ABSTRACT

Title of Thesis: PHARMACEUTICAL INNOVATION THROUGH LASER LITHOGRAPHY STRATEGIES

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The Human Immunodeficiency Virus is an autoimmune disease which targets the body's immune system, leaving individuals at risk of worse infections, such as AIDS. Currently, HIV is treated via drug cocktails limited by synthesized particle size, architecture, material composition, and lack of controlled drug release. Advancements in additive manufacturing through the use of Direct Laser Writing (DLW) and biodegradable structures have allowed for new methods of drug cocktail delivery. This project utilizes additive manufacturing to develop a biodegradable capsule with geometry that can be easily modified to control the release of the HIV drug cocktails. A mixture of PEGDA 250 and PETA was optimal for fabricating microcapsules, a teardrop design was tested for shell performance, and microchips facilitate targeting liquid to the shell. Our research shows promise towards the delivery of medications via controlled release mechanisms by allowing for continuous administration of medication to maintain the therapeutic window.

PILLS

Pharmaceutical Innovation through Laser Lithography Strategies

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Chapter 1: Introduction

Human immunodeficiency virus (HIV) damages the body's immune system by destroying white blood cells that help the body fight off disease [1]. Because HIV destroys the body's immune system, patients are vulnerable to other infections, such as Acquired Immunodeficiency Syndrome (AIDS), cancers, and other complications. According to the Centers for Disease Control and Prevention (CDC), 1.2 million people in the US were infected with HIV in 2019. Research has indicated that HIV prevalence rates in urban poverty rates are inversely related to socioeconomic status (SES): the lower the SES, the higher the HIV prevalence rates [2]. Across the world, HIV affects 38 million people, with 690,000 people dying from AIDS-related illnesses in 2019 alone. The area of the world most affected by HIV is the Eastern and Southern Africa region, which makes up 43% of all new cases of HIV annually [3]. Out of the approximate 39 million people with HIV in 2022, 1.5 million were children from ages 0-15 affected by the disease [4].

Currently, there is no cure for HIV, but there are treatments that focus on decreasing the viral load to stop virus progression and keep it from spreading to others. The current treatment of HIV consists of three major classes of drugs: NRTI, NNRTI, and PI's. These drug components work in tandem with each other in a drug cocktail to reduce the viral load within patients and increase their overall health [5]. With no known individual drug to fight the HIV infection, the cocktail components must be used together to best combat the virus. This highlights the importance of the creation of a biodegradable pill that can be geometrically modified to specifically control the release of the drug cocktail.

The goal of the project will be to create a biodegradable pill shell using microparticles to control the release of HIV drug cocktails, enabling the use of one pill to fight the HIV infection. Microparticles, which range in size from 10-100 μm, have been previously used in a wide range of biomedical applications, such as in controlled drug delivery. For our project,

we researched biodegradable microparticles that can be used to control the release of the HIV cocktail. We can modulate the release time of the different drugs by varying the thickness of the polymer that we use which determines how long it takes the different drugs to diffuse out. This is crucial for the treatment of HIV because the levels of the drug in the bloodstream need to be maintained within a specific range. There must be a high enough level of the drug to be effective, but there must not be too much that it becomes unsafe. The focus of this project is to implement 3D printing as a new method to create microparticles with controlled biodegradation.

Chapter 2: Literature Review

Within the literature review, we will go over the HIV virus and its subsequent treatment methods. Focus will be shifted to the HIV cocktail and the drugs that make up the treatment. We will discuss the definition of microparticles in our context and their application to drug delivery. The literature review will then cover the term “biodegradable caps” and discuss their significance to the biomedical field. The literature review will end by discussing both the process of 3D printing and how it can be used to build these biodegradable microparticles, and how 3D printing can be used to control the release of drugs in the pill against various environmental conditions.

HIV Cocktails and Drugs Used

I. HIV Cocktails and Inhibition

Current medicinal techniques fight the HIV infection with a combination of drugs that work in tandem to stop both HIV viral replication and infected cells from reproducing in order to increase the overall health of patients and decrease their viral load. Currently, HIV drug cocktails consist of three components, taken as three different pills, known as the antiretroviral therapy regime (ART). A one-pill solution that delivers the necessary drugs needed to fight HIV has yet to be developed [5]. Typically, two out of the three components in ART are made from nucleoside reverse transcriptase inhibitors (NRTIs), with the third from a class of protease inhibitors (PI) or non-nucleoside reverse transcriptase inhibitors (NNRTIs) as shown in **Figure 2-1**. With over 24 drugs approved by the Food and Drug Administration to fulfill these requirements, many variations of cocktails have been created to fight HIV infection including Atripla and Complera, each composed of two NRTIs and one NNRTI [6].

Cabenuva is one of the most recent advancements in HIV treatment. The drug consists of a combination of synergistic medications cabotegravir and rilpivirine. This

combination consists of an NNRTI and an integrase strand transfer inhibitor, distributing the drugs via injection. This has the potential to be extremely beneficial, however, consistent injections must be adhered to in order to maintain the therapeutic capacity of the medication. Further, Cabenuva reduces the efficacy of different anti-seizure medications and corticosteroids [7].

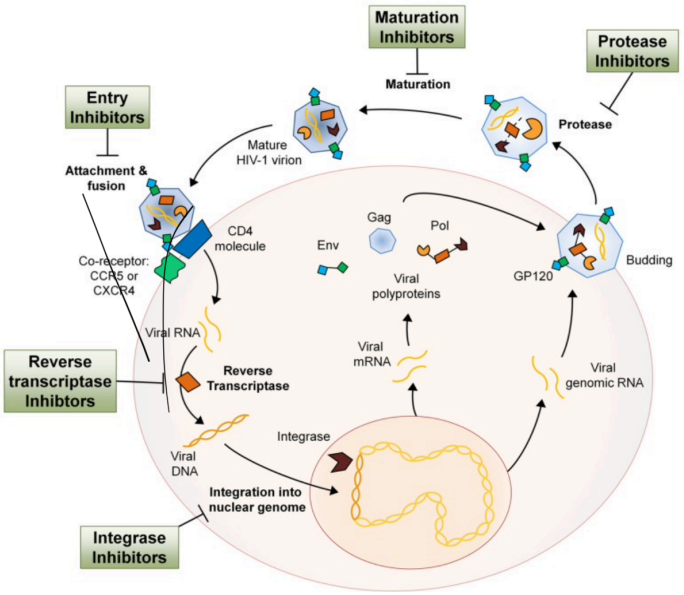

Figure 2-1. *Schematic illustrating the different drug classes combating various stages in HIV viral replication. Adapted from [8].*

II. Nucleoside Reverse Transcriptase Inhibitors (NRTI)

NRTIs serve a crucial role in the HIV cocktail. Responsible for halting the HIV virus from replicating, the drug is heavily involved in the reduction of transmittance from mother to child during pregnancy [9]. The drug's main mechanism for replication reduction involves stopping the phosphodiester bond from forming in growing DNA strands [9], thus halting DNA formation and preventing viral replication.

III. Non-nucleoside Reverse Transcriptase Inhibitors (NNRTI)

The HIV cocktail must consist of other parts in order to fully fight the HIV infection. The second drug comes from the class known as (NNRTIs. As stated earlier, HIV production

occurs largely through the enzyme reverse transcriptase. As opposed to NRTIs, NNRTIs are non-competitive inhibitors, meaning they do not directly bind to the enzyme's active site. NNRTIs disfigure the enzymes, slowing the process by which HIV can be replicated, as DNA replication would also be slowed [9].

IV. Protease Inhibitors (PI)

PIs act as the third major drug class in HIV cocktails. The main function of protease is to break down proteins through hydrolysis of peptide bonds [10]; however, some proteases can also act as viral proteases, which help the virus create new infectious virions. This occurs when viral proteases break down proteins and use them to create more virus particles and infect the body. HIV infects CD4 cells (also known as T-cells) and instructs these cells to make more proteins which are broken down by viral proteases to reproduce more of the virus. PIs target these viral proteases, stopping them from breaking down proteins [11]. Thus, PIs serve a complementary and important role in the obstruction of viral cell reproduction. PIs must work in tandem with NNRTIs and NRTIs as they inhibit protein formation rather than DNA replication.

