ENGINEERING ANTIRETROVIRAL ELUTING INTRAVAGINAL RINGS FOR HUMAN IMMUNODEFICIENCY VIRUS PREVENTION

by

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ABSTRACT

Over three decades have passed since the discovery of human immunodeficiency virus (HIV), and with no viable vaccine, new technologies to prevent the spread of the virus are urgently needed. Pre-exposure prophylaxis, the concept of chemically interrupting HIV transmission, effectively reduced HIV incidence in a number of clinical trials. However, it has been difficult to achieve high and consistent effectiveness in clinical trials due to variable adherence to the dosing regimens requiring frequent administration. This, along with the disproportionate burden of the HIV/AIDS pandemic in young women in sub-Saharan Africa, has catalyzed the research into long-acting, antiretroviral eluting drug delivery systems like intravaginal rings (IVR) in an attempt to increase adherence over dosage forms that require frequent administration, and provide a more consistent drug concentration where transmission occurs.

However, IVR technology is generally limited to releasing small quantities of hydrophobic, low molecular weight species that can diffuse through the IVR elastomer. This dissertation describes two adaptable drug delivery platforms that increased the diversity of the drugs that can be delivered from IVRs. Polyether urethane reservoir IVRs were engineered for the delivery of the thermally and hydrolytically unstable prodrug tenofovir disoproxil fumarate. This drug presents
a unique design challenge to minimize the hydrolytic drug degradation in the IVR core while also promoting hydration to solubilize and release the drug. This design delivered mg/day quantities of drug and conferred complete protection in macaques from multiple vaginal viral exposures. Next, we engineered a system that uncouples the mechanism of drug release from the interaction of the drug with the elastomer and provides nearly constant release of any stable molecule. In this system, orifices control the hydration rate of the hydrophilic matrix contained within the core of the IVR, and release of the drug-containing gel. We evaluated the utility of this system for the topical delivery of macromolecules, and multiple different small molecule antiretrovirals, specifically for the delivery of drug microparticles. This work describes the design and evaluation of antiretroviral eluting IVR systems that provide adaptable platforms for vaginal drug delivery.
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3  **INTRAVAGINAL FLUX CONTROLLED PUMP FOR SUSTAINED**
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>A</td>
<td>Area</td>
</tr>
<tr>
<td>ABS</td>
<td>Acrylonitrile butadiene styrene</td>
</tr>
<tr>
<td>AIDS</td>
<td>Acquired immunodeficiency syndrome</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>APCI-ESI</td>
<td>Atmospheric pressure chemical ionization-electrospray ionization</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
</tr>
<tr>
<td>b</td>
<td>Constant that characterizes the water concentration dependence of the diffusion coefficient</td>
</tr>
<tr>
<td>C</td>
<td>Concentration</td>
</tr>
<tr>
<td>CAPRISA</td>
<td>Centre for the AIDS Programme of Research in South Africa</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
</tr>
<tr>
<td>CG</td>
<td>Carrageenan</td>
</tr>
<tr>
<td>Cmax</td>
<td>Maximum observed concentration</td>
</tr>
<tr>
<td>C₀</td>
<td>Initial drug concentration</td>
</tr>
<tr>
<td>CTMR</td>
<td>5-(and-6)-carboxytetramethylrhodamine</td>
</tr>
<tr>
<td>CVL</td>
<td>Cervicovaginal lavage</td>
</tr>
<tr>
<td>D</td>
<td>Diffusion coefficient</td>
</tr>
<tr>
<td>DESI</td>
<td>Desorption electrospray ionization</td>
</tr>
<tr>
<td>dm/dt</td>
<td>Drug release rate</td>
</tr>
<tr>
<td>DMPA</td>
<td>Depo-medroxyprogesterone</td>
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</tbody>
</table>
DPV  Dapivirine
FCP  Flux controlled pump
h  Thickness
H&E  Haematoxylin and eosin
HEC  Hydroxyethyl cellulose
HIV  Human immunodeficiency virus
HPC  Hydroxypropyl cellulose
HPEU  Hydrophilic polyether urethane
HPMC  Hydroxypropyl methylcellulose
HSV  Herpes simplex virus
IVR  Intravaginal ring
K  Water permeability
LC  Liquid chromatography
LLOQ  Lower limit of quantification
LLQ  Lower limit of quantification
MALDI  Matrix-assisted laser desorption/ionization
MP  Model protein
MPT  Multipurpose prevention technologies
MS  Mass spectroscopy
M_t  Cumulative amount of drug that diffused across
MVC  Maraviroc
NNRTI  Nonnucleoside reverse transcriptase inhibitor
PD  Pharmacodynamics
PEO  Polyethylene oxide
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>PEU</td>
<td>Polyether urethane</td>
</tr>
<tr>
<td>PK</td>
<td>Pharmacokinetics</td>
</tr>
<tr>
<td>PrEP</td>
<td>Pre-exposure prophylaxis</td>
</tr>
<tr>
<td>R²</td>
<td>Coefficient of determination</td>
</tr>
<tr>
<td>RMSD</td>
<td>Root mean square deviation</td>
</tr>
<tr>
<td>SHIV</td>
<td>Simian-human immunodeficiency virus</td>
</tr>
<tr>
<td>SIV</td>
<td>Simian immunodeficiency virus</td>
</tr>
<tr>
<td>SMP</td>
<td>Shape-memory polyether urethane</td>
</tr>
<tr>
<td>STI</td>
<td>Sexually transmitted infection</td>
</tr>
<tr>
<td>TCID₅₀</td>
<td>Tissue culture infectious dose 50</td>
</tr>
<tr>
<td>TDF</td>
<td>Tenofovir disoproxil fumarate</td>
</tr>
<tr>
<td>TFV</td>
<td>Tenofovir</td>
</tr>
<tr>
<td>TFV-DP</td>
<td>Tenofovir-diphosphate</td>
</tr>
<tr>
<td>Tg</td>
<td>Glass transition temperature</td>
</tr>
<tr>
<td>UNAIDS</td>
<td>The Joint Program United Nations Program on HIV and AIDS</td>
</tr>
<tr>
<td>UV-HPLC</td>
<td>Ultraviolet high performance liquid chromatography</td>
</tr>
<tr>
<td>ΔΠ</td>
<td>Osmotic pressure difference</td>
</tr>
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CHAPTER 1

INTRODUCTION

1.1 HIV/AIDS Pandemic, Therapy, and Pre-exposure Prophylaxis

Despite a 38% reduction of new HIV infection since 2001, the number of new HIV infections remains alarmingly high with over 2 million occurring annually, continuing to drive biomedical advances to control the pandemic (1). The reduction in new infections is due in part to a number of factors including expansion of HIV testing, oral antiretroviral therapy, in addition to increased counseling and education (2). An important factor that increases the risk of sexual transmission of HIV is viral load in blood plasma (3). Therefore, effective viral suppression achieved with successful implementation of antiretroviral therapy is associated with vastly reduced HIV transmission rates and is termed treatment as prevention (4, 5). Combination antiretroviral therapy regimens, initially developed in the early 1990s, can effectively suppress viral replication in most individuals transforming HIV infection from a progressive illness to a chronic but manageable illness. However, despite improvements in potency and safety of antiretroviral drugs, HIV infection and life-long antiretroviral therapy is costly, leads to an accelerated immune senescence, and is associated with a number of side effects (6-8). Despite vast increases in HIV testing and access to
antiretroviral therapy, an estimated 19 million of the total 35 million HIV-infected individuals do not know their HIV status and 22 million HIV-infected individuals lack access to antiretroviral therapy (1). For example, only 37% of HIV-infected individuals in sub-Saharan Africa receive antiretroviral therapy (1). Due to a number of issues with current antiretroviral therapy, 20% of treated HIV-infected individuals in the US are not in long-term treatment associated with complete viral suppression (9). The Joint United Nations Program on HIV and acquired immunodeficiency syndrome (UNAIDS) has set the goal of achieving diagnosis of 90% of HIV infections, achievement of 90% engagement with care, and suppression of viremia in 90% of the treated individuals by 2020 (10). However, even if this goal was obtained, greater than 30% of HIV-infected individuals will remain viremic and potentially continue to spread the infection since even a small undiagnosed cohort is capable of sustaining and even driving increases in HIV incidence (9, 11, 12). Treatment as prevention is having a significant effect in reducing the number of new HIV infections (13, 14), but without other prevention interventions, scaling up HIV testing and antiretroviral therapy is unlikely to control the HIV epidemic (15, 16); therefore, additional methods are required to further control the pandemic.

Since an effective HIV vaccine (17, 18) or cure (19, 20) remains elusive, there has been a significant effort towards developing pre-exposure prophylactic (PrEP) strategies to prevent HIV transmission where antiretroviral are dosed prior to sexual exposure (21, 22). PrEP is designed to chemically prevent HIV transmission where transmission occurs or before systemic infection can be
established and can empower at-risk individuals to protect themselves from HIV infection. Approximately 70% of the global population living with HIV reside in sub-Saharan Africa (25 of 35 million), where biological and social gender inequalities continue to drive HIV incidence rates that are twice as high in young women (15-24 years) compared to young men, motivating the development of women-controlled HIV prevention modalities (2, 23). Additionally, men who have sex with men account for 63% of new HIV infections in the US (24), are 20x more likely to become infected than the general population (25), and young men who have sex with men experienced a 22% increase in new HIV infections per year from 2008 to 2010 in the US (26). Therefore, PrEP development has focused particularly on young women at high risk, men who have sex with men, and heterosexual serodiscordant couples, i.e. couples with one member who is HIV-infected. PrEP would likely be a time-limited strategy compared to antiretrovirals for HIV treatment that is a life-long intervention, and PrEP has been demonstrated to be cost-effective in sub-Saharan Africa where HIV is endemic (27).

Initial PrEP development for women focused on vaginal gels containing nonspecific agents focusing first on membrane solubilizing surfactants (e.g. nonoxynol-9, C31G) and then anionic polymers that act as entry inhibitors (e.g. cellulose sulfate, carrageenan). Unfortunately all of the nonspecific agents that were clinically tested showed no reduction in HIV acquisition rates, likely due to low potency (28-32). In fact, there were a number of safety concerns with the surfactants, most notably detected as an increased HIV susceptibility observed in
a clinical trial (33). The low \textit{in vivo} potency and safety issues associated with
nonspecific agents lead the development efforts towards oral and topical
products containing antiretrovirals specific against HIV.

A number of recent clinical trials evaluating oral and topical administration
of antiretrovirals have proven that PrEP strategies can be effective in reducing
HIV acquisition rates (34-39). Specifically, a number of clinical trials have shown
that oral administration of tenofovir disoproxil fumarate (TDF) with and without
emtricitabine can protect from sexual HIV exposure (34-37, 39). Based on a
number of successful clinical trials evaluating oral PrEP, the FDA approved oral
TDF and emtricitabine to reduce the risk of HIV infection in 2012. However,
uptake of oral PrEP has been slow (40) and is due to a number of issues
including a lack of awareness of oral PrEP, a low perceived risk of HIV infection,
concerns regarding potential adverse effects, cost of the drugs and follow-up
visits, and repeated HIV testing (41, 42). Moreover, particularly in clinical trials
evaluating oral PrEP in women in sub-Saharan Africa, there is a stigma
associated with taking oral antiretrovirals. Individuals who are not HIV-infected do
not want to be perceived as HIV-positive by taking oral antiretroviral therapy (43).
Taken together, it has been difficult to achieve consistent and high levels of
protection in clinical trials due largely to variable adherence that corresponds to
insufficient drug concentrations where transmission events occur. An important
outcome of PrEP clinical trials has been the correlation of adherence determined
from the drug concentration in blood plasma and effectiveness in reducing HIV
incidence. (44-48) In an attempt to increase adherence and to improve drug
pharmacokinetics (PK), i.e. provide a more consistent drug concentration in the mucosal tissue, there has been a shift towards long-duration delivery systems such as intravaginal rings (IVRs) (49-51) that can be used for a month or more. Currently, there are two clinical trials evaluating the ability of IVRs delivering dapivirine to prevent HIV infection in women. Generally, as the duration between dosing events increases, the adherence increases as well (52, 53), and this has been well understood in contraceptive delivery systems for four decades (54). As an example, the most effective contraceptives are long-acting subcutaneous implants or intrauterine systems that are effective for multiple years after a single administration (55). The idea that limiting or simplifying user intervention to achieve better effectiveness is one of the main drivers for innovative and improved drug delivery technologies.

1.2 Vaginal Drug Delivery

This manuscript focuses on vaginal drug delivery from IVRs in the context of HIV prevention; however, even though vaginal drug dosing is less common than oral or dermal routes of administration, there are a number of advantages to vaginal drug delivery for other indications than prevention of HIV infection in women. Advantages of vaginal drug delivery include self-administration, avoidance of first-pass hepatic drug metabolism, potentially minimized systemic toxicity effects for local delivery, and prolonged retention and drug delivery to minimize fluctuations of drug concentration over time (56, 57). Typically, vaginal drug delivery systems focus on indications that are gender specific including
contraceptives, atrophic vaginitis, vaginal bacterial or fungal infections, and precancerous lesions in the female reproductive tract. Vaginal drug delivery systems are available in a number of forms including IVRs, gels, creams, films, tablets, pessaries, and suppository. The global health community has hypothesized that including a contraceptive with a HIV PrEP agent into a single product will increase user demand and uptake (58, 59).

In addition to the motivation to develop long-acting drug delivery systems for HIV prevention in an attempt to improve adherence and drug PK, there is a compelling interest for on-demand HIV prevention modalities due to the varied preferences for prevention products voiced by the diverse population of individuals at high risk for HIV acquisition (60, 61). Simply put, choice between prevention products remains important to successful implementation of HIV prevention. Due in part to the low adherence to frequent gel administration observed in HIV prevention clinical trials, researchers are investigating other short to medium duration delivery systems that could be easier to use in an attempt to improve adherence. These formulations include films, tablets, electrospun fibers, and drug nanoparticles. Films (62-64), tablets (65), and electrospun fibers (66, 67) are typically designed to release drug immediately. To increase the drug dissolution rate, it is desirable for the drug to be amorphous in the polymer matrix, and particularly in the case of electrospun fibers, to maximize the surface area available for dissolution. Advantages of films, tablet, and electrospun fibers compared to a gel include a smaller volume for more discreet use and/or easier administration, and potentially improved drug stability.
compared to aqueous gels for hydrophobic drugs. Nanoparticles are often investigated to improve drug uptake into tissue and to date have been delivered to the vagina as a solution or semisolid gel (68-70). However, an intravaginal delivery system capable of delivering drug particulates would enable the sustained delivery of nanoparticles to complement the increased drug uptake into tissue.

**1.3 Vaginal and Oral Pre-Exposure Prophylaxis**

Vaginal gels to prevent HIV transmission in women containing the nucleotide analogue reverse transcriptase inhibitor, tenofovir, have displayed no or modest effectiveness in clinical trials (38, 45). There are a number of social and biological explanations for the limited effectiveness of the 1% tenofovir gel; however, the most plausible and leading causes of these clinical failures are thought to be low adherence (22) to either coitally associated or daily gel dosing coupled with the application-dependent drug pharmacokinetics (47, 71). In the clinical trial CAPRISA 004, the success was modest and correlated with user adherence with a 39% overall effectiveness (38). Women with tenofovir concentration of 1 μg/mL or greater in vaginal fluid exhibited a significantly lower HIV incidence corresponding to 74% effectiveness (48). However, a confirmatory trial displayed no effectiveness (72). In a different clinical trial (VOICE) evaluating the same 1% tenofovir gel with a daily compared to coitally associated dosing, no protection was observed (45). It was established from pharmacokinetic data of drug concentration in blood plasma that participants poorly adhered to the dosing
regimen, leading to the lack in effectiveness (22). Moreover, the episodic nature of gel dosing confers a limited window of protection after gel dosing. This idea was confirmed in efficacy studies in macaques that showed that protection against vaginal virus challenge conferred by tenofovir or maraviroc containing vaginal gels depends on the relative timing of the gel and virus application (73, 74). The small window of protection provided by the drug containing vaginal gels necessitates strict adherence to frequent dosing and has motivated the development of sustained topical delivery strategies that can provide consistent drug release to the tissue where HIV transmission occurs.

In HIV PrEP development, unlike HIV treatment, there is no clear biomarker for the drug effect to guide the drug product design and clinical trial design. It has been hypothesized that the drug concentration in the mucosal tissue where the initial HIV infection occurs (75) may better reflect prophylactic effect than systemic drug concentration for topical drug delivery. Currently, it is unknown how oral drug dosing is preventing infection, whether preventing early transmission events at the portal of viral entry, or preventing the establishment of a systemic infection. The role of mucosal drug concentration for oral drug dosing is less clear since tenofovir and tenofovir diphosphate, the active molecule, display differential accumulation at rectal and vaginal mucosal tissue. Oral dosing of TDF results in ~100-fold lower vaginal tenofovir concentration and ~50-fold higher plasma tenofovir concentration than vaginal tenofovir gel (47, 76), and oral TDF was shown to be highly effective in preventing HIV transmission in women (77). On the other hand, tenofovir and tenofovir diphosphate concentrations in
both rectal mononuclear cells and tissue was higher than that achieved in peripheral blood mononuclear cells (PBMCs) after oral dosing of TDF in both macaques and humans (78-80). Interestingly, parity between rectal viral challenge studies in macaques and clinical studies of men who have sex with men has been observed for oral dosing of TDF and emtricitabine, i.e. a similar tenofovir diphosphate concentration in PBMCs was associated with a 90% risk reduction from both clinical and animal data (81, 82). If the drug concentration in mucosal tissue is the most relevant, topical dosing which confers higher tissue drug concentrations should be more effective in preventing the sexual transmission of HIV than oral dosing. Topical drug delivery also has the advantage of lower plasma drug concentrations compared to oral dosing which should reduce the risk of selecting for drug resistant virus. Nevertheless, clinical trials of vaginal gels exhibited the lowest effectiveness levels compared to oral trials, but inconsistent adherence levels between trials further complicate this. To effectively design and optimize HIV PrEP strategies, how different antiretrovirals delivered via different routes of administration prevent HIV infection must be known.

