer or other substrates, facilitating asymmetric functionalization of exposed device regions (22). This asymmetric surface modification can be used to promote binding of the drug-releasing side of the device, providing unidirectional drug release toward epithelial tissue (20, 21). Lectins, carbohydrate-binding proteins capable of binding to glycosylated proteins and cell membrane components to provide mucocytadhesion (14), have been functionalized onto drug delivery systems to promote adhesion to the lining of the GI tract (6, 69, 71, 74, 77-79). PMMA microdevices modified with tomato lectin, which binds selectively to the epithelium of the small intestine (80), demonstrated 92 ± 4% retention in an in vitro Caco-2 adhesion assay, whereas devices lacking modification showed 29 ± 9% retention (71). In vivo, lectin-conjugated PMMA microdevices showed 41% retention in the proximal small intestine of mice two hours following oral administration as opposed to 27% for bare devices (69). Biochemical adhesion utilizing high-affinity interactions between a targeting ligand and specific moieties can provide highly specific binding to the small intestine or diseased tissue. However, one drawback to the use of biomolecules and other surface modifications to promote adhesion is degradation as a result of the low pH of the stomach and proteolytic and metabolic enzymes throughout the GI tract (81). Therefore, molecular stability must be considered for surface modification of oral drug delivery platforms.

1.4.4 Micro- and nanotopography-mediated adhesion

Topography-mediated adhesion presents an alternative approach to promote bioadhesion that is dependent upon geometry rather than degradable surface modifications. By increasing surface area, micro- and nanofeatures increase the interfacial surface adhesion (82-85). Cylindrical pills coated with microneedles designed for physical penetration of epithelial tissue to increase drug permeation are also likely to provide the additional benefit of increased adhesion to the GI tract (86). As with asymmetric surface functionalization, asymmetric topographical modifications have potential to promote unidirectional drug release toward epithelial tissue. In an example of hierarchical microdevice structure, multi-layer fabrication was employed to modify one
surface of 150 × 150 µm microdevices with microposts 10 µm in diameter (87). While not yet applied to oral drug delivery, this approach has potential to combine the benefits of asymmetric, planar microdevices with nanowire-mediated adhesion while utilizing polymers with FDA approval for medical applications (88, 89).

1.4.5 Mucoadhesive bulk materials

As an alternative to surface modification, a number of mucoadhesive materials including alginate (90, 91), chitosan and chitosan derivatives (92-94), hyaluronic acid (95, 96), gelatin (97, 98), cellulose derivatives (99, 100), and a number of synthetic polymers (101) are available for use as a bulk material in fabrication of oral drug delivery systems. Among these materials, chitosan has been highly utilized in a number of oral drug delivery systems, including chitosan-based micro- and nanoparticulate drug delivery systems (102-107), chitosan-drug conjugates (108, 109), and chitosan macroscale patches (110, 111). Chitosan is an attractive material for micro/nanofabricated platforms as it is compatible with a number of microfabrication approaches (112, 113), is stable through pH values relevant to GI physiology (114), and has been utilized in microfabricated oral drug delivery systems (115, 116).

1.5 LOADING OF MICRO- AND NANOSCALE ORAL DRUG DELIVERY PLATFORMS

Ideally, the loading of drugs into carrier systems should be (i) efficient, minimizing the amount of drug wasted during the loading process, (ii) high-throughput, allowing for scalability in production, and (iii) able to maintain drug integrity under loading and storage conditions. The versatility of using polymers in combination with MEMS technology has led to several innovative ideas of loading novel drug delivery carriers for controlled release applications (117-119). Most of these innovative carriers load and release drugs using photolithography, inkjet printing, or capillary action.

1.5.1 Photolithography

The most common and successful MEMS technique to load miniaturized carriers with a variety of drugs is by using photolithography. Photolithographic crosslinking of polymers in the presence of a photoinitiator proves useful in tailoring specific material properties such as hydrophobicity, biodegradability, and biocompatibility that play a role in drug release kinetics, cellular interaction, and immunogenicity. These properties can also be modified by varying the chemical structure/functionality of the monomer used, molecular weight, and/or crosslinking density (72, 115, 120, 121). The process involves spin casting a photoinitiator mixed pre-polymer solution as shown in Fig. 1-4A. Polymerization is then carried out through the localization of light using an appropriate photomask. The drug is loaded inside the polymer matrix either by mixing it with the pre-polymer solution or via responsive swelling-diffusion-collapse method (122-125). While spin casting is rapid in loading microreservoirs with drug solutions, a significant amount of drug is lost during the spin-casting and development steps, which makes the platform not viable for expensive drugs. Furthermore, drugs loaded by photolithographic approaches must be able to remain intact following UV exposure.
1.5.2 Inkjet printing

A more precise low-wastage drug loading method can be achieved by individually loading each reservoir with the appropriate volume of drug-polymer solution. Boisen and co-workers recently employed an inkjet printer to load a drug solution into microdevice reservoirs (68, 126), which is a quasi-no-waste performance technique as shown in Fig. 1-4B. They also loaded microdevice reservoirs with hydrophobic drugs in the absence of toxic organics by incorporating supercritical fluid impregnation with inkjet printing (127). In this method, well defined quantities of poly(vinyl pyrolidone) (PVP) were dispensed into microcontainers/microreservoirs. Then the poorly soluble drug ketoprofen was impregnated into the polymer matrix using supercritical carbon dioxide as the loading medium. The amount of drug loaded or dosage achieved in microcontainers is tuned by varying the impregnation parameters. Compared to solid dispersions of the same drug, supercritical impregnated microdevices exhibit more reproducible drug loading and faster dissolution of drug, which allows for the modulation of drug release. While this method is capable of precise, relatively zero-waste performance, the sequential loading of each microdevice in a semi-automated manner makes it a low-throughput technique relative to spin casting.

Simpler techniques for loading drugs into reservoirs have been utilized to overcome the low-throughput issue associated with microinjection/inkjet printing and high-wastage issue involved with spin casting. Nielsen et al. used a modified screen printing technique that involves the use of a stencil mask pre-fabricated by laser machining that aligns on top of microdevices for accessing the vacant reservoirs (128). The stencil has pores/holes matching the microdevice reservoirs with a high level of precision. Once the stencil is aligned using an optical microscope, powdered amorphous drug is pressed through the stencil into the microreservoirs (Fig. 1-4C). Any excess powdered drug not located within the microwells is removed along with the stencil and is reused for loading more devices, thereby reducing drug waste. An alternative wet loading technique developed by Guan and co-workers uses a discontinuous de-wetting technique to collect and crystallize model drugs via solvent evaporation into device reservoirs to provide a high throughput drug loading method (115).

1.5.3 Other approaches

A similar high-throughput and rapid approach to loading microdevices with drug is achieved by harnessing the phenomenon of capillary action. Several researchers have fabricated nanowire-coated oral microdevices and used the high surface area of nanowires to effectively load both aqueous and non-aqueous drugs via capillary action. After solvent evaporation, the drug crystallizes over the microdevice surface at the base of the nanowires (Fig. 1-4D) (61, 129). Because solvents of drug solutions can evaporate in a manner of minutes with >90% of drug collecting over nanowire-coated microdevices, loading via capillary action is efficient in both throughput and minimizing drug waste. However, because the drug is surface-loaded rather than loaded within a matrix with tunable properties, this approach may present challenges in tuning release rates.

A more controlled approach to loading drugs into oral devices involves using modified soft lithography techniques. Micromolding in capillaries (MIMIC) uses a PDMS mold that comes into contact with the substrate surface. A low-viscosity prepolymer
solution is then placed at the open end of the channel, wherein the solution is transferred to specific locations on the substrate by fluid flow or capillary action (130, 131). A modified version of MIMIC is currently being studied to load drugs into microdevice reservoirs. Lee et al. loaded small amounts of model drugs methylene blue and tetracycline hydrochloride into microreservoirs using wet microcontact printing method (132). In this process, a liquid drug-carrier solvent mixture was transferred to the reservoir by contact printing process as shown in Fig. 1-4E. By prolonging the time of contact printing in the presence of a non-volatile drug carrier solvent such as PEG, a higher dosage of drug was loaded into the microreservoirs. Although research on loading drugs into oral drug delivery microplatforms is at its relative inception, well established semi-conductor industry fabrication techniques combined with polymer technology is bound to improve the mass fabrication of novel microplatforms loaded with a variety of oral drugs.

1.6 INCORPORATION OF TUNABLE AND BIORESPONSIVE SMART MATERIALS FOR SUSTAINED AND CONTROLLED DRUG RELEASE

Ideally, oral administration should deliver drug to a specific target of the intestine at the required concentration within the therapeutic window. In addition, the drug should be delivered at the right time in a safe and reproducible manner. Currently, with a variety of loading techniques available for introduction of drugs into oral microplatforms, the future of oral microdevices involves the appropriate selection of polymer systems and other materials that can be tuned to modulate the release kinetics of entrapped drug. The development of such devices is made possible through use of different polymeric matrices that entrap the drugs of interest. Polymers have varying chain length, hydrophilicity, and ionic charge, granting ample control when tailoring drug release kinetics. Additionally, advances in the field of polymer science have fostered the design and preparation of polymers with sensitivity to pH, temperature, and a variety of additional stimuli, which confers added specificity. Use of these materials would allow for targeted and independent release of multiple drugs, resulting in the widespread application of miniaturized oral platforms that release a wide array of drugs including sensitive drugs and macromolecules that are currently administered non-orally.

1.6.1 Swellable polymers

The first systems developed for oral drug delivery were based on hydrophilic polymers that swelled in the presence of water to allow release of drug (133, 134). Such systems were appealing due to previously demonstrated material biocompatibility and FDA approval, and as such, currently form the bulk of oral drug delivery strategies. However, a variety of complications limit the effectiveness of these systems for oral drug delivery. Due to the swelling-based mechanism of drug release, these systems often experience an initial burst release of drug before the drug delivery system reaches its intended site of action. Additionally, such systems are susceptible to bulk degradation by hydrolysis, altering drug release kinetics and creating another challenge in controlling drug release. The use of hydrophobic materials circumvents several of these issues, as they primarily degrade via surface erosion, maintaining drug release kinetics (133). However, poor efficacy due to limited specificity and low bioavailability remain significant challenges in these systems.
1.6.2 Stimuli-responsive materials

Stimuli-responsive materials are promising for controlled drug release because they specifically regulate drug release by actively sensing and responding to external conditions. When exposed to a specific stimulus, including temperature, pH, light, electric field, magnetic field, ultrasound and binding of biomolecules (135-137), these materials undergo conformational changes that alter their hydrophilicity, affecting bulk matrix properties that modify drug release. Of these, pH- and enzyme-sensitive systems are the most relevant for oral drug delivery, as they detect and respond to signals along the GI tract without the need for external intervention.

pH-responsive hydrogels are a class of stimuli-responsive systems that have great potential in oral drug delivery due to dynamic changes in pH along the GI tract. Researchers have taken advantage of this variability in pH to design drug delivery systems that release therapeutic payloads specifically in the stomach, intestine, or colon. These systems are generally made of synthetic polyacids or polybases (138-141), although naturally occurring pH-sensitive polymers (e.g., alginate, chitosan, hyaluronan) have also been investigated (135, 136, 138-141). Of particular note is a class of polymers known commercially as Eudragit. Eudragit is a copolymer made of methyl methacrylate and ethyl acrylate as is primarily used as an enteric coating. By varying the carboxylic groups, it is possible to control the dissolution pH to 5-7 (142). Eudragit polymers are primarily used as pharmaceutical pill coatings and matrix formulation.

Hydrogels that swell/shrink in response to a biological cue have also been developed. For example, glucose-responsive hydrogels containing phenylboronic acid or concanavalin A (ConA) are able to bind glucose and swell to release entrapped insulin (143). These moieties generate a closed-feedback loop that senses and responds to the demands of the environment. While these systems are appealing, such biological interactions are not always easily found and incorporated into drug delivery systems. To this end, antibody- and aptamer-containing hydrogels have been engineered to respond to a multitude of biological stimuli (144, 145).

1.6.3 Incorporating “smart” polymers into micro- and nanofabricated oral drug delivery systems

While many of these materials are promising for oral drug delivery, relatively few have been used in combination with micro- and nanofabrication technologies to combine the chemical and biological responsiveness with micro/nanotopography and other forms of geometric engineering. By incorporating these “smart” materials into these microscale devices, it may be possible to tailor the extent and specificity of drug loading and drug release for oral drug administration. The most facile method for achieving this is to use a matrix system where drugs of interest are entrapped in these materials and then tune the release rates at the relevant physiological conditions. Additionally, it is possible to use a reservoir system where these polymers can be used as a cap that will either control diffusion for the lifetime of the device or block drug release until it is dissolved in the presence of low pH or gastrointestinal enzymes. This ability to design microdevices on a molecular scale as well as on the micro- and nanoscale enables precise control over where and how drugs are delivered to the GI tract, suggesting the great potential of microdevices as oral drug delivery platforms.
1.7 EPITHELIAL PERMEATION ENHANCEMENT

1.7.1 Advantages and risks of epithelial permeation enhancement

While polymer chemistry allows us to tailor drug release kinetics with significant control, there remain barriers to drug uptake once the drug leaves the microdevices, such as the existence of a thick mucus layer and the presence of tight junction proteins between epithelial cells. In order to address the physiological barriers posed by the intestinal epithelial layer, chemical and nanotopographical permeation enhancers have been explored in the field of drug delivery. Permeation enhancers temporarily disturb epithelial tightness, allowing greater transepithelial transport of therapeutics. With proper use, permeation enhancers can greatly increase bioavailability of orally administered drugs and allow more efficient delivery of therapeutics. However, since a normal epithelial layer serves as a natural barrier to harmful foreign agents, disruption of the membrane may have potentially dangerous side effects. Therefore, it is important to ensure that the effects of permeation enhancers are reversible and do not cause any permanent cellular damage.