Microparticles

The term microparticle (MP) generally describes particles between 1-100 μm. Engineered microparticles have a variety of applications in the medical field and can be categorized into four distinct groups: cell carriers, identifiers for biological assays, actuators for biomedical applications, and drug delivery agents [12]. In the case of drug delivery, the ability of MP containers to carry drugs, deliver them to a target site, and release them in a controlled manner (i.e. controlled release drug delivery) has motivated researchers to develop techniques for manufacturing these microparticles with specified functionalities. MPs have properties that raise several interesting possibilities in the application of drug delivery.

Introducing biodegradable material into MPs allows for the controlled release of drugs contained within the MP by varying the thickness of the material used [13].

Typically, microparticles have been produced through emulsion polymerization, but these approaches encounter issues such as polydispersity (nonuniformity in particle sizes and shapes), poor reproducibility, and lack of precision in creating complex morphologies. Since there has recently been a growing recognition for the necessity of custom-designed and non-spherical microparticles for various applications (e.g. biomedical applications), many have researched other techniques such as electrohydrodynamic (EHD) systems and microfluidics-assisted droplets.

EHDs are relatively uncommon as there are more advanced techniques for fabricating MPs. EHDs involve the usage of a high-voltage electric field to break up a solution into MPs. While it can produce MPs with low feature sizes, it does not allow for flexibility in the morphology of fabricated MPs. The microfluidic-assisted droplet technique for engineering MPs offers finer control over the morphology, size, and substance of the spherical MPs. However, it is also hampered by the inflexibility of MP morphology [12].

The shortcomings of these selected techniques give some idea of the challenges that the greater variety of techniques face. Current technology for creating microparticles remains limited in regard to particle polydispersity, reproducibility, and the construction of sophisticated morphologies. An alternative set of techniques that have gained attention involves additive manufacturing (3D Printing). In particular, “direct laser writing” (DLW) allows for high feature resolution and complicated particle architecture. It involves focusing a laser within a photocurable material to induce polymerization in a layer-by-layer manner to produce a 3D object. DLW has been used to fabricate liquid-core-shell MPs with orifices and separately added caps [14].

Biodegradable Caps

The biomedical field has sought to use 3D printing as a reliable source for creating micromaterial, such as biodegradable microneedles. M. A. Luzuriaga et al. have been working towards creating this microparticle biomedical material. Due to print resolution, they discovered that cylindrical shapes worked better than traditional conical-shaped needle heads. More importantly, they used polylactic acid (PLA), a biodegradable substance that can be used in 3D printing at a small scale (~100 μm) [15]. L. A. Beardslee et al. researched the utilization of polycaprolactone (PCL), poly lactic-co-glycolic acid (PLGA), and polyglycidyl methacrylate (PGMA) as biodegradable polymers [16]. These materials were promising for working with human trabecular meshwork cells (HTMCs). This research group also used a method called the "sacrificial process," in which a thin layer of a substance was 3D printed into a mold. Then it etched away to produce a very thin layer of biodegradable material. This process could be used to create a very thin biodegradable cap. A third study conducted by F. Claeyssens et al. achieved the creation of a biodegradable polycaprolactone-based triblock copolymer [17]. They also performed initial cytotoxicity tests, which can be used to see if the material would interfere with cell division, and thus throw off the cell growth rate, and therefore be toxic. Fortunately, the initial tests showed that the material did not interfere with cell division, which promises another safe, biodegradable, 3D-printable substance.

Looking at Two-Photon Polymerization, research has shown that certain materials, such as Irgacure 2959 and Irgacure 369-SO3NA, have proven to work in certain human cell types [18]. Additionally, enzymes could be used to speed up dissolution times, such as chymotrypsin, aminolysis, and a basic pH environment; however, acidic environments were not a good environment to promote the dissolution of the biodegradable substances [19].

3D Printing

3D nanoprinting multidrug delivery capsules are minimally addressed in available literature. Advances in 3D printing such as DLW and microfluidic strategies have opened up the possibility to mimic drug cocktails and other multidrug delivery capsules in a much more efficient manner. These advances offer new methods for controlled drug delivery through biodegradable caps and multidrug deliveries, similar to current HIV cocktails. Currently, other proposed methods of creating similar drug cocktail particles, such as centrifugal-based synthesis, gas-shearing fabrication, and microfluidic droplet synthesis utilizing oils contain significant drawbacks that limit their control of the synthesized particles' size, architecture, material composition, and the ability for controlled multidrug release [20].

I. Alternative Methods

Alternative proposed methods for multidrug delivery capsules other than 3D printing include centrifugal-based and microfluidic droplet synthesis. Centrifugal-based droplet synthesis allows for the formation of compartmentalized and orientable particles through the utilization of centrifugal forces ejecting pre-polymerized solutions through multi-capillary arrays. While this process has the advantage of having relatively simple parameters that allow for the control of particle size, the shape and material composition of the final product is limited to spherical-based architectures composed of a solution of polymers [21]. Gas-shearing methods are similar to centrifugal processes, instead utilizing a shear force from the ejection of gas to emit particles. This process contains the same drawback of limited particle shape and material composition [22]. Alternatively, microfluidic droplet methods that utilize oils to transport particles can be used to create particles with a more varied selection of structures. Droplets propelled by parallel oil flow allow for the creation of layered and compartmentalized particles; however, the presence of crude oils that are not bio-friendly as

well as the restriction to spherical-based architectures restricts the use of this method for our applications [23].

II. Advancements in 3D Printing

Advancements in printing speed and 3D volume elements (“Voxels”) have allowed for more flexibility in 3D printing [24]. In addition, DLW can be used to fabricate microparticles with anisotropic shapes, such as circular disks [25]. Traditional DLW techniques rely on manual alignment and thus must account for the significant potential for human error. Some have improved on multi-material microfluidic printing abilities by developing a stainless steel substrate mounting component and customized pressure-flow control units to dispense the fluids; however, techniques like these are incompatible with in situ DLW [26]. To address this concern, researchers have developed a new microfluidic multi-material direct laser writing (μFMM-DLW) technique that improves fabrication and processing time, registration accuracy, and repeatability [27]. These advancements allow for more efficient and flexible 3D printing options. This allows for the creation of a micro-scale capsule that can be used in multidrug delivery methods. The capsule can be fully printed and using microfluidic techniques, and the liquid or pharmaceutical can then be placed inside the capsule.

III. 3D printing biodegradable structures

Further advances include innovative geometric designs for 3D-printed biodegradable structures that allow for more precise modulation of late-stage biodegradation [13]. Combining these advances with the previous ones allows for a fully 3D-printed capsule with a biodegradable cap. This enables the controlled release of the drug, as the biodegradable caps can vary in size thus allowing for different times of degradation. These advances allow for the rapid design of unique multidrug capsules, controlled release of the drug through a biodegradable cap, and μm-scale resolution through DLW techniques.

Controlled Release

A key component of our biodegradable cocktail will be the release mechanisms used to deliver the drug into the body. To have a drug released into the body, it must flow down its concentration gradient. This follows the law of diffusion, as the drugs exit down a concentration gradient from their container until they reach equilibrium. The initial release sees a sharp rise in concentration until it can plateau at equilibrium, one which must be maintained within a therapeutic window [28]. Controlled release systems are used in drug delivery to ensure that the drug level in the blood stays within the therapeutic window. The therapeutic window is the range of drug concentration between the minimum effective concentration and the minimum toxic concentration as shown in **Figure 2-2** [29]. Targeting the therapeutic window in drug delivery makes the drug more effective by improving its bioavailability and reducing many toxic side effects [30]. Many single-dose drugs raise the drug level in the blood above the minimum toxic concentration level and then cause a rapid drop below the minimum effective concentration [29]. This fluctuation in drug levels in the blood can cause many toxic side effects in those taking single-dose drugs or medications.