1.4 Drug Release from IVRs

Intravaginal rings are torus-shaped, elastomeric devices that are inserted by the user to deliver drug for weeks to months to provide sustained drug delivery. There are five rings currently on the global market that deliver hormones for the prevention of unwanted pregnancy and hormone replacement therapy.
In contrast to frequently applied dosage forms such as gels, IVRs may provide increased effectiveness resulting from higher user adherence due to infrequent user intervention (44, 84). Specifically the contraceptive NuvaRing® demonstrated 96% user satisfaction in international acceptability studies (84). The most relevant evaluation of user adherence of IVRs for HIV prevention involved African women using a placebo IVR. This study found that >95% of women wore the IVR every day for at least 12 hours and 82% never removed the IVR for the 12-week period of use (50). User motivation and education are essential to high uptake and adherence, particularly in the case of populations where IVRs are not commonly used. Further clinical evaluations must be performed in a larger population; however, the 82% perfect use far exceeds adherence in gel clinical trials for HIV prevention and suggests that certain populations will be motivated to use IVRs that protect against HIV infection (44, 45).

Drugs are released from nondegradable, elastomeric polymers by a permeation-controlled mechanism that depends on the solubility and diffusivity of the drug within the IVR elastomer (85, 86). The two main ring designs are matrix and reservoir. A matrix ring has drug homogeneously dispersed throughout the cross-section with a drug release profile that typically is proportional to t^{-1/2} and continually reduces with time. This continual reduction in the drug release rate from a matrix IVRs occurs due to an increased diffusional distance with time. After a sufficient amount of drug is released, an annulus of polymer not containing drug can be observed upon visualization of the ring cross-section (87).
The thickness of the drug-depletion zone increases with time and is the cause of the decreasing drug release rate. Reservoir type IVRs have a drug loaded core covered with a rate-controlling membrane and can have a time independent, constant drug release rate (88). Time independent, zero-order drug release only occurs with reservoir type devices if the drug concentration within the IVR is maintained at a constant value (89). Often the drug release rate continually decays with time due to a decreasing drug concentration within the reservoir (88, 90). Also reservoir type devices are often characterized by a burst release in the first few days caused from a higher drug concentration in the rate-controlling membrane or drug crystallized on the surface of the device (91, 92).

Until recently, all clinically tested IVR technologies could only achieve low, µg/day release rates of small hydrophobic drugs due to the limited drug solubility and diffusivity in silicone and poly(ethyl-co-vinyl acetate) elastomeric matrixes (49, 51, 93). These polymers are particularly useful for the delivery of hormones, whose relatively small molecular size and hydrophobic nature results in adequate solubility and permeation of the hormone through the elastomer. To overcome the low solubility and diffusivity in commonly utilized IVR elastomers, a high (30-50 wt%) loading of hydrophilic excipient or drug can be utilized that exceeds the percolation threshold to form a connected, porous structure (94-97). Dissolution of hydrophilic drug or excipient from the polymer matrix leaves an interconnected water filled, porous network for drug to solubilize and diffuse. The drug release from this type of device is typically proportional to $t^{-1/2}$. An additional drawback to this design is the device swells resulting in a significant reduction in stiffness.
during use making long-term device retention in the vagina difficult to impossible to achieve.

There has been a resurgence in the development of new IVR technologies due to the need for new HIV prevention technologies to control the pandemic. Initially, matrix IVRs were evaluated due to their simple design and manufacturability. However, often the large reduction in the drug release rate characteristic of matrix IVRs has driven the field towards reservoir and other new designs to achieve a higher drug release rate at the end of the IVR duration and a more controlled drug release rate. This has led to utilizing new polymers, in particular polyether urethanes (90, 98-100), and a number of new ring designs (101-104) to enable the delivery of hydrophilic and macro-molecules at mg/day delivery rates that are impossible to achieve with EVA or silicone and still maintain the necessary IVR stiffness for proper retention in the vagina. Polyether urethanes are a diverse class of polymers and are synthesized by condensation polymerization of diisocyanate, such as 4,4′-Methylenebis(cyclohexyl isocyanate), poly(ether diols) such as poly(tetramethylene oxide) and poly(ethylene oxide), and chain extenders such as 1, 4-butandiol. The equilibrium water absorption can be controlled by altering the ratio of poly(tetramethylene oxide) and poly(ethylene oxide) where an increase in the poly(ethylene oxide) content corresponds to an increased water uptake. Recently, Kiser and colleagues have been investigating IVRs composed of extruded polyether urethane tubing filled with a dry powder or semisolid paste of drug and excipient. An IVR of this design delivering TDF is described in Chapter 2. The drug release
and ring mechanical properties can be tuned by altering the hardness and equilibrium water uptake of the polymer, IVR dimensions, and formulation of the core. (89, 100) This has enabled the delivery of more polar drug molecules at higher release rates that are presumed to be necessary for HIV prevention (87, 89, 99, 100). However, the polymer must still be matched to the drug and the drug release is typically limited by the drug solubility and diffusivity in the polymer.

Another new ring design is insert vaginal rings where small drug delivery systems are contained or held within the ring body (101-103, 105). This separates the drug delivery requirements from the mechanical properties necessary for proper ring insertion, retention, and biocompatibility. A number of drug delivery inserts have been embedded into IVR including drug-containing matrices consisting of compressed pellets or lyophilized solutions of water-soluble polymer, and silicone (101). Another example consists of compressed drug pellets coated with polylactic acid that acts as a rate-controlling membrane for drug release. The coated pellets are then embedded into body of the ring with one or more delivery channels. In this system, drug release is controlled by the solubility and diffusivity of the drug in the membrane, thickness and exposed area of the membrane, the number of pods embedded into a ring, and the area of the delivery channel(s). (102) To achieve sustained drug delivery, these alternative IVR delivery systems utilize permeation-controlled delivery systems where the drug must still diffuse through the polymer(s) of the insert (101, 102, 105). This still leads to the requirement to match the drug to the polymer to
achieve the desired release rate, and limits the surface area for the drug release compared to more traditional IVR designs where most of the ring surface area is available for drug release, making it difficult to achieve the high, mg/day release rates necessary with a number of the leading antiretroviral agents for HIV prevention. We were interested by osmotic pump technology where a concentration gradient of the osmotic agent, and not necessarily the drug, drives water entry across a semipermeable membrane to drive drug release (106). Until recently, osmotic pump technology had only been suggested (107) and not utilized for vaginal drug delivery (104). We designed an osmotic pump tablet composed of a compressed pellet of water swellable polymer, hydroxypropyl cellulose, and drug coated with a semipermeable membrane with an orifice, and achieved controlled drug release over a week duration (104). The equation describing release from an osmotic pump is shown below (Eq. 1.1) where \( \frac{dm}{dt} \), the release rate, is proportional to the area and thickness of the semipermeable membrane, \( A \) and \( h \); the water permeability, \( K \); and importantly the osmotic pressure difference (\( \Delta \Pi \)) and the total concentration of drug, \( C \).

\[
\frac{dm}{dt} = K A h \Delta \Pi C \tag{Eq. 1.1}
\]

We were inspired by the osmotic pump and wondered if it could be integrated into an IVR for long-term delivery. One of the main goals of this dissertation was to create drug delivery devices where the drug release rate was not dependent on the solubility or diffusivity of the polymers of the ring enabling the delivery of drugs irrespective of their chemical properties (Chapters 3-5).
1.5 Mechanical Properties of IVRs

IVRs are drug-device combinations and the performance is innately connected to the dimensions and mechanical properties of the ring. IVRs are inserted by the user in a compressed or figure eight configuration. Upon insertion, the IVR is retained by a force balance between the elastic recoil of the ring under compression by the vaginal walls. The ease of insertion and retention is determined by the ring geometry, design, and elastic modulus of the elastomer. Despite a range of vaginal shapes and sizes (108-110), IVRs typically have an outer diameter of 55 mm and the cross-section is determined by the polymer hardness and drug release considerations. A ring that is too soft results in an insufficient recoil force, and the IVR is not retained well and is easily expelled (111, 112). However, if the ring is too stiff, it can be difficult to insert and remove, and can cause damage to the vaginal epithelium (113, 114). These clinical studies provide the upper and lower bounds of acceptable ring stiffness and compressibility. Moreover, a large clinical study evaluated four different IVR representing a wide range of ring stiffness found all designs were well retained and found acceptable by the users with no safety issues (115). To date, typically a force to compress the ring 10 and 25% of the outer diameter is used as a quantitative measure of ring compressibility (87, 89, 90, 99, 116-118) with the goal of achieving values comparable to IVRs currently on the market. This, however, is a simplified measure not taking into account the ease or difficulty of insertion, or the hysteresis typically observed during a compression and relaxation cycle. Nevertheless, IVR mechanical properties are typically a
secondary concern to the drug release due to relatively broad ranges of ring stiffness that have been found acceptable despite a large variation in vaginal size and shape (108-110).

1.6 Modeling Drug Release from Hydrophilic Matrix Tablets

There are many examples of drug release kinetics from IVRs being mechanistically described by solutions to the diffusion equation (85, 87, 90, 119-123). However, new types of delivery systems can necessitate new or modified drug release models to facilitate the design and development of these devices. Early research into sustained release strategies largely focused on developing oral formulations to extend the duration of drug release and control the drug release kinetics to result in more consistent drug PK. Many of these oral formulations are compressed pellets or tablets containing water-soluble polymers, e.g. hydroxypropyl methylcellulose, natural polysaccharides, polyethylene oxide, and hydroxypropyl cellulose (124, 125). Drug release from these hydrophilic matrix tablets occurs through a combination of drug dissolution and diffusion from the swollen polymer matrix, and polymer dissolution. Moreover, the interplay between these two mechanisms depends in part on the drug solubility in the release media, drug loading in the polymer matrix, and polymer chemistry and molecular weight. Particularly with hydroxypropyl methylcellulose, upon polymer hydration, a swollen polymer layer persists on the outer edge of the tablet acting as a rate-controlling membrane for drug diffusion. There are a number of models of drug release from hydrophilic solid matrices
describing the mechanism and kinetic of water diffusion into the hydrophilic matrix, the resulting polymer swelling and dissolution, in addition to drug diffusion through the swollen polymer (126-131). The diffusion of the water into and drug release from hydrophilic matrix tablets is described by the diffusion equation (Eq. 1.2) (132).

\[
\frac{\partial C_k}{\partial t} = \frac{\partial}{\partial x} \left( D_k \frac{\partial C_k}{\partial x} \right) + \frac{\partial}{\partial y} \left( D_k \frac{\partial C_k}{\partial y} \right) + \frac{\partial}{\partial z} \left( D_k \frac{\partial C_k}{\partial z} \right) \quad \text{(Eq. 1.2)}
\]

Following the free volume theory, the diffusion coefficient of water and drug in hydrophilic polymers displays a strong dependence on the water concentration (133). That is, as the polymer is hydrating and the water concentration increases, the free volume available for the water to diffuse increases, resulting in an increase in the water diffusivity in the polymer. A similar phenomenon is observed for drug diffusion. The diffusivity of water in a swollen polymer matrix increases exponentially as the water concentration increased until the self-diffusion is reached and the polymer concentration is too low to hinder water diffusion (Eq. 1.3). \(D_k\) is diffusion coefficient of each species and \(\beta_k\) is a constant that characterizes the water concentration dependence of the diffusion coefficient. \(D_{k, eq}\) represent the diffusion coefficients of each species at the maximum water concentration, \(C_{water, eq}\) (in equilibrium with the swollen matrix) (133).

\[
D_k = D_{k, eq} \exp \left( -\beta_k \left( 1 - \frac{C_{water}}{C_{water, eq}} \right) \right) \quad \text{(Eq. 1.3)}
\]

Typically, ideal swelling is assumed, and the volume of the swollen polymer equals the sum of the components. The system of equations is solved
with a moving boundary to account for expansion due to polymer hydration and swelling. Often the parameters are fit to experimental data and the model is then utilized to estimate the effect design changes have on the drug release profile. However, the water and drug diffusivities as a function of polymer concentration can be directly measured experimentally utilizing pulsed-field-gradient spin-echo nuclear magnetic resonance for the water and drug diffusivity (134, 135), Franz cell diffusion experiments for the drug diffusivity (136), and fluorescence recovery after photobleaching for polymer and drug diffusivity (137, 138). Much is known regarding the mechanism and kinetics of drug release from hydrophilic matrix tablets that can be utilized to model drug release from new drug delivery systems where a similar mechanism occurs. Chapter 4 of this manuscript describes a mechanistic model of drug release that includes the hydration, swelling, and diffusion of the water-soluble polymer from flux controlled pumps.

1.7 PK of Antiretrovirals Delivered from IVRs

In clinical trials evaluating the performance of dapivirine eluting IVRs (139-141) and animal studies of IVRs releasing another hydrophobic antiretroviral (142), typically a higher concentration of drug in vaginal fluid was observed proximal to the ring compared to distal locations. Since the sexual transmission of HIV can occur along the female reproductive tract (143, 144), the drug concentration must be sufficient to prevent HIV transmission throughout the canal. A matrix IVR with dapivirine, a potent non-nucleoside reverse transcriptase inhibitor, homogenously dispersed in silicone is currently in Phase 3
clinical studies in two separate trials. The results of the clinical trials are expected in 2015 and will be the first example of clinical effectiveness of HIV PrEP from an IVR. The \textit{in vitro} drug release rate from this matrix IVR exhibited a significant reduction in the release rate over time characteristic of this design, with potential excess drug release early on and insufficient drug release near the end of the 28-day duration (118). This reduction in release rate \textit{in vitro} over a month corresponded to a reduction in dapivirine concentration in vaginal fluid. However, the reduction of the dapivirine concentration in vaginal fluid and blood plasma was slight, suggesting that the large reduction of the drug release rate observed \textit{in vivo} was attenuated compared to the \textit{in vitro} release conditions (141). A similar phenomenon was observed with a matrix ring eluting IQP-0528, another hydrophobic, non-nucleoside reverse transcriptase inhibitor. In this study, the authors argued that the drug release from the ring was partition-controlled and not diffusion-controlled, meaning the rate-limiting step for drug release was the drug dissolution into the vaginal fluid instead of the drug solubility and diffusivity in the elastomer of the ring. This resulted in a reduced release rate \textit{in vivo} compared to \textit{in vitro} release conditions, and also likely resulted in a nearly constant \textit{in vivo} drug release rate over a month duration. (142) In addition to the matrix dapivirine IVR, a reservoir dapivirine IVR has also been evaluated in humans consisting of a dapivirine containing silicone core covered with a silicone rate-controlling membrane. This reservoir IVR achieved more consistent dapivirine concentration in vaginal fluid and tissue compared to the matrix IVR during the 14-days of ring use. However, the advantage of more consistent
release from the reservoir IVR was offset by the overall reduction in drug release compared to the matrix design. (140)

Moreover, sustained drug release from IVRs can achieve more consistent drug levels in vaginal fluid and tissue with a smaller daily dose compared to daily-applied gels. Recently, the PK of a tenofovir eluting IVR was compared to the 1% tenofovir gel in sheep (103). The IVR consisting of hydrophilic polyurethane tubing filled with a tenofovir/glycerol paste achieved zero-order, 90-day tenofovir release. The authors demonstrated the IVR delivering a daily tenofovir dose ~10x less than a daily application of a 1% tenofovir vaginal gel achieved comparable tenofovir concentration in vaginal tissue and fluid in sheep. Moreover, the tenofovir concentrations in vaginal fluid and tissue were more consistent with the IVR compared to the gel. (103) These findings strongly support the development of IVRs for topical HIV PrEP since improved drug PK and possibly improved adherence can be achieved compared to a vaginal gel. This TFV IVR, a combination contraceptive and HIV prevention IVR, along with a TDF IVR of a similar design are currently being evaluated in small Phase 1 clinical trials.

1.8 Animal Models to Evaluate IVR Performance and Efficacy

There are a number of relevant animals model for evaluating safety, drug PK, and pharmacodynamics of vaginal products. The most important animals include rabbits, sheep, and monkeys, predominately pigtailed and rhesus macaques. Rabbits are typically used to assess safety and local toxicity of vaginal products (145-147). Rabbits are a relatively easy to use and low-cost
small animal model for testing vaginal products; however, the vaginal anatomy and physiology of rabbits is quite different compared to humans (148, 149). There are also a number of PK studies of vaginal solid dosage forms in rabbits. Typically, the device is in the form a segment of an IVR. To ensure retention, the rod is surgically inserted and sutured to the vaginal wall (90, 116, 121, 150). Not only is this procedure difficult and expensive, but the effects of the healing response on the device performance in unknown potentially confounds the analysis of any possible inflammatory response to the drug delivery system. In Chapter 3, we describe the design and evaluation of a retainer device that enables the long-term evaluation of drug-eluting segments in the rabbit model that does not require surgical insertion or retention of the device (103). Sheep possess a number of advantages for testing vaginal products stemming from their similar vaginal anatomy and stratified squamous epithelium. In addition to being utilized for systemic and local safety of vaginal products (89, 151, 152), in contrast to nonhuman primates and rabbits, most sheep breeds are of a similar body mass and vaginal dimensions, enabling evaluation of human-sized IVRs to avoid or minimize scaling issues such as the effect of ring size on the ring stiffness, drug dosing, and PK (153, 154).

Despite the difficulty in proving the effectiveness of PrEP in humans, there are many examples of HIV chemoprophylaxis in nonhuman primates. For these studies, macaques, either pigtailed or rhesus macaques, are challenged with simian or simian-human immunodeficiency virus (SIV or SHIV) while administered with the PrEP formulation and compared to either nontreated or
placebo-treated control animals. Rhesus and pigtailed macaques are particularly relevant since they possess similar vaginal anatomy and physiology (155). There are two major regimens of SIV/SHIV efficacy studies, a single high-dose challenge or a repeat low-dose challenge. The repeat low-dose challenge is preferred over the single high-dose model since it provides a better representation of infection and pathogenesis of HIV in humans (156, 157). A number of topical and systemic formulation of antiretrovirals have provided protection against vaginal and rectal virus challenges including vaginally applied gels containing tenofovir, maraviroc, and anti-HIV antibodies against vaginal transmission (73, 157, 158); oral administration of TDF with and without emtricitabine against both vaginal and rectal transmission (159, 160); parental administration of cabotegravir in the form of a intramuscular long-acting injectable against rectal transmission (161); and a number of antiretroviral releasing IVR formulations (which is the focus of this manuscript). To date, a number of different IVRs delivering antiretrovirals have provided complete or partial protection against high-dose vaginal SIV or SHIV challenge in rhesus macaques receiving high-dose depot medroxyprogesterone acetate, a long-acting hormonal contraceptive that results in thinning of the vaginal epithelium associated with increased viral transmission in animals (117, 162, 163). However, a TDF IVR described in Chapter 2 demonstrated 100% protection from 16 weekly low-dose vaginal viral exposures in normally cycling pigtailed macaques (100). Normally cycling pigtailed macaques, in similar way as humans, are more susceptible to vaginal SHIV infection during the luteal phase (156). This
same ring was also tested for efficacy in pigtailed macaques receiving depot medroxyprogesterone acetate and protected 5 of 6 macaques from 12 weekly vaginal virus exposures (164). The combination of repeated vaginal viral exposures in the context of medroxyprogesterone acetate treatment represents the most stringent animal model to date for PrEP efficacy with the animals in an exaggerated and sustained luteal phase. There is considerable debate whether depot injection of medroxyprogesterone acetate increases HIV incidence in women; however, the high-dose administered to macaques results in a more severe thinning of the mucosal epithelium.