1.7.2 Mechanisms of permeation enhancement

While many different mechanisms may be utilized to achieve increased permeation, one of the most common modes by which permeation enhancers function is through modulation of tight junctions. Tight junctional complexes greatly inhibit paracellular drug permeation, lowering bioavailability, and often serve as the rate-limiting barrier to various hydrophilic, large therapeutic agents. Another common mode of permeation enhancement includes modulation of transcellular drug transport by disruption of the cell membrane using excipients, such as fatty acids. In addition, some studies have utilized efflux inhibitors in order to reduce efflux of therapeutics by membrane transporters, such as p-glycoprotein (146-148). We refer to the reviews by Aungst (149, 150) for more detailed information regarding various types of permeation enhancers that have been used as excipients in clinical trials as well as their mechanism of action.

1.7.3 Co-delivery of chemical permeation enhancers

Drug release profiles of co-delivered drugs from micro/nanofabricated drug delivery systems can be individually tailored to facilitate optimal chemical permeation enhancement. For example, as mentioned earlier, work by Chirra et al. enabled co-delivery of several therapeutics by loading them into multi-reservoir bioadhesive microdevices (60). Using this approach, permeation enhancers described above, such as efflux inhibitors, can be co-delivered with the therapeutic drug of interest in order to provide localized permeation enhancement at the site of delivery. This localized co-delivery of permeation enhancer may potentially reduce side effects associated with universal enhancement of transepithelial permeation along the intestinal lining. In addition, since the release of drugs from each reservoir is independent of each other, this technology allows the therapeutic to be delivered at a different rate than the permeation enhancer, potentially providing epithelial disruption prior to the release of the bulk of the therapeutic drug. This approach can also be used with the design of multilayered polymeric microdevice by Ainslie et al. (76). Their study showed that their microdevices
can provide simultaneous, unidirectional release of multiple therapeutics as well as achieve a significant increase in transepithelial permeation. By adding a layer that simultaneously releases a permeation enhancer along with the therapeutic of interest, localized permeation enhancement and consequent reduction in side effects may be possible.

1.7.4 Topographical permeation enhancement

Similarly to a subset of chemical permeation enhancers that rely on perturbation of tight junctional proteins, micro- and nanotopography have been shown to achieve tight junctional reorganization, which resulted in enhanced transepithelial permeability in cell culture models (129, 151, 152). For instance, studies by Uskokovic et al showed that silica microparticles covered with PEGylated silicon nanowires are able to increase transport of fluorescein-Na across a layer of Caco-2 cells compared to bare particles. Moreover, the nanowire-coated particles were able to decrease transepithelial electrical resistance (TEER), which indicates epithelial tightness, to a greater extent than bare particles when tested on Caco-2 cells (151). This platform has further advanced to the form of planar nanowired microdevices that are made with biodegradable polymers for enhanced biocompatibility in oral drug delivery applications (61).

Additional studies have focused on the role of nanostructured polymeric films in increasing transepithelial transport (152). Unlike the nanoengineered microparticles described above, this study focused on the effects of nanotopography in the form of nanostructured thin films that were relatively large (>2 mm in dimension) compared to the microparticles. Nanostructured polypropylene thin films were fabricated using nanoimprint lithography and placed on top of Caco-2 cells that were cultured in transwell inserts. In line with work by Uskokovic et al (151), this study showed a significant decrease in TEER with nanostructure contact, which was reversible upon removal of the film. As mentioned earlier, the transient nature of TEER reduction upon nanostructure contact is noteworthy, as it is important to ensure lack of long-term disruption of the epithelial layer. The reversibility of tight junction modulation was also evident in immunohistochemical staining images, which show ruffled ZO-1 pattern upon nanostructure contact, which was reversible upon removal of the film. Moreover, the study noted a significant increase in transport of large molecules, such as etanercept (MW = 150 kDa), across a Caco-2 layer upon contact with nanostructured films. While its underlying mechanism has yet been fully elucidated, the notable increase in transport is thought to be due to active modulation of tight junctional complexes by formation of focal adhesion complexes at the site of nanostructure-epithelial cell contact (152, 153).

1.8 CONCLUSION

The GI tract presents a complex set of physiological barriers that limit drug uptake. Micro- and nanotechnology provide flexibility in microdevice design, allowing for fabrication of drug delivery platforms that specifically address these barriers. The efficacy of micro/nanofabricated oral drug delivery systems may be enhanced by incorporating (i) tunable and/or responsive drug reservoir polymers for targeted release of intact drug, (ii) adhesive polymers, surface modifications, and topographies to enhance adhesion, and (iii) chemical and topographical permeation enhancers to increase drug permeability. With
recent success in vivo, these technologies show promise for clinical trials. However, many of the top-down approaches used to fabricate these platforms for proof of concept are low-throughput and expensive relative to bottom-up fabrication techniques. To scale these technologies to the clinic, efficient, low-cost fabrication and drug loading approaches are being developed. Furthermore, to maximize cost-efficiency, these platforms may be used with highly potent drugs to minimize the number of devices required per dosage. This dissertation work focuses on developing novel micro- and nanofabrication approaches to create oral drug delivery systems that utilize and combine smart materials, bioadhesive functionalization, nanotopography, planar shape, and asymmetric design to address the many barriers to oral drug uptake.
1.9 REFERENCES


1.10 LIST OF FIGURES

Figure 1-1 Schematic representing the routes of drug transport through the physiological barriers of the small intestine. Drug molecules can be transported passively via paracellular or transcellular diffusion or actively via receptor-mediated endocytosis and carrier-mediated transport.
Figure 1-2 Schematic of the photolithography process. (A) Photoresist is deposited onto a silicon wafer and baked. (B) A mask covers the photoresist and radiation (i.e. ultraviolet light, ion-beam, electron-beam, etc.) exposes the uncovered regions. The photoresist is then developed and removed. Depending on the photoresist, the region exposed is (C) degraded for positive photoresist lithography or (D) cross-linked for negative photoresist lithography.
In contrast to spherical microparticles, planar, asymmetric microdevices provide proximal, unidirectional drug release and increased residence time in the GI tract. A planar microdevice shape reduces the force experienced from intestinal fluid flow (blue arrows) and increases surface area available for binding to epithelial tissue, increasing device adhesion to the lining of the GI tract and prolonging drug exposure. Devices can be asymmetrically fabricated with a drug reservoir on one side of the device, allowing for proximal, unidirectional release of drug (green) toward epithelial tissue.
Figure 1-4 Schematic representation of loading drugs into microdevice platforms. (A) Rapid loading of reservoirs via spin casting followed by drug entrapment inside a photopolymerized polymer matrix. (B) Precise loading of microreservoirs using microinjection/inkjet printing and then entrapping the drug within the reservoir via (i) introduction of a cap layer on top or (ii) supercritical impregnation. (C) A high-throughput screen printing method involves the use of a transparent stencil that aligns with the microdevices to the reservoirs. (D) Drug loading via capillary action of drug solution during solvent evaporation. (E) Novel soft lithography methods of wet microcontact printing or microtransfer printing to load drugs into multiple devices simultaneously.
CHAPTER 2: PICOLITER-VOLUME INKJET PRINTING INTO PLANAR MICRODEVICE RESERVOIRS FOR LOW-WASTE, HIGH-CAPACITY DRUG LOADING
2.1 INTRODUCTION

Oral drug administration is less invasive and has better patient compliance than parenteral methods making it the preferred route for both patients and physicians. However, there are significant physiological barriers present in the gastrointestinal (GI) tract, which lower the bioavailability of many drugs. The low pH of the stomach and intestinal digestive enzymes cause drug breakdown before absorption (1, 2). Furthermore, many drugs possess low solubility and/or low permeation through the epithelial mucosa of the small intestine, further reducing bioavailability of many drugs. Thus, the vast majority of biological therapeutics currently require parenteral administration due to their high molecular weights and low stability (3).

Over the past decade, patches, hydrogels, and micro- and nanoparticles have been explored as oral drug carriers (4-6). However, issues with polydispersity and drug dosing have limited the translation of these delivery methods. Previous work in our lab has demonstrated that polymeric, micron-scale planar devices, henceforth referred to as microdevices, are capable of enhanced adhesion in the GI tract due to their high surface-area-to-mass ratio and flat shape that minimizes the amount of shear stress from intestinal fluid flow (7). Additionally, a drug reservoir can be fabricated on one side of the disc-shaped microdevice to further improve drug bioavailability by providing unidirectional release rather than the omni-directional release of drug from conventional microparticles. The presence of a reservoir can protect drug payload from the harsh microenvironment of the GI tract until release is desired. The benefits of microdevice delivery have been previously demonstrated in vivo with acyclovir where the bioavailability was observed to be 4.5-fold higher in mice when administered in microdevices relative to a bolus dose of oral solution (8). These devices can be manufactured en masse in a reproducible manner by taking advantage of well-established microfabrication techniques. The advantages of microdevices make this technology an attractive candidate for oral drug delivery of pharmaceuticals.

While microdevices are a promising technology, loading drug into the micron-scale reservoirs of these devices in an efficient manner remains challenging. Traditional oral dosage requires large amounts of drug, which can be cost prohibitive. Previously, microdevices have been loaded by spin-casting a drug-hydrogel solution over the microdevices and then selectively crosslinking the solution within device reservoirs by ultraviolet light (UV) exposure (8). However, drug loading efficiency is decreased due to losses during the spin-casting step, and the volume occupied by the hydrogel reduces the drug capacity of the devices. Furthermore, UV light can damage photosensitive molecules and lead to degradation of the active compound. More recently, Fox et al. demonstrated the use of nanostraw membranes in order to passively take up drug into microdevice reservoirs via diffusion (9). Unfortunately, this method requires the use of concentrated drug solution that is usually discarded after loading, thus leading to waste, and drug loading capacity is limited to the product of the drug solubility and microdevice reservoir volume. Therefore, alternative loading methods need to be considered.

Inkjet printing is a technique that has been used for microarray spotting, surface functionalization, cell culturing, and drug formulation (10-13). A major advantage of inkjet printing is its drop-on-demand mode, which allows for spotting of precise volumes of liquid onto a surface. Previously, it has been shown that small-volume dispensing systems can
be used to print polymer solutions into micro-scale containers (14, 15). When printing drugs, the solutions ideally would not contain polymer to maximize the free volume available for drug. These previous methods were used for tall micro-containers measuring over 250 µm in height with aspect ratios typically >1 (14, 15). However, planar microdevices for oral drug delivery are typically designed with heights <10 µm and aspect ratios <0.1 (9, 16). To our knowledge, this method has not been used with thin (<10 µm thickness) microdevices, which typically have reservoir volumes in the tens of pL range rather than the ≥500 pL volume reservoirs previously utilized for inkjet printing (7, 14).

Beyond drug loading, it is important to protect the drug payload and ensure that it is delivered to the correct location. If the site of delivery is the small intestine, any release to an outside region such as the stomach or large intestine is detrimental for drug bioavailability as the drug is degraded and/or unable to pass through the epithelial cell lining and into the bloodstream for absorption (17). Furthermore, it is important to have control over the release kinetics. Depending on the drug candidate, burst release to slower, zero-order release is necessary to achieve the desired therapeutic window.

Chitosan is a material that has been explored in oral drug delivery (18). It is derived from chitin, a polysaccharide commonly found in fungi and arthropods. One advantage chitosan possesses is the ability to preferentially bind to the intestinal mucus layer due to its inherent positive charge, which can enhance retention time in the GI tract. Second, chitosan coatings and particles have been utilized to load peptides and proteins and stabilize them for delivery (19). The ability to both encapsulate and protect drug while controlling drug release makes chitosan an intriguing material for oral drug delivery.

In this study, we demonstrate the use of picoliter-volume inkjet printing to efficiently load planar microdevices with topotecan, a small molecule chemotherapeutic agent, and insulin, a peptide hormone. Both drug solutions were prepared in acidic solutions and were directly spotted into the reservoirs of each microdevice with high precision and accuracy in an automated fashion. We also introduce a simple surface modification step to improve the surface hydrophobicity of the microdevices, ensuring reservoir loading without overflow. We demonstrate that microdevices that can be loaded with nearly 100% of the free total volume space filled with both small molecule and biological therapeutics in an essentially zero-waste manner. Finally, we cap the insulin-loaded microdevices with chitosan and show controlled release over the course of multiple hours. The pharmacokinetics of insulin release from chitosan-capped microdevices was investigated using Caco-2 cells to model the epithelium of the gastrointestinal tract.

2.2 MATERIALS AND METHODS

2.2.1 Materials for device fabrication and drug loading

Topotecan hydrochloride, insulin (recombinant human), hydrochloric acid (HCl), acetic acid, trichloro(1H,1H,2H,2H-perfluorooctyl)silane, simulated intestinal fluid (SIF), Hank’s balanced salt solution (HBSS), human insulin ELISA kit, and chitosan (low Mw) were purchased from Sigma-Aldrich, USA. Poly(methyl methacrylate) (950 kDa in anisole), Shipley 1818 positive photoresist, Microposit 351 developer, and 1112A photoresist remover were purchased from MicroChem, USA. CyQUANT direct cell proliferation assay kit and micro BSA assay kit was purchased from Thermo Fisher Scientific, USA. Silicon
wafers were purchased from Addison Engineering Inc, USA. Caco-2 cells were purchased from ATCC, USA.

2.2.2 Microdevice fabrication

Microdevices with reservoirs were fabricated as previously described (9). Briefly, a silicon wafer was spin-coated with poly(methyl methacrylate) (PMMA) followed by a baking step. The PMMA layer was spin-coated with positive photoresist followed with another baking step. The wafer was then exposed to UV light through a photomask to form the device body features. The exposed wafer was developed and post-baked. The PMMA surrounding the masked region was dry etched with oxygen plasma in a Surface Technology PE1000 AC Plasma Source Reactive Ion Etcher. Remaining photoresist was removed by incubation in photoresist remover. To fabricate the reservoirs, the microdevice bodies were spin-coated with photoresist followed by UV exposure through a second photomask with a reservoir pattern aligned over the microdevice bodies. The microdevices were then developed and dry etched, and excess photoresist was removed to form microdevices with reservoirs. The final microdevices were disc-shaped, 200 µm in diameter and 8 µm in height. Each device contained a central reservoir 100 µm in diameter and 5.5 µm in depth, corresponding to a volume of 43.2 pL. Each 3-inch silicon wafer contained a 4 × 4 array of 20 × 20 microdevice subarrays for a total device count of 6400.