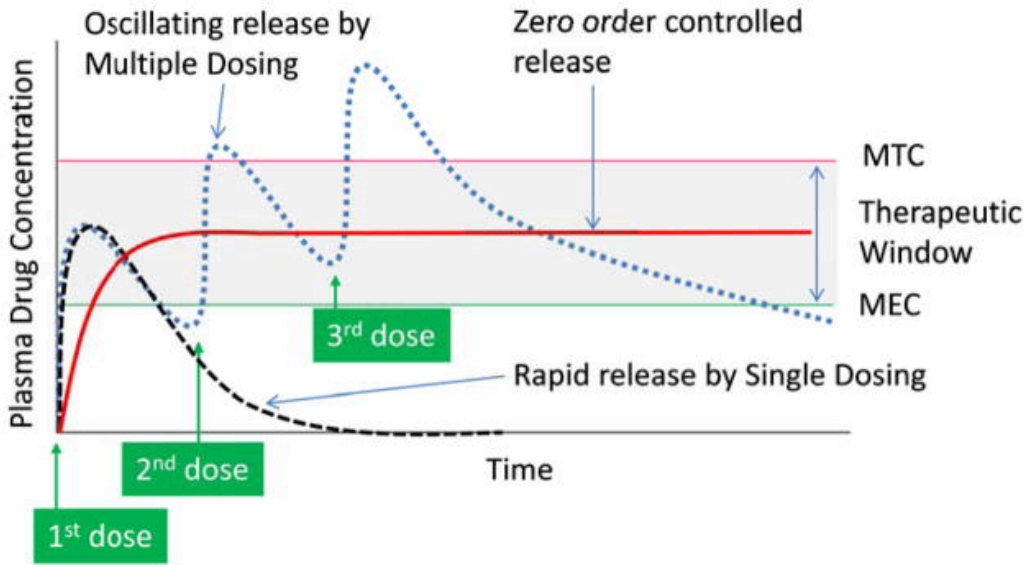

Figure 2-2. *Graph visualization of single dosing, multiple dosing, and controlled release drug concentration over time compared to the minimum toxic concentration (MTC) and minimum effective concentration (MEC). Adapted from [29].*

The dosage must be maintained at a constant therapeutic level for proper delivery of any particular drug to the body. As any concentration is broken down, it must be replenished for as long as desired by keeping the drug concentration at a healthy equilibrium. Subsequently, the drug must neither dip below a non-therapeutic level nor must it enter a toxic dosage range. Controlled release is a mechanism of action that allows dosage concentrations to remain within a therapeutic window [28,29]. To maximize the effectiveness of the drug while minimizing the time when the concentration is toxic to the body, the release of the drug must be maintained within the therapeutic window. Drugs can be released episodically, where the drug is re-administered as metabolism mechanisms in the body begin to degrade the drug, thus allowing the concentration to be maintained beneath the minimum toxic concentration (MTC) and above the minimum effective concentration (MEC) [28]. A controlled delivery also reduces the number of doses needing to be administered, as shown in **Figure 2-3** from Adepu, S. & Ramakrishna, S. (2021) [28].

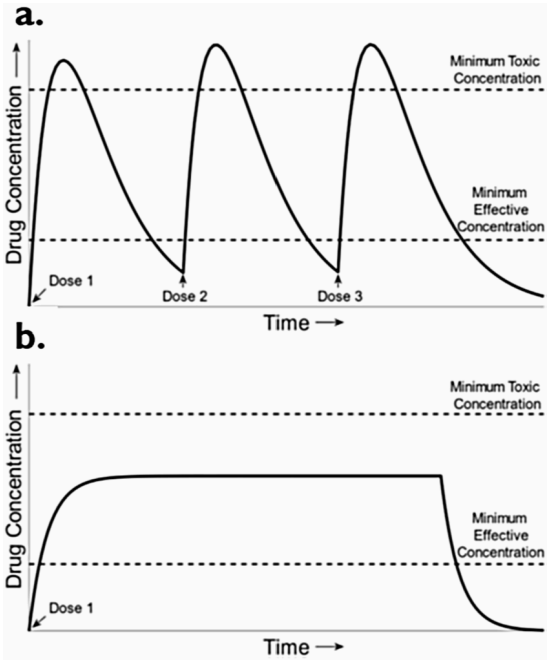

Figure 2-3. *Graph comparing traditional drug delivery systems to controlled release systems. Adapted from [28].*

I. Factors that Influence Drug Release

Many factors need to be considered when designing a controlled drug release system including the composition of the drug, polymer, and other additives; the ratio of drugs to biodegradable material; the physical and chemical interactions among the components of the drug, and the preparation methods that are used [31]. An experiment by Professor Innocent Macha et al. (2019), was conducted to test the dissolution behavior of two drugs: GentaMicin (GM) and clodronate disodium BisPhosphonate (BP). Coraline HAp was used for drug loading and release within a polymer-based matrix. They were able to obtain diffusion coefficients based on the geometry of the drug containers. They then assert from their results that diffusion coefficients change over time and are not constants independent of the geometry of the polymer carrier; they are also concentration-dependent [32]. Thus, the diffusion of the drugs from their containers was not only based on container configuration; it was also influenced by the concentration of the drug within the vessel.

II. Mechanisms of Controlled Release Systems

A few drug delivery systems commonly used are chemical reaction, diffusion, and stimuli-controlled release. Chemical reaction drug release involves biodegradable polymers that release the drug causing enzymatic degradation of the backbone. Diffusion and stimuli-controlled release take place in response to the drug carrier's surrounding environment, which can include temperature, pH, and concentration of the drug [29]. Many controlled release systems used in drug delivery today make use of several different mechanisms of release. Our main approach is to use a chemical reaction release in the form of biodegradable caps.

Polymers are used in controlled release systems because of their biocompatibility and degradability. The process of degradation of polymers depends on the surface erosion of the polymer matrix, cleavage of polymer bonds, and the diffusion of the entrapped drug [31]. The

actual degradation of polymers follows three phases beginning with an initial burst due to the diffusion of the drug, a lag phase, and then the controlled release of the drug due to the degradation of the polymer [32]. Another consideration when implementing polymers for controlled release systems is the properties of the polymers. Polymer crystallinity, for example, describes the degree of crystalline regions within a polymer sample in relation to amorphous regions. This is important because only amorphous regions are permeable and therefore accessible to water molecules. A lot of different factors are dependent on polymer crystallinity including polymer mechanical strength, swelling, and biodegradation rates. Another property of polymers is their glass transition state which determines whether the amorphous region is in a “glasslike” or “rubberlike” state [31].

Drug delivery can also be achieved using an osmotic agent, one in which osmosis controls the movement of the drug out of its container. The osmotic containers contain a water-permeable membrane, with one side of the membrane having an osmosis agent while the other side contains the drug. As the water diffuses into the side with the osmotic agent, the membrane pushes against the side with the drug, forcing it out through an orifice in the capsule [28]. Other mechanisms result in the biodegradation of the capsule, with the release of the drug occurring with the erosion of the container [28]. Such biodegradable materials are decomposed via enzymatic and non-enzymatic reactions that yield harmless waste products to be excreted. Using nanomaterials over larger compounds has been shown to increase the efficacy of these biodegradable deliverables, especially as many of the biodegradable materials are not toxic to the human body [33]. For the context of this thesis, a biodegradable shell will be used as the primary mechanism of action.

Microparticle Fabrication

A considerable amount of research has been conducted on the fabrication of core-shell microparticles for their application in drug delivery. In particular, research and findings from

Acevedo et. al. informed our defined methodology and is the work we will be building off of throughout this thesis. The main focus of their research was to address specific challenges related to the fabrication of 3D printed microparticles including the diversity among products in terms of size and shape as well as a limited ability to customize the geometry of these microparticles. Due to these challenges, Acevedo et. al. employed a DLW approach that allowed for tunable shell geometry and architecture.

The methodology for printing the microparticle shell, loading in the liquid core, and sealing the core by printing the cap on top, is similar to the methods outlined in the next section. The fabricated microparticle shells shown in **Figure 2-4** were designed with varying top orifice diameters ranging from 2 μm (left) to 12 μm (right), increasing by 2 μm from one column to the next.