1.9 Long-acting Injectables for HIV Therapy or Prevention

Most recently, there have been a number of interesting manuscripts evaluating long-acting parenteral formulations of antiretrovirals. These are of particular interest for their potential capacity for both HIV treatment, and HIV prevention by multiple routes including rectal and vaginal intercourse, and intravenous exposure. There are still many questions to be answered regarding the acceptability of an intramuscular injection for HIV therapy or prevention, the optimal drug dose and duration between injections, and the ability to prevent HIV infection. Currently, there are long-acting injectables under clinical investigation for cabotegravir, an integrase strand transfer inhibitor, and rilpivirine, a non-nucleoside reverse transcriptase inhibitor. Both of these drugs are formulated as a nanosuspension of drug crystals and as a result of the hydrophobicity, long half-life, and high potency of the drugs, therapeutic drug concentrations in
plasma are maintained for 3-4 months after a single injection (165-168). Additionally, the cabotegravir injectable completely protected macaques from multiple rectal and vaginal SHIV challenges (161, 169, 170). Despite that therapeutically relevant drug concentration is maintained for multiple months, the PK profile of the current parenteral antiretroviral formulations in the clinic are characterized by high plasma drug concentrations early on, with the maximum concentration ($C_{max}$) observed days after administration, followed by a continual decrease in the drug concentration in plasma with time (165-168). This long tail in the drug concentration with time profile could potentially be an issue since low plasma drug concentration can select for drug resistant virus, making future HIV therapy more difficult. However, currently in clinical trials, no resistance has been observed, partially due to repeat administration of the parenteral formulation or oral drug administration to maintain therapeutic drug concentrations (171). There are a number of different parenteral antiretroviral formulations currently under preclinical evaluations including degradable and nondegradable implants, and injectables to improve drug PK and extend the duration compared to the nanosuspensions of cabotegravir and rilpivirine. The high burst release of drug due to the high surface area available for drug dissolution means excess drug is being released early on that could be used to extend the duration. Implants displaying controlled drug release would lack the burst release or possess an attenuated burst release which can be used to extend the duration of protective drug levels beyond those attainable by the current long-acting injections formulations.
1.10 Dissertation Chapter Overview

An overview and publication information of each chapter is included below.

1.10.1 Chapter 2 overview

Chapter 2 describes the design and evaluation of a reservoir IVR for the delivery of the thermally and hydrolytically unstable prodrug TDF. We hypothesized that IVRs that achieve a more controlled drug release rate than matrix designs will provide a more consistent drug concentration in the tissue where infection occurs, resulting in complete protection from vaginal viral exposure. Due to the large reduction of the drug release rate characteristic of a matrix IVR, and to achieve a more controlled and increased TDF release rate, we designed a reservoir IVR consisting of a hot-melt extruded polyether urethane tube formed into a ring filled with a mixture of drug and excipient. This IVR delivered mg/day quantities of TDF over a month duration from a macaque-sized IVR. We evaluated the PK of TDF, tenofovir, and tenofovir-diphosphate (the active compound) in the vaginal fluid and surrounding tissue of macaques. The tenofovir concentration in vaginal fluid and tenofovir diphosphate concentration in tissue was similar or higher than previous benchmarks of efficacy established in this animal model. This IVR completely protected macaques from 16 weekly vaginal viral exposures over the course of 4 monthly IVR changes. This was the first IVR to completely protect macaques from multiple vaginal SHIV challenges, the most stringent animal model for PrEP efficacy.

This chapter was highly collaborative in nature and was not possible
without colleagues at the Center for Disease Control and Prevention who performed all of the macaque studies. My contributions to this manuscript include the design, in vitro evaluation, and fabrication of the IVRs with Dr. Rachna Rastogi. I assisted in writing and editing the manuscript including data analysis and assembling all of the figures.


1.10.1 Chapter 3 overview

The key features of Chapter 3 are first, the description and testing of a new drug delivery system for the sustained, vaginal delivery of macromolecules, and second, a nonsurgical technique for vaginal implantation in the rabbit model using a shape memory polyurethane ring. There are a number of promising anti-HIV macromolecules; however, sustained delivery of macromolecules has been difficult to achieve since their size makes diffusive release through elastomers challenging, and their activity depends on complex intra- and intermolecular structures.

We described the mechanism of drug release, and the effect of various polymeric osmotic attractants that allow for near zero-order release of
macromolecules compounded into the pump. The device is made of a compressed water-soluble polymer pellet compounded with a macromolecular drug and enclosed in a hard polymer casing. The casing contains orifices to allow influx of fluid and efflux of the hydrated contents. We hypothesized that the drug release would be predominately controlled by the hydration and diffusion of the hydrophilic matrix from the core of the device and not the diffusivity of the model macromolecules. The orifice size and swelling kinetics of the hydrophilic polymer controlled the hydration rate and thereby drug release from the device. Another important part of this chapter is the description and use of the holder we engineered to retain the pump, or other solid dosage forms, in the abdominal vagina of the rabbit. The use of a shape memory polyurethane retainer to facilitate the nonsurgical intravaginal implantation of devices in the rabbit model is new and can enable safety and performance evaluations of vaginal drug delivery systems without tissue damaging survival surgery in this important animal model.


1.10.2 Chapter 4 overview

In Chapter 4, we sought to further design and characterize the flux controlled pump reported in Chapter 3. Our main aims were to 1) evaluate the
device with other small molecule antiretrovirals to better understand the
mechanism of drug release, and 2) develop a mechanistic model of drug release
from this device to aid in the design. We achieved controlled drug delivery
ranging from μg/day to mg/day of multiple antiretrovirals by altering the orifice
design, drug loading, and mass of pellets loaded in the device. This chapter
further supports the idea that drug release is predominately controlled by the
chemical properties, namely the swelling kinetics of the water-soluble polymer
and not the drug diffusivity in the polymers of the ring. We hypothesize that the
limited water solubility of IQP-0528 would result in the release of drug
microparticles entrained within the swelling polymer from the flux controlled
pump, and therefore, the drug release would be a function of the hydration and
diffusion of the hydrophilic polymer from the core of the ring and not drug
solubility and diffusivity in the swollen polymer matrix. A mechanistic model
describing the hydration and diffusion of the hydroxypropyl cellulose matrix is
presented, and good agreement between the quantitative model predictions and
the experimental studies of drug release from this system was achieved. This
device provides an adaptable platform for the vaginal drug delivery of many
antiretrovirals with release kinetics nearly independent of the molecule being
delivered.

Publication: Teller RS, Malaspina DC, Rastogi R, Clark JT, Szleifer I, and Kiser
PF. 2015. *Controlling the hydration rate of a hydrophilic matrix in the core of an
intravaginal ring determines antiretroviral release.* Journal of Controlled Release.
1.10.3 Chapter 5 overview

Chapter 5 continues to describe the flux controlled pump, specifically evaluating the PK of IQP-0528, a potent non-nucleoside reverse transcriptase inhibitor, in a sheep model. Our main aims were to 1) measure the drug distribution along the vaginal tract, and 2) quantify the reduced release rate in vivo compared to in vitro release conditions. Previously, a higher drug concentration of hydrophobic antiretrovirals delivered from a ring was observed proximal to the ring compared to distal locations. This system releases a semisolid gel containing microparticles of the drug substance, and we hypothesized the water-soluble polymer would aid in distributing the drug along the vaginal canal. We characterized the reduced in vivo release rate and provide evidence that it can be overcome by increasing the orifice area.

1.10.4 Chapter 6 overview

Chapter 6 includes brief conclusions of the dissertation's work in addition to recommendations for future research into vaginal drug delivery specifically and drug delivery for the prevention of HIV transmission in general.
1.11 References


26. **Maulsby C, Sifakis F, German D, Flynn CP, Holtgrave D.** 2013. HIV risk among men who have sex with men only (MSMO) and men who have sex with men and women (MSMW) in Baltimore. J Homosex 60:51-68.


CHAPTER 2

INTRAVAGINAL RING ELUTING TENOFOVIR DISOPROXIL FUMARATE

COMPLETELY PROTECTS MACAQUES FROM MULTIPLE

VAGINAL SIMIAN-HIV CHALLENGES


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Intravaginal ring eluting tenofovir disoproxil fumarate completely protects macaques from multiple vaginal simian-HIV challenges

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Topical preexposure prophylaxis interrupts HIV transmission at the site of mucosal exposure. Intermittently dosed vaginal gels containing the HIV-1 reverse transcriptase inhibitor tenofovir protected pigtailed macaques depending on the timing of viral challenge relative to gel application. However, modest or no protection was observed in clinical trials. Intravaginal rings (IVRs) may improve efficacy by providing long-term sustained drug delivery leading to constant mucosal antiretroviral concentrations and enhancing adherence. Although a few IVRs have entered the clinical pipeline, 100% efficacy in a repeated macaque vaginal challenge model has not been achieved. Here we describe a reservoir IVR technology that delivers the tenofovir prodrug tenofovir disoproxil fumarate (TDF) continuously over 28 d. With four monthly ring changes in this repeated challenge model, TDF IVRs generated reproducible and protective drug levels. All TDF IVR-treated macaques (n = 6) remained seronegative and simian-HIV RNA negative after 16 weekly vaginal exposures to 50 tissue culture infectious dose SHIV162P3. In contrast, 11/12 control macaques became infected, with a median of four exposures assuming an eclipse of 7 d from infection to virus RNA detection. Protection was associated with tenofovir levels in vaginal fluid [mean 1.8 × 10^5 ng/mL (range 1.1 × 10^5 to 6.6 × 10^5 ng/mL)] and ex vivo antiviral activity of cervicovaginal lavage samples. These observations support further advancement of TDF IVRs as well as the concept that extended duration drug delivery devices delivering topical antiretrovirals could be effective tools in preventing the sexual transmission of HIV in humans.

Significance

Topical prevention of HIV is designed to pharmacologically interrupt sexual transmission at the genital mucosa. Attempts at preventing transmission in women using vaginal gels have yielded disappointing results in part because of poor rates of adherence. Controlled topical drug delivery using intravaginal ring technology should improve efficacy and adherence by providing sustained mucosal delivery of antiretrovirals. In this paper, we describe a reservoir intravaginal ring that delivers tenofovir disoproxil fumarate (TDF) for 1 mo. The ring protected pigtailed macaques from weekly vaginal simian-human immunodeficiency virus challenges (12). Reasons for failure of these device-drug combinations to provide complete protection in macaque models are complex and may reflect differences in the model systems (species, use of depo-medroxyprogesterone (DMPA), viral dose, and strain), pharmacokinetics of the drugs tested, and controlled drug delivery.

The prodrug tenofovir disoproxil fumarate (TDF) and its hydroxyl product tenofovir (TFV) have been the major focus of HIV prevention research. TDF is one component of the two-drug mixture Truvada, the first US Food and Drug Administration–approved oral preexposure prophylaxis (PrEP) agent against HIV acquisition in discordant couples and other high-risk populations (1), and TFV is the active pharmacological ingredient in the first vaginal gel to show partial protection in female macaques (2). Both molecules have a long safety record and have been widely used in humans with minimal toxicity. The 1% (w/w) TFV vaginal gel formulation administered 30 min before virus exposure provided complete protection (6/6) against multiple simian-HIV (SHIV) challenges (3, 4) and retained partial activity protecting four of six macaques that were treated once weekly with gel and exposed to virus twice weekly; the second exposure being 3 d after gel application (4). Unfortunately, the TFV gel provided only partial protection in clinical trials when women were asked to use the gel before and after coitus and no protection when daily dosing was evaluated (2, 5). There are many factors, both social and biological, that may have contributed to the TFV gel clinical trial outcomes. However, as far as we know the most important factor in all gel trials to date is the difficulty of sustaining high adherence to frequent dosing (6). Simply put, if the course of protective antiretroviral levels does not match the kinetics of viral exposure, PrEP will not be highly effective. This motivates the development of long duration, drug delivery systems such as intravaginal rings (IVRs) that may overcome these limitations by facilitating adherence and providing less variation in mucosal drug levels (7, 8).

Matrix IVRs delivering the nonnucleoside reverse transcriptase inhibitor (NNRTI) dapivirine and the entry inhibitor maraviroc are being advanced through the clinical pipeline. Neither of these rings, which are currently in phase 3 and phase 1 trials, have been evaluated for efficacy in macaques. Among all antiretroviral IVR efficacy studies conducted to date, only one formulation conferred protection against a single, high-dose viral challenge in nonhuman primates, whereas the remainder yielded partial protection (9–11). None has been evaluated in repeated vaginal challenge models designed to mimic the repeated viral exposures during human sexual transmission (12). Reasons for failure of these device-drug combinations to provide complete protection in macaque models are complex and may reflect differences in the model systems (species, use of depo-medroxyprogesterone (DMPA), viral dose, and strain), pharmacokinetics of the drugs tested, and controlled drug delivery.

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and/or inadequate drug release profile of the IVRs. We hypothesize that IVRs that maintain a consistent level of mucosal TDF may provide greater protection. This notion is supported by the higher cell permeability and at least 100 times greater potency of TDF compared with TFV against HIV in vitro (13). The bioactive form, TFV diphosphate (TFV-DP), has the further advantage of a long intracellular half-life (60–150 h) (14, 15) that could mitigate lapses in adherence and may prove advantageous compared with antiretrovirals (e.g., dapivirine) that can readily diffuse from the intracellular to the extracellular compartments in response to drug concentration gradients in time and space (16).

Developing IVRs to elute the thermally and hydrothermally unstable TDF at therapeutically relevant rates is a challenge. Although TDF is more hydrophilic than TFV, its polarity is too high for adequate polymer solubility to deliver protective doses of the drug from common elastomers used in IVR technology such as silicone and poly(ethylene-covinyl acetate) (13). Furthermore, TDF is susceptible to hydrolysis, making formulation of this drug in any aqueous topical dosage form impossible (17, 18) and precluding its use in gels. We engineered a class of IVRs capable of achieving high fluxes of hydrophilic antiretrovirals such as TFV and TDF using hollow hydrophilic polyether urethane (HPEU) elastomeric tubings (Fig. 1 A and B) (19). The hydrophilic-hydrophobic balance of HPEU can be tailored to solubilize and deliver drugs with a range of physical properties (20) and at fluxes that achieve clinically relevant concentrations (21).

We assessed the ability of TDF IVR designs to achieve monthly sustainable protective levels of TDF in vaginal fluid and tissue by performing pharmacokinetic (PK) studies in pigtailed macaques. Pigtailed macaques were chosen for the study because of their similarities in hormonal cycling, vaginal architecture, and microflora to women, and previous studies in this model provide data on intracellular TFV-DP levels that correlate with protection (4). The IVR design that generated high and consistent drug levels in vaginal fluids and tissues was then evaluated in a more extensive terminal PK study to determine the levels of TFV-DP that would maintain therapeutic concentrations in vaginal fluid for multiple months (24). An osmotic agent was used to attract vaginal fluid into the core to solubilize TDF and rapidly establish a concentration gradient of soluble and dif fusible drug to drive release.

**Results and Discussion**

**TDF IVR Design.** IVRs are divided into two well-known controlled release device categories: matrix and reservoir. Matrix devices exhibit drug release rates that decrease with time, whereas reservoir devices have a rate controlling membrane that allows for more consistent drug flux (Fig. 1 A) (8). Because matrix devices are simple and more easily fabricated, we originally explored the same matrix design strategy as the dapivirine ring (22, 23). Polyether urethane (PEU) matrix TDF devices containing 10 mg/mL of TDF alone resulted in a long drug release lag time of more than 20 d (Fig. 2B). Therefore, we included osmotic agents to attract vaginal fluid into the core to solubilize TDF and rapidly establish a concentration gradient of soluble and diffusible drug to drive release.

**Drug PK Study in Pigtailed Macaques.** We investigated the TDF reservoir IVRs designed of HPEU tubing with a drug-bearing core sealed by induction-melt welding into a torus (19). The tubing acted as a rate controlling mechanism for drug release while providing the necessary mechanical support for ring retention in the vaginal canal. Simply filling the device with TDF alone resulted in a drug release lag time of more than 20 d (Fig. 2B). Therefore, we included osmotic agents to attract vaginal fluid into the core to solubilize TDF and rapidly establish a concentration gradient of soluble and diffusible drug to drive release (Fig. 1 A).

**Fig. 1. TDF IVR design and in vitro release.** (A) Schematic depicting mechanism of drug release from TDF reservoir IVR. Vaginal fluid hydrates the swellable HPEU tubing (left) and water is driven into the osmotically active drug NaCl core along a gradient, resulting in TDF dissolution and elution from the IVR (right). NaCl aids in establishing soluble drug in the core and achieving equilibrium drug release (Fig. 2B). (B) Photograph of macaque (left) and human (right) TDF IVR. Scale bar, 1 cm. (C) Comparison of 28-d in vitro TDF release rates from matrix (n = 3) and reservoir (n = 6) IVRs under simulated vaginal conditions (2). Data represented as mean ± SD. The SD of some of the collected data are too small to be visualized.
distribution and concentration in vaginal fluid and tissue (Fig. 3A and B and Fig. S2). The TDF IVR provided high TFV (Fig. 3A, Epper) and TDF (Fig. 3A, Lower) mean vaginal fluid concentrations of 7.2 \times 10^{10} \text{ ng/mL} (range 7.1 \times 10^{10} to 3.5 \times 10^{10}) and 1.0 \times 10^{10} \text{ ng/mL} (range 5 to 6.1 \times 10^{9}) respectively. The former consistently exceeded the TFV concentration of 1000 ng/mL, recovered in cervicovaginal aspirates that correlated with protection in women receiving 1% TFV gel (24). In addition, we detected comparable but more variable concentrations of the more potent TDF. Levels of both drugs appear stable in vaginal fluid (days 3 to 28 and levels seemed proportional and distally (Fig. 3A). TFV levels also appeared stable over time in proximal and distal tissues (Fig. 3C). We observed similar TDF and TFV levels in vaginal fluid and tissue to those observed in pigtailed macaques (Fig. 3A and B). These data indicate that the IVRs provide TFV-DP concentrations that exceed protective levels observed previously in macaques, suggesting that the ring could confer protection against vaginal SHIV challenge.