2.2.3 Microdevice surface modification

Prior to drug printing, the silicon wafer with fabricated microdevices was silanized with trichloro(1H,1H,2H,2H-perfluorooctyl)silane via vapor deposition under vacuum at room temperature for 30 min. Wafers were printed the same day as silanization.

2.2.4 Drug loading into microdevices

Drug printing was performed using the sciFLEXARRAYER S3 (Scienion AG, Johannisthal, Germany) (Fig. 2-1). This system is an automated picoliter drop-on-demand piezoelectric printing system suitable for deposition of 50-800 pL drops at up to 1000 Hz with positional accuracy of ± 20 µm. The printer is composed of an XYZ movable head with a mounted piezo-driven dispenser. Topotecan and insulin solutions were prepared on the day of printing at 10 mg/mL in 10 mM HCl and filtered through a 100 kDa centrifuge tube for 10 min at 5000 RCF. The freshly prepared drug solutions were then loaded into a microtiter plate. Prior to printing, drug solution was aspirated into the dispenser nozzle. During setup, the printer’s control unit was aligned to the fiducaries on the silicon wafer, which enabled programmable automatic dispensing. The printer is equipped with a camera to visualize drop volume, stability, and trajectory, which can be adjusted by changing the piezo voltage, pulse width, and frequency. These parameters were optimized to obtain drops with volumes of ~400 pL. Single 400 pL drops of either drug solution were printed into device reservoirs at a rate of ~400 devices per minute. The printing process was performed in multiple cycles to allow the solvent to completely evaporate between each cycle, preventing solution spillover.
2.2.5 Scanning electron microscopy

Samples were prepared for SEM by sputter coating with 8 nm of gold followed by mounting onto carbon tape. The samples were imaged with a Carl Zeiss Ultra 55 field emission scanning electron microscope.

2.2.6 Cellular toxicity studies

To determine the cytotoxicity of silanized microdevices, samples were analyzed using a proliferation assay from the CyQUANT direct cell proliferation assay kit. Prior to use, drug loaded microdevices were stored desiccated at 4°C. Caco-2 cells were grown to confluency in 12-well tissue culture plates. All media was aspirated from wells and one of the following was added in triplicate: media containing 400 silanized and insulin-filled microdevices, media only, or 20% DMSO in media. Microdevices were scraped from the wafer with a razor and added to the media. The microdevices sunk in the media to come into direct contact with cells. The cells were then incubated at 37°C, 5% CO₂ for 4 hours. At the end of the incubation, cells were trypsinized and spun down to pellets via centrifugation. Trypsinizing and pelleting the cells allowed for collection of any cells that had lost adherence due to death or damage, which increased the sensitivity of the assay. The supernatant was discarded, and the cells were resuspended in PBS. Detection reagent with background suppressor was then added to each tube. The samples were incubated at 37°C for one hour and then plated in a 96-well plate in triplicate. Fluorescence of samples was measured using a spectrophotometer with cells in suspension.

2.2.7 Insulin stability assay

Microdevices were printed with 12 layers of insulin as previously outlined and stored under desiccated conditions at 4°C until stability analysis (within 1 week). Immediately before analysis, insulin was extracted from two sub-arrays containing a total of 800 microdevices by incubating in 10 mL 0.1 N HCl at 4°C for 1 hour. Samples were then analyzed with reverse-phase high-pressure liquid chromatography (HPLC). For each sample, 200 μL were injected into a 50 × 2 mm Proto 200 5 μm C18 column (Higgins Analytical, U.S.A.). A linear gradient from 30% to 35% 0.1% (v/v) trifluoroacetic acid (TFA) in water: 0.08% TFA in acetonitrile was applied over 10 minutes at a flow rate of 0.7 mL/min. Insulin and insulin degradation products were detected over time my measuring UV absorbance at 214 nm. Insulin stability was calculated as the ratio of the area of the known insulin peak to the total area of all detected peaks.

2.2.8 Chitosan capping of microdevices and PK in vitro studies

Chitosan caps were fabricated via imprinting. Chitosan was dissolved in a 2.5% (v/v) acetic acid to form a 3% (w/v) solution. The solution was then filtered using centrifugation at 20,000 RPM for 1 h. Two mL of chitosan solution was deposited onto a 3’ silicon wafer and was spun-cast at 1600 RPM for 2 min. An array of insulin-loaded microdevices was placed onto the chitosan solution for 20 s followed by separation. A thin layer of chitosan was imprinted onto the microdevices.
Devices were incubated in SIF at 37 °C to assess insulin release. Samples were collected at 0.25, 0.5, 1, and 2 hours. A micro BSA protein kit was used according to the manufacturer’s protocol to assess the amount of insulin release at each time point.

To assess insulin permeation in an in vitro model, Caco-2 cells were prepared as described earlier. 100 chitosan-capped microdevices were scraped from a silicon wafer and added to the apical chamber for a total insulin dose of 5.6 µg per transwell. An equivalent dose of free drug insulin was added in the apical chamber of control samples. All apical chambers were brought to a total volume of 500 mL with pH 6.5 HBSS. The basolateral chamber was filled with 500 mL of pH 7.4 HBSS. The total volume from the basolateral chamber were collected and replaced with fresh HBSS at 0.25, 0.5, 1, 2, and 4 h time points. Insulin concentrations were determined by ELISA according to the manufacturer’s protocol.

2.3 RESULTS

2.3.1 Fabrication and surface modification of microdevices

We fabricated planar microdevices 200 µm in diameter and 8 µm in height. This low aspect ratio is necessary to resist shear flow from peristalsis and increase residence time. Each microdevice possesses an inner reservoir measuring 100 µm in diameter and 5.5 µm in depth. Furthermore, the reservoir is etched into only one side of the device, allowing for unidirectional drug release toward intestinal tissue and high local drug concentration at the epithelium.

Before printing, the microdevices were silanized via vapor deposition of trichloro(1H,1H,2H,2H-perfluorooctyl)silane, a silane commonly used to render surfaces hydrophobic (20, 21). The hydrophobic surface on the microdevices allowed printed drops to collect inside the reservoir (Fig. 2-2). When loading microdevices without the silane treatment, the drops would collect outside the reservoir, which compromised loading efficiency. Following silane treatment, the drops localized into the reservoir without overflow. Silanized microdevices were used henceforth for topotecan and insulin loading.

2.3.2 Cytocompatibility of chemically-modified microdevices

To confirm that silanized microdevices do not cause cytotoxicity and are thus biocompatible, we conducted a CyQUANT assay using Caco-2 cells, a heterogeneous human epithelial colorectal adenocarcinoma cell line that is used as a model for the gastrointestinal tract (22). Here, we show that silanized, drug loaded microdevices do not significantly decrease live cell counts compared to cells incubated in media alone (Fig. 2-3). The DMSO group showed an expected decrease in cell viability. Assuming all microdevices lie flat on the cells, there is 38.1% coverage of the total area, which is much higher than would be expected in vivo. Either side of the microdevice could come into contact with the cells, so 50% of the microdevices have their silanized surface in direct contact with the cells.

2.3.3 Picoliter inkjet printing of topotecan and insulin into microdevices

As low-viscosity solutions (<10 cP) are most compatible with the printer, we sought to find suitable solvents for both drugs. Topotecan is soluble in aqueous solutions and is most
stable at low pH values (23). Insulin is not soluble at neutral pH but increases solubility in acidic solutions. Thus, we dissolved topotecan and insulin at 10 mg/mL in 10 mM HCl. The measured viscosity was found to be less than 10 cP (data not shown). A silicon wafer with silanized microdevices was then placed into the printer and aligned via image recognition of the fiducial markers on the wafer before use.

Following loading of solution into the nozzle, the size of the topotecan and insulin drops was tracked by the camera and software of the printer. Before printing, 100 drops were dispensed at 200 Hz to calculate the average volume. The average drop size was determined to be ~400 pL. Camera images taken post-printing revealed that one 400 pL drop was sufficient to fill the entirety of the microdevice reservoir (Fig. 2-4). When more than one drop was printed at a time, we observed overflow beyond the reservoir. Thus, one drop per microdevice was used during a single printing run. In order to fill the entirety of the microdevice reservoir with dry, packed drug, multiple runs were conducted with drying time between each pass.

To determine the optimal number of single drops that can be loaded into each microdevice, we systematically increased the number of drops up to 16 total drops per microdevice and characterized loading efficiency via SEM (Fig. 2-5). For both topotecan and insulin, we observed gradual filling of the reservoir with increasing cycles. Imaging revealed that approximately 10 drops was the ideal number for loading topotecan while 12 drops was ideal for insulin. Topotecan loading beyond 10 drops began to overfill the microdevice reservoir until the topotecan pellet delaminated from the reservoir. Insulin loading beyond 12 drops showed overflow, but no delamination was observed. The differences in drug loading between topotecan and insulin is likely due to the two drugs’ differing bulk densities.

2.3.4 Insulin stability post-printing

Reverse-phase HPLC was used to determine the stability of insulin printed into microdevices (Fig. 2-6). Insulin stored in microdevices showed 21.5 ± 0.3 µg per 20×20 array (of the expected 19.2 µg insulin). Differences in loading may be caused by variations in droplet size during printing. Stability, calculated as the ratio of the area of the known insulin peak to the area of all peaks, was determined to be 96.0 ± 0.6% after 28 days of storage at 4ºC, indicating that the printing approach has a limited impact on insulin integrity.

2.3.5 Chitosan capping of insulin-loaded microdevices and insulin release

We spun-cast chitosan on a separate wafer and then placed insulin-filled microdevices into contact with the chitosan for imprinting. After separating the microdevices from the chitosan, a chitosan cap was left behind on the microdevices. SEM imaging was used to assess the visual formation of the caps (Fig. 2-7A and 2-7B). Based on our imaging, it is evident that there is a cap on each microdevice.

We tested our chitosan-capped microdevices in SIF to assess how insulin was released (Fig. 2-7C). At 0.25 h, we saw approximately 10-15% of insulin release. However, at 0.5 h, the insulin release reached closer to 50% of the total drug payload. The release rate slowed down at 1 h with ~80% total insulin released. Total release was achieved at approximately 2 h.
2.3.6 Insulin permeation through an in vitro model

The next step after determining the PK profile of our chitosan-capped microdevices was to test them in an in vitro cell system that is designed to mimic the small intestine. Here, we used the Caco-2 transwell system to assess insulin permeation over the course of 4 h (Fig. 2-8A and 2-8B). The percent insulin permeation ranged from 0.15% at 0.25 h to 0.70% at 4 h for chitosan-capped microdevices and 0.05% at 0.25 h to 0.3% at 4 h for insulin bolus. This represents a 2-3-fold improvement in insulin permeation.

2.4 DISCUSSION

2.4.1 Choice of therapeutic agent

Because planar microdevices can be used for oral delivery of both small molecule drugs and biological therapeutics, drug loading techniques will ideally be compatible with both small molecules and biologics in addition to being compatible with techniques for microdevice fabrication. We chose topotecan, an inhibitor of DNA enzyme topoisomerase I used as a chemotherapeutic, as a model small molecule drug (24, 25). Orally administered chemotherapy could reduce hospital admissions or visits to outpatient infusion centers for parenteral administration. However, oral formulations require higher amounts of drug to be delivered compared to the intravenous route, which can lead to higher off-target effects and toxicity. Preparing chemotherapeutic agents in microdevice form can potentially reduce the dosage needed.

We sought to expand the utility of this platform by also examining insulin as a model biologic. Insulin is an important peptide hormone that is secreted by the beta cells in the Islets of Langerhans within the pancreas to signal for cellular glucose uptake from the bloodstream. Insulin is currently administered by multiple subcutaneous injections per day or via insulin pumps. This is not ideal due to issues such as non-compliance, cost, and tissue damage at injection sites. Oral delivery of insulin is preferable as it is a less painful administrative route and can reduce peripheral hyperinsulinemia. However, previous research investigating insulin for oral drug delivery has not been able to translate clinically due to its low stability and gastrointestinal permeability (26, 27). Microdevices loaded with a high density of insulin may potentially overcome bioavailability limitations.

The ability to load relatively large amounts of drug with minimal drug waste holds many advantages. The inkjet printer enables printing of drug directly into the reservoir and obviates the need of other methods such as spin-casting or supercritical impregnation which can result in significant drug loss and does not guarantee uniform loading conditions.

2.4.2 Microdevice toxicology and residence time

Drug delivery systems must also display a favorable toxicological profile in addition to demonstrating efficacy. Although we have tested microdevices in animal models before, this is the first time we have utilized silane deposition on microdevices. Silane in large quantities can be toxic to cells. However, previous studies using silanized nanoparticles did not show cell cytotoxicity suggesting the silanization process uses volumes small enough to be biocompatible (28). Additionally, previous work in our lab has shown these planar microdevices to have no effect on cell viability prior to drug loading (8).
Here, we used a surface deposition technique to coat the microdevices in a hydrophobic silane. The silane is not cross-linked to the surface, but the cytotoxicity data suggests that any dissociation is not toxic at these levels. If toxicity is seen in the future, the silane could be cross-linked. The results indicate that the silanized, drug loaded microdevices display negligible cytotoxicity, a necessary feature when translating to animal models.

The microdevices are not biodegradable on the time scale anticipated for residence in the gastrointestinal tract. These devices are expected to pass completely and be excreted prior to any meaningful degradation. Additionally, the turnover time of the intestinal epithelium is 2-3 days (29). Even accounting for the expected increase in residence time of the devices, they would shed with the epithelium and exit the body prior to significant degradation.

2.4.3 Scaling to clinically relevant doses

Loading drug by printing 400 pL droplets in multiple passes allowed for significantly higher loading capacity than previous loading approaches utilizing spin casting and photolithography. For example, spin-casting and UV-crosslinking a 10 mg/mL insulin hydrogel solution to fill the 43.2 pL device reservoir volume would allow for loading of 0.432 ng of insulin per device. However, with inkjet printing, twelve 400 pL drops of 10 mg/mL insulin were printed into each device for a total of 48 ng insulin per device, a >100-fold increase in loading capacity.