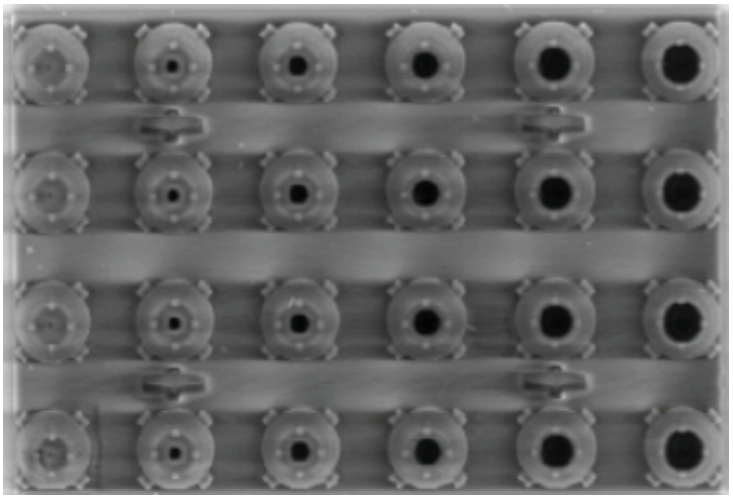

Figure 2-4. *Printed microparticle shells with varying orifice diameters. [12]*

Following visual inspection of the fabricated microparticle shells using Scanning Electron Microscopy (SEM), an evaluation was done to measure the relative fluorescence intensity (RFI) in relation to the loading potential of the shells and determine if the diameter of the top orifice affected that potential. Acevedo et. al. found that microparticles with a top orifice diameter of 4 μm or greater, could effectively be loaded with methylene blue-dyed DI water while loading was inhibited in microparticles with a topo orifice diameter of 2 μm. The

RFI values for each orifice diameter is shown in **Figure 2-5c.** They also saw that after once the microparticle shells were successfully loaded with the liquid core, the microparticles with a top orifice diameter between 4 and 8 µm, retained their liquid core as shown in **Figure 2-5d.**

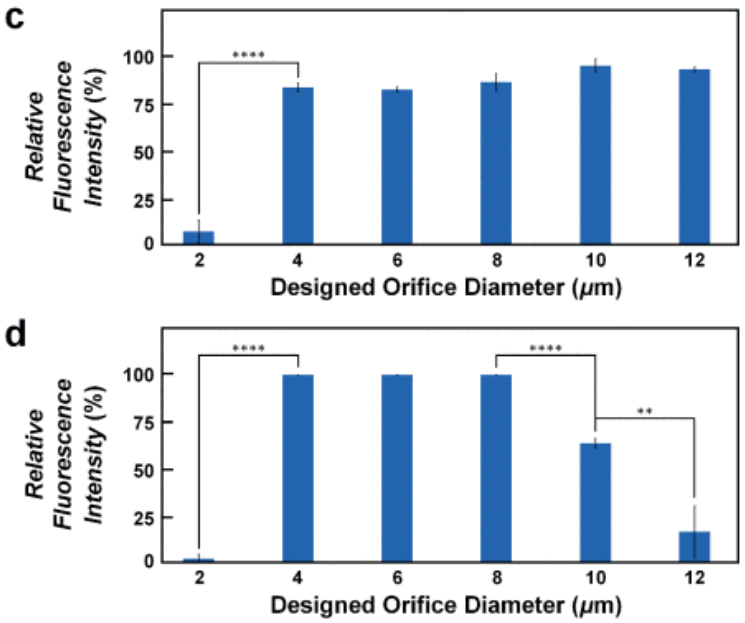

Figure 2-5. *Relative fluorescence intensity measurements for quantification of (c) core loading potential and (d) liquid core retention without a cap. [12]*

To determine how the cap of the microparticle affects its retention potential, Acevedo et. al. added rhodamine B-dyed IPA to microchannels that contained microparticles both with (**Figure 2-6b**) and without caps (**Figure 2-6a**). By again using RFI calculations as shown in **Figure 2-6c**, they found that the microparticles without caps (negative control) filled with rhodamine B-dyed IPA, while those with caps effectively sealed the liquid cores. The microparticle with a top orifice of 4 µm had the highest retention rate compared to the other microparticle orifice diameters and is suggested to be the best choice for this application.

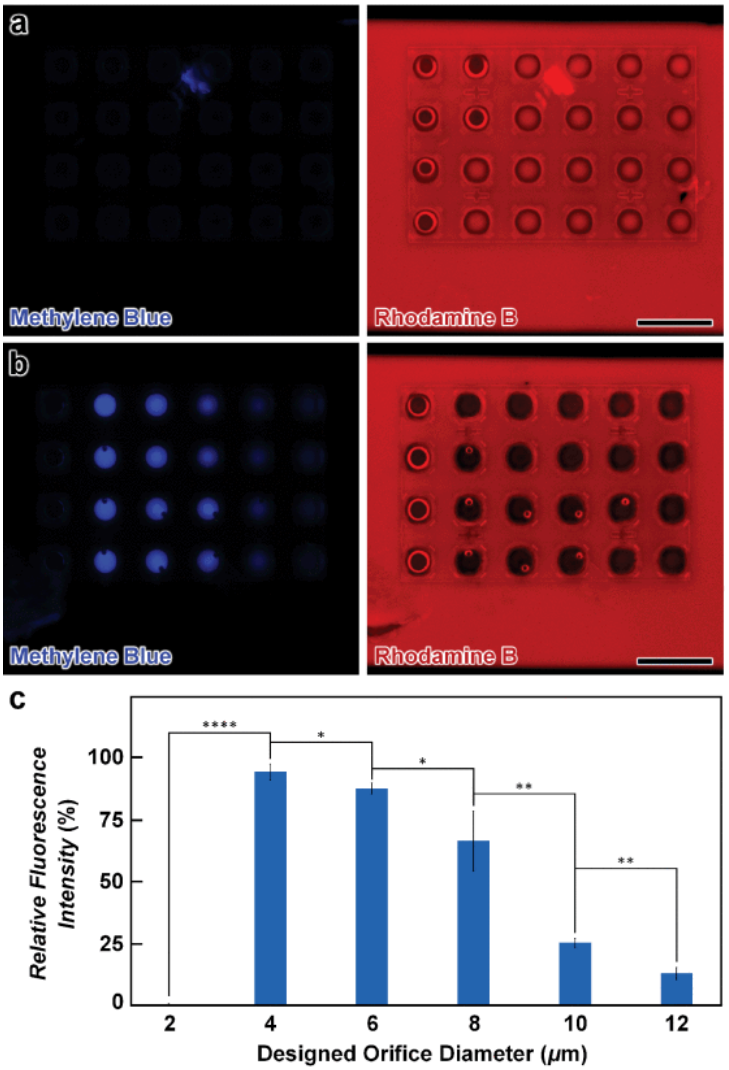

Figure 2-6. *Retention of liquid core following capping of the micropartice shells. [12]*

The results from the research conducted by Acevedo et. al., were insightful into the fabrication process of liquid core-shell microparticles as well as into the effect that the orifice diameter has on the core loading and retention potentials. To expand on this research, it will be important to determine if the 4 µm orifice diameter is still effective for different shell geometries and outer shell diameters. It will also be important to look into how an actual therapeutic liquid core will interact with the shell and cap materials and if it will affect the integrity of these materials at all. Research by Acevedo et. al. provided an all-important foundation for the research and results detailed in the following sections.

Chapter 3: Methodology

I. Research Questions

From our literature review, we established research questions that will drive our methodology. We will first address whether direct laser writing-based strategies can be extended to create multi-material microcapsules containing drug cocktails. Second, we will determine what biodegradable materials will be compatible with this process. There are several factors we have to consider for what materials we will use. The material must be photocurable allowing for laser printing, and it must not interact with the drugs. Finally, we will look into how precisely controlled-release kinetics can be tuned via the geometric customization of biodegradable caps. Once we have biodegradable capsules with multiple separate cap and shell materials, we can move on to modifying the cap thickness to control the drug release rate. For example, thicker caps would result in longer release times.