**Terminal PK Study in Rhesus Macaques.** To evaluate intracellular TFV-DP concentrations, we administered TDF IVRs to rhesus macaques that were scheduled to be euthanized because they had been previously infected with SHIV in other studies (n = 3). In this 14-d study, we had the opportunity to evaluate levels of the bioactive metabolite TFV-DP in lymphocytes from vaginal, cervical, and rectal tissue as well as lymph nodes, which are sites where HIV transmission and dissemination is presumed to occur. Previous challenge studies in pigtailed macaques with 1% TFV vaginal gel suggested protection from SHIV infection correlated when TFV-DP levels in vaginal lymphocytes exceeded the IC_{50} of 1.4 \times 10^{9} \text{ ng/mL} cells (4). Mean TFV-DP levels after 14 d of IVR application were highest in vaginal and cervical lymphocytes, 3.3 \times 10^{9} \text{ ng/mL} cells (range 1.5 \times 10^{9} to 7.5 \times 10^{9}) and 1.7 \times 10^{9} \text{ ng/mL} cells (range 8.4 \times 10^{9} to 3.2 \times 10^{9}) respectively. The IC_{50} of the intracellular levels exceeds the TFV-DP IC_{50} of 1.4 \times 10^{9} \text{ ng/mL} cells and is comparable to levels that showed complete protection in macaques (4) (Fig. 3C). Relative to vaginal and cervical lymphocytes, lower drug concentrations were detected in rectal and inguinal lymphocytes, 13 fmol/10^6 cells (range 6 to 1.3 \times 10^6) and 81 fmol/10^6 cells (range 17 to 1.3 \times 10^6), respectively (Fig. 3C). Additionally, we observed similar TDF and TFV levels in vaginal fluid and tissue to those observed in pigtailed macaques (Fig. 3A and B). These data indicate that the IVRs provide TFV-DP concentrations that exceed protective levels observed previously in macaques, suggesting that the ring could confer protection against vaginal SHIV challenge.
by 86% (Fig. 5B). The anti-HIV activity correlated with both TDF and TFV levels in the CVL (Spearman PK/pharmacody-

dicated tissues of rhesus macaques after 14-d IVR administration (n = 3).

Inconsistent with the persistence of TFV in vaginal fluid, CVL collected 1 d following removal of the IVR inhibited HIV infection

from two additional TDF IVR-treated pigtailed macaques not exposed to SHIV in parallel to the challenge study. CVL col-

lected from these two macaques over the course of 28 + 1 d

sequential months of TDF IVR administration. The protection is also consistent with ex vivo antiviral activity of CVL samples

from two additional TDF IVR-treated pigtailed macaques not exposed to SHIV in parallel to the challenge study. CVL col-

lected from these two macaques over the course of 28 + 1 d

(TFV-DP levels in lymphocytes isolated from the in-

dicated time points. (C) TFV-DP levels in lymphocytes isolated from the in-

icated tissues of rhesus macaques after 14-d IVR administration (n = 3).

Fig. 3. Drug PK in pigtailed (28 + 2-d) and rhesus (14-d) macaques. Each
data point represents a single sample and the bar corresponds to the mean

for that dataset. (A) TFV (Upper) and TDF (Lower) concentrations in pigtailed

mackaque vaginal fluid with 28-d TDF IVR administration (n = 6). Samples

were collected proximal (open symbols) and distal (closed symbols) to IVR
placement for the indicated time points. (B) TFV concentrations in vaginal

detection limit [n = 102, lower limit of quantification (LLOQ) = 1

ng/mL], throughout the efficacy study. Detectable TFV levels

(median 8 ng/mL; range 7–19 ng/mL; n = 102, LLOQ = 5 ng/mL)

were observed in five of 102 blood samples collected with 4 se-

quential months of TDF IVR administration. The protection is also consistent with ex vivo antiviral activity of CVL samples

from two additional TDF IVR-treated pigtailed macaques not exposed to SHIV in parallel to the challenge study. CVL col-

lected from these two macaques over the course of 28 + 1 d

was defined as infected and exposures discontinued if vRNA was detected

for presence of SHIV by RT-PCR and confirmed by Western blot. Macaques

were monitored weekly (until week 20)

Fig. 4. TDF IVR protects macaques from repeated vaginal viral challenge.

(A) Six TDF IVR-treated cycling female macaques received weekly 50 TCID

SHIV162p3 vaginal exposure removed

IRV insertion

Time (wk)

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consider the need for higher concentrations of drug in settings of possible increased risk of HIV, among women using DMPA, women with other sexually transmitted infections, and following exposure to acutely infected males with high viral loads in semen (34). Here we report full protection in normally cycling macaques, but the SHIV/macaque susceptibility model using DMPA or coinfections with STIs have been established and the effect of each of these conditions on transmission in the context of this ring can be modeled in future macaque studies (35).

The pigtailed macaque model used here (12) is one of the most rigorous experimental systems available to model vaginal HIV exposure and infection in women because of the repeated exposures and a probability of infection that is at least 200 times that of human unprotected intercourse. The model is able to predict a drop in efficacy resulting from intermittent adherence as well as providing a range of drug levels in vaginal fluids and target cells that correlate with protection (4, 12, 36). Although the model may not fully predict clinical outcomes, exposures and intensive PK/PD and efficacy studies can be performed that are simply not possible in women.

In summary, we report on an antiretroviral eluting IVR conferring complete protection in a nonhuman primate model against frequent vaginal viral challenges. This TDF reservoir IVR is designed to provide drug release rates that generate high and consistent drug concentrations in vaginal fluid and tissue. The design of this reservoir IVR is simple and can be manufactured cost-effectively. We have developed the analogous human-sized IVR (Fig. 1B) that is being considered for clinical evaluation.

**Methods**

IVR Fabrication and in Vitro Studies. Hydrophilic elastomer HydroThane AL 25 93A (Advantec Biomaterials, Inc.) tubing (wall thickness: 0.7 mm) was extruded as described previously (19). Tubing was cut to a 76 ± 0.5-mm length and the end sealed in an indirect tip-forming welder (Plastic Weld Inc.) (19). The open tube was filled with TDF only or with a mixture of TDF (National Standards) with NaCl (US Pharmacopeia (USP) grade, Spectrum Chemicals) or sodium acetate (anhydrous, USP grade, Spectrum Chemicals) in differing ratios (Fig. 5A). The final formulation of TDF and NaCl (86:14) was filled to achieve a final concentration of 130 ± 10 mg TDF and 20 ± 2 mg NaCl per IVR. For a placebo formulation, one-end sealed tubes were filled with 20 ± 2 mg NaCl per IVR. The open end was sealed in a second inductive welding step to form a sealed nod. To form reservoir IVRs, the ends were butt-welded with a thermoplastic welding blade to form a ring with an average diameter of 25 mm as previously described (21, 37). The devices were packaged in heat-sealed pouches (QPS Industries) and were placed at 56 °C for 5 d to load the wall of the IVR with TDF. To fabricate matrix TDF IVRs, TDF-loaded IPFU, ATPU-1 (DSM Biomedical) segments was extruded as described (13), cut to a length of 66 ± 0.5 mm followed by butt-welding as described previously. Formulations were tested for in vitro drug elution under physiologically relevant conditions in 25 mM acetate buffer (PH 4) at 37 °C. NaCl release was measured using a chloride ion selective electrode (Mettler Toledo) coupled to a Seven Multi pH meter (Mettler Toledo). IVRs were extruded as described (13), cut to a length of 66 ± 0.5 mm followed by butt-welding as described previously. Formulations were tested for in vitro drug elution under physiologically relevant conditions in 25 mM acetate buffer (PH 4) at 37 °C. NaCl release was measured using a chloride ion selective electrode (Mettler Toledo) coupled to a Seven Multi pH meter (Mettler Toledo). IVRs were analyzed for residual drug content after in vitro and in vivo studies by chemical extraction followed by methods reported previously (13).

Drug PK. All macaques were housed at the Centers for Disease Control and Prevention (CDC) (Atlanta, GA). All procedures were conducted under approved CDC Institutional Animal Care and Use Committee protocols 2003DO08 (IVR-PK) and 2004DO15 (IVR-PK and efficacy) in accordance with the standards incorporated in the Guide for the Care and Use of Laboratory Animals (National Research Council of the National Academies, 2010). Matrix IVRs were administered to female piggledy macaques (TDF IVRs, n = 6 and placebo n = 2; r = 2 ± 2 ± 2, d) and female rhesus macaques of Indian origin (TDF IVRs, r = 3, r = 14, 0). The latter rhesus macaques were infected with SHIV012D3 virus in a previous study and were used for the terminal PK experiment after virus was no longer detectable in plasma. All sampling procedures were performed under anesthesia with isoflurane in the pigtailed macaque PK study. IVRs were inserted at day 0 and removed at day 14 just before being euthanized, with samples taken on days 0, 7, and 14. Collection and processing of vaginal fluids and biopsies were performed as...
previously described (21, 39). TDF and TFV levels in vaginal fluid collected using Widell-Coble (Beaver Vكت, CVL) brushes of genital tract with 5 mL PBS, and vaginal tissue (days 7, 21, and 30) were determined using liquid chromatography-tandem mass spectrometry (LC-MS/MS) as described (21, 39). Procedures involving euthanasia and evaluation of intracellular TFV-DP were performed as described previously (6).

LLOQ for TDF was 5 ng/mL, which is equivalent to ~13 fmol/10^{6} cells (40). The average fluid and tissue mass was 0.04 g and 0.01 g, respectively. The concentration of drug in vaginal fluid was determined by converting the change in the mass to volume, assuming the density of vaginal fluid was 1.0 g/mL. Samples below LLOQ were assigned values midway between zero and LLOQ and then dividing by the mass or volume of the sample.

Efficacy Studies. TDF-IVRs were administered to normal cycling, non-synchronized female pigtailed macaques (n = 6) followed by weekly inoculation (12) vaginally with 50 TCID_{50} SHIV162P3 virus (12). Virus exposures were started on vaginal tissue and lymphocytes by LC-MS/MS methods as described previously (38, 39). The first viral inoculation was supported by the National Institutes of Health Grant U19 AI076980. The findings and conclusions in this paper are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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Supporting Information

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Fig. S1. (A) Maximum, mean, and minimum in vitro tenofovir disoproxil fumarate (TDF) release rates over 30 d from hydrophilic polyether urethane reservoir intravaginal rings (IVRs) filled with TDF and NaCl or NaAc formulations. TDF mass per IVR was kept constant and the NaCl and NaAc amounts varied (TDF:NaCl: 93:7, 86:14, and 79:21; TDF:NaAc: 93:7, 85:15, and 74:26; w:w, n = 3). Data represented as mean ± SD. (B) Amount of TDF delivered from TDF IVRs determined from the residual drug in IVR after 28-d in vivo (n = 36) and in vitro (n = 6) studies. Each data point represents a single sample and the bar and whiskers correspond to mean and SD, respectively.

Fig. S2. TDF concentrations in pigtailed macaque vaginal tissue (28 ± 2-d pharmacokinetic study). TDF concentrations in pigtailed macaque tissue with 28-d TDF IVR administration (n = 6). Samples were collected proximal (open symbols) and distal (closed symbols) to IVR placement for the indicated time points. Each data point represents a single sample and the bar corresponds to mean for that dataset.
CHAPTER 3

INTRAVAGINAL FLUX CONTROLLED PUMP FOR
SUSTAINED RELEASE OF MACROMOLECULES

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**Intravaginal Flux Controlled Pump for Sustained Release of Macromolecules**

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**ABSTRACT**

**Purpose:** To design a flux controlled pump (FCP) capable of 30-day, controlled release of macromolecules to the vaginal mucosa.

**Methods** The FCP is composed of a single chamber fabricated from a rigid thermoplastic with orifices and encloses a pellet of water-swellable polymer containing the drug substance. We performed testing both in vitro and in rabbits. To ensure vagina retention in the rabbit, we designed and attached an oval shape-memory polyether urethane retainer to the FCP allowing for long-term intravaginal evaluation of a solid dosage form without invasive surgical implantation.

**Results** The orifices and swelling properties of the polymer pellet control water entry for polymer hydration and expansion, and subsequent extrusion of the drug-containing gel from the orifice. A FCP device containing a pellet composed of hydroxypropyl cellulose compounded with a model macromolecule, achieved controlled in vitro release for 30 days with an average release rate of $24 \pm 2 \mu g/day$ (mean ± SD) and range of 16 to 42 $\mu g/day$. We observed a slightly lower average release rate in vivo of $20 \pm 0.6 \mu g/day$ (mean ± SD).

**Conclusions** The size of the orifice and nature of the swelling polymer controls the hydration rate and thereby macromolecule release rate and duration from this FCP.

**KEY WORDS** in vivo evaluation - macromolecules - non-surgical implantation - vaginal delivery

**ABBREVIATIONS**

- ABS  Acrylonitrile butadiene styrene
- CG  Carrageenan
- CTMR 5-(and-6)-carboxytetramethylrhodamine
- FCP  Flux controlled pump
- HEC  Hydroxyethyl cellulose
- HPC  Hydroxypropyl cellulose
- IVR  Intravaginal ring
- MP  Model protein
- PK  Pharmacokinetics
- SMP  Shape-memory polyether urethane
- Tg  Glass transition temperature

**INTRODUCTION**

The science of using intravaginal drug delivery is less advanced for macromolecules like proteins, oligonucleotides and synthetic polymers than for small molecules (1). There have been promising results in the potential use of macromolecules for the prevention of sexually transmitted infections (2-6), vaccination strategies (7), and treatment of endometriosis or uterine fibroids (8). Macromolecular classes for vaginal delivery include peptides and proteins (2-6), antibodies (9-11), oligonucleotides (12), small-interfering RNA (13), and synthetic and natural polymers (6,14). However, to deliver these molecules the most common approach has been to utilize short duration formulations (2,3,6) like semi-solid gels that may require frequent administration, and that then may result in poor user adherence and variable pharmacokinetics (PK). Engineering long-acting, vaginal drug delivery systems may increase user adherence and provide favorable PK over frequently dosed formulations (1,15,16).

Intravaginal rings (IVR) are commercially successful long duration, topical delivery vehicles for small molecules, and can have high user compliance (17,18). However, macromolecules are difficult to formulate compared to small molecules given their...
low solubility in biomedical elastomers from which the IVRs are made, their size makes diffusive release through elastomer walls challenging, and their activity depends on complex intra and intermolecular structures. Pioneering work by Saltzman (9,10) and Malcolm (11) addressed the need for new solid dosage forms delivering macromolecules to the vaginal mucosa. This work displayed first-order release kinetics proportional to t1/2, characterized by an initial burst followed by a continuous reduction in release rate. In many therapeutic situations this is an acceptable release profile but in some applications like topical prevention of HIV acquisition or in cases where the drug is toxic at high levels, better control of the drug release rate is required. To overcome the low solubility and inadequate diffusivity in commonly utilized elastomers, they incorporated a high (50–50 wt.% loading of drug and/or hydroscopic excipient exceeding the percolation threshold, to form a connected porous structure (9–11,19,20). Here, a potential drawback of high drug or porogen loading is the decrease in device stiffness upon drug release, potentially leading to poor vaginal retention. Morrow et al. improved upon previous designs and engineered a new intravaginal device with small inserts incorporated into an IVR to decouple macromolecule delivery from IVR stiffness (11). Therefore, a need exists for intravaginal delivery systems with improved control of the drug release rate and device mechanical stiffness. This study presents a new method of delivering macromolecules intravaginally we call a flux controlled pump (FCP). The FCP is made of a compressed water soluble polymer pellet (11) compounded with a macromolecular drug and enclosed in a hard polymer casing. The casing contains orifices to allow influx of water/vaginal fluid and efflux of the pellet (11) compounded with a macromolecular drug and/or hydroscopic excipient exceeding the percolation threshold, to form a connected porous structure (9–11,19,20). Here, a potential drawback of high drug or porogen loading is the decrease in device stiffness upon drug release, potentially leading to poor vaginal retention. Morrow et al. improved upon previous designs and engineered a new intravaginal device with small inserts incorporated into an IVR to decouple macromolecule delivery from IVR stiffness (11). Therefore, a need exists for intravaginal delivery systems with improved control of the drug release rate and device mechanical stiffness.

Preparation of Compressed Pellets

Model compounds, MP, 5-(and-6)-carboxytetramethylrhodamine (CTMR) labeled insulin (see supplemental materials or 10 kDa rhodamine B dextran (Sigma Aldrich, St. Louis, MO), at 1 wt.% were mixed with water-swellable polymers: LF, JF and GF hydroxypropyl cellulose (HPC: Klucel Pharma, Norwood, MA) and hydroxyethyl cellulose (HEC: Natrosol™ 250 HR; Ashland Chemicals, Covington, KY), or carragenan (CG: Gekarin® PC 379: FMC BioPolymer, Philadelphia, PA) using a homogenizer (Qiagen Tissuelyser II, Valencia, CA) for 9 min at 50 Hz. Pellets were formed by compression on a manual bench top press (Carver, Wabash, IN) at 2 metric tons for 20 s or 2 min using a punch and die set specifically designed for 3 mm diameter pellets (Fig. S1); Rapid Machining, Nashua, NH). Barium sulfate at 35 wt.% (USP grade, Spectrum Chemicals, Gardena, CA) was compounded with SMP category MM-3520 with glass transition temperature (Tg) of 35°C (SMP Technologies, Tokyo, Japan) using a Haake-Minilab twin screw extruder (Thermo Scientific, Newington, NH). The extruder barrel temperature was set to 200°C with the screw speed at 70 rpm. To ensure homogeneity, the mixture was extruded twice into strands that were pelletized using a variable-speed pelletizer (Randcastle Extrusion Systems, Cedar Grove, NJ). The subsequent pelletized extrudate was injection molded into an ellipsoidal shaped stem-attached devices (25 mm major axis, 17 mm minor axis with a 2 mm cross-section ellipse and a 5 mm long, 4 mm cross-section attached rod; Fig. 1a) using a Babylast 6/10F micro-injection molding system ALBA Enterprises, Rancho Cucamonga, CA and a custom-fabricated aluminum mold (Sorenson Mold Inc., Midvale, UT). Injection molding temperatures ranged from 120°C to 190°C and mold temperatures ranged from 12°C to 14°C. After device fixtures were injection molded, SMP devices were heated to 37°C in an oven and the ellipsoidal flattened to the other axis (in-line with stem) to allow for insertion into the vaginal catheter (Fig. 1c). The devices were allowed to cool to room temperature while held in this position. The details of SMP retainer device development are described in supplemental material.