Topotecan is dosed based on body surface area and can be expected to be delivered in the range of 1-4 mg/day given intravenously. Oral dosing is given for specific clinical circumstances and is 3-fold higher (30). Each microdevice contains ~40 ng of topotecan, so administering a maximum oral dose of 4 mg of topotecan would be contained by 75,000 devices. Each 3-inch wafer contains 6,400 microdevices, thus approximately 15 wafers would contain the equivalent dose. Insulin dosing is approximately 0.5-1 U/kg/day divided over 3 or more subcutaneous injections (31). Thus, a 70-kg person could expect to use 35-50 U/day (1.2-1.7 mg/day). Each microdevice contains ~48 ng of insulin, so administering a similar amount of drug would be contained in 25,000-37,500 microdevices or approximately 4-6 wafers per day.

2.4.4 Insulin stability

Drug stability following loading into microdevices is a concern, and biological therapeutics such as insulin are more prone to degradation than small molecule drugs due to their large molecular weight and complexity (32). Therefore, we used HPLC to determine that our loading and release method does not cause degradation of insulin. The stability of insulin was determined to be ~96% after one-month post printing, indicating that this method can be used in a manufacturing process with little worry about insulin degradation. Other proteins, such as antibodies, will be assessed in the future for their compatibility with this approach.

2.4.5 Oral drug pharmacokinetics

Controlling the pharmacokinetics of oral drug delivery is important to ensure that we can deliver drug to the proper location and at the right dose concentration. To protect the
insulin payload and control its release, we developed a method to fabricate chitosan caps onto the microdevices. Our release data suggests that it is possible to slow the release of insulin to create a profile that would release over the course of hours. With thicker or thinner chitosan caps, we believe that we can further tune the release of insulin if necessary.

Next, we tested our chitosan-capped microdevices in an established in vitro Caco-2 transwell model to assess insulin permeation. We observed that the presence of microdevices can enhance the permeation of insulin across the Caco-2 monolayer compared to a bolus dose. This result was unexpected as we released insulin in a static environment without flow. However, our previous work suggests that the presence of topographical cues may remodel tight junctions and allow a higher insulin permeation rate (33). In addition, other researchers have shown that chitosan can also induce the opening of tight junctions as a permeation enhancer (34). In an in vivo setting where fluid flow is present, we anticipate improved utility of our microdevices due to the unique geometry of our microdevices.

2.5 CONCLUSION

In this work, we demonstrate the use of inkjet printing to efficiently load two different drugs, topotecan and insulin, into microdevices. The advantages of the inkjet printer system lie in its high-throughput loading efficiency, accuracy, and programmability. Spotting drug directly into the reservoir minimizes drug waste. Additionally, multiple printing and drying cycles allow for significantly higher drug loading capacity than that achieved by currently available techniques limited to loading drug at its solubility limit. Furthermore, this method does not require UV light or heat, which can damage sensitive therapeutics, and measurements of the stability of printed insulin demonstrate that biologics can be printed without significant degradation. Surface functionalization increased surface hydrophobicity, which allowed printed drug solution to localize into microdevice reservoirs and did not show significant cytotoxicity. This inkjet printing approach could be adapted for low-waste, high capacity loading of a number of drugs into planar microdevices for oral drug delivery. Chitosan imprinting allowed us to form caps on insulin-filled microdevices and control drug release in a simulated system. In addition, we saw enhanced insulin permeation in an in vitro Caco-2 cell model using our microdevices compared to a bolus dose of insulin.
2.6 REFERENCES


22. Hidalgo IJ, Raub TJ, Borchardt RT. Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. Gastroenterology. 1989;96(3):736-49.


30. Topotecan: Drug Information [Internet]. Lexi-Comp, Inc. [cited December 16, 2016].

31. Insulin: Drug Information [Internet]. Lexi-Comp, Inc. [cited December 16, 2016].


2.7 LIST OF FIGURES

Figure 2-1 Schematic of printer configuration. Prior to each run, drug is drawn from the microtiter plate into the nozzle, and the fiduciary markers on the wafer are recognized by automated recognition of images taken by the alignment camera. Drug is then printed into microdevice reservoirs in an automated sequence. For each printing pass, the nozzle is dipped into the wash basin, and the drop camera is used to confirm successful formation of 400 pL droplets for the specified piezo settings. The printer then dispenses a single droplet into the reservoir of each microdevice on the wafer. Droplets quickly dry, and additional drug solution is printed over solidified drug in future passes. Finally, the alignment camera captures quality-control images of all devices.
Figure 2-2 Microdevice silanization enhances drug localization into device reservoirs. (A) Fluorescence microscopy of devices (outlined in green dashed lines) loaded with topotecan (blue) indicates that drug spotting size was too large in non-silanized devices, with drug being deposited both inside device reservoirs and onto the device body outside of the drug reservoirs. (B) Devices silanized with trichloro(1H,1H,2H,2H-perfluoro-octly) silane became more hydrophobic, providing efficient loading into device reservoirs. Scale bars are 500 mm.
Figure 2-3 Microdevice toxicity assay. *In vitro* CyQUANT viability data showing that silane-coated microdevices do not show significant toxicity to Caco-2 intestinal epithelial cells.
Figure 2-4 Quality control images of (A) fiducial alignment marker, (B) insulin droplet spotting from nozzle, and (C) insulin spotting into microdevice reservoirs.
Figure 2-5 SEM images of topotecan and insulin printing. SEM images of representative microdevices loaded with increasing number of 400 pL drops of (A) 10 mg/mL topotecan and (B) 10 mg/mL insulin.
Figure 2-6 Insulin retains its stability (>96%) during the printing process. Collected samples were analyzed using the described HPLC method.
Figure 2-7 Chitosan-capped microdevices and insulin release. (A) SEM image of an array of chitosan-capped microdevices. (B) Zoomed-in SEM image of one chitosan-capped microdevice. (C) Cumulative insulin release from an array of 400 chitosan-capped microdevices in SIF. Insulin is fully released after 2 h. Scale bars are 200 µm.
Figure 2-8 Insulin permeation assay using a Caco-2 transwell model system. 100 chitosan-capped microdevices were used in each sample with a dose of 5.6 µg per transwell. (A) Total mass permeation of insulin through the Caco-2 cell monolayer. (B) Percent of insulin permeating the Caco-2 cell monolayer based on original dose in the apical chamber. Microdevices show improved drug transport of insulin compared to bolus control dose.
CHAPTER 3: BOTTOM-UP FABRICATION OF MULTILAYER ENTERIC DEVICES FOR THE ORAL DELIVERY OF PEPTIDES
3.1 INTRODUCTION

Oral drug delivery is preferred by both providers and patients as a method of therapeutic administration due to ease of use and high patient compliance (1). Despite many advances in oral delivery systems, peptides and proteins are restricted to parenteral administration due to their high molecular weight, degradation by proteolytic enzymes, and extreme pH conditions in the gastrointestinal (GI) tract. Limited permeability across the GI mucosa further exacerbates the problem leading to oral bioavailabilities of less than 1% (2–4). Therefore, there is a need for innovative delivery systems that can simultaneously protect the sensitive protein cargo while improving the oral bioavailability of the active pharmaceutical ingredient (API).

Lipid and polymer micro/nano-particles have been used to improve the dispersion of APIs with low solubility in the GI tract and to protect encapsulated materials against pH and enzymatically driven proteolysis to improve the efficiency of oral delivery (5,6). However, these spherical particles release the encapsulated drug omni-directionally, which is inefficient and leads to reduced local concentration of drug molecules at the apical surface of the intestinal epithelium where the absorption process occurs, resulting in a lower overall oral bioavailability (5).

To overcome these delivery challenges, many groups have turned to a microtechnology approach (6). Microfabrication has traditionally been used in the semiconductor industry to create structures on the size scale of one micron or smaller (7). Microfabrication principles have also been heavily employed in advancing healthcare technologies including the fabrication of microneedle arrays for dermal drug delivery, microfluidics devices for organ on a chip systems, and micro-machined particles for oral administration (5,8,9). Our group and others have demonstrated the use of fabricated micron-scale devices with customized size, geometry, and aspect ratios to overcome the anatomical and physiological barriers impeding oral drug delivery (6,10). The majority of these fabrication approaches have utilized conventional materials such as poly(methyl methacrylate), SU-8, silicon, or polydimethylsiloxane for the encapsulation of therapeutic molecules (10–12). However, the safety and compatibility of these materials for clinical use, particularly via the oral route, have not been confirmed, which presents a significant regulatory barrier limiting the translation of these microdevice products to the clinic (13).

Additive manufacturing, a potential microfabrication alternative, describes a broad range of processes that involve the layer-by-layer assembly of 3D structures. Based on the desired application, additive manufacturing can be applied to a number of different materials, including plastics, metals, and photo-crosslinkable resins (14,15). Compared to traditional microfabrication approaches, additive manufacturing offers flexibility in scaling device parameters and speed of iterations (14,16). Most current approaches that utilize additive manufacturing to produce pharmaceutical products, including Levetiracetam®️ from Aprecia, use a binder jetting technique to achieve rapidly dissolving 3D structures to improve the ease of use and onset time (17–19). This is achieved through sequentially depositing a water-soluble binder onto a powder bed dispersion of the API and other formulation agents. However, this strategy is not amenable for controlled or delayed release formulations because of the lack of a diffusion-limiting layer. To develop controlled or delayed release formulations using additive manufacturing, other groups have demonstrated the use of a continuous or drop-on-demand material jetting approach,
using filaments composed of API and polymer (19). One limitation of translating these strategies is the reliance on hot-melt extrusion to homogenize APIs and formulating polymers into the desired form factor with the required mechanical properties. This limits the API selection to molecules that can survive relatively high temperatures or APIs that are miscible with low melting temperature binding materials (20,21).

Materials and techniques used in the fabrication of oral protein/peptide delivery systems must maintain the stability of their cargo during fabrication, processing, and administration. Among the most common materials used in the controlled oral delivery of APIs is the Eudragit family of polymers. Eudragit polymers contain a range of linear methyl methacrylate polymers with differentiated pH-sensitive dissolution based on the ratio of acrylate groups to free carboxylic acids. Eudragit polymers are ideal for oral protein delivery due to their extensive characterization and their ability to be processed without harsh organic solvents or excessive heat (22,23).

Our approach is similar to a drop-on-demand additive manufacturing system and inkjet printing, where APIs and enteric coatings are additively assembled in a dropwise, layer-by-layer process to achieve micron scale, multilayer structures (24). Similar techniques have been applied for more than a decade in the commercial scale fabrication of DNA and RNA microarray chips (25,26). Because our approach utilizes piezoelectric-based droplet formation of aqueous solutions, there is no sample heating, enabling us to deposit biologics and other heat-sensitive materials (27–29). First, Eudragit FS 30 D is deposited onto a silicon wafer and evaporated utilizing the “coffee-ring” drying effect to form concave device bodies. Next, solutions of insulin as a model peptide API are deposited into the wells of the device bodies, resulting in loaded devices. Compared to alternative strategies for microdevice loading (e.g. super critical impregnation, hot embossing, spray coating, spin coating, or powder filling), droplet inkjet printing has dramatically reduced drug waste and is amenable to many APIs (12,30–35). This makes inkjet printing the ideal method for loading costly but soluble protein and peptide therapeutics as our group and others have shown previously (27–29,36). Finally, we are able to fully encapsulate APIs in multiple capping layers of enteric polymer, enabling controlled and delayed release of APIs, tunable to the desired pharmacokinetic profile. This strategy also enables on-the-fly tuning of device parameters, including device size, the mass of API and capping material deposited, and the capping material formulation. This allows for greater flexibility and adaptability compared to traditional microfabrication approaches that rely on costly photomasks and replica molding equipment.

3.2 MATERIALS AND METHODS

3.2.1 Materials

Lyophilized recombinant human insulin (CAT: 91077C), hydrochloric acid (HCl), HPLC grade trifluoroacetic acid (TFA), HPLC grade acetonitrile (AcN), ethyl alcohol, simulated intestinal fluid (SIF) and trichloro(1H,1H,2H,2H-perfluorooctyl)silane were purchased from Sigma-Aldrich (St. Louis, MO). Triethyl citrate (TEC) was purchased from Thermo Fisher Scientific, USA. Eudragit FS 30 D, S 100 and L 100 were graciously provided by Evonik Industries (Essen, Germany). 3” silicon wafers were purchased from Addison Engineering Inc, USA. India Ink was purchased from KOH-I-NOR (Bloomsbury, NJ).
3.2.2 Microdevice reservoir fabrication

The overall fabrication, loading, and capping process is shown in Fig. 3-1A. First, a silicon wafer was oxygen plasma treated for 3 min at 50 W followed by silanization with trichloro(1H,1H,2H,2H-perfluorooctyl)silane via vapor deposition under vacuum at room temperature for 25 min. India Ink stock was diluted 10-fold with deionized water. Eudragit FS 30 D dispersion was diluted with deionized water to form a 5% (w/v) solution with 0.5% (w/v) TEC. All solutions were filtered through 0.22 μm polyvinylidene difluoride (PVDF) syringe filters (Millipore, Burlington, MA). All microdevices with reservoirs were fabricated using a sciFLEXARRAYER S3. Prior to printing devices, India Ink was aspirated into the dispenser nozzle and was used to print fiducial markers on the silicon wafer. The FS 30 D formulation was then aspirated into the dispenser nozzle, which was aligned to the fiducial markers, to begin printing the microdevices. Voltage, pulse width, and frequency parameters were optimized to obtain drops with volumes of ~400 pL. To form the microdevice body, a specific amount of solution was spotted on the wafer and allowed to air dry. Total volume of dispensed droplets was controlled by the number of drops dispensed. Drop volumes of 10, 20, 40, 80, and 160 drops were used. Furthermore, the stage holding the wafer was temperature controlled. Tested temperatures included 14°C, 16°C, 18°C, 20°C, and 22°C. Relative humidity was maintained between 50-55% in the printing chamber. Each 3” silicon wafer was printed with an array of 26×26 microdevices for a total of 676 devices and was baked post-printing at 100°C for 20 min. The height profile of microdevices was measured using an Ambios Technology XP-2 profilometer.