II. Printing Pill Capsules

The method of fabrication chosen for the experiments is Microfluidic Multi-Material Direct Laser Writing. The first step involves the creation of 3D models of the outer shell in Computer Aided Manufacturing (CAM) software as shown in **Figure 3-1**. This process will rescale the models, allowing us to print with the Nanoscribe Photonic Professional GT DLW 3D printer. In preparation for printing, glass slides need to be silanized for better adhesion between the print and substrate. The material for the capsule will then be loaded onto the slides, and the shells will be printed with a top orifice. After the outer shell cores are printed, the next step will be to load liquid cores into the outer shell. A polydimethylsiloxane (PDMS) microchannel molded after the shape of the outer shell will be created with inlet and outlet ports punched into the PDMS microchannel.

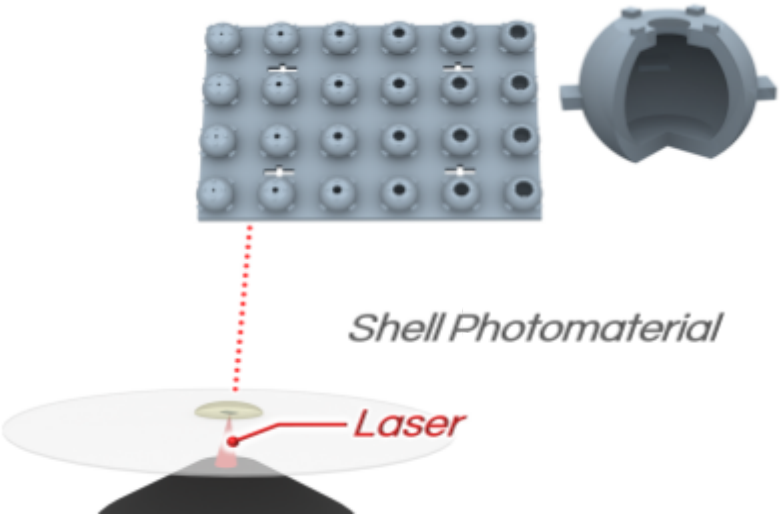

Figure 3-1. *First step of printing microcapsules with the DLW approach. Laser photo-cures the cap material. [14]*

III. Microchip Fabrication

Fabrication of polydimethylsiloxane (PDMS) microchips, with an embedded microchannel, is essential to the printing process of our biodegradable pill capsules as shown in **Figure 3-1**. The microchannel allows for vacuum loading of the therapeutic drug core into the microcapsule as well as subsequent printing of the cap material, enclosing the therapeutic core in the microcapsule shell. Fabrication of the microchip begins with a mixture of PDMS in a 10:1 ratio. This ratio consists of ten ten-part base and one-part curing agent. After this mixture is obtained, it is placed into a vacuum chamber to remove any air bubbles. The PDMS mixture is then poured into existing microchannel molds, placed again in the vacuum chamber, and cured on a hot plate overnight as shown in **Figure 3-3**. Once the PDMS is cured, the microchannel is cut away from the mold, and the inlet and outlet holes of the microchannel are punched into the PDMS shown in **Figure 3-4**. In order to prepare the microchannel for a vacuum seal, the outlet port will be covered with tape, allowing the channel to hold a vacuum outside of the vacuum chamber. The PDMS microchannel is then aligned and thermally bonded to the substrate slide before being placed in a vacuum chamber.

The vacuum chamber is then turned on, creating a seal within the microchannel that is aligned with the substrate slide and preparing the microchannel to draw in the target liquid.

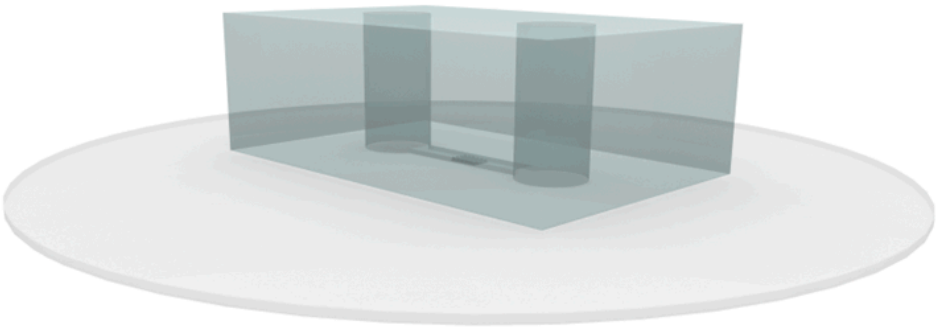

Figure 3-2. *Model of PDMS microchannel displaying the inlet and outlet channels used to vacuum load the shell capsules. [14]*

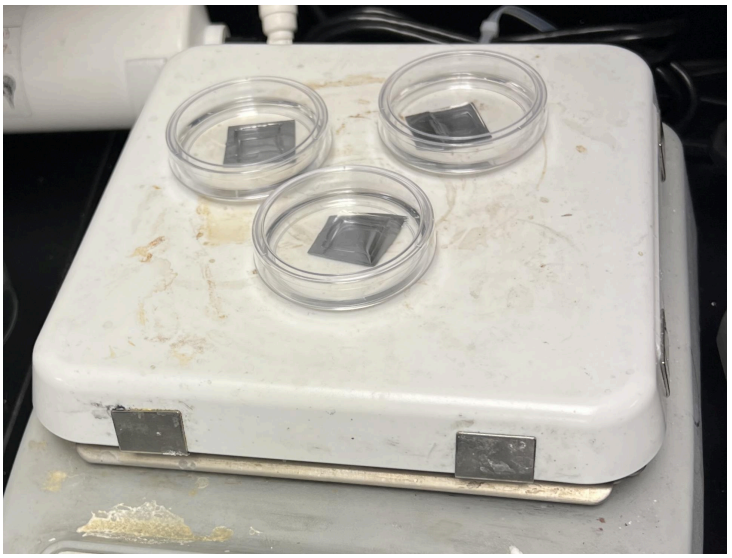

Figure 3-3. *PDMS curing in the microchannel molds. Curing is accomplished by placing the PDMS on a hot plate overnight.*

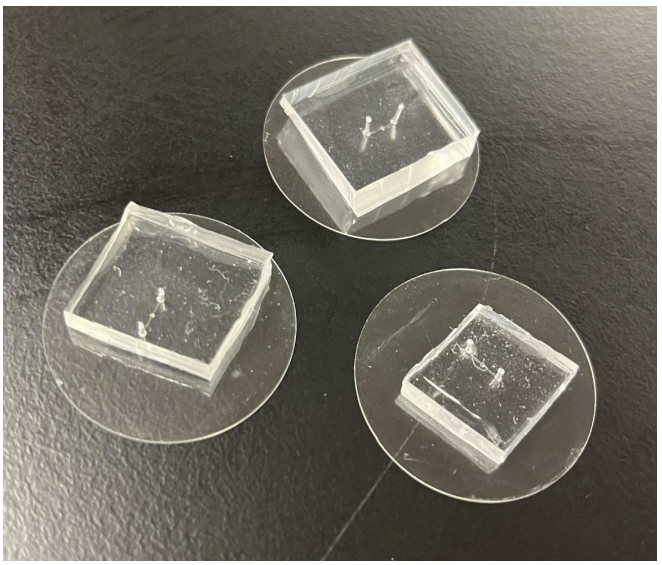

Figure 3-4. *Fully cured PDMS microchannels. Inlet and outlet holes were punched on either side of the channel to allow for vacuum loading of the microcapsule.*

IV. Liquid Core Loading and Printing of Cap

After the microchannel-substrate is removed from the vacuum, the target liquid is placed on the microchannel. The liquid will then be drawn into the microchannel and shell capsules, filling them as shown in **Figure 3-5**. Once the shell cores are filled with the target liquid, *in situ* direct laser writing (*is*DLW) will be used to print identical caps using a very similar procedure as the one for outer shells. However, an additional step of aligning the full fluidic sealing of the caps on the printed shells is needed. Once printing of the caps is complete, the print is to be chemically developed, with propylene glycol methyl ether acetate (PGMEA) and IPA as shown in **Figure 3-6**.