Fabrication of Flux Controlled Pump

FCPs (16 mm long, 5 mm outer diameter and 3.1 mm inner diameter) were designed in SolidWorks® and fabricated on a lathe from 1/4” acrylonitrile butadiene styrene (ABS) rod stock (McMaster-Carr, Robbinsville, NJ). The dimensions of the FCPs were based on previous reports testing intravaginal devices (23–25). Additionally, the dimensions of the FCP were designed based on the length of the rabbit abdominal vagina in vivo. PK study using our SMP vaginal device retainer system. Orifices were manually drilled, and the diameter of the orifices was measured using a stereomicroscope. Digitized images of the devices are shown in the digital images (Fig. 1d). The FCPs were loaded with a high (30–50 wt.%) loading of drug and/or hydroscopic excipient exceeding the percolation threshold, to form a connected porous structure (9–11,19,20). Here, a potential drawback of high drug or porogen loading is the decrease in device stiffness upon drug release, potentially leading to poor vaginal retention. Morrow et al. improved upon previous designs and engineered a new intravaginal device with small inserts incorporated into an IVR to decouple macromolecule delivery from IVR stiffness (11). Therefore, a need exists for intravaginal delivery systems with improved control of the drug release rate and device mechanical stiffness.

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Fig. 1. FCP-SMP retainer device. a Photograph showing the FCP-SMP retainer device with two 1.5 mm diameter orifices, containing 1 wt.% MP in GF HPC, with a schematic depicting polymer swelling and chain relaxation. b X-ray of device retained in a rabbit. The SMP retainer device contained 35 wt.% barium sulphate as the radiocontrast agent. c Vaginal catheter to insert the FCP-SMP retainer device composed of clear tubing with marks showing insertion length (10 cm) and plunger (white rod) included with an FCP-SMP device in the compressed configuration.

Images were taken and distances were measured by comparing to a scale. For example, 1.5 mm diameter orifices measured 1.58 ±0.097 mm (n=8 orifices with 3 measurements per orifice). The compressed pellets were placed inside the FCP and the ends were closed with two 3 mm diameter ABS plugs of equal length and secured with ABS cement (ACE hardware, Oak Brook, IL) followed by curing overnight at room temperature. For in vivo studies, the FCPs were butt-welded to the SMP retention devices (Fig. 1a) and cured overnight at room temperature. The joint flashing was then trimmed flush. The device was wiped with isopropyl alcohol, dried overnight in a sterile laminar flow hood and sealed in pouches until the study.

Measure Swelling Rate of Polymers

Swelling studies were performed in DDI water at room temperature on 100 mg pellets of water-swellable polymers (CG, HEC or LF, JF or GF HPC) made with a 1/4" pellet press at 2 metric tons for 20 s. Time dependent increases in the axial dimension and mass of pellets were measured for LF, JF and GF HPC, and CG, HEC and HPC. Swelling index was calculated as a fractional increase in either mass or axial dimension. Swelling rate was calculated as the slope of the swelling index vs. time (70 graph).

In Vitro Release of Rhodamine B Dextran and MP

Release studies were performed in 25 mM aceate buffer pH 4.2 and PBS pH 7.4 at 37°C and shaken at 80 rpm (25, 27). Drug content was measured by dissolving a known amount of model compounds in release media and creating a standard curve by serial dilutions. Fluorescence measurements were performed using a Synergy2 plate reader (BioTek, Winooski, VT) at 540±20 nm excitation and 620±40 nm emission wavelengths. Total pellet content at each of the studies was measured in terms of mass of polymer remaining. Cumulative release was calculated from measured daily release using the trapezoidal rule to calculate the area under the curve. Average release rates were calculated as the cumulative release divided by the elapsed time.

Rabbit Studies

In vivo studies were performed according to the guidelines established by National Institutes of Health and Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). The study protocol was approved by the Institutional Animal Care and Use Committee (IACUC; Protocol No. 09-11013) at the University of Utah. New Zealand White adult female rabbits (N=6; 15-16 week old, 3.5-4 kg) were quarantined for 1 week for acclimatization before the beginning of the study. The devices were inserted using a custom-designed vaginal catheter system. The system is composed of a catheter made using a 18 cm long Tygon® tubing (3606; ID 1/4", OD 5/16"; Cole Parmer, Vernon Hills, Illinois) and a plunger with a flat end. We inserted the catheter 10 cm beyond the introitus (28) and used the plunger to expel the FCP-SMP device into the upper vaginal tract beyond the urinary sphincter (Figure 1c). The animals were anaesthetized using 35 mg/kg ketamine and 5 mg/kg xylazine intramuscularly maintained with inhalation of 1.0-2.5% isoflurane during device insertion. The vaginal catheter was lubricated with universal placebo gel (21) to facilitate insertion. Upon completion of the study, and confirmation of device retention by X-ray, the animals were euthanized with 3 ml saturated potassium chloride intracardiac. The complete vaginal tract
was resected; a transverse section was cut and fixed in 10% formalin. H&E staining was performed by ARUP Laboratories (Salt Lake City, UT).

**Statistics**

Single factor ANOVA and two-tailed t-test assuming equal variances were used to compare three or greater, and two different sets of values respectively. All data has been represented as mean ± SD, N=3, except N=4 for the 20 and 30-day arm of the rabbit study, with p-values denoting significance. In vitro release of rhodamine B dextran was plotted as N=12 until day 10, N=6 for days 15 and 20, and N=3 for days 25 and 30.

**RESULTS**

**Effect of Type and Molecular Weight of Swelling Polymers on In Vitro Release**

The in vitro release profiles were determined for FCPs with two 1.5 mm diameter orifices and a range of different swelling polymers: CG, HEC and three different molecular weight HPCs (LF, JF and GF; 95, 140, and 370 kDa respectively). The release rate depended greatly on the polymers used, with polymers with higher swelling rates imbibing more water in a given time and resulting in higher release rates. We initially compared the swelling rates of CG, HEC and GF HPC and observed that CG swells the most and GF HPC the least (mass swelling rates: CG 1.8±0.1 s−1/2 > HEC 1.4±0.09 s−1/2 > GF HPC 0.38±0.02 s−1/2; p<0.01; Fig. 3a). With the CG formulation, 390±31 µg of MP was observed on day 1 in comparison to 110±39 µg and 41±6.6 µg from HEC and GF HPC containing FCPs respectively (p<0.01) resulting in nearly 100% release from CG FCPs in 5 days (CG 100±0%; > HEC 53±19%; > GF HPC 10±2%); p<0.01; Fig. 3a). The average MP release (t=5 days) was CG 210±8.0 µg/day, HEC 110±10 µg/day, and GF HPC 38±6.2 µg/day (p<0.01; Fig. 3b).

Comparison of swelling rates of HPCs showed high molecular weight HPC (GF) had the maximum swelling rate but disintegrated and dissolved slower than JF and LF HPC (axial swelling rates: GF 6.8±1.5 s−1/2 > JF 5.5±1.1 s−1/2 > LF 2.1 ±1.4 s−1/2; p<0.05; Fig. 2b and c). LF HPC disintegrated most rapidly as seen by quickly asymptoting followed by a decline in mass upon dissolution (Fig. 2c). This led to higher initial MP release with up to 45±16% released by day 5; in comparison, moderate and high molecular weight HPCs (JF and GF respectively) resulted in 27±14% and 19±3.1% released in the same duration (p<0.05; Fig. 3c). The average MP release (t=10 days) was 69±18 µg/day LF, 51±11 µg/day JF and 41±2.4 µg/day GF (p=0.07; Fig. 3b). Due to the high initial release rate for the LF and JF formulations, the release dropped significantly after day 10 (data not shown).

**Drug release was observed to vary with orifice size, with a smaller diameter orifice resulting in lower release (Fig. 4). For the orifice sizes tested; 1.0, 1.5 and 2.0 mm diameters; the average MP release (t=10 days) was 36±5.0, 41±4.2 and 51 ±2.0 µg/day respectively (p<0.01; Fig. 4b). Interestingly, a linear relationship between total orifice area and average release was observed (R²=0.976; Fig. 4b). Furthermore, we performed a power law fit of the cumulative release (Fig. 4a) with an exponent value of 1 representing zero-order release (29). The exponent values were 1.2, 0.99 and 0.88 for the 1.0, 1.5 and 2.0 mm orifices respectively (R²>0.98).

**Effect of Orifice Size on In Vitro Release**

Comparison of MP and Rhodamine B Dextran Release

FCPs with two 1.5 mm diameter orifices and GF HPC as the swelling polymer were tested in a 30-day in vitro release study with rhodamine B dextran as the model compound resulting in an average of 2±0.05 µg/day and 79% cumulative release with a maximum and minimum daily release rates of 42±8.2 µg/day on day 3 and 16±1.8 µg/day on day 20 respectively (Fig. 5). The average release rate of rhodamine B dextran was statistically comparable to MP (t=10 days) with 35±7.0 µg/day and 41±4.2 µg/day of rhodamine B dextran and MP respectively (p=0.2, Fig. 5 and Fig. 5a and b). A power law curve fit of the cumulative release (Fig. 5a) validated that near zero-order release was achieved in the first 10 days (Fig. 5b and Fig. S2a and b). The release rate over 30 days appeared biphasic with an increased release rate in the first 10 days compared to beyond day 10 (Fig. 5a). This was apparent by comparing the calculated average release rate over different time periods. The average release rate was 35±7.0 µg/day from days 1–10, 29±1.8 µg/day from days 10–30, and 24±2.1 µg/day from days 1–30. Depending on the device design and polymer used there are multiple processes that are driving release, however if the rhodamine B dextran and GF HPC release correlate this suggests that hydration and transport of the HPC gel out of the FCP was the major factor (p=0.2, Fig. 5a and Fig. S2a and b). A single factor ANOVA and two-tailed t-test assuming equal variances was performed (29), with exponent values of 0.99, 0.83 (rhodamine B dextran, R²=0.99 over 10 and 30 days respectively), and 0.99 (MP over 10 days, R²=0.99) validating that near zero-order release was achieved in the first 10 days (Fig. 5b and Fig. S2a and b).

**Comparison of MP and Rhodamine B Dextran Release**

FCPs with two 1.5 mm diameter orifices and GF HPC as the swelling polymer were tested in a 30-day in vitro release study with rhodamine B dextran as the model compound resulting in an average of 2±0.05 µg/day and 79% cumulative release with a maximum and minimum daily release rates of 42±8.2 µg/day on day 3 and 16±1.8 µg/day on day 20 respectively (Fig. 5). The average release rate of rhodamine B dextran was statistically comparable to MP (t=10 days) with 35±7.0 µg/day and 41±4.2 µg/day of rhodamine B dextran and MP respectively (p=0.2, Fig. 5 and Fig. 5a and b). A power law curve fit of the cumulative release (Fig. 5a) validated that near zero-order release was achieved in the first 10 days (Fig. 5b and Fig. S2a and b). The release rate over 30 days appeared biphasic with an increased release rate in the first 10 days compared to beyond day 10 (Fig. 5a). This was apparent by comparing the calculated average release rate over different time periods. The average release rate was 35±7.0 µg/day from days 1–10, 29±1.8 µg/day from days 10–30, and 24±2.1 µg/day from days 1–30. Depending on the device design and polymer used there are multiple processes that are driving release, however if the rhodamine B dextran and GF HPC release correlate this suggests that hydration and transport of the HPC gel out of the FCP was the major factor controlling rhodamine B dextran flux. Cumulative percent release of GF HPC was 40±4.1%, 88±5.9% and 78±1.9% on days 10, 20 and 30. This was comparable to the cumulative percent release of rhodamine B dextran of 39±7.0%, 54±7.4% and 79±6.9% on days 10, 20 and 30 (Fig. 3a).

The cumulative percent release of rhodamine B dextran calculated from the
Fig. 2. Swelling properties of hydrophilic polymers. a Swelling rate of GF HPC, HEC and CG determined by mass. b Swelling rate of LE JF and GF HPC determined by axial dimensions. c Mass swelling indices of LE JF and GF HPC demonstrating that LF asymptotes the quickest because of dissolution compared to the others.

In vitro elution profile correlated to cumulative percent release of GF HPC measured from residual content on days 10, 20 and 30 (Spearman correlation of ρ=0.99, p<0.001; Fig. S2c).

Furthermore, we performed in vitro release on the rhodamine B dextran containing FCPs in two release media for 10 days, PBS pH 7.4 and 25 mM acetate buffer pH 4.2, to mimic pH conditions in the rabbit and human vaginal tracts (25,30). No statistical difference was observed in the two cases; therefore an average of the two is reported (p=0.8; Fig. 3). For assessment of how the FCP functions, we fabricated a clear, acrylic device to visualize polymer hydration and dissolution. Water entered through the orifices with initial polymer hydration visually observed around the orifices which spread longitudinally towards the ends by day 5. The photographs illustrate the time-dependent hydration and release of the MP containing HPC gel during the initial 15 days. (Figure 6)

Fig. 3. In vitro MP release. a Cumulative release of MP and (b) average release rate and cumulative release of MP over 5 days from FCPs with GF HPC, HEC and CG as the swelling polymers. c Cumulative release of MP and (d) average release rate and cumulative release of MP over 10 days from FCPs with LE JF and GF HPC as the swelling polymers.
**Sustained Vaginal Delivery of Macromolecules**

**In vitro** MP release. a Cumulative in vitro release of MP from FCPs as a function of orifice size: 1.0, 1.5 and 2.0 mm. b In vitro average release rate and cumulative release compared to orifice area. A linear correlation between orifice area and average release \( t = 10 \text{ days} \) was observed \( (R^2 = 0.976) \).

**In Vivo Rabbit Study**

In a 30-day study we tested the safety and PK of rhodamine B dextran loaded FCP-SMP retainer devices in adult female New Zealand White rabbits (Fig. 1). Drug release calculated by residual polymer extraction after 10, 20 and 30 days *in vivo* application was lower compared to *in vitro* samples. We noted a marked difference for all time points \( (p=0.023, 0.002 \text{ and } <0.0001 \text{ for } 10, 20 \text{ and } 30\text{-day arms}) \). The diminished release measured *in vivo* compared to *in vitro* resulted in a calculated average rhodamine B dextran release rates of 20 ±0.6 *in vivo* and 24 ±0.8 *in vitro* \( \mu g/day \) over 30 days, a 16% reduction. The latter is not significantly different from the average release rate calculated from the 30-day cumulative profile \( (24 ±2.1 \mu g/day, \ p=0.96) \). No irritation or immune cell infiltration was observed in tissue sections from treated animals in comparison to the naive suggesting that the devices were well tolerated (Fig. 7 and Fig. S6).

**DISCUSSION**

The main objectives of these studies were to first determine if the FCP could be used for prolonged vaginal delivery of macromolecules based on simultaneous control of water entry into the device and polymer gel extrusion out of the device. Next, we sought to improve testing of vaginal solid drug delivery systems in the rabbit model by engineering a retainer device that could be used without surgery. To achieve these objectives, we fabricated a series of prototype devices which we evaluated *in vitro* for drug release properties and then for *in vivo* performance in the rabbit. Our initial design was inspired by the semipermeable membrane of the osmotic pum p that controls the hydration of the core pellet and thereby the release rate of its contents \( (31) \). We substituted the function of semipermeable membrane for an orifice of variable size in a non-permeable rigid polymer casing imparting control of hydration and release of the gel from the device and providing mechanical integrity for long duration delivery. Our device consists of a cylindrical ABS thermoplastic casing with two orifices for opposing water diffusion in and drug-loaded gel extrusion out of the casing (Fig. 1a). In the case of pellets made from high molecular weight polymers like CG, the polymer swelling is driven by osmotic gradients between the hydrating gel and water, and solvent-polymer mixing \( (32) \); in non-ionic polymers like HEC and HPC, pellet hydration and swelling is driven by water-polymer mixing only \( (29) \). Our data show that by modulating the size of the orifice and swelling properties of the polymers we can modify the release rate of the molecules entrained in...
Fig. 6 Mechanism of release from the FCP. Photographs showing acrylic MP loaded FCP depicting polymer hydration and release during the initial 15 days. The devices had two 1.5 mm diameter orifices and contained a GF HPC pellet with 1 wt.% MP. Water entered through the orifices due to a concentration gradient resulting in polymer hydration, expansion and release.

The devices had two 1.5 mm diameter orifices and contained a GF HPC pellet with 1 wt.% MP. Water entered through the orifices due to a concentration gradient resulting in polymer hydration, expansion and release.

Our observations support that polymer hydration and swelling rate are critical variables in controlling the drug release rate. We observed dramatic differences in in vivo release rates from FCPs containing three polymers with different kinetics and extent of hydration: CG, HEC, and GF HPC. All polymers selected have been tested for safety in vaginal formulations (6,21,22). CG formulations exhibited the greatest swelling rate and extent followed by HEC and GF HPC, resulting in the highest release rate with approximately 100% drug released by day 5 (Fig. 3a and b). We previously published on a vaginal osmotic pump that is conceptually similar to this FCP and delivered a small molecule drug in a crystalline form (33). Formulations containing CG or HEC that displayed nearly complete release over 5 and 10 days could be suitable for several important indications in women’s health, including antibiotics for treatment of bacterial vaginosis, antifungals for vaginal candidiasis, and antiprogestins for cervical ripening. GF HPC formulations showed controlled release over the initial 5 days with the largest amount of hydrogel remaining within the casing compared to CG and HEC; therefore we tested the effect of HPC molecular weight on in vivo release for longer duration release. Generally, polymer dissolution depends on chain length, with shorter polymer chains dissolving more rapidly (29). Among the three molecular weights tested, high molecular weight HPC (GF) exhibited greater polymer swelling rate and lower dissolution rate (Fig. 2b and c). Low (LF) and median (MF) molecular weight HPC displayed higher initial release that decreased beyond day 10, compared to GF (Fig. 3c and d). Since the lower molecular weight LF and MF HPCs swelled less and dissolved more readily (Fig. 2b) the mechanism of release may be more influenced by diffusion of the model drug through the swollen material in the casing and out of the orifice than the simultaneous co-transport of the gel and its contents from the orifice as in the case of GF HPC, HEC and CG.