For stability studies, wafers with printed devices were diced into smaller sub-quadrants containing 169 devices. Each quadrant was placed into a petri dish and exposed to SIF for 60 to 90 min at 37°C on an orbital shaker. Samples were removed and rinsed with deionized water to remove salts from solution before being dried under a stream of dry N2 gas. Dried samples were then mounted and prepped for scanning electron microscopy (SEM) imaging.

3.2.3 Insulin loading

Insulin solution was prepared fresh at 10 mg/mL in 10 mM HCl and filtered through a 100 kDa centrifuge tube for 5 min at 5000 RCF (Pall Corp, New York, New York). Insulin printing was performed using the sciFLEXARRAYER S3. Prior to printing, drug solution was aspirated into the dispenser nozzle. During setup, the printer’s control unit was aligned to the fiducaries on the silicon wafer, which enabled programmable automatic dispensing. The stage temperature was set to 22°C. Parameters were optimized to obtain drops with volumes of ~400 pL as described earlier. A sufficient volume of insulin solution was printed into device reservoirs to maximize loaded volume inside the reservoir and minimize solution spillover. The printing process was performed in multiple cycles to allow the solvent to completely evaporate between each cycle. Microdevices were loaded with ~100 ng insulin per device and stored under desiccated conditions at 4°C until further use.

3.2.4 Fabrication of a sealing cap

Eudragit L 100 and Eudragit S 100 were dissolved in ethyl alcohol at 1% (w/v) with 0.1% (w/v) TEC and filtered through 0.22 μm PVDF syringe filters. Microdevice capping was
performed using the sciFLEXARRAYER S3. The printer was primed with ethyl alcohol prior to printing, and the stage temperature was set to 22°C. The system was aligned to the fiduciaries on the silicon wafer as described earlier. Parameters were optimized to obtain drops with volumes of ~300 pL. Either Eudragit L 100, Eudragit S 100, or a 1:1 blend was aspirated into the nozzle and a sufficient amount of 300 pL drops was dispensed onto each device to form caps. The printing process was performed in multiple cycles to allow the solvent to completely evaporate between each cycle. Devices were capped with between 200 ng to 220 ng of material, depending on the diameter of the device. Capped microdevices were stored under desiccated conditions at 4°C for further use.

3.2.5 Morphology of planar microdevices

A Carl Zeiss Ultra 55 field emission scanning electron microscope (Carl Zeiss, Oberkochen, Germany) was used to assess the morphological and structural characteristics of the microdevices, before and after printing, API loading, capping, mechanical removal from the silicon substrate, and before and after exposure to SIF. Prior to imaging, devices adhered to a silicon wafer were mounted on aluminum stubs using conductive carbon tape (Ted Pella, Redding, CA) and desiccated under vacuum overnight. Devices were sputter coated with 20 nm of gold, imaged at a 10° tilt and a 2 keV accelerating voltage with magnifications ranging from 150x-250x.

3.2.6 Characterization of insulin release

Insulin release from uncapped and capped sub-arrays, each containing a total of 169 microdevices, was performed by incubating the devices in SIF for 5, 15, 30, and 60 min on an orbital shaker at 37°C. Samples were then analyzed using high-performance liquid chromatography (HPLC). Insulin release was quantified using a 1260 Infinity Quaternary LC System (Agilent Technologies, Santa Clara, CA) using a 50x2.0 µm Proto 200 C18, 5 µm column (Higgins Analytical, Mountain View, CA) at room temperature with a 100 µl injection. Insulin was detected at 214 nm using an MWD. Quantification was performed using a reverse-phase HPLC method where mobile phase (A) is deionized water with 0.1% (v/v) TFA and mobile phase (B) is HPLC grade acetonitrile with 0.08% (v/v) TFA. For min 0-50, the fraction of mobile phase A decreased linearly from 100% to 0% and maintained from min 50-55. In min 55-60 the mobile phase A fraction increased from 0% to 100% and maintained until min 70. Between samples, the autosampler was dip washed in methanol. Insulin was observed to elute at 19.1±0.1 min.

3.3 RESULTS

3.3.1 Microdevice integrity and need for plasticizer

Device bodies were fabricated from Eudragit FS 30 D, a low viscosity aqueous anionic polymer dispersion developed for colonic delivery and dissolution at pH ≥ 7.0 (22). Eudragit FS 30 D was diluted to 5% (w/v) with deionized water to meet the viscosity limitations of the printer system. During Eudragit FS 30 D printing, the chamber was maintained between 50-55% relative humidity, which was found to yield the most reproducible results.
It was necessary to incorporate a plasticizer (0.5% w/v TEC) into the Eudragit FS 30 D formulation to improve the mechanical robustness of the material (Fig. 3-2). Microdevices printed without TEC became brittle after evaporation of solvent, and upon removal, devices fractured, whereas devices with incorporated TEC were mechanically robust upon removal. The addition of TEC improved device integrity and brittleness was no longer observed after solvent evaporation.

Wafers printed with Eudragit FS 30 D microdevices were baked at 100°C for 20 min. Without a bake step, the devices were soft, susceptible to deformation, and difficult to remove from the wafer. After the bake step, the microdevices hardened and were easily removed from the silicon wafer via scraping with a sharp blade (Fig. 3-2).

3.3.2 Scaling microdevice size with number of drops

By controlling the volume of Eudragit FS 30 D dispensed, it is possible to scale microdevice size. Wafers were chilled on a stage at 14°C prior to dispensing droplets. Increasing the number of dispensed droplets from 10-160 leads to microdevices with increasing diameter and height, ranging from ~198-573 µm and 9-19 µm respectively (Table 3-1, Fig. 3-3A). Reservoir volumes increased from 90-1821 pL for 10-160 droplets respectively, correlating to a range in loading capacity from 100 ng of insulin per device to upwards to 2 µg of insulin per device. There is a positive correlation between the number of drops and the size and volume of the device (Fig. 3-3C, Table 3-1).

3.3.3 Controlling microdevice morphology with temperature

Temperature plays an important role in the evaporation rate of water, the transport kinetics of solutes in solution, and the formation of the coffee-ring effect (37,38). To determine the role of temperature in our system, 40 droplet devices were printed while adjusting the stage temperature (Table 3-2, Fig. 3-3B). As the temperature increased from 14°C to 22°C, accelerated drying resulted in a device morphology with thinner reservoir bases, ranging from ~1.75 µm to less than 1 µm respectively. Profilometry revealed sloped reservoir walls that increased from ~177 to 250 µm as temperature increased from 14°C to 22°C. The result is a larger volume capacity, 314 pL vs. 598 pL for 14°C vs. 22°C respectively (Fig. 3-3D, Table 3-2). However, the thin reservoir bases of devices printed at greater than 14°C are susceptible to rupture when mechanically perturbed by a razor blade for removal (Fig. 3-4). As a result, we maintained the use of a 14°C stage temperature throughout the rest of our studies, as it provided the most mechanically robust devices.

3.3.4 Microdevice material stability

Microdevice bodies were exposed to simulated intestinal fluid (SIF) for 90 min to simulate release in the small intestine and the local pH conditions. SIF is a buffer with a pH of 6.8, below the theoretical threshold of dissolution for Eudragit FS 30 D. To assess the integrity of the FS 30 D polymer, we took SEM images to visualize surface dissolution. While there was some noticeable surface erosion, device morphology was relatively unchanged, suggesting functional stability in a relevant time frame for delivery (Fig. 3-5).
3.3.5 Insulin loading and controlled release

Microdevices were loaded as described previously (29). Filtered insulin formulated in 10 mM HCl was aspirated into the capillary and aligned to the fiducials on the silicon wafer before beginning an automated print run. Approximately 100 ng of insulin was deposited into each device. Insulin was stable upward of 94% after loading, capping, and in vitro release (Fig 3-6). This insulin stability is similar to previous work using the printing process (29). SEM images of each stage of device fabrication can be seen in Fig. 3-1B.

Two types of Eudragit were used for capping: L 100 and S 100. Eudragit L 100 is a co-polymer designed for dissolution in solutions pH 6.0 or higher, whereas Eudragit S 100 is a similar polymer designed for release in environments above pH 7.0. Prior to SIF exposure, capped devices appear to be similar in material deposition (Fig. 3-7A). To simulate oral drug delivery to the small intestine, microdevices were exposed for 60 min in SIF. Release studies were performed at 37°C to simulate physiological conditions for release kinetics and protein stability. In order to normalize release profiles between wafers, some devices are left uncapped. After exposure, it is evident from profilometry data that dissolution of the capping material and loaded insulin occurred in L 100 caps while 1:1, and particularly, S 100 caps, show resistance to dissolution (Fig. 3-7B). Devices are allowed to fully dry between sequential insulin and cap printing steps. After printing, the devices are stored under desiccating conditions, limiting the presence of residual solvent in the final devices.

Based on capping formulation, microdevices exhibited a range of release kinetics (Fig. 3-8). L 100 capped devices show purely burst release properties, releasing more than 80% of their loaded insulin within 5 min, and more than 90% at 15 min. S 100 capped devices alternatively showed almost no insulin release, with less than 10% of the loaded insulin released by 60 min. The 1:1 capped devices have a release profile that is a hybrid of the L 100 and S 100 caps. By 5 min, the 1:1 capped devices have released less than 3% of the loaded insulin, suggesting a temporal delay that closely matches the kinetics of the S 100 capped devices. Then between 15 min and 60 min, the 1:1 capped devices gradually released an additional 70% of the loaded insulin, suggesting the presence of a more effective diffusion-limiting barrier as compared to the L 100 capped or uncapped devices.

3.3.6 Mechanical integrity of capped microdevices

Insulin-loaded, capped Eudragit FS 30 D devices printed onto a silicon wafer (Fig. 3-9A) maintained their structural integrity through mechanical removal from the wafer using a razor blade (Fig. 3-9B). The caps remain intact during removal even without a heat processing step.

3.4 DISCUSSION

3.4.1 Device fabrication and characterization

In this work, we demonstrate an additive manufacturing approach using droplet deposition from a picoliter dispenser to fabricate micron-scale devices of varying geometries in a scalable manner. The same picoliter dispenser is used to load sensitive peptides in a low-waste manner followed by enteric polymer capping for controlled release of peptide for
oral delivery. The drug carrier and cap components are formulated out of Eudragit-based materials, which are commonly used polymers for GI targeting, sustained drug-release, and enhanced solubility (22).

An ideal drug carrier will protect its payload during gastrointestinal transit. Eudragit FS 30 D was the ideal candidate for printing the microdevice body due to its resistance to degradation in pH conditions < 7.0, thereby producing a carrier that will persist in the stomach for oral delivery to the small intestine. It is formulated as a low pH, aqueous dispersion that is amenable to dilution, blending, and additional formulation with plasticizers like TEC. Furthermore, the low-viscosity of the solution, < 10 cP, makes it amenable for printing out of a picoliter ink-jet printer.

The microdevice body shape is formed via a phenomenon in material deposition during drop-drying known as the coffee-ring effect (38–40). Edge pinning and convective transport of dispersed material causes material deposition preferentially at the drops' edge. The result is a device with a thick ring on the outside and a thinner base in the center to form a reservoir. This unique geometry allows us to take advantage of the interior space for drug loading. In addition, the geometry of our devices can be tuned on-the-fly by adjusting the total number of drops dispensed, an improvement to previous methods utilizing microfabrication or batch processes. We found that it is possible to fabricate devices with a wide range of volume capacities, the most important parameter for drug loading, that span two orders of magnitude from tens to thousands of pL. Common clinical doses of insulin are on the order of hundreds of µg, which would correlate to delivering hundreds of the 160-droplet sized devices, a feasible dose target for both fabrication and delivery. For human-scale trials, even larger devices could be implemented thereby drastically reducing the number of devices required per dose (41).

In addition, by controlling the kinetics of drying and transport, it is possible to control deposition morphology. By precisely controlling the chamber humidity and substrate temperature, we generated devices that have a defined reservoir and base after material deposition onto a silicon substrate. In addition, we discovered that it is critical that microdevices are formed at low temperatures, 14°C, to ensure that microdevice reservoirs are thick enough to prevent fracture when removed from the silicon wafer. Drying at higher temperatures result in devices with thin bases that cannot be removed from the silicon substrate without shattering, due to accelerated convective transport of the FS 30 D dispersion to the pinned edges of the drop during drying. Conversely, lower temperatures approaching the environmental dew point prevent fluid evaporation and device drying, a requirement for device formation.