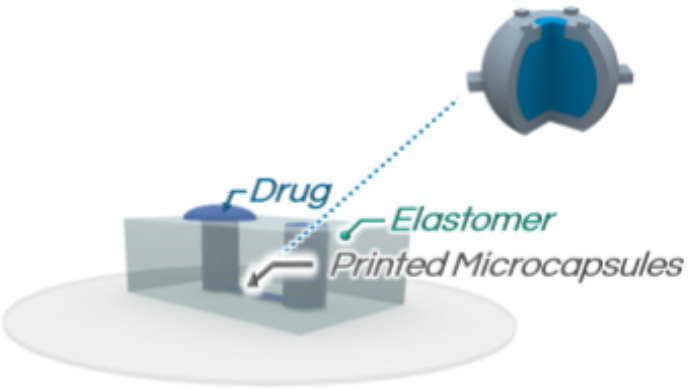

Figure 3-5. *Loading of the liquid core into the microcapsule using PDMS microchannel. [14]*

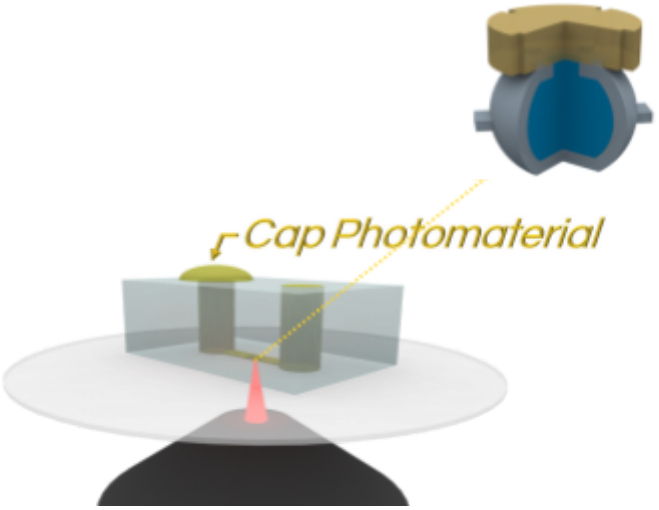

Figure 3-6. *Printing of cap material on top of the microcapsule loaded with a therapeutic core. [14]*

V. Cap Degradation

Cap degradation was attempted by submerging the caps into a 1 normal (N) sodium hydroxide solution. 1 N solution is created by mixing one molar equivalent of sodium hydroxide in one liter of water, equating to 40.00 grams of sodium hydroxide in the liter of water. Prints of the cap material were submerged for at least 8 hours and monitored through Scanning Electron Microscopy (SEM) for changes in structure.

VI. Analyzing Results

Experimental results will be quantified using analytic software, such as ImageJ, to measure the orifice and capsule diameters from captured SEM micrographs. Quantification enables the evaluation of the precision of varying objective lenses (ex. 25x and 63x) in creating desired diameter measurements in the microscale. Through the use of brightfield and fluorescence microscopy, microparticles can be monitored through the microfluidic vacuum-loading process and subsequent infusion processes to provide insight into potential sources of error. Subsequently, the efficacy of varying orifice diameters in retaining the liquid core can be evaluated following the photo-material loading process. By the end of the analysis, the ideal orifice diameter size for the microcapsules will be determined.

VII. Applications/Tools Used

This outlined procedure required a few different applications and tools. The first application we used was Computer Aided Design (CAD). CAD was used to create the shell and cap designs. Once the designs were completed in CAD, we used CAM to convert the CAD model into a laser writing path file in order to use the Direct Laser Writer, Nanoscribe. Once the capsule designs were printed we used ImageJ software and SEM to better visualize the degradation of the pills.

Chapter 4: Results

I. Initial designs

The initial proposed design of our drug delivery system consisted of a spherical shell with variable cylindrical cap thicknesses. The shells were printed from a biodegradable, photoresin known as DEGRAD INX sourced from the company BIO INX. The caps were printed using a 90:10 ratio of polyethylene glycol diacrylate (PEGDA) and pentaerythritol tetraacrylate (PETA) along with a photoinitiator compatible with the Nanoscribe.

Later designs of our drug delivery system included experimenting with different opening diameters for the shells, changing the geometry of the shells, and varying the mixture ratio of our PEGDA-PETA cap material.

II. Initial Results

Our initial experimentation steps began with printing multiple test slides to determine the degradation rate of our cap material. A PEGDA-PETA mixture was used as the cap material as mentioned and different prints were made to test the degradation rate of this material. As shown in **Figure 4-1**, the CAD design is seen to have numbers printed on squares of varying thicknesses and also on an angle to visualize the degradation. The printed designs as seen through an SEM is also shown. However, in this initial design there were inconclusive complications that resulted in no degradation rate to be found. In the design itself, there were issues with the visualization of the degradation. This ultimately led to the fabrication of a new design as seen in **Figure 4-1c**.

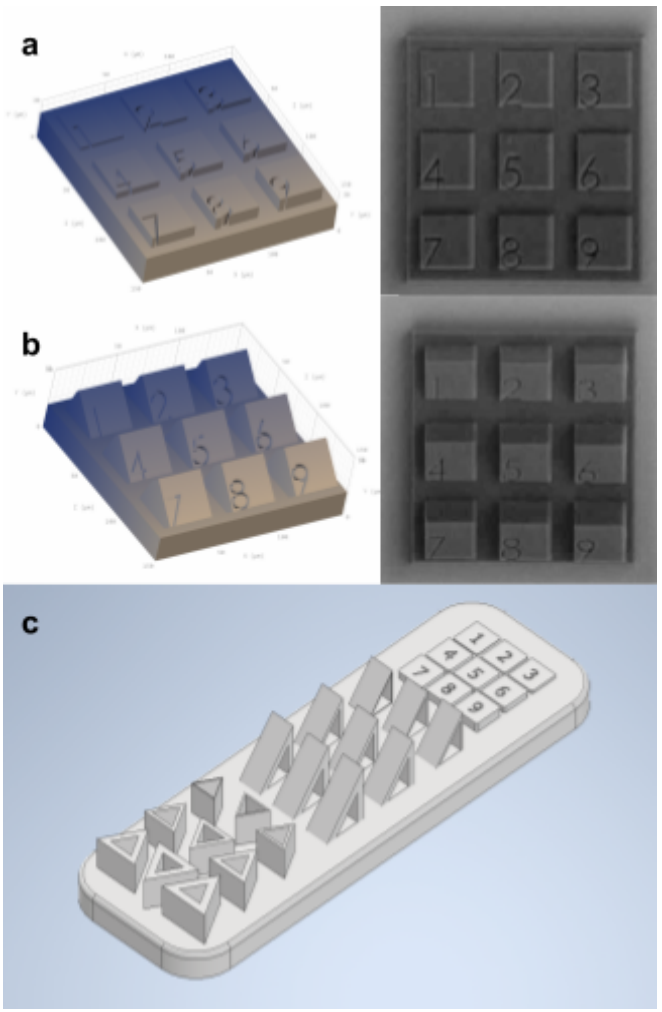

Figure 4-1. *(a) and (b) show the initial CAD models and prints of the design that were used for degradation testing. (c) shows the new design that was used for further degradation rate testing.*

The original PEGDA-PETA mixture being used had swelling properties when tested and did not degrade as anticipated. The cap was found to absorb the material placed in the shells. Furthermore, due to printing malfunctions there was an adhesion problem with the material to the base which caused the prints to float away as seen in **Figure 4-2**. This resulted in an inconclusive degradation rate as the prints could no longer be visualized. In addition, the solution used to dissolve the material was changed. Initially, the samples were tested in basic, neutral, and acidic solutions, but in order to mimic the digestive path of a pharmaceutical, a more neutral solution was used similar to the pH of the intestines. A degradation test was eventually performed, but the results showed little to no degradation in the first three days. This led to researching a different material for the cap itself. The new

material had to be biodegradable, biocompatible, photocurable/photopolymerizable, and hydrophobic.