We established that orifice size was another important design variable impacting release. The average release rate linearly depended on orifice area suggesting drug release rate was dependent on the rate of water entry and polymer swelling and that these rates can be modulated with orifice area. We selected the 1.5 mm diameter orifice FCP for 30-day in vitro release studies since the 2.0 mm diameter orifice FCP released approximately 50% of the model compound in 10 days, leaving an insufficient amount to achieve controlled release over 30 days. Moreover, the 1.5 mm orifice FCP exhibited a more zero-order release profile in the first 10 days of release compared to the 1.0 and 2.0 mm orifices. (Figure 4)

We evaluated the release of two model macromolecules, rhodamine B dextran [10 kDa] and MP [7.4 kDa], in the design with GF HPC as the swelling polymer and two 1.5 mm diameter orifices. In vivo studies with the extended high radius of gyration rhodamine B dextran molecule and compact MP molecule demonstrated release rate was comparable and was

Fig. 7 In vivo rabbit study. a. Mass and percent of polymer pellets released under in vivo and in vitro conditions determined by residual content. Representative H&E histology sections of vaginal tissue from (b) Naive and (c) FCP-SMP device treated animals (30 days). No significant immune cell infiltrates, epithelial inflammation or disruption was seen.
a function of polymer swelling and dissolution (Fig. S2a and b). The 30-day release profile (Fig. 5) of rhodamine B dextran was biphasic with a higher, nearly zero-order release rate in the first 10 days followed by a reduced and nearly constant release rate for the remaining duration. The decreased release rate after day 15 likely occurred from the decreasing amount of hydrated polymer in the casing causing slower extrusion and dissolution of the gel from the device (Fig. 5). Often a time-independent release rate is desired to maintain a protective and safe drug concentration in the therapeutic window. However, a release profile with an increased release rate early on could provide a loading dose to more quickly attain high drug concentration in tissue while the lower release rate later on could provide a maintenance dose. (1) We propose that drug release was initiated by water entry through the orifices diffusing down its concentration gradient. As water continues to enter, the polymer expands and exerts an internal pressure on the rigid polymer casing, causing extrusion of the viscoelastic drug-loaded gel from the orifice (Fig. 6). In addition to the extrusion of the polymer with the entrained model compound, it is possible for the model compound to diffuse through the swollen polymer out of the device. The diffusion coefficient of the entrained compound in the polymer will increase as the water content increases (29). However, the cumulative percent release of rhodamine B dextran correlated to GF HPC from the FCP on days 10, 20 and 30 (Fig. S2c). This supports that the predominant mechanism of rhodamine B dextran release was from extrusion of HPC gel from the FCP and not diffusion of the rhodamine B dextran through the swollen HPC gel within the FCP casing.

Development of new intravaginal drug delivery technologies necessitates small animal models to assess safety and PK as a function of device design. The rabbit model is the standard non-rodent species approved for vaginal irritation studies (34—36) during preclinical testing of vaginal products (37). However, the application of this model for understanding intravaginal device safety and PK is limited by the invasive surgical procedure needed to saturate the device to the vaginal wall to ensure retention (23—25). While perturbations and inflammation were not reported, it is unknown if the trauma and resulting healing response influences drug release and PK. Furthermore, a non-surgical approach could significantly improve and expand the use of the rabbit model for intravaginal device testing. Accordingly, we designed an ellipsoidal SMP vaginal device retainer attached to the FCP for non-surgical retention in the rabbit cervicovaginal tract (Fig. 1 and Fig. S5). Prior to insertion, the device was warmed to 37°C, above the SMP Tg, and the major axis flattened for placement into a vaginal catheter used to introduce the drug delivery system. We allowed the device to equilibrate to room temperature resulting in a device maintaining the elongated, flat arrangement (Figure 1c). Upon insertion, the SMP transitioned from room to rabbit body temperature, and the straight arrangement necessary for device insertion relaxed into the native, ellipsoidal shape for device retention (Fig. 1a and b). Utilizing the SMP elastomers with a Tg between room and body temperature facilitated easy insertion through the catheter. We observed attenuated release in vivo compared to in vitro conditions with a 10% reduction in total release over 30 days. Possibly the reduced release rate observed in vivo resulted from insufficient fluid to drive polymer hydration and drug release compared to in vitro sink conditions. Additionally, no significant immune cell infiltrates or inflammation was observed with the device, suggesting good tolerance. (Figure 7 and Fig. S6)

A sustained, solid dosage form for vaginal delivery has multiple design inputs including three key requirements: desired drug release rate and duration, device retention, and manufacturability. However, except for a recent publication (11), prior reports in the field emphasized macromolecular delivery with less attention towards device retention or ease of fabrication. These device designs demonstrated high hydrophilic drug and/or excipient loading (50—59 wt.% in hydrophilic polymers can form a connected porous structure resulting in osmotically driven controlled and sustained release (9—11, 19, 20). Morrow et al. presented the first human device for intravaginal macromolecular delivery, displaying 28-day in vivo release of BSA, with a daily release profile approximately proportional to 1/2 that decreased constantly with time (11). By adjusting the design of the FCP, we were able to obtain a more constant release rate over a month duration (Fig. 5). Additionally, the FCP is simple to manufacture, including thermoplastic parts suitable for injection molding or hot-melt extrusion, and pellets by tablet manufacturing technology.

Increasing interest in developing multipurpose prevention technologies for sexually transmitted infections and unwanted pregnancy prevention can benefit from a platform capable of simultaneous delivery of both small and macromolecular drugs (38). Our results provide motivation for integrating up to four FCPs into an IVR as pods or segments, for simultaneous, controlled delivery of macromolecular and small molecular weight drugs from the FCP and IVR respectively (11, 39—41). While the mechanical stiffness of the IVR will be altered by the incorporation of one or multiple FCPs, this design would not exhibit reduced rigidity upon macromolecule release as observed with previous matrix designs with high loadings above the percolation threshold (9, 10, 19, 20). The FCP design provides a tunable platform where drug release rate can be controlled by altering the number of FCPs per IVR and drug loading. Furthermore, altering the swelling polymers and orifice size modifies the release rate and duration. Moreover, by incorporating multiple FCPs into an IVR, it would be possible to deliver different drugs at individual release rates from each FCP. Pellet hydration is critical for...
CONCLUSIONS

This study presents the use an FCP device for delivery of macromolecules to the vaginal mucosa for prolonged duration. The simple design involves a single chamber with two orifices, enclosing a drug loaded polymer pellet that when swollen pressurized the FCP chamber. In this first study, we observed a small decrease in drug release rate as compared to in vitro. Further, this study describes an improved in vivo methodology for testing vaginal delivery of macromolecules to the vaginal mucosa for prolonged duration. Therefore, the activity and physical stability of the macromolecule released from this system needs to be assessed.

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REFERENCES


SUPPLEMENTAL MATERIALS

MATERIALS AND METHODS

Fluorescent labeling of Insulin

Bovine insulin was fluorescently tagged with 5-(and-6)-carboxytetramethylrhodamine (CTMR) succinimidyl ester for ease of detection. CTMR succinimidyl ester was prepared by reacting CTMR (3.0 mg, 7.0 µmol), N, N'-Diuccinimidyl carbonate (2.2 mg, 8.5 µmol) (Sigma Aldrich, St. Louis, MO), and 4-Dimethylaminopyridine (0.93 mg, 7.6 µmol) (Acros Organics, Morris Plains, NJ) in 0.43 ml of dry DMF. The reaction was run at RT for 4 hrs. The percent esterification was determined by HPLC using relative peak areas of CTMR before and after the reaction. The reaction mixture was run on a Hewlett-Packard 1050 Series HPLC on a Jupiter C18 column (4.6 x 250 mm, 5 µm; Phenomenex, Torrance, CA) at 25°C at a flow rate of 1.0 ml/min using an acetonitrile (ACN)-water (0.1% TFA) gradient (0-2 min: 10% ACN, 2-7 min: 10-73% ACN, 7-9 min: 73% ACN, 9-10 min: 73-10% ACN, 10-12 min: 10% ACN). Data collected at 254 nm was used to calculate a percent esterification of 90%. Bovine insulin (25 mg, 0.88 mmol; Sigma Aldrich, St. Louis, MO) was dissolved in 5 ml of 0.1 M NaHCO₃ solution with 1.25 M urea, pH 8. This solution (1.5 ml) was added to the CTMR ester in a molar ratio of 1.4.8. The reaction was continued for 3 hrs at RT, followed by addition of 0.1 M HCl (2 ml) to stop the reaction. The mixture was stored at 4°C until separation. The unreacted dye was separated using HiTrap™ desalting columns (GE Healthcare, Bellefonte, PA) according to manufacturer’s protocol, and the fractions containing labeled insulin were lyophilized. The labeled product was mixed with insulin in a 1:50 ratio by suspending in DDI water followed by lyophilization and stored at -20°C until the study.

Fabrication of O-shaped vaginal retainer devices

O-shaped devices were produced similarly to oval SMP devices as described previously in the main methods section, with the following alteration. Tecoflex® EG-85A polyether urethane
Lubrizol, Wickliffe, OH) was compounded with barium sulphate (35 wt%) using a Haake-Minilab at 145°C and 70 rpm. A custom-fabricated aluminum mold was used to produce a device with 17 mm outer diameter and 2 mm cross-section ring with an attached rod, 5 mm long and 4 mm cross-section. Injection molding temperatures ranged from 120°C to 180°C and mold temperatures ranged from 12°C to 14°C during fabrication.

**Fabrication of T-shaped vaginal retainer devices**

5.5 mm diameter Tecoflex® EG-85A polyether urethane rod was extruded using a Haake-Minilab at 145°C and 70 rpm. The extruded rod was cut into 12 mm segments. Then 8 mm of the rod was bisected. Each side of the bisection were held apart in a T-shape with a mold and annealed at 65°C for 15 mins and cooled to RT.

**RESULTS AND DISCUSSION**

**Effect of compression time on release**

The release rate did not depend on compression time for pellet fabrication under the conditions tested: compression times of 20 s and 2 min (p = 0.6 comparing 10 day cumulative release; Fig. S1). For further studies, all FCPs had pellets compressed for 20 s with a force of 2 metric tons. We hypothesize that due to constraints with tooling used, we could not make a harder and stronger pellet that could result in a reduced release rate.
**Figure S1.** (a) *In vitro* release rate and (b) cumulative release of MP from FCPs comparing different compression times for pellet fabrication: 20 s and 2 min.

**Figure S2.** (a) *In vitro* release rate and (b) cumulative release of MP and rhodamine B dextran over 10 days. The line on the cumulative release graph (b) represents a power law curve fit of the data with the associated exponent and $R^2$ values. (c) Cumulative rhodamine B dextran release calculated from *in vitro* elution profile correlated to GF HPC release from residual content on days 10, 20 and 30 (Spearman correlation of $r = 0.90, p = 0.002$). FCPs were 1 wt% model compound in GF HPC with two 1.5 mm diameter orifices.

**Design of vaginal device retainer for in vivo studies**

To improve upon previous surgical implantation procedures utilized for vaginal devices, initial rabbit studies tested three different retainer designs, O, T and oval shaped elastomeric devices, for device retention in the cervicovaginal tract and adverse effects for 14 days (Fig. S3). The design requirements for the retainer devices include: (1) device insertion without surgery, (2) proper retention in the cervicovaginal tract without rotation, (3) easy incorporation of a representative segment of an IVR formulation, and (4) no observable mucosal irritation or inflammation. In the first study, we tested two different retainer device configurations, O’s and T’s ($n = 3$). Only one O-device was properly retained, the second was expelled and the third was retrieved from the urovaginal tract. No T-devices were expelled, however all devices
rotated 90° with two retrieved from the cervicovaginal tract and one from the urovaginal tract. (Fig. S3) Moreover, marked epithelial disruption was noted with T-devices (Fig. S4).

**Figure S3.** Summary of the retention and orientation of different designs of retainer devices during 14 day studies, including O, T and oval-shaped devices.

The second study evaluated a higher number of O-devices (n = 12) to further elucidate critical parameters in device retention. Since no T-devices were retained without rotation and tissue disruption was observed, the design was discontinued. Out of the 12 O-devices, six were located in the cervicovaginal tract, five were properly retained and one was found rotated 180°; two retrieved from the urovaginal tract with one rotated 180°; and four expelled (Fig. S3). Measurements of resected vaginal tracts and their correlation to device retention suggested increasing the retainer device width without altering the overall length may lead to less expulsion. Furthermore, the retainer devices were difficult to compress and push through a catheter. Therefore, we made two design modifications: (1) altered the shape from circular to ellipsoidal and (2) the retainer was fabricated from SMP instead of Tecoflex. The SMP utilized with a Tg of 35°C resulting in a malleable, elastomeric device at body temperature and a stiff
device at RT facilitating easy insertion with the vaginal catheter. Prior to insertion, retainer devices were warmed to 37°C, the major axis flattened to ease insertion through the catheter. Upon introduction in the vaginal tract, the SMP transitioned from room to body temperature, and relaxed into the native, ellipsoidal shape necessary for device retention. In study 3, we tested the oval-SMP retainer devices (n = 8). All devices were properly retained without rotation for 14 days (Fig. S3 and S5). No epithelial disruption or inflammation was noted (Fig. S4) leading to oval-SMP retainer devices to be utilized for further in vivo experiments described in the main text of the paper. Additional H&E micrographs from the 20 and 30-day time points evaluating the FCP-oval SMP retainer device are presented in Fig. S6.
Figure S4. Representative images of H&E stained vaginal sections from the different study groups: (A, B) naive, (C, D) T-device, (E, F) O-device and (G, H) oval-SMP device. Epithelial disruption noted for panel D.
Figure S5. Time dependent x-ray of barium sulphate loaded oval retainer devices attached to a drug delivery segment during a 14 day study.
Figure S6. Representative images of H&E stained vaginal sections from the 20 and 30 day endpoints evaluating the FCP-oval SMP retainer device.
CHAPTER 4

CONTROLLING THE HYDRATION RATE OF A HYDROPHILIC MATRIX IN THE CORE OF AN INTRAVAGINAL RING DETERMINES ANTIRETROVIRAL RELEASE

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4.1 Abstract

Intravaginal ring technology is generally limited to releasing low molecular weight species that can diffuse through the ring elastomer. To increase the diversity of drugs that can be delivered from intravaginal rings, we designed an IVR that contains a drug matrix encapsulated in the core of the IVR whereby the mechanism of drug release is uncoupled from the interaction of the drug with the ring elastomer. We call the device a flux controlled pump, and it is comprised of compressed pellets of a mixture of drug and hydroxypropyl cellulose within the hollow core of the ring. The pump orifice size and chemistry of the polymer pellets control the rate of hydration and diffusion of the drug-containing hydroxypropyl cellulose gel from the device. A mechanistic model describing the hydration and diffusion of the hydroxypropyl cellulose matrix is presented. Good agreement between the quantitative model predictions and the experimental studies of drug release was obtained. We achieved controlled delivery of multiple antiretrovirals ranging from μg/day to mg/day release rates by altering the orifice design, drug loading, and mass of pellets loaded in the device. This device could provide an adaptable platform for the vaginal drug delivery of many molecules.

4.2 Introduction

Over 2 million new HIV infections occur annually, predominately through sexual transmission (1), urging the development of new technologies to control the pandemic. Recently, several clinical trials have provided evidence that prophylactic oral and vaginal administration of antiretrovirals can prevent the
sexual transmission of HIV (2, 3). In particular, clinical studies evaluating a 1%
tenofovir vaginal gel resulted in modest to no reduction in HIV incidence (4, 5).
The clinical failures of the tenofovir gel likely stem from low user adherence and
application-dependent drug pharmacokinetics of the gel formulation (5, 6) leading
to an insufficient drug concentration in the local tissue where the initial
transmission events occur (7, 8). This drives the developmental efforts towards
longer duration delivery systems like intravaginal rings (IVRs) that may provide
higher adherence than gels (9-11) and improved drug pharmacokinetics as a
result of the sustained drug delivery (8, 12). Despite more than four decades of
research into IVR technology, the chemical properties and delivery requirements
of the drug typically governs the elastomer selection, previously limiting this
product for delivery of hydrophobic molecules (8, 13, 14). However, the need for
new HIV prevention technologies resulted in a resurgence of IVR development in
the last decade. This has led to new ring designs and utilizing new polymers to
enable the delivery of hydrophilic and macro-molecules at mg/d delivery rates
that were previously unachievable (12, 15-18). Of particular interest to this work
are pod/insert vaginal rings where small drug delivery systems are held within the
ring body, separating the drug delivery requirements from the mechanical
properties (15, 18-20). Nevertheless, even with these new materials and designs,
to achieve extended duration release, the drug molecule must still diffuse through
either the ring elastomer or the polymer of a drug delivery device embedded in
the ring body leading to the requirement to match the polymer to the drug. New
IVR designs where drug release does not depend on the drug solubility and
diffusivity in the polymers of the IVR are needed and would enable the delivery of drugs irrespective of the chemical properties of the drug.

Osmotic pumps can achieve controlled and sustained release of drugs with a range of chemical properties and molecular weights, with the drug potentially acting as the osmotic agent or simply releasing as a suspension of particles in a solution or semisolid gel (21, 22). However, osmotic pump technology has been under-utilized for topical drug delivery. We designed the first intravaginal osmotic pump tablet for multiday antiretroviral delivery and achieved controlled release over 10 days in sheep (17). We then modified the osmotic pump design by substituting the function of the semipermeable membrane with orifices in a rigid non-water permeable casing. The orifices and swelling properties of the polymer pellet control both water entry into and release of a drug containing semisolid gel from the orifices of this flux controlled pump (FCP) (23). The design and function of the FCP is fundamentally different from an osmotic pump. Fluid entry occurs through the semipermeable membrane of an osmotic pump, whereas fluid entry only occurs through the orifices of a FCP, reducing the hydration kinetics and thereby resulting in longer duration controlled release. However, in both osmotic pump tablet and FCP designs, we exploited the expansion of a high molecular weight polymer caused by hydration to deliver a drug-loaded gel through an orifice (17, 23).