Still, micronized Eudragit FS 30 D may be more susceptible to accelerated dissolution due to increased surface area compared to the bulk material. Thus, we wanted to ensure that the Eudragit FS 30 D microdevices do not dissolve prior to the release of insulin. By exposing the devices to SIF, which mimics a pH environment similar to that of the small intestine, we found minimal evidence of surface erosion with our microdevices. This is important for two main reasons. First, the device will protect the API payload throughout transit and second, the device body will not lead to critical failure that would cause burst release of the API.
3.4.2 API loading and release

We loaded our microdevices with insulin as our model API. While the loading process is similar to our previous work, the ability to print fiducial markers and microdevices with the same platform used to print API improves the throughput of the entire fabrication process. Controlled drug release in the gastrointestinal tract is a necessary benchmark for improved pharmacokinetic properties in oral delivery systems (42,43). Effective targeting of the small intestine can maximize absorption but requires a delivery vehicle that can release the drug in the correct region of the small intestine, usually in a pH sensitive manner. Our controlled release and dissolution studies demonstrate the utility of different formulations of Eudragit caps to enable tunable drug release. The goal of this process was to produce a device cap with different dissolution properties compared to the device body, which prompted the use of Eudragit S 100 and L 100, both of which erode at a lower pH compared to Eudragit FS 30 D. In normal use cases, Eudragit L 100 is ideal for small intestinal delivery. However, our microdevices have a much higher exposed surface area compared to that of the bulk material, leading to more rapid dissolution. Conversely, Eudragit S 100 is designed for colonic delivery. And in our study, we show that a micronized form of Eudragit S 100 is very robust against exposure to a pH less than 7, leading to effectively no erosion or drug release. In addition, we formulated a 1:1 (w/w) blend of both Eudragit L 100 and S 100 to create caps which feature characteristics of both materials. Our results demonstrate that altering the capping formulation can change both the temporal delay and kinetics of drug release. This demonstrates that controlling the ratio of L 100 to S 100 in the capping materials allows for tunable control of peptide release kinetics. Micronized forms of both L 100 and S 100 are expected to undergo dissolution in SIF, but these assumptions are based on studies of macroscale bulk materials. It is reasonable to assume that the low aspect ratio and the form factor of the S 100 and the 1:1 blend led to the observed deviations from the expected dissolution profiles of these two capping materials. The release data are further supported by SEM images of both uncapped and capped devices with all three Eudragit formulations before and after exposure to SIF for 60 min (Fig. 3-7). It is likely the case that through further tuning of the blending and layering of the capping material, it will be possible to achieve an even wider range of API release profiles including pulsatile release profiles. Furthermore, our drug release studies in SIF demonstrated that we were able to recover an intact, stable insulin product after deposition into the devices and post-capping. This demonstrates that the printing process and subsequent capping process can be used for sensitive drug molecules, an advantage over other systems that lead to drug degradation during the fabrication process.

From a translational point of view, it is important that we are able to extract the microdevices from the silicon wafer and formulate them into a solution for oral drug delivery. However, our extraction process does not require use of water or solvent, an advantage over previous microdevice technologies that require a wetting solvent to ensure clean extraction of devices, which can lead to premature drug release during the device removal process.
3.5 CONCLUSION

We demonstrated a bottom-up, layer-by-layer approach for fabrication of microdevices for oral peptide delivery. We take advantage of drop-drying physics and the coffee-ring effect to fabricate micron-scale devices with reservoirs. The microdevices consist of Eudragit FS 30 D as a device body having a reservoir to allow encapsulation of insulin as a model peptide, followed by a capping polymer. The microdevice size can be tuned by controlling the substrate temperature and the number of printed drops. More than 94% insulin remained intact during the fabrication process, demonstrating that the process does not appreciably degrade protein cargo. Furthermore, we show that by altering the capping material, it is possible to achieve a range of release kinetics from our devices. This method enables rapid iteration and optimization of new materials, APIs, and device geometries during fabrication and has potential utility beyond drug delivery in microelectronics and microfluidic systems.
3.6 REFERENCES


List of Figures

Figure 3-1. Schematic of the printing process. (A) (1.) A silicon wafer is silanized to achieve a hydrophobic surface. (2.) The polymer dispersion is ejected from the picoliter dispenser onto the silicon wafer. (3.) Evaporation of solvent results in the formation of device body. (4.) The dispenser is then used to print API formulation into each device. (5.) After devices are loaded with API, a second polymer is printed on top of devices to form a cap. (6.) Devices can then be removed from wafer for use. (B) Scanning electron microscopy (SEM) images of representative devices throughout the fabrication, loading, and capping process. All scale bars are 100 µm.
Figure 3.2 SEM imaging shows microdevices are robust against mechanical extraction from a silicon substrate with the addition of a plasticizing agent and a bake step. Devices printed without the addition of a plasticizing agent (Left) have a roughened morphology and are prone to shattering upon removal. Devices that are not baked (Center) before removal adhere to each other and do not disperse effectively after removal. Devices printed with added triethyl citrate (10% w/w by polymer mass) and baked at 100°C for 20 min have a smooth surface, are robust post-extraction, and disperse effectively (Right). All scale bars are 100 µm.
Figure 3-3 Microdevices composed of Eudragit FS 30 D have tunable morphologies and reservoir volumes. Device body profiles can be tuned based on the number of drops deposited (A) and the stage temperature (B) as shown by profilometry and SEM. Device volume increases in correlation with increasing volume (C) and stage temperature (D). Data shows mean ± 1 SD, n=5 measurements of separate devices for all groups. All scale bars are 100 µm.
Figure 3-4 SEM images of devices dried at an elevated temperature (22°C). Devices have a thin reservoir base and tend to fracture upon extraction. All scale bars are 100 µm.
Figure 3-5 SEM imaging at 200x and 250x magnification shows that devices retain their integrity after prolonged exposure to simulated intestinal fluid (SIF). Compared to control devices (Left), exposed devices (Right) show signs of surface erosion but retain their morphology and integrity. All scale bars are 100 µm.
Figure 3-6 Stability of insulin is maintained throughout device fabrication, loading, and capping. Insulin was either prepared fresh in 10 mM HCl at a concentration of 10 µg/mL (Insulin Control), aspirated into the sciFLEXARRAY S3, and deposited into devices before being eluted into SIF for one hour (Post Print), or capped prior to elution (Post Capping). Collected samples were analyzed using the described HPLC method.
Figure 3-7 Capping formulation impacts dissolution kinetics and morphologies as shown through SEM imaging and profilometry. (A) Insulin loaded devices either left uncapped, capped with L 100, S 100, or capped with a 1:1 blend of L 100 and S 100 before exposure to simulated intestinal fluid (SIF). (B) Insulin loaded, capped devices exposed to SIF for 60 min. As expected, caps that incorporate S 100 undergo less erosion than purely L 100 caps. All scale bars are 100 µm.
Figure 3-8 Release of insulin from capped microdevices over the course of 1 h in SIF. Three different caps were tested: (i) Eudragit S 100, (ii) 1:1 blend of Eudragit S 100 and Eudragit L 100, and (iii) Eudragit L 100. Caps containing Eudragit S 100 show temporal and kinetic delay of insulin release. Data represents mean ± 1 SD (No Cap Control; L 100; 1:1, n=3; S 100, n=4).
Figure 3.9 Loaded and capped microdevices are mechanically robust and dispersible. (A) Representative image of 3” silicon wafer with 676 printed devices and India ink fiduciary marks. (B) Insulin loaded, 500-capped devices mechanically scraped from silicon substrate using a razor blade remain intact for subsequent processing and delivery. Scale bar is 100 μm.
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Table 3-1 Effect of volume deposited on final device body morphology. Measurements made via contact profilometry. Data represents mean ± 1 SD, n=5 measurements of separate devices for all groups. Wafer temperature was set at 14°C for all groups. Reservoir volume represents a derived value based on the reservoir diameter and height estimated as a cylinder.

<table>
<thead>
<tr>
<th>Volume Dispensed</th>
<th>Device Diameter (µm)</th>
<th>Device Height (µm)</th>
<th>Reservoir Diameter (µm)</th>
<th>Reservoir Height (µm)</th>
<th>Reservoir Thickness (µm)</th>
<th>Volume (pL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 drops</td>
<td>198.04±2.30</td>
<td>9.14±0.11</td>
<td>114.00±4.89</td>
<td>8.80±0.07</td>
<td>0.34±0.13</td>
<td>89.82±15.43</td>
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<tr>
<td>20 drops</td>
<td>247.60±4.48</td>
<td>11.42±0.13</td>
<td>145.52±5.03</td>
<td>10.88±0.08</td>
<td>0.54±0.15</td>
<td>180.95±25.04</td>
</tr>
<tr>
<td>40 drops</td>
<td>298.96±3.66</td>
<td>13.72±0.40</td>
<td>178.02±12.70</td>
<td>12.18±0.31</td>
<td>1.54±0.50</td>
<td>303.16±86.84</td>
</tr>
<tr>
<td>80 drops</td>
<td>394.48±4.28</td>
<td>16.74±.48</td>
<td>244.00±8.97</td>
<td>14.62±0.46</td>
<td>2.12±0.67</td>
<td>683.62±102.77</td>
</tr>
<tr>
<td>160 drops</td>
<td>573.00±6.98</td>
<td>19.15±0.22</td>
<td>366.00±7.79</td>
<td>17.31±0.14</td>
<td>1.841±0.26</td>
<td>1821.16±173.11</td>
</tr>
</tbody>
</table>
Table 3-2 Effect of substrate temperature on final device body morphology. Measurements made via contact profilometry. Data represents mean ± 1 SD, n=5 measurements of separate devices for all groups. 40 drops were dispensed per device for all groups. Reservoir volume represents a derived value based on the reservoir diameter and height estimated as a cylinder.

<table>
<thead>
<tr>
<th>Stage Temperature (°C)</th>
<th>Device Diameter (µm)</th>
<th>Device Height (µm)</th>
<th>Reservoir Diameter (µm)</th>
<th>Reservoir Height (µm)</th>
<th>Reservoir Thickness (µm)</th>
<th>Volume (pL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14°C</td>
<td>305.88±6.94</td>
<td>14.48±0.31</td>
<td>176.98±10.02</td>
<td>12.78±0.24</td>
<td>1.7±0.39</td>
<td>314.39±71.41</td>
</tr>
<tr>
<td>16°C</td>
<td>351.60±9.84</td>
<td>14.58±0.63</td>
<td>230.58±11.19</td>
<td>13.66±0.56</td>
<td>0.92±0.84</td>
<td>570.41±113.18</td>
</tr>
<tr>
<td>18°C</td>
<td>360.72±7.00</td>
<td>14.00±0.39</td>
<td>241.16±1.52</td>
<td>13.08±0.41</td>
<td>0.92±0.57</td>
<td>597.46±23.97</td>
</tr>
<tr>
<td>20°C</td>
<td>360.26±3.09</td>
<td>13.88±0.41</td>
<td>241.56±1.61</td>
<td>13.10±0.47</td>
<td>0.78±0.63</td>
<td>600.36±26.98</td>
</tr>
<tr>
<td>22°C</td>
<td>359.78±4.33</td>
<td>13.08±0.92</td>
<td>250.38±1.70</td>
<td>12.14±1.06</td>
<td>0.94±1.41</td>
<td>597.73±54.85</td>
</tr>
</tbody>
</table>
CHAPTER 4: INVESTIGATION OF NANOTOPOGRAPHICAL GEOMETRIC PARAMETERS TO OPTIMIZE ORAL PROTEIN DRUG DELIVERY
4.1 INTRODUCTION

Large molecular weight therapeutics, such as proteins and peptides, represent some of the most novel and biologically specific classes of drugs being developed. However, many never reach commercial status due to poor drug distribution and low absorption levels (1, 2). Etanercept, for example, is a highly effective tumor necrosis factor inhibitor used to treat autoimmune disorders such as rheumatoid arthritis but is currently limited to delivery via intravenous administration and/or subcutaneous injection (3). To improve the distribution and absorption of therapeutics, new approaches to enhance epithelial permeability are needed.

The epithelium is a thin tissue that lines the cavities and surfaces in the eyes, nose, mouth, lung, and gastrointestinal system. It is designed to allow uptake of certain nutrients while preventing the entry of pathogens and foreign molecules. There are two main routes of transport across the epithelia: (i) transcellular delivery through cells via membrane vesicles or ion/protein channels and (ii) paracellular delivery between cells through the intercellular tight junctions (4-6). Transcellular delivery is limited, however, by the surface area of the lipid membrane and by the transporter proteins available. The paracellular route is limited to small hydrophilic molecules and ions due to tight junction strands that form a selective barrier between neighboring cells. This epithelium is a substantial barrier that deters absorption of drugs, particularly high-molecular weight molecules such as proteins and peptides.

Chemical permeability enhancers have been employed to help increase biologic permeability across the intestinal epithelium. In general, chemical permeation enhancers exert their effects by disrupting the tight junctions and increasing the area available to facilitate enhanced diffusion (7). Examples of chemical permeability enhancers include chitosan, chelators, and toxins that are used to induce the opening of tight junctions (8, 9). However, these permeation enhancers suffer from quality of administration and toxicity issues. Therefore, there is a need for a permeation enhancer that can not only consistently modify tight junction permeability but also be done safely without permanently disrupting cell function.

It is well known that cells can respond to purely topographical cues from nanostructures and alter their behavior, not unlike cell responses to exogenous chemical cues. These cues play an important role in all cell functions including adhesion, migration, proliferation, and differentiation (10, 11). Previously, we have demonstrated that it is possible to reversibly open tight junctions between epithelial cells through the introduction of nanostructures to improve drug delivery of large molecular weight therapeutics (12). Low-aspect ratio nanostructured films made from polypropylene resulted in a decrease in transepithelial electrical resistance (TEER) and increase in permeation of BSA, IgG, and etanercept. However, when a nanostructured film was used with a high-aspect ratio, there was a reduced change in TEER and permeation of drug.

This approach to drug delivery represents a paradigm shift from the traditional biochemical strategies because it utilizes inert mechanical cues derived from nanostructures as a means to enhance permeability to alter drug transport. However, the specific geometric parameters required to elicit this increase in permeability are unknown.
We explored UV lithography as a cost-effective technique to systematically fabricate nanostructures of different geometries (13). This allowed us to expand our library of structures that have not been studied previously while improving the throughput of fabrication and experimentation. Here, we test various nanostructured films using an established Caco-2 transwell system to assess their effects on enhancing IgG antibody permeation. Finally, we explore how surface-treated films can further play a role in regulating transport.

4.2 MATERIALS AND METHODS

4.2.1 Materials

Poly(urethane acrylate) (PUA)-based polymer NOA 86 was purchased from Norland Optical Adhesive, USA. Trichloro(1H,1H,2H,2H-perfluoroctyl)silane, fluorescein isothiocyanate conjugated to immunoglobulin G (FITC-IgG, 150 kDa), Hoechst 33342 nucleic acid stain, collagen type I, ethanol, triton-X, goat serum, paraformaldehyde, methanol, acetone, glycine, and dichloromethane (DCM) were purchased from Sigma-Aldrich, USA. Polyethylene terephthalate (PET) was purchased from Tekra, USA. OrmoStamp and poly(methyl methacrylate) (PMMA) was purchased from MicroChem, USA. Caco-2 cells were purchased from ATCC, USA. PrestoBlue cell viability assay kit, chromium etchant, minimum essential medium (MEM), fetal bovine serum (FBS), phosphate buffered solution (PBS), sodium pyruvate, penicillin-streptomycin, trypsin-EDTA, and high-density Transwell plates and all antibodies were purchased from Thermo Fisher Scientific, USA. 4” silicon wafers were purchased from Addison Engineering Inc, USA.