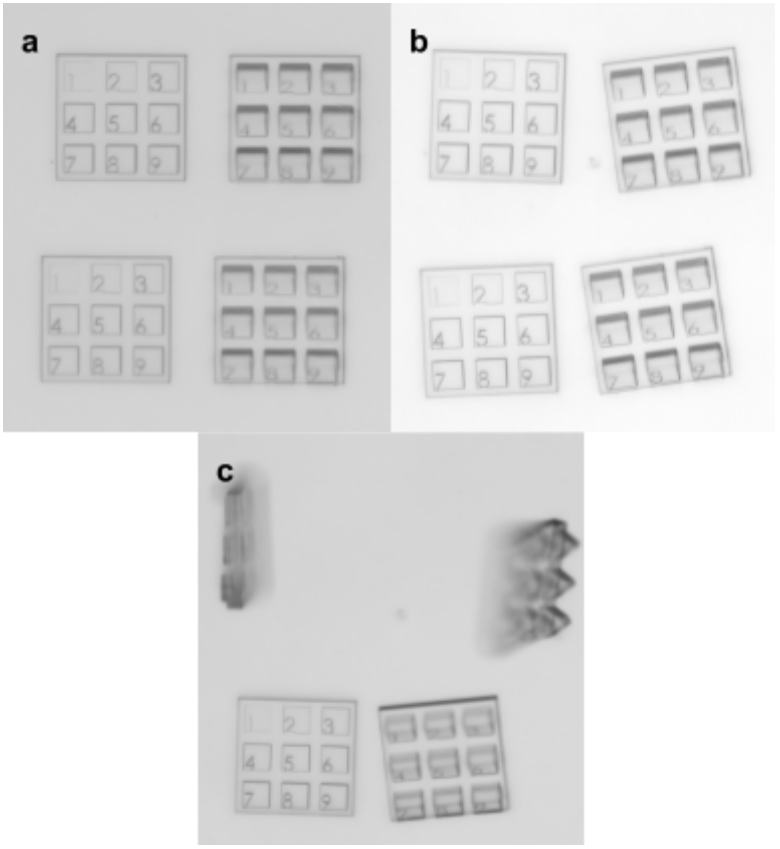

Figure 4-2. *Shows the initial failed degradation tested. Adhesion problems are seen.*

New materials for the cap that met the requirements were then researched. Initially, an entirely new material was researched. Some options included the use of polycaprolactone due to its hydrophobicity and biocompatibility, however, the degradation rate was longer than desired. Poly(L-lactide) acid was also considered due to similar characteristics. However, during this process of researching different materials, it was discovered that reducing the molecular weight of PEGDA decreased the absorption of the liquid. A PEGDA 250 and PETA mixture was then used as the final cap material.

Simultaneously, for the cap material experimentation, initial prints for the shell design were conducted. The spherical design also encountered significant failures upon print validation. The plate upon which the shells were printed was not adhering to the shells,

leading us to be unable to properly vacuum load the shells with dye for validation. We theorize that these adhesion problems were linked to issues associated with the Nanoscribe as shortly after these prints were complete, and many times subsequently, the printer was shut down for maintenance. However, within a small batch of our successful prints, we were able to properly load the shells. The spherical shells were loaded with a fluorescent red dye known as rhodamine which allowed us to analyze the shells under an SEM (**Figure 4-3**).

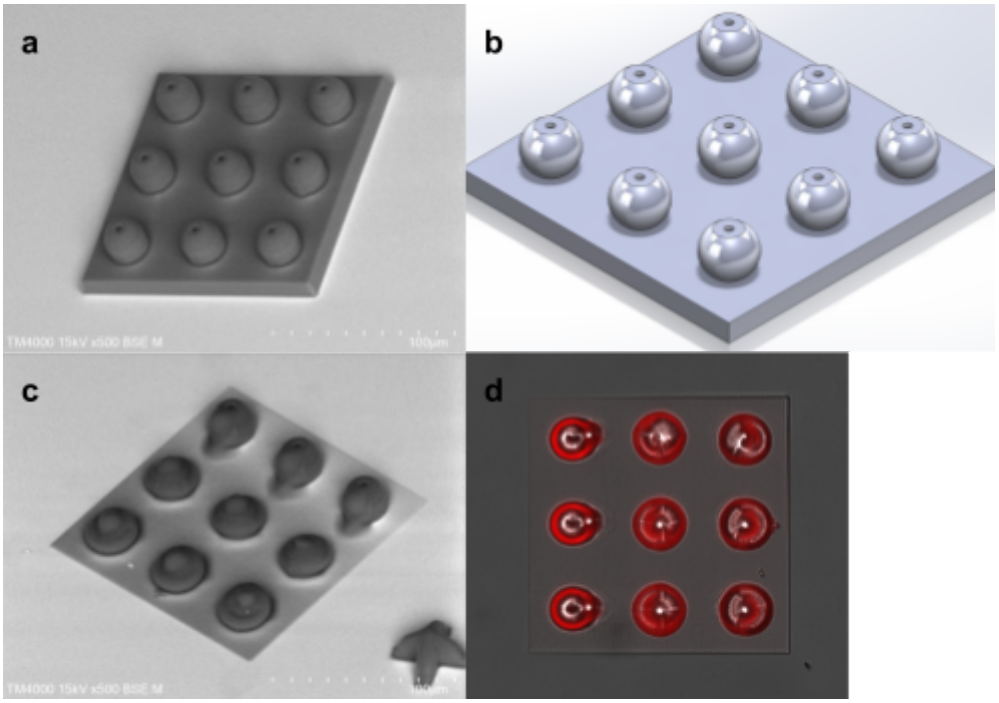

Figure 4-3. *Initial shell prints and loading with rhodamine.*

The spherical shells were observed to leak out their contained liquid over time, even before the caps could be printed. This led to many redesigns until we established a final geometry shape along with an opening size that would reduce the loss of liquid from the shells. This redesign consisted of a teardrop-shaped shell with an opening diameter of 4 μm. CAD models were created for this new design as seen in **Figure 4-4**. However, due to issues linked with the Nanoscribe machinery that rendered it non-functional for many months at a time, the designs were unable to be printed or validated.

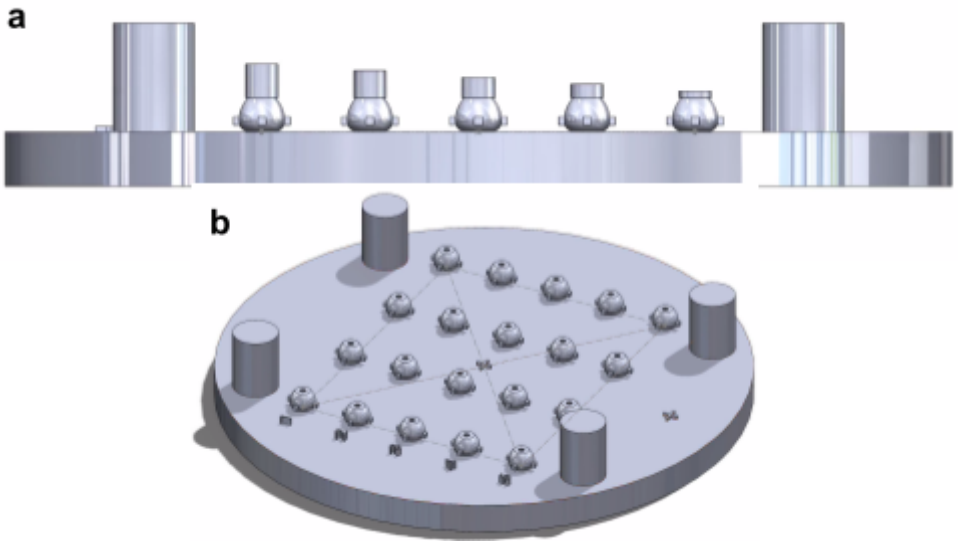

Figure 4-4. *Final designs of the shells to be used for testing.*

III. Microfluidic Channel Fabrication

The microfluidic channels were fabricated as discussed in the methods section. They were then used for the testing of these prints to allow for the controlled addition of solution and visualization of the prints. A large number of these channels were made and were functional in their application.

Chapter 5: Discussion

I. Discussion

Utilizing 3D printing as a new method to create compartmentalized microparticles with controlled biodegradation holds promise for the future of drug delivery, especially for HIV. In order to explore this idea, we created a methodology for creating the pill capsules. This involved creating models, fabricating microchips to load the target liquid, printing the cap, and then evaluating the results. Experimentally, we tested a variety of cap materials for their material properties, explored various designs for the shell, and used microchips to load test liquids into the shells. Despite various difficulties encountered along the way, we were able to find that a PEGDA 250 and PETA mixture would work well as a cap material. We were also able to find a teardrop-shaped design for the shell, with an opening diameter of 4 μm. Finally, we were able to show how the microchips could be used to add a target liquid to the shell.