Herein we present the design and evaluation of a FCP integrated into an IVR with pseudo zero-order release kinetics of many leading HIV prevention antiretrovirals. We demonstrated a linear relationship between the release rate of
the hydrophobic drug IQP-0528 from the device to drug loading and orifice area \textit{in vitro}. Moreover, depending on the molecule delivered, we observed that the hydration kinetics and swelling properties of the polymer pellet control drug release. This suggests that for hydrophobic and macro-molecules, the drug solubility and diffusivity in the swollen polymer matrix has a negligible influence to the overall drug release, whereas for more hydrophilic molecules, the drug diffusion through the polymer matrix contributes to drug release. This differentiates the FCP from other IVR platforms where drug release depends entirely on drug diffusion and solubility in the ring elastomer (8, 13, 14). In addition, to aid in the design of this system and to understand the mechanism of drug release, we developed a finite element model that reproduces the drug release behavior for different orifice geometries, and drug type and loading. This model results in a fast and accurate tool to design this FCP device and supports the hypothesis that polymer hydration controls drug release from these systems.

4.3 Materials and Methods

4.3.1 Fabrication of flux controlled pumps

IQP-0528 (ImQuest Biosciences, Frederick, MD), dapivirine and maraviroc (DPV and MVC respectively; International Partnership for Microbicides, Silver Spring, MA), tenofovir and tenofovir disoproxil fumarate (TFV and TDF respectively; Gilead Sciences, Foster City, CA), and 10 kDa rhodamine B dextran (Sigma Aldrich, St. Louis, MO) were each geometrically mixed with hydroxypropyl cellulose (HPC; GF, MW = 370 kDa; Klucel Pharm, Hercules,
Wilmington, DE) to achieve a range of loadings from 0.9-15 wt% IQP-0528 in HPC, 10 wt% DPV, MVC, TFV, and TDF in HPC, and 0.9 wt% rhodamine B dextran in HPC. Pellets, 4 mm in diameter and 51 ± 1 mg, were formed by compression as previously described (23). Tecoflex aliphatic polyether thermoplastic polyurethane (EG-65D; Lubrizol Advanced Materials, Wickliffe, OH) tubing was fabricated by hot-melt extrusion to create final tubing dimensions of 0.7 mm wall thickness and 5.5 mm diameter (12). The tubing was cut to a length of 36, 40, and 44 ± 0.5 mm for FCP with 2, 3, and 4 pellets, respectively. Tubing lengths were chosen so that 2, 3, and 4, 50 mg pellets fit snugly inside the device. One end was sealed by inductive tip-forming welding (PlasticWeld Systems, Inc., Newfane, NY). The orifices were manually drilled and the diameter was measured using a stereomicroscope and compared to a scale. The standard deviation of the orifice diameter was 3-4% of the diameter (at least 3 orifice with 3-4 measurements each). Next 2, 3, or 4 compressed pellets were inserted into the open end of the device followed by sealing the second end by inductive tip-forming welding similarly to the first weld.

4.3.2 In vitro drug release testing

In vitro drug release was measured from individual FCPs in 20 mL of 25 mM acetate buffer pH 4.2 at 37°C and 80 rpm shaking (N = 3). The release media was replaced daily. To measure the release rates of IQP-0528 and DPV, the complete release media was collected on days 1, 2, 3, 5, 7, 10, 15, 20, 25, and 30 and diluted with methanol to dissolve the released drug. To measure the
release rates of TFV, TDF, MVC, and rhodamine B dextran, an aliquot of the release media was collected for analysis on the same schedule and the remainder discarded. Cumulative release was estimated by integration of the release rate profile using a trapezoidal approximation. Average release rates were calculated as the cumulative release divided by the elapsed time. For FCPs with four to one, 1.5 mm orifices, the mass of the device was measured on the same days the media was collected.

To measure the decay of the pseudo zero-order portion of the release rate profile, dimensional analysis was performed. The IQP-0528 release was plotted with both variables normalized to the maxima of the experiment. Then a linear fit from the maximum release rate to the end of the release curve was performed and the dimensionless slopes were compared. The day 30 point was excluded for the FCPs containing two, 50 mg pellets with two, 1.5 mm orifices, and four, 50 mg pellets with four, 1.5 mm orifices since the release on that day was drastically different from the preceding days. All linear and power law curve fitting were performed using OriginPro8 (OriginLab Corporation, Northampton, MA).

4.3.3 Drug extraction from pellets and FCPs

For determination of drug loading in HPC pellets, pellets were placed in a volumetric flask and dissolved overnight in methanol or 1:1 water:methanol mixture for TFV. Upon completion of in vitro release studies, FCPs were placed in a 50 mL centrifuge tube with methanol or 1:1 water:methanol mixture for TFV and shaken overnight. The solution was transferred to a volumetric flask and the
FCP casing was rinsed at least 5 times and drug content was determined by UV-HPLC. To determine the amount of pellet, i.e. the sum of drug and HPC, remaining, a portion of the extraction solution was dried to constant mass. To confirm drug recovery, known amounts of drug and HPC were dissolved in parallel with a similar amount of Tecoflex EG-65D present in the case of FCP extractions.

4.3.4 Measure drug diffusivity in HPC solutions

The diffusivity of TFV, TDF, and MVC were measured as a function of HPC concentration using Franz cells (Permegear, Hellertown, PA). The solutions were made with 0.1 wt% drug and 1.2, 2, 3, 5, and 10 wt% HPC in 25 mM acetate buffer pH 4.2. The concentration of each drug in the HPC solutions was determined by dissolving 100 μL of gel in a 10 mL volumetric flask in methanol for TDF and MVC or methanol:water for TFV. Durapore membrane filters (hydrophilic PVDF, 25 mm diameter, 0.45 μm pore size; Millipore, Billerica, MA) were fitted to a Franz cells with a 20 mm orifice diameter and receptor compartment with 15 mL of 25 mM acetate buffer pH 4.2. Then 1.5 mL of each drug-HPC solution at 37°C was placed on the donor compartment and covered with parafilm to minimize evaporation. Samples of 0.5 mL were taken from the receiver compartment with an analytical syringe at predetermined time points; 10, 20, 30, 45, 60, 75, and 90 min; and then replaced with fresh buffer. The drug concentration at each time point was measured by UV-HPLC (methods below). The cumulative amount of drug that diffused from the donor compartment to the
receptor \((M_t)\) with respect to the square root of time was plotted and the slope of
the line was used to calculate the diffusion coefficient, \(D\), according to the
diffusion equation (Eq. 4.1) solved for semi-infinite geometry and a completely
dissolved solute (24) where \(C_0\) is the initial drug concentration in the HPC
solution, and \(A\) is the exposed area. All data are presented as the mean ± SD.

\[
M_t = 2C_0A\sqrt{Dt/\pi}
\]  
(Eq. 4.1)

4.3.5 Drug content analysis

IQP-0528 (25), TDF (26), and TFV (12) concentration for extraction and \textit{in
vitro} release studies was measured by UV-HPLC methods described previously.
The same method was used for TDF and MVC as described previously with the
addition of monitoring 197 ± 4 nm to quantify MVC (26). DPV was quantified by
UV-HPLC using an Agilent 1260 Infinity series system with an Eclipse XDB-C18
column (4.6x150 mm, 5 ßm; Agilent, Santa Clara, CA) at 25°C at a flow rate of
1.5 mL/min Data were collected at 280 ± 8 nm. A gradient of 0.1% TFA in
acetonitrile (ACN) - 0.1% TFA in water (0-1 min: 30% 0.1% TFA in ACN, 1-9 min:
30-95% 0.1% TFA in ACN, 9-10 min: 95-30% 0.1% TFA in ACN) was used for
elution. Rhodamine B dextran concentration was determined by reading the
fluorescence using a Synergy2 plate reader (BioTek, Winooski, VT) at 540 ± 20
nm excitation and 620 ± 40 nm emission wavelengths.
4.3.6 Model of drug release

The release kinetics of FCPs under in vitro conditions was modeled using a transport model based on a generalized diffusion equation. For the model calculations, we implemented a finite element analysis in a cylindrical geometry following the dimensions described above (Fig. 4.1c). The mass transport is modeled with a diffusion equation of the form (Eq. 4.2):

$$\frac{\partial C_k}{\partial t} = \frac{\partial}{\partial x} \left( D_k \frac{\partial C_k}{\partial x} \right) + \frac{\partial}{\partial y} \left( D_k \frac{\partial C_k}{\partial y} \right) + \frac{\partial}{\partial z} \left( D_k \frac{\partial C_k}{\partial z} \right)$$  (Eq. 4.2)

where $k$ represents each of the diffusing components of the system, and $C_k$ and $D_k$ are their concentration and diffusion coefficients, respectively.

The model assumes that the release kinetics of the FCP system is mainly governed by the hydration and diffusion of the polymeric matrix (HPC). Thus, the two components that we consider are 1-water and 2-HPC. As drug transport is not directly considered in this model, drug release profiles were obtained by applying the weight fraction of drug in the HPC released.

We assumed that the release dynamics of the HPC depend on the extent of hydration, thus, more hydrated regions diffuse faster than less hydrated ones. To describe the water concentration dependence of the diffusion coefficients, we used a Fujita dependence (27), which is based on a free volume approach. This type of model has been successfully used to describe drug diffusion in hydroxypropyl methylcellulose (HPMC) (28, 29), polymer diffusion in polyethylene oxide (PEO) (30), and other similar systems (31-33). In this approach, diffusion coefficients are dependent on the water concentration according to (Eq. 4.3):
\[ D_k = D_{k,eq} \exp \left( -\beta_k \left( 1 - \frac{c_{water}}{c_{water, eq}} \right) \right) \]  
(Eq. 4.3)

where \( C_k \) are the concentrations of each species \((k)\), \( D_k \) are diffusion coefficients, and \( \beta_k \) is a constant that characterizes the water concentration dependence of the diffusion coefficient. \( D_{k, eq} \) represent the diffusion coefficients of each species at the maximum water concentration \( c_{water, eq} \) (in equilibrium with the swollen matrix).

The solution of the diffusion equation in the geometry of the device was obtained using the COMSOL Multiphysics package (COMSOL Inc., Burlington, MA). We used the following assumptions and boundary conditions:

i. Diffusion is isotropic and there is no convective flow.

ii. There is no water in the device at time equal to zero (dry matrix condition).

iii. The concentration of water outside the device is constant and equal to \( c_{water, eq} \).

iv. The concentration of HPC and drug outside the device is equal to zero (perfect sink conditions).

v. The water and drug transport through the casing is negligible.

4.3.7 Comparison between model and experiments

For the model to help in the design of FCPs, we fitted the parameters of Eq. 4.3 to experimental data for a device with three orifices of 1.5 mm in diameter and four, 50 mg pellets containing 10 wt% IQP-0528 in HPC. This allowed us to test the flexibility of the model under new experimental conditions. The fitted parameters are: \( D_{HPC, eq} = 1.02 \times 10^{-10} \text{ m}^2/\text{s}, \beta_{HPC} = 2.1 \) and \( \beta_{water} = 0.5 \) and \( D_{water} \).
\( e_0 = 5.6 \times 10^{-10} \text{ m}^2/\text{s} \). All other device configurations are predicted using these parameters.

To test the performance of the model, we compared the values of cumulative release at different experimental conditions. The comparison was made by the coefficient of determination \( (R^2) \) and by the root mean square deviation (RMSD) between the model and the experimental data. Additionally, we consider that the experimental average release rate and the model release rate are not a good quantity to be compared. This is due to the fact that the experimental release rate is a measure of amount released per day while the model release rate is the derivative of the cumulative release with a higher time resolution. Therefore, we included the values of release rate for the model but the comparison of the performance of the model was done on the cumulative release only.

### 4.4 Results

#### 4.4.1 Effect of orifice size and number on the IQP-0528 release rate

We measured the in vitro drug release kinetics as a function of orifice size on FCPs with four, 50 mg pellets of 10 wt% IQP-0528 in HPC with different orifice configurations, one, two, three, and four orifices of 1.5 mm in diameter (Fig. 4.2a and b) and three orifices of 2.3 and 2.7 mm in diameter (Fig. 4.2d and e). As expected, a larger orifice area generally resulted in a higher release rate due to the higher rate of water entry. However, after \( \sim 75\% \) cumulative release was attained, the drug release rate dropped dramatically. In the first 10 days, the
average release rates for FCPs with four, three, two, and one, 1.5 mm orifices were 700 ± 60, 570 ± 14, 410 ± 33, and 170 ± 32 µg/d, respectively. This corresponded to peak drug release rates of 900 ± 38, 670 ± 29, 480 ± 110, and 224 ± 40 µg/d for the devices containing four to one orifices, respectively. However, the drug release rates from the FCPs with four and three orifices reduced to the level of the two orifice FCPs by day 25. This resulted in 30-day average drug release rates of 610 ± 12, 490 ± 12, 350 ± 15, and 190 ± 19 µg/d, and calculated cumulative drug releases of 93 ± 1.8, 75 ± 1.8, 54 ± 2.3, and 30 ± 2.9% for four, three, two, and one, 1.5 mm orifice FCPs, respectively (Fig. 4.2b, p < 0.001, single factor ANOVA). The 30-day average release rates and orifice areas presented a linear dependence for FCPs with one to four, 1.5 mm orifices (Fig. 4.2f, Spearman correlation of r = 0.97 and p = 0.0002). The 30-day IQP-0528 cumulative release calculated from the release rate was comparable to values measured by residual drug extraction for FCPs with one to four, 1.5 mm orifices (p = 0.33, 0.65, 0.042, and 0.12 for one to four, 1.5 mm orifice FCPs, respectively; paired t-test for means). This confirms the discrete integration method utilized for calculating cumulative release profiles.

Comparing the IQP-0528 release rate from FCPs containing three orifices of 2.7, 2.3, and 1.5 mm in diameter (Fig. 4.2d and e), we found the peak release rates were 1.9 ± 0.055, 1.3 ± 0.075, and 0.70 ± 0.014 mg/d, respectively. This corresponded to 10-day average release rates of 1.4 ± 0.16, 1.1 ± 0.027, and 0.57 ± 0.014 mg/d for three, 2.7, 2.3, and 1.5 mm orifice FCPs, respectively. The drug release rate for FCPs with three, 2.7 and three, 2.3 mm orifices dropped to
nearly 0 on day 20 and day 25, respectively, and remained low for the study duration. The drastic reduction of the release rate on days 20 and 25 corresponded to calculated cumulative release of 90 ± 8.0 on day 20 for the three, 2.7 mm orifice FCP and 99 ± 6.8% on day 25 for the three, 2.3 mm orifice FCP (Fig. 4.2e). This confirms that the severe decrease of the release rate resulted from an insufficient amount of drug remaining within the device.

The release rate displayed a biphasic behavior with a few day lag time to reach a maximum release followed by a pseudo-steady state that decayed for the remainder of the 30 days (Fig. 4.2a). For FCPs with four to one, 1.5 mm orifices, the 10-day average release rate was higher than the 30-day average release rate for each design except the one, 1.5 mm orifice devices. This difference resulted from a more extreme maximum release rate for the four, three, and two, 1.5 mm orifice FCPs and the differences in the decay of the release rate after the maximum release rate was achieved. To enable comparisons of the release rate decay in the later portion of the curve between devices with different release rates, we performed dimensional analysis by normalizing the IQP-0528 release rate and time to the maximum values. Then we performed a linear fit of the portion of the plot after the initial lag from the peak release rate and beyond. A steeper slope, i.e. a slope with a reduced negative value, represents increased release rate decay. The slopes of such fits were -1.0, -0.93, -0.57, and -0.36 for the four, three, two, and one, 1.5 mm orifice FCPs, respectively \((R^2 = 0.961, 0.992, 0.998, \text{ and } 0.549)\). For FCPs with three, 2.3 and 2.7 mm orifices, the decay slopes were -1.4 and -1.9, respectively \((R^2 = 0.992, \text{ and } 0.998)\).
and 0.875). The lower $R^2$ value for the one, 1.5 mm orifice FCPs ($R^2 = 0.549$) suggests that orifice configuration did not exhibit a linear decay. The 30-day time point was excluded from the fitting for the four, 1.5 mm orifice FCPs due to the anomalous behavior. Similarly, days 25 and 30 for three, 2.3 mm, and days 15-30 for three, 2.7 mm FCPs were excluded since the release dropped to negligible levels. Generally, a larger orifice area corresponded to a greater decay of the release rate during the later portion of the release profile.

To understand hydration kinetics, we measured the mass of the FCPs with one to four, 1.5 mm orifices on the same days media was collected for drug concentration analysis. We estimated the cumulative media uptake in the FCPs (Fig. 4.2c) by adding the cumulative pellet release, assuming the HPC and IQP-0528 were released concurrently, to the increase in FCP mass. An increased orifice area, changed here by increasing the number of 1.5 mm orifices, correlated to increased hydration kinetics. The only exception was on day 30 for the four, 1.5 mm orifice devices where we hypothesize that the HPC polymer remaining in the FCP was sufficiently small to lead to a dilute solution inside the FCP that readily diffused out of the device. Next the cumulative media uptake curves were fitted to a power law equation ($y = a^t^b$ where $y$ is the cumulative media uptake and $t$ is time). The exponent $b$, which determines the shape of the curve, was similar for the different devices containing one to four, 1.5 mm orifices ($p = 0.11$, single factor ANOVA). However, the hydration-scaling factor, $a$, that determines the magnitude of the curve exhibited a linear relationship to the total orifice area (Fig. 4.2f, Spearman correlation of $r = 0.91$ and $p < 0.0001$). This
provides evidence that the dependence on the release with respect to the orifice area correlates with the hydration kinetics.

4.4.2 Effect of orifice size and number in the model

The model parameters were optimized for the FCP with three orifices of 1.5 mm in diameter and four, 50 mg pellets of 10 wt% of IQP-0528, as mentioned above. Using these parameters, we calculated the effect of the orifice number shown in Fig. 4.2a and b (solid lines). The model predictions are in good agreement with the experimental data. The comparison of the cumulative release between the model and the experiments (Fig. 4.2b) exhibited an $R^2$ of 0.983, 0.998, 0.990, and 0.975 for four, three, two, and one orifice, respectively. The RMSD of the model with respect to experiments was ±5.2, ±1.3, ±2.0, and ±1.7% for four, three, two, and one orifice, respectively. When we investigated the effect of the orifice size on the behavior of the model (Fig. 4.2d and e, solid lines) we observed that the model was again in good agreement with the experimental data. The comparison of the cumulative release between the model and the experiments (Fig. 4.2e) had an $R^2$ of 0.998, 0.992, and 0.957 for the devices with three orifices of 1.5, 2.3, and 2.7 mm, respectively. The RMSD of the model was ±1.3, ±4.5, and ±6.9% for 1.5, 2.3, and 2.7 mm, respectively. As you can observe, there are slightly higher deviations when we modify the orifice diameter compared to altering the orifice number (Fig. 4.2a and d). Nevertheless, the $R^2$ shows that the deviations are still comparable with the deviations from the experimental data.
4.4.3 Effect of the drug loading on the drug release rate

We observed a linear increase in drug release with the increase of IQP-0528 loading in the pellets (Fig. 4.3a, b, and c). This was readily apparent when the cumulative drug release was normalized to the IQP-0528 loading and presented as a percent (Fig. 4.3b), and from the linear correlation of the 30-day average IQP-0528 release rate and IQP-0528 loading (Fig. 4.3c, Spearman correlation of $r = 0.98$ and $p < 0.0001$). The 30-day average release rates for FCPs with 0.9 and 15 wt% IQP-0528 were 40 and 700 μg/d, respectively. The 30-day cumulative release was 68 ± 2.0, 77 ± 3.3, 74 ± 3.6, 81 ± 0.93, and 72 ± 1.9% for FCPs containing 0.9-15 wt% IQP-0528 in HPC pellets (Fig. 4.3b).

Additionally, upon dimensional analysis, comparable release rate decay beyond day 5 was observed for all IQP-0528 loadings tested. Furthermore, for FCPs with 10 wt% IQP-0528 in HPC and three, 1.5 mm orifices, the cumulative percent release of IQP-0528 correlated in a linear fashion to the cumulative percent release of the pellet, i.e. the sum of IQP-0528 and HPC measured from residual content on days 10, 20, and 30 (Fig. 4.3c, Spearman correlation of $r = 0.95$ and $p = 0.004$). Taken all together, the data suggest that the release rate of insoluble IQP-0528 was controlled by the polymer hydration and diffusion with negligible contributions from drug diffusion through the hydrated HPC semisolid.

We then evaluated the release kinetics of other antiretrovirals, DPV, TDF, TFV, and MVC in addition to the model macromolecule rhodamine B dextran loaded into the pump with three, 1.5 mm orifices (Fig. 4.3e). To compare the release of the different compounds, the release was normalized to the total drug
load. The average release calculated over 30 days was $2.5 \pm 0.06$, $2.7 \pm 0.08$, $2.6 \pm 0.04$, $3.0 \pm 0.20$, $3.1 \pm 0.37$, and $3.5 \pm 0.01\%$/d for IQP-0528, DPV, rhodamine B dextran, TDF, TFV, and MVC, respectively. The average release rate and cumulative release of TFV ($p = 0.06$), TDF ($p = 0.01$), and MVC ($p = 0.001$) were all higher than that of IQP-0528; however, only MVC and TDF were significantly higher than IQP-0528 (paired t-test for means). MVC, TFV, and TDF are ~100,000x more water soluble than IQP-0528 with solubilities in 25 mM acetate buffer pH 4.2 of 11 mg/mL for MVC and 7 mg/mL for TDF and TFV compared to 0.14 µg/mL for IQP-0528, suggesting the hydrophilic drugs can diffuse independently of the HPC polymer. The antiretrovirals with a higher aqueous solubility were observed to solubilize within the device during in vitro release testing with dissolution initial observed near the orifice and then spread along the length of the device. We measured the diffusivity of the three more hydrophilic antiretrovirals as a function of the HPC concentration. We observed a comparable trend of an exponential decrease of the diffusivity of MVC, TDF, and TFV in HPC solutions as the HPC content was increased (Fig. 4.3d), suggesting differences in diffusivity do not explain the different drug release rates observed.

4.4.4 Effect of the drug loading in the model

We show in Fig. 4.3a and b the comparison between experimental observations (symbols) and the model predictions (solid lines) for different initial loadings of IQP-0528 in the HPC pellets. The model was in good agreement with the experimental data for different loadings of IQP-0528 (Fig. 4.3a and b). The $R^2$
of the model for the cumulative release was 0.998, 0.996, 0.995, 0.998, and 0.997 for 0.9, 2, 4, 10, and 15 wt% IQP-0528 in HPC, respectively. The RMSD of the model for the cumulative release was ±3.5, ±2.2, ±3.7, ±1.3, and ±2.0% for 0.9-15 wt%, respectively. These results are in line with our postulate that the polymer dissolution and the constraint imposed by the orifice control the mechanism of drug release for this device. This is supported by the experimental results (Fig. 4.3c) and is evidenced in our model by the good predictive power, as compared to the experimental observations, when we conserve the same geometry. Furthermore, Fig. 4.3e shows that the drug release profile displays an almost identical behavior for DPV as with IQP-0528, despite the difference in the chemistry between the two drugs. This suggests that for small hydrophobic drugs, the variables that control release are the geometry and hydration dynamics of the polymeric matrix. The $R^2$ between model and DPV was 0.998 with an RMSD of ±3.7% for the cumulative release. In the case of rhodamine B dextran, the hydrophilic character of the molecule probably does not play a role since the high molecular weight of the molecule limits the diffusivity in the HPC matrix. The $R^2$ between model and rhodamine B dextran was 0.997 with an RMSD of ±1.3 % for the cumulative release. However, we see deviations between the model predictions and the experimental results for TDF, TFV, and MVC, suggesting that for hydrophilic, small molecule drugs, the drug diffusivity in the swollen HPC matrix modify the release mechanism adding an extra contribution. The $R^2$ between model and TDF, TFV, and MVC was 0.984, 0.964, and 0.992, respectively with an RMSD of ±12.0, ±7.9, and ±21.3% for the
cumulative release. Not surprisingly, the hydrophilic drugs were released faster than IQP-0528, supporting an additional diffusive contribution to release of the soluble molecules.

4.4.5 Effect of number of pellets on the drug release rate

We measured the release kinetics of IQP-0528 from FCPs with two, 1.5 mm orifices and two, three, and four, 50 mg pellets of 10 wt% IQP-0528 in HPC and observed an increased rate of decay of the release rate for devices loaded with a smaller number of polymer-drug pellets (Fig. 4.4). We reduced the FCP length to correspond to the reduced length of less pellets. The 30-day cumulative release from the FCPs with two, 1.5 mm orifices and two, three, and four, 50 mg pellets were 9.8 ± 0.35, 9.9 ± 0.07, and 11 ± 0.35 mg corresponding to 110 ± 4.0, 73 ± 0.49, and 60 ± 1.9%, respectively (Fig. 4.4b). By performing dimensional analysis of the IQP-0528 release rate, the FCPs with two pellets exhibited an increased decay in the release rate compared to the three pellet FCPs that in turn decayed more readily than four pellet FCPs. This can be seen particularly on days 15, 20, and 25 as the differences in the drug release rates between the three configurations increased. The slopes of the normalized drug release and time plots for FCPs containing two, three, and four pellets were -0.90, -0.76, and -0.50, respectively ($R^2 \geq 0.98$). On day 30, the FCPs with two pellets and two, 1.5 mm orifices demonstrated a burst similar to FCPs with four pellets and four, 1.5 mm orifices (Fig. 4.2a and 4.4a). This was likely caused by the low concentration and viscosity of the polymer solution remaining within the device and easily
diffusing out of the FCP from shaking during *in vitro* release testing. Therefore, we did not include day 30 for this device configuration for the calculation of the release rate decay.

4.4.6 Effect of number of pellets in the model

Reducing the number of pellets within the device was modeled by reducing the length of the cylindrical geometry that represents our system. The results are presented in Fig. 4.4 (solid lines). Since the experimental data were measured in a FCP with an orifice configuration (two, 1.5 mm orifices) that differed from the design used to fit the model parameters (three, 1.5 mm orifices), some differences appear between the model predictions and the experimental results. Nevertheless, the model predictions are in good agreement with the experimental data. This can be explained in terms of our postulate that the release of hydrophobic molecules like IQP-0528 is controlled by the release of the HPC polymer and the constraint imposed by the orifices. This implies that the length of the device is not one of the controlling parameters for the release and the differences between the experimental data and the model are not significant. The $R^2$ of the model for the cumulative release was 0.990, 0.995, and 0.988 for four, three, and two pellets, respectively. The RMSD of the model for the cumulative release of four, three, and two pellets was ±2.0, ±3.4, and ±4.5%, respectively, for the cumulative release.
4.5 Discussion

We designed a device for the sustained and controlled drug delivery to the vaginal mucosa. This design presents a number of advantages compared to previous IVR delivery technologies, particularly for the delivery of hydrophobic drugs. First the mechanism of release from this IVR can decouple drug release from the drug solubility and diffusivity in the ring elastomer and the polymer of the insert, which can lead to high and controlled drug release rates irrespective of the solubility of drug molecule in the elastomer. The release rate can easily be modulated over a significant range of low μg/d to mg/d quantities by altering the orifice size and number, and drug loading in the swellable polymer pellets. Additionally, the polymer chemistry and molecular weight of the hydrophilic polymer within the device can be altered to achieve vastly different release rates and durations (23). As a potential approach to improve user demand and adherence, there is a compelling interest to develop multipurpose technologies for the prevention of HIV, unwanted pregnancy, and/or other sexually transmitted infections (34). Such multipurpose technologies will likely require segmented IVR incorporating segments of dissimilar materials and designs to tailor the drug release rate for drugs with disparate physical properties and delivery requirements (12, 15, 16, 35, 36). We designed the FCP to be incorporated into a ring as a segment occupying less than a quarter of the total ring with the remainder of the ring containing another drug delivery segment (Fig. 4.1a). Additionally, since polymer hydration rate is the predominate factor controlling drug release, one can deliver one or multiple drugs at differing fluxes by
changing the drug loading in the hydrophilic swelling polymer pellet. This could be utilized to delivery multiple antiretrovirals for improved potency and a higher barrier to development of drug-resistant virus strains (37, 38).

We present a model based on a finite element calculation of the diffusion of the water into and drug containing semisolid gel from the device that supports the idea that the swelling properties of the polymer and the orifice area are the controlling factors for drug release in this FCP design. When the FCP contacts *in vitro* release media, a steep water concentration gradient exists at the interface of the polymer pellet at the orifice resulting in water entry into the polymer contained in the device (Fig. 4.1b). As the polymer hydrates, polymer chain relaxation occurs and the semisolid gel containing the drug diffuses from the device. Following this idea, our model uses a Fujita free-volume theory (27); as the water concentration increases and the polymer concentration decreases inside the device, the free volume available for diffusion increases, resulting in an increase in the diffusivity of the water and polymer. The model performed remarkably well for different configurations and offers a fast and efficient tool to aid in the design of new FCP geometries and configurations.

We determined that the total orifice area (varied herein by changing both orifice diameter and number) (Fig. 4.2) as well as the drug loading in the HPC pellets (Fig. 4.3) are important design parameters controlling the IQP-0528 release rate and duration. We observed a linear dependence between orifice area and average drug release rate (Fig. 4.2f), and IQP-0528 loading and average drug release rate (Fig. 4.3c). The good agreement of the model with the
experimental data when we varied IQP-0528 loading in the HPC from 0.9 to 15 wt% (Fig. 4.3a and b) and the number of 1.5 mm orifices from one to four (Fig. 4.2a and b) implies that the model mechanistically captures the hydration and diffusion of the polymer with these configurations. However, we observed increased deviations (RMSD) between the model and experimental results for the largest orifice areas tested compared to altering the IQP-0528 loading in the HPC or the number of 1.5 mm orifices (Fig. 4.2 and 4.3). This can be interpreted as a missing term due to the transport resistance imposed by the orifice that is implicitly taken in account into the parameters $D_{\text{HPC}}$ and $\beta_{\text{HPC}}$. Re-fitting of these parameters to a new geometry can minimize the error in the predictions from geometry to geometry. Nevertheless, providing the design critical parameters of the device (type of drug, orifice size, and number), the model represents a robust, fast, and accurate way to predict the drug release performance of these FCPs, with a deviation in the prediction lower than 7% and $R^2$ higher than 0.9 for all of the configurations tested with IQP-0528.

The daily release rate of IQP-0528 appeared biphasic, with a lag time in the first few days to reach a maximum followed by near constant drug release that decayed with time (Fig. 4.2a and 4.3a). We quantified the release rate decay by performing a linear fit of the decay portion of the normalized release profile. Generally, larger orifice areas that corresponded to higher release rates were also associated with increased release rate decay (Fig. 4.2a and d). From monitoring the cumulative uptake of release media into the FCP, we found that the increased drug release rate associated with a larger orifice area
corresponded to an increased hydration rate (Fig. 4.2c). Interestingly, we observed a linear dependence the hydration-scaling factor and orifice area (Fig. 4.2f). These observations together with the correlation of the cumulative release of IQP-0528 and the pellet, i.e. IQP-0528 and HPC (Fig. 4.3c), support the hypotheses that the release of IQP-0528 was controlled by the swelling properties of the polymer and the orifice area, and not the drug diffusivity in the swollen polymer pellet.

Additionally, we tested a number of more water-soluble antiretrovirals in this system to further understand the mechanism of drug release, specifically to increase the drug solubility and diffusivity in the hydrated HPC matrix and contribute to drug release from the FCP (Fig. 4.3e). The experimental release data of MVC, TDF, and TFV were higher than both the measured and model predicted IQP-0528 release. This likely occurred due to an additional mechanism of drug release from the drug diffusing through the HPC matrix. This can be explained by the much higher aqueous solubility of MVC, TDF, and TFV compared to IQP-0528. Furthermore, the diffusivities of MVC, TDF, and TFV as a function of HPC concentration were comparable (Fig. 4.3d). To accurately predict the drug release for MVC, TDF, and TFV using the model, we need to add an additional component of the drug dissolution and diffusion in the hydrated polymer matrix. The model was in good agreement with the release of the high molecular weight compound rhodamine B dextran, suggesting that despite the hydrophilic nature of the molecule, the diffusivity in the swollen HPC matrix was sufficiently hindered to not contribute to the overall release. The model only takes
into account the hydration and diffusion of the HPC polymer and therefore accurately predicts the drug release when the drug diffusion through the swollen polymer network does not contribute to the overall release (Fig. 4.3e).

We observed a relationship between the IQP-0528 release rate and the total polymer and drug loading (Fig. 4.4). Devices with a smaller drug and polymer load exhibited a slight increase in the decay rate of the release rate from day 10 and beyond, and the model accurately reproduced this change in the drug release rate due to the change in the number of pellets (Fig. 4.4). Specifically, this signifies that FCPs with two pellets did not contain sufficient water-swellable polymer inside the device to sustain the same release rate as the FCPs with four pellets. A similar behavior existed with FCPs with the two largest orifice areas tested: three, 2.3 or 2.7 mm orifices. After ~75% of the drug was released, the drug release rate drastically reduced (Fig. 4.2b). Together, these observations support our initial hypothesis that release was controlled by the polymer hydration and diffusion from the device. After a certain cumulative release was achieved, the polymer concentration within the device was insufficient to drive the diffusive release of the drug-containing semisolid gel out of the FCP orifice.

4.6 Conclusions

We describe the design of an extended duration, vaginal drug delivery system where the drug release is predominately controlled by the hydration, swelling, and dissolution of a hydrophilic matrix contained within the IVR. This is in contrast to other IVR designs where drug release occurs by drug diffusing
through the IVR elastomer. We argue because of the unique mechanism of drug release, this type of system is capable of high, mg/d drug release rates of both hydrophobic small molecules and macromolecules, whereas previous IVRs were incapable of achieving this. Finally we also provide a model to aid in the design of future FCP configurations, providing accurate information of the release dynamics with an easy implementation.

4.7 Acknowledgements

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Figure 4.1. Photograph and schematics of the flux controlled pump-IVR. (a) The photograph depicts an FCP (top), and an FCP integrated into an IVR (bottom). The FCP contains compressed pellets of drug and hydrophilic polymer within a sealed polymer tube with orifices. (b) Schematic of drug (orange dots) release from an FCP with water entry through the orifices causing polymer hydration and expansion resulting in release of a drug-loaded gel. (c) Scheme of the geometry implemented in the finite element calculation (three orifices of 1.5 mm diameter).
Figure 4.2. *In vitro* release of IQP-0528 comparing different orifice configurations. (a and d) IQP-0528 release rate and (b and e) cumulative release from FCPs with four, three, two, and one, 1.5 mm diameter orifices and three, 2.7, 2.3, and 1.5 mm diameter orifices containing four, 50 mg pellets of 10 wt% IQP-0528 in HPC. Panels a, b, d, and e compare the experimental (symbols) and model (solid lines) results. (c) Cumulative water uptake as measured by device mass increase and calculated cumulative pellet release. (f) 30-day average release rates and the hydration-scaling factor correlated linearly with orifice area for FCPs with four, three, two, and one, 1.5 mm orifices (Spearman correlation of $r = 0.97$ and $p = 0.0002$, for the 30-day average release rate, and $r = 0.91$ and $p < 0.0001$ for the hydration-scaling factor). The hydration-scaling factor was from fitting the cumulative water uptake of each device to a power law equation.
Figure 4.3. Loading dependent *in vitro* release of IQP-0528 and other antiretrovirals. (a) IQP-0528 release rate and (b) cumulative release from FCPs with three, 1.5 mm orifices with pellets of 0.9-15 wt% IQP-0528 in HPC represented in µg/d (a), and as a percent of loaded IQP-0528 (b). (c) 30-day average IQP-0528 release rate linearly correlated to the IQP-0528 loading with devices containing pellets with 0.9-15 wt% IQP-0528 in HPC (Spearman correlation of $r = 0.98$ and $p < 0.001$). The cumulative pellet release linearly correlated to the cumulative IQP-0528 release after 10, 20, and 30 days of release for FCPs with three, 1.5 mm orifices and 10 wt% (Spearman correlation of $r = 0.95$ and $p = 0.004$). (d) Diffusivity of TDF, MVC, and TFV as function of HPC concentration. (e) Cumulative percent release of multiple different drugs loaded into FCPs. Panels a, b, and e compare the experimental (symbols) and model (solid lines) results.
Figure 4.4. *In vitro* release of IQP-0528 comparing four, three, and two pellets per FCP. (a) IQP-0528 release rate and (b) cumulative amount release from FCPs with two, 1.5 mm orifices and