4.2.2 Fabrication of nanostructured films

The overall process of nanoscale fabrication of silicon master molds is shown in Fig. 4-1. Nanostructured silicon master molds were fabricated using electron beam lithography (Vistec VB300) to form six patterned arrays on a PMMA resist spun-cast onto a silicon wafer. Table 4-1 shows the desired geometric parameters for fabrication. The diameter of nanopillars within each array was set to 100 nm, 200 nm, or 500 nm with a density of either 200 nm or 500 nm. A 10 nm chrome layer was deposited onto samples before liftoff was performed using dichloromethane. Reactive ion etching (Oxford PlasmaLab 150 Inductively Coupled Etcher) was conducted to etch a fixed depth of >200 nm into the arrays (-100°C stage cooling, 15 sccm O2, 38 sccm SF6, RF forward power 20 W, 24 sec etch time). This etch distance produced nanostructures with aspect ratios ranging from 0.4-2 at minimum depending on the diameter. The chrome was removed by submersion in chromium etchant solution and under sonication for 10 min. Silicon master molds were rinsed in water and dried under N2 gas. To prepare silicon molds for replica molding, an anti-stick coating was applied. The silicon masters were exposed to oxygen plasma for 5 min before being placed in an oven at 80°C for vapor deposition of trichloro(1H,1H,2H,2H-perfluoroctyl)silane for 30 min.

First-generation nanostructured films were fabricated using OrmoStamp polymer. PET films were cleaned with 100% ethanol and dried under O2/N2 gas before being exposed to oxygen plasma for 5 min to prime the surface for bonding the OrmoStamp
polymer. OrmoStamp was dropdispensed onto a nanostructured silicon master. The PET film was placed into contact with the dispensed OrmoStamp and was cured under UV light (λ = 365 nm) for 120 sec. Following curing, the PET film was peeled away with the OrmoStamp attached to the film as the negative of the nanostructured silicon mold. Films were cured under UV light for an additional 60 min. OrmoStamp films were silanized similarly to the silicon master molds to improve anti-stick properties.

Positive NOA 86 (~2.5 GPa) nanostructured films were fabricated from OrmoStamp first-generation nanostructured films. PET films were prepared as described above to improve adhesion of NOA 86 to the PET film surface. NOA 86 was dropdispensed onto an OrmoStamp mold. The PET film was placed into contact with the dispensed NOA 86 polymer and was cured under UV light for 120 sec. Following curing, the PET film was peeled away with the NOA 86 attached to the film as the positive of the nanostructured silicon mold. Films were cured under UV light for an additional 60 min. Flat NOA 86 films were fabricated by using a PET film made without a nanostructured mold.

4.2.3 Characterization of nanostructured films

Nanostructured PUA films were prepared for scanning electron microscopy (SEM) by sputter coating with 10 nm of gold followed by mounting onto carbon tape. The samples were imaged with a Carl Zeiss Ultra 60 scanning electron microscope.

4.2.4 Caco-2 cell culture

Human intestinal Caco-2 cells (passage number <15) were cultured in MEM with 20% FBS (v/v), 1% (v/v) sodium pyruvate, and 1% (v/v) penicillin-streptomycin, and 1% sodium pyruvate in an atmosphere containing 5% CO₂ at 37°C. The cells were subcultured at 60% confluency by trypsinization with 0.05% trypsin-EDTA.

4.2.5 Cell viability assay

Caco-2 cells were grown to confluence on a 96-well cell culture plate. Nanostructured films, with nanostructures facing the cells apical side, were directly applied to cells for 60 min. PrestoBlue reagent was added directly to cell media and incubated at 37°C for 10 min. Fluorescence was measured at an excitation of 560 nm wavelength.

4.2.6 Drug permeation assay

Transwell permeable inserts were coated with collagen type I (10 μg/cm²) solution in ethanol. Ethanol was allowed to evaporate before use. Caco-2 cells were seeded onto Transwell inserts at a density of 75K cells/cm². Tight junction presence was confirmed when the transepithelial electrical resistance (TEER) was >400 ohm X cm². After formation of a tight barrier, nanostructured films were placed into direct contact with the cell monolayer in the apical chamber of the Transwell insert. The negative control was an untreated monolayer. The positive control was a flat film with the same material and weight properties. FITC-IgG was suspended in phenol red-free MEM (0.1 mg/mL) and was introduced into the apical chamber of the Transwell insert. A PBS solution was used as the sink condition in the basal chamber. The basal solution was sampled at 30 min, 60
min, and 120 min. PBS was replaced for each time point. Detection of FITC-IgG concentration was conducted using a spectrophotometer plate reader with an excitation wavelength of 490 nm and emission wavelength of 520 nm.

4.2.7 Immunocytochemistry

Caco-2 cells were washed and fixed at room temperature in 2% (v/v) paraformaldehyde after film treatment for 2 h. The cells were incubated in 1 M glycine/PBS solution for 10 min. Cells were permeabilized with 1:1 methanol/acetone for 2 min at room temperature. Following PBS wash, the cells were further washed with a solution comprised of PBS, 0.5% (v/v) triton-x, and 2% (v/v) goat serum before incubation with primary antibodies for 1 h at room temperature. The cells were washed with PBS and 2% goat serum followed by secondary antibody incubation for 1 h at room temperature. Cells were then washed in PBS and mounted onto coverslips prior to imaging using a confocal microscope. Z-stack images were produced using a confocal microscope. Post-processing was done in FIJI and contrast/brightness values were normalized across all images for each channel.

4.2.8 Surface modification of films and characterization of films

Flat and HD 200 films were surface treated with oxygen plasma to produce hydrophilic surfaces that were used within 10 min of treatment. Hydrophobic films were produced using the silanization method as described earlier. Surface energy of films was measured using a goniometer.

4.2.9 Statistical analysis

All statistical analysis was performed using Prism (GraphPad Software). Two-way analysis of variance (ANOVA) tests with suitable post hoc test for multiple comparisons were used to compare the performance of nanostructured films in TEER and drug permeation assays. In all experiments, a p value less than 0.05 was considered statistically significant.

4.3 RESULTS

4.3.1 Fabrication of nanostructured silicon master molds

Six nanostructured patterns were fabricated onto silicon. Electron beam lithography was used to etch the desired nanostructure dimensions into a PMMA resist due to its ability to create features at the nanoscale with high-resolution. Chrome deposition was used to protect the regions that are to become nanostructures during the etching process. To etch the silicon, inductively coupled plasma and reactive ion etching processes were utilized.

4.3.2 Replica molding via capillary photolithography and SEM characterization

OrmoStamp polymer was used to fabricate the first-generation replica film from the silicon master molds. OrmoStamp possesses low surface energy which promotes the release of other polymers that make contact with it, thus making ideal for fabricating second-generation nanostructured films out of other materials (14). The first generation of each film formed a ‘negative’ of the nanostructure pattern. Instead of forming nanopillars,
nanoholes were formed instead. As a result, the replica molding process must be performed twice in order to obtain the original ‘positive’ nanostructure pattern.

SEM imaging of the films revealed that fabrication of the silicon masters was successful (Fig. 4-2). We measured the diameter, height, and pitch of each nanopattern to measure the success of fabrication (Table 4-2). The diameters of each nanostructure pattern were observed to be within 10% of the desired value. The pitch of each nanostructured film was within 2% of the expected value. The low variability of the pitch indicates that the spread of the nanopatterns are well-controlled. The heights of each nanostructure pattern ranged from ~200-400 nm with standard deviations of up to 10% within each group. The variability in height is expected due to slight changes in the ICP RIE parameters each time the etching process was conducted. We were able to meet a threshold of at least 200 nm for all films, thereby producing aspect ratios in the range from ~0.77-3.18 for the various groups.

4.3.3 Cytotoxicity of nanostructured films

We used PrestoBlue, a resazurin-based live cell assay that works as an oxidation-reduction indicator in the presence of cell metabolism. Here, we show that flat and nanostructured films made from six different nanostructure patterns do not significantly impact cell viability after two hours of contact with cells (Fig. 4-3).

4.3.4 Effect of nanostructured films on transepithelial electrical resistance

When we applied flat and nanostructured films to cells, we observed an immediate decrease in TEER of ~30-60% at 0.5 h for all groups (Fig. 4-4). At later time points of 1 and 2 h, TEER continued to decline slowly to ~40-60% of the original TEER. The decrease in TEER indicates that there is an increased flow of ions crossing the cell monolayer in the presence of these treatment groups. Compared to the control group without any film applied, the TEER remained consistent throughout the study.

4.3.5 IgG drug permeation through intestinal model system

In our first IgG drug permeation study, we studied the effect of flat PUA and six different nanostructured PUA films on the effects of IgG permeation through a Caco-2 transwell system at three time points of 0.5, 1, and 2 h (Fig. 4-5). At each time point, the IgG permeation trended higher compared to the control group. The fold-increase at 0.5 h ranged from 2-7 among all groups. However, at 1 h, the HD 200 group showed statistical significance over the control group with an average drug permeation increase of 7.5-fold. The other groups stayed within similar ranges compared 0.5 h. At 2 h, all experimental groups showed an increase in drug permeation with ranges from 4-11. All nanostructured groups showed statistically higher drug permeation compared to control. In addition, the LD 500, HD 200, and HD 500 groups showed the highest permeation overall. Over the course of 2 h, it appeared that nanostructured groups with higher density of nanostructures showed a higher rate of IgG permeation.

In our next study, we focused on high-density groups only (Fig. 4-6). At 0.5 h, we observed that all groups increased drug permeation from 2.5-10-fold over control with HD 200 and HD 500 films showing the strongest effect. At 1 h, the effect became stronger in all groups but only HD 200 films showed a significant improvement over control. Finally,
at 2 h, the extent of drug permeation extended to an average increase of 19-fold for the HD 200 group. While HD 200 and HD 500 films showed significant improvement in drug permeation over control, only HD 200 showed a significant improvement over flat and HD 100 films as well, thus indicating that HD 200 represents the best improvement to drug permeation of all nanostructured groups followed by HD 500.

4.3.6 Immunocytochemistry of Caco-2 cells

Caco-2 cells with no treatment, treated with flat films, and treated with HD 200 films, were stained using Hoechst 33342 nucleic acid stain and primary/secondary antibodies for the nucleus and ZO-1 and actin proteins respectively. Maximum pixel intensity representative images are shown in Fig. 4-7. Fluorescent intensity of ZO-1 and actin appear reduced in Caco-2 cells treated with flat and HD 200 films.

4.3.7 Surface modification and characterization of nanostructured films

Flat and HD 200 films were plasma treated for 5 min and placed on Caco-2 cells within 10 min of surface treatment. Silanized flat and HD 200 films were prepared using the same silanization protocol for chemically-modify the silicon masters. Films were washed in PBS the day prior to being placed on cells.

Separate surface-modified flat and HD 200 films were prepared for contact angle characterization (Table 4-3). Plasma-treated flat and HD 200 films had a half-contact angle measurement less than 10 degrees, which is consistent with other findings. Unmodified flat and HD 200 films showed measurements of ~66º and 72.3º respectively. Silanized flat and HD 200 films increased contact angles to ~99.7º and 108.7º respectively.

4.3.8 Surface-modified nanostructured films impact on cytotoxicity and TEER

Flat films and HD 200 films that were plasma-treated, silane-treated, or underwent no treatment were assessed for their cytotoxic effects in Caco-2 cells. PrestoBlue results did not indicate significant cytotoxicity in any experimental groups compared to no treatment control over the course of 2 h (Fig. 4-8).

TEER was also used to determine the effects of surface-modified nanostructured films (Fig. 4-9). An immediate decrease of 55-70% was observed at 0.5 h for experimental groups. The TEER further decreased to 55-80% by 2 h.

4.3.9 Effects of surface-modified nanostructured films on IgG drug permeation

We assessed the effects of plasma-treated and silanized HD 200 films and their effects on IgG drug permeation in the Caco-2 cell transwell system at 0.5, 1, and 2 h (Fig. 4-10). IgG permeation trended higher for all experimental groups at 1 and 2 h. However, significantly higher permeation was only observed at 2 h for HD 200 silane, fold-change of 7.5 over control, and its plasma-treated counterpart.
4.4 DISCUSSION

4.4.1 Fabrication of nanostructured films for cell studies

In this work, we developed six nanostructure molds that would be used to form the nanostructured films through a series of steps utilizing e-beam lithography, metallic deposition, lift-off, and etching steps. E-beam lithography enables precise control over density and diameter of features whereas the height can be tuned based on the temperature, oxygen content, and time duration of reactive ion etching. SEM imaging revealed that we were able to meet our parameter targets. The requirement for producing films with at least a 200 nm height is important to differentiate the nanostructured films sufficiently from the flat control group. If the aspect ratio is too small, cells may not be able to perceive the nanotopographical cues imparted by the nanostructures. Another important consideration is the density and pitch of the nanostructure patterns. A high density of nanostructures will greatly increase the surface area available for cells to interact with the nanostructured film.

NOA 86 PUA polymer was used to form the ‘positive’ nanostructured film. It was chosen for three main reasons based on previous literature (15). First, it is a photo-crosslinkable resin with a favorable cytotoxicity profile. Second, it has a high Young’s modulus and stiffness similar to the nanostructured films that were used in prior studies that demonstrated drug efficacy. Third, it is simple to use for replicating the precise nanoscale features of the master silicon mold. As replica molding is a high-throughput and reproducible process, we can be confident that the nanostructures we use in our studies look and behave consistently with little batch variation.

4.4.2 Cytotoxicity of nanostructured films

Drug permeation enhancers should offer a favorable cytotoxicity profile. Therefore, we wanted to ensure that our nanostructured films, which primarily exert mechanical and topographical cues, were not causing cell death and impairing barrier function. While nanostructured PUA has been used as a substrate for cell studies in the past, no one has confirmed the effects of nanostructured PUA films when placed into contact with cells (15). Our data showed no significant differences in cell toxicity for all groups. This reveals two things. First, PUA as a material is cytocompatible, and the different nanotopographies do not exert toxic effects. Second, the cell exposure to films for 2 h is a significant amount of time for oral drug delivery, and we expect physical contact would be made with cells on a shorter duration in animals or humans. This is important to note as chemical permeation enhancers disrupt tight junction barriers between cells to enhance drug permeation but also cause cell death at the same time.

4.4.3 Impact of nanostructured films on Caco-2 barrier function and IgG permeation

Transepithelial electrical resistance is a useful measurement to determine the presence of barrier function in epithelial cells. At a threshold above 400 ohm cm$^2$, cells form tight junctions similar to those found in vivo (16). In the presence of permeation enhancers, we would expect to see a decrease in TEER due to the reorganization of the tight junction complex. When flat and nanostructured films were placed onto cells, we observed an immediate drop in TEER at 0.5 h. The TEER continued to drop and stabilized after 1 h.
This decrease is consistent with what we have observed previously when utilizing DN2 and DN3 films (12).

Next, we sought to determine the effects of different nanostructured films on the permeation of IgG antibody. Oral antibody drug delivery is of particular interest because current drug administration is applied intravenously. Here, we used a FITC-tagged IgG antibody as our model drug. At a molecular weight of ~150 kDa, IgG is 2-3 orders of magnitude larger than typical substrates that can transport through the intestinal lining such as glucose or mannitol (17). At 0.5 h, we noticed a trend of enhanced IgG permeation for all groups. However, it isn’t until 1 h where HD 200 nanostructured films, which are most similar to DN2 films in nanostructure geometry, showed significant permeation over the no treatment control. At 2 h, all films showed significantly higher enhanced permeation of IgG over the control although the high-density nanostructured films trended higher compared to their low-density counterparts. Therefore, we focused further on the high-density films to determine whether there was a best candidate.

In our follow-up drug permeation study with high-density nanostructured films, we observed that both HD 200 and HD 500 films tended to promote higher permeation, almost 2-fold, compared to flat and HD 100 films at all time points. Still, of the candidates tested, we determined HD 200 films to be the best candidate for enhancing IgG permeation. At 1 h, HD 200 significantly improved IgG drug permeation over the no treatment control whereas other films did not show significance. Furthermore, at 2 h, HD 200 and HD 500 films both promoted significantly higher IgG permeation over the control. However, only HD 200 was significant compared to flat and HD 200 films. Thus, we believe that HD 200 nanostructured films are within the ideal range of geometric parameters necessary to enhancing antibody permeation.

To assess how Caco-2 cells may be responding to our films, we conducted immunocytochemistry using our best candidate, HD 200, and comparing to the flat condition and no treatment control. After staining the cytoskeleton actin and ZO-1, a key protein in the tight junction complex, we observed a decrease in fluorescent intensity of both types of proteins in the flat and HD 200 groups (18). Interestingly, we noticed lower intensity in the HD 200 groups compared to flat groups. This may suggest protein remodeling that occurs in the presence of a physical surface on top of the cells and may further be enhanced by the presence of nanostructure features. Future work will look into other proteins of the tight junction complex and determine the how nanostructured films exert their effects from a mechanistic point of view.

4.4.4 Characterization and cytotoxicity of surface-modified nanostructured films

After assessing the geometric parameters that can best enhance antibody permeation, we turned to other potential parameters that may play a role, such as surface energy. It has been established that cells will respond to surfaces differently depending on surface energy (19, 20). Here, we tested plasma-treated and silanized films to explore the two extremes of surface energy. Plasma-treatment is an established method to increase the surface energy of a surface, typically resulting in contact angle measurements less than 10° (21). To decrease surface energy, we used a silanization approach with a hydrophobic silane chemical comprised of fluorine and methyl groups. This approach can produce surfaces with contact angles well over 100° (22). Half-contact angle
measurements confirmed that our plasma-treated and silanized films were successfully modified.

As we move forward with chemically-modifying the surface of our films, we need to reestablish that the chemical changes are not promoting cytotoxicity for the same reasons stated earlier. Of concern is silane, a chemical that is known to be cytotoxic when it is not bound to a surface. Our data shows that both silanized and plasma-treated flat and HD 200 films do not cause significant cytotoxicity in the span of 2 h.

4.4.5 Impact of surface-modified films on Caco-2 barrier function and IgG permeation

We assessed the effect of surface-modified films on Caco-2 barrier function through TEER. Similar to our previous experiments with films, we observed a sharp decrease in TEER for all film groups compared to the no treatment control at 0.5 h. In addition, the TEER decreased further at 1 h before stabilizing.

Next, we used the surface-modified HD 200 films in our transwell model system to determine the impact of surface energy on enhancing IgG permeation. While no differences were observed at 0.5 h, a trend began to emerge at 1 h where silanized HD 200 films began to show enhanced IgG permeation compared to all other groups. This effect was further enhanced at 2 h in which we observed a significantly higher IgG permeation in cells treated with the silanized HD 200 film compared to the no treatment control and HD 200 plasma-treated group. This suggests that surface energy may play a role in facilitating IgG transport across Caco-2 cells. Future work will explore the potential mechanism while assessing whether this phenomenon can be translated to other drug classes.

4.5 CONCLUSION

In summary, six different nanostructured silicon molds were fabricated using e-beam lithography and ICP RIE. Nanostructured PUA films were produced via replica molding and were utilized to determine their efficacy as an epithelial permeation enhancer for IgG. High-density films showed improved transport compared to their low-density counterparts. Furthermore, nanostructured films possessing 200 or 500 nm diameter pillars showed significantly improved IgG transport compared to control groups. These results indicate that there is a geometric parameter optimum that can be used to enhance the transport of high-molecular weight therapeutics. Finally, investigation of surface-treatment of nanostructured films revealed that hydrophobic, low-surface energy films improved transport of IgG. Future work will consider the effects of other key parameters such as material stiffness while investigating the cell molecular processes responsible for promoting antibody permeation enhancement.
4.6 REFERENCES


4.7 LIST OF FIGURES

Figure 4-1 Schematic of the fabrication of nanostructured films. (A) Photoresist PMMA is spun-cast onto a silicon wafer to form an even, thin coating on the surface. (B) Electron-beam lithography is used to etch the desired nanostructure pattern into the PMMA. (C) A thin layer of chrome is deposited onto the surface of the remaining PMMA and silicon. (D) PMMA is removed by dissolving in DCM. (E) The wafer is exposed to inductively coupled plasma reactive ion etching to anisotropically etch through the silicon. Chrome protects non-exposed regions. (F) Chrome is removed in an acid etchant solution. (G) The surface of the nanostructured silicon is rendered hydrophobic via silane vapor deposition of a hydrophobic silane such as trichloro(1H,1H,2H,2H-perfluorooctyl)silane. (H) Nanostructured films are fabricated by applying a polymer solution of polyurethane acrylate to the silicon wafer. A backing layer comprised of polyethylene terephthalate is applied and the polymer crosslinks under UV light. (I) The backing layer is peeled from the silicon mold to reveal the nanostructured film.
Figure 4-2 Top-down and cross-section SEM images of six different nanostructure patterns. Nanostructures with (A) diameter of 100 nm and pitch distance of 300 nm; (B) diameter of 200 nm and pitch distance of 400 nm; (C) diameter of 500 nm and pitch distance of 600 nm; (D) diameter of 100 nm and pitch distance of 700 nm; (E) diameter of 200 nm and pitch distance of 800 nm; (F) diameter of 500 nm and pitch distance of 1000 nm. Scale bars are 200 and 500 nm respectively.
Figure 4-3 Nanostructured film toxicity assay. *In vitro* PrestoBlue viability data showing that nanostructured films do not show significant toxicity to Caco-2 intestinal epithelial cells after contact for 2 h. Error bars are shown in SD. n = 3
Figure 4-4 Transepithelial electrical resistance assay over 2 h. The presence of any film, flat or nanostructured, significantly lowers TEER by 25-75% at 0.5, 1, and 2 h time points. Error bars are shown in SD. *** indicates p-value < 0.001; n = 3.
Figure 4-5 IgG drug permeation assay over 2 h. The presence of any film, flat or nanostructured, increases drug permeation at all time points. HD 200 films show significantly higher drug permeation at 1 h over control. All groups show statistically significantly higher drug permeation over control at 2 h. Error bars are shown in SEM. ** indicates p-value < 0.01; *** indicates p-value < 0.001; n = 4-6
Figure 4-6 IgG drug permeation assay over 2 h with HD films only. The presence of any film, flat or nanostructured, increases drug permeation at all time points. HD 200 films show significantly higher drug permeation at 1 h over control. HD 200 and HD 500 films show significantly higher drug permeation over control at 2 h. HD 200 films show significance over flat and HD 100 films at 2 h. Error bars are shown in SEM. * indicates p-value < 0.05; *** indicates p-value < 0.001; n = 9-12
Figure 4-7 Immunostaining of Caco-2 cells with or without treatment. Fluorescent intensity is observed to decrease in both ZO-1 and actin staining for flat and HD 200 experimental groups compared to no treatment control. Nucleus = blue; ZO-1 = green; Actin = Cytoskeleton.
Figure 4-8 Nanostructured film toxicity assay. *In vitro* PrestoBlue viability data showing that surface-treated flat and nanostructured films do not show significant toxicity to Caco-2 intestinal epithelial cells after contact for 2 h. Error bars are shown in SD. n = 3
Figure 4-9 Transepithelial electrical resistance assay over 2 h. The presence of any chemically-treated film, flat or nanostructured, significantly lowers TEER by 55-80% at 0.5, 1, and 2 h time points. Error bars are shown in SD. ** indicates p-value < 0.01; *** indicates p-value < 0.001; n = 3
Figure 4-10 IgG drug permeation assay over 2 h with HD 200 films only. The presence of any film, plasma, untreated, or silanized, increases drug permeation at 1 and 2 h time points. HD 200 silane films show significantly higher drug permeation at 2 h over control. HD 200 silane show significantly higher drug permeation over plasma-treated HD 200 films at 2 h. Error bars are shown in SEM. * indicates p-value < 0.05; *** indicates p-value < 0.001; n = 9-12
4.8 LIST OF TABLES

Table 4-1 Desired nanostructure geometric parameters for each pattern.

<table>
<thead>
<tr>
<th>Nanopattern Geometry</th>
<th>Diameter (nm)</th>
<th>Height (nm)</th>
<th>Pitch (nm)</th>
<th>Aspect Ratio</th>
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<tr>
<td>LD 100</td>
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<td>&gt;200</td>
<td>600</td>
<td>&gt;2</td>
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<tr>
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<tr>
<td>HD 200</td>
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<td>700</td>
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Table 4-2 SEM geometry measurements of six different nanostructure arrays.

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<th>Nanopattern Geometry</th>
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<th>Height (nm)</th>
<th>Pitch (nm)</th>
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<tr>
<td>LD 100</td>
<td>110.91±11.55</td>
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<td>403.34±12.22</td>
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Table 4-3 Water contact angle measurements on flat and nanostructured films. n = 3

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<tr>
<td>HD 200 Plasma</td>
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<td>HD 200</td>
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<td>Flat Silane</td>
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CHAPTER 5: SUMMARY AND CONCLUSIONS
5.1 CONTRIBUTIONS TO THE FIELD

The presented dissertation work investigated new methodologies that can overcome the core challenges facing oral drug delivery. Those obstacles are as follows: (i) protect drug payload, (ii) efficiently load drug without minimal loss while retaining stability, (iii) control rate of drug release at the desired delivery location, (iv) enhance drug permeation, and (v) scale device fabrication.

In previous work, efforts to use micro/nano fabrication technologies yielded the development of low-aspect ratio microdevices that possessed a reservoir designed for unidirectional delivery. These microdevices acted as effective drug carriers and had increased retention rate inside of the small intestine due to their geometry and ability to resist shear stress. Further modifications that added chemical moieties or nano-scale topographical protrusions to the surface of the microdevices further enhanced the adhesion of these devices to the small intestine.

In chapter 2, we developed a method to load drug inside microdevices in a highly efficient, low-waste manner. Stability of insulin, our model API, was retained when printed from an inkjet printer. Other drugs, such as small molecule topotecan, were loaded successfully in the microdevices as well. Furthermore, microdevices were capped with a biocompatible, muco-adhesive polymer known as chitosan. Chitosan caps were able to control the release of insulin in simulated intestinal model systems.

In chapter 3, we established a bottom-up fabrication approach that was able to produce microdevices, load drug, and cap drug-loaded microdevices with an enteric polymer. This approach, building off of our previous work using an inkjet printer, was able to successfully fabricate microdevices out of a widely-used pharmaceutical polymer coating known as Eudragit. Furthermore, we were able to load insulin into said Eudragit microdevices and cap using various formulation blends of different Eudragit polymers. Different blends yielded different insulin release kinetics in simulated intestinal conditions, thereby allowing us to control drug release.

In chapter 4, we investigated mechanical cues as a means to enhance oral protein delivery. Through a systematic investigation of different nano-scale geometric parameters (e.g. diameter, height, pitch), we report an optimal set of parameters that were able to enhance IgG permeation through an intestinal model system. Furthermore, we found that the chemical properties and surface energy of our materials played an important role in enhancing drug permeation.

5.2 FUTURE DIRECTIONS

Through the development of different technologies, we have been able to address the various challenges in oral drug delivery. Still, there is significant work left to be done, as much of the work described was conducted in in vitro model systems. The next logical step would be to test our microdevice technology in rodent models to assess if the technology is translatable in vivo. In addition, the development of an all-in-one microdevice that can address each oral drug delivery obstacle simultaneously would be desirable. We envision such a device would incorporate the existing microdevice design with biocompatible materials, utilize inkjet printing for efficient delivery of drug, enteric/mucoadhesive materials as a capping agent, and a nanostructured surface that can promote adhesion and drug permeation in the small intestine.