II. Project Weaknesses

Difficulties related to the printing process occurred throughout this project. As mentioned in the results, the Nanoscribe printer faced many issues for nearly a year. The problems were initially unknown and issues with the methodology were questioned to begin with. This resulted in time spent attempting to solve these issues. However, it was realized that there were no problems with the methodology but with the proper function of the printer. For example, the materials did not adhere to the printer. This greatly hindered the process as the degradation tests would give inconclusive results due to floating out of view. Overall, the printer was down for approximately one year, with the only available printer being at the University of Princeton.

To combat the errors in the printer, graduate students in the BAM lab were sent to Princeton for printing. This also delayed the process as the prints would have to wait until a student could travel and make the prints. Eventually, the printer was fixed at the University of Maryland for a time, but even then, it was not fully functional. We identified errors present in the prints even after following proper methodology. This resulted in poor prints with inaccurate results until the printer was deemed fully unfunctional for the second time.

Furthermore, there are limitations in the materials that can be used in this process. As mentioned the materials in the printing process have to be biodegradable, biocompatible, hydrophobic, and photocurable. This could limit what materials are feasible to use and in turn limit the applications of this methodology. In addition, there were issues found in the printing of the caps onto the shells. The cap material was not able to print onto the open shell and solidify. It would print into the shell, where the pharmaceutical would be loaded. This would require future research to understand why this occurs and how to fix the issue.

III. Future Directions

Future work has a variety of ways to expand upon this research. For example, some treatment techniques utilize a combination of medications, such as the treatment technique used with HIV. [6]. A technique called compartmentalization can be used to put all of the medications into a single pill capsule as seen in **Figure 5-1**. Determining whether multi-material direct laser writing-based strategies can be extended to create compartmentalized microcapsules containing drug cocktails is the next step that can be taken to further our research. This method of laser writing has already been shown to be capable of creating micro-shells with one compartment, so fabrication of a multi-compartmentalized capsule would be an improvement on current research. If this research is successful, medication cocktails, such as the one used for HIV treatment, could be taken in a single capsule. Additionally, it could be investigated how the materials used throughout the

fabrication process interact with the HIV medications. The process could also be applied to other treatments, so research could be made into how the fabrication materials interact with various other medications. Hopefully, this research can enable easier drug delivery and improve the lives of those taking HIV medications.

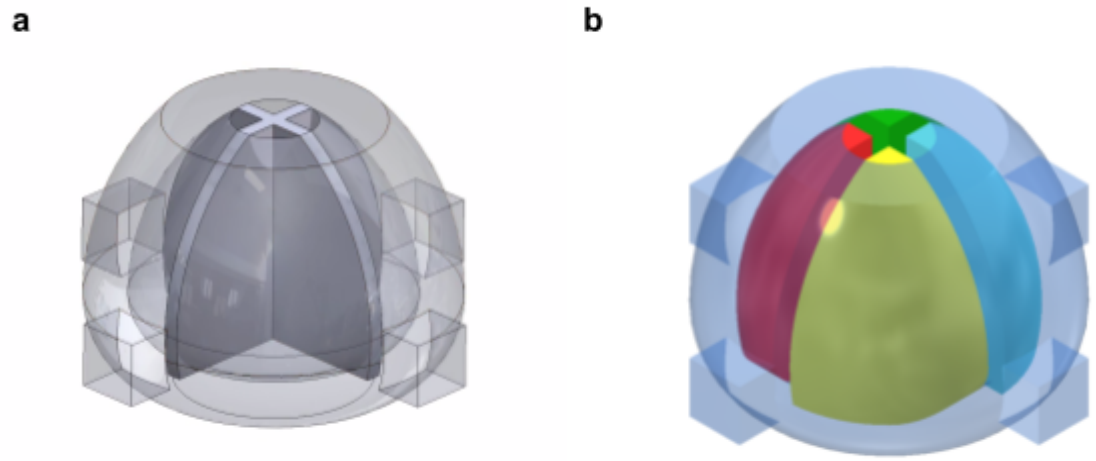

Figure 5-1*. Compartmentalized capsule. Allows for multiple pharmaceuticals to be put into one capsule for easier manufacturing and greater control of delivery.*

Lastly, different applications of this method could be researched. This method could potentially be applied to different diseases or viruses. For example, many elderly individuals require different pharmaceuticals to be taken daily for various reasons. This technique could be applied to these patients to minimize the frequency of the drug administration. Notably in patients who suffer from Alzheimer's or are more prone to forget taking their medications this technique could increase patient compliance. In addition, research could be done for best administration of the capsules, whether through an injection, patch, or in some cases orally. These different routes of administration would seek to reduce toxicity and systemic side effects as well as maintain constant drug concentrations.

Chapter 6: Conclusions

The application of controlled release is particularly valuable to HIV medication administration. Current protocols require a medication cocktail mixture of different enzymatic inhibitors. Subsequently, these medications must be maintained within a small therapeutic window, maintaining the dosages above the minimum effective range and below the toxic dosage range. Controlled release can allow for the maintenance of healthy medication concentrations, which can maximize the effective range of medications. Further, as medication administration technologies develop, this technology could be used with an intramuscular or intravenous delivery system in order to deliver a consistent stream of medications. Such an application could allow medication delivery to occur for months or even years, as the device constantly delivers a dosage that can maintain the therapeutic window of treatment.

While initial designs of our project hold promise for the controlled release of medications through additive manufacturing techniques, previously discussed project limitations halted further data and evidence to adequately determine the efficacy of such a project. That being stated, much of our research agrees with and expands prior research. We found some issues with a prior design for the shells and created a new design that resolved these issues [14]. However, different materials were used, so the material properties could have caused the difficulties encountered. In that same paper, they used a microchip liquid loading procedure, which we were able to use successfully [14]. When analyzing cap materials, there was research into the biocompatibility of materials that could be used in DLW [18], but not much research into the degradation rate. As such, we were able to expand prior research by creating methods to test and calculate the degradation rate and determine the degradation rate of the tested materials.

Finally, we do admit that such a project may be inaccessible to lower income populations due to the cost of creating a print. However, if this research were to be developed for mass production, the cost of medications could substantially decrease, providing a wider application of our research. In HIV for example, this can broaden the scope of access to low socioeconomic communities, as HIV disproportionately affects the population in the United States [2]. Mass production of this research could drastically decrease cost of treatment, providing more equitable care to the community. This can also improve access for children, as they would not be required to take many pills nor weekly frequent injections.

Glossary

AIDS - Acquired Immunodeficiency Syndrome

ART - Antiretroviral Therapy

BAM Lab - Bioinspired Advanced Manufacturing Lab

BP - BisPhosphonate

CAD - Computer Aided Design

CAM - Computer Aided Manufacturing

CDC - Centers for Disease Control and Prevention

DLW- Direct Laser Writing

EHD - Electrohydrodynamic

GM - GentaMicin

HIV - Human Immunodeficiency Virus

HTMS - Human Trabecular Meshwork Cells

INSTI - Integrase Strand Transfer Inhibitor

μFMM-DLW - Microfluidic Multi-Material Direct Laser Writing

MEC - Minimum Effective Concentration

MP - Microparticle

MTC - Minimum Toxic Concentration

NNRTI - Non-Nucleoside Reverse Transcriptase Inhibitors

NRTI - Nucleoside Reverse Transcriptase Inhibitors

PCL - Polycaprolactone

PDMS - Polydimethylsiloxane

PEGDA - Polyethylene Glycol Diacrylate

PETA - Pentaerythritol Tetraacrylate

PGLA - Poly Lactic-co-glycolic Acid

PGMA - Polyglycidyl Methacrylate

PGMEA - Propylene Glycol Methyl Ether Acetate

PI - Protease Inhibitors

PLA - Polylactic Acid

SEM - Scanning Electron Microscopy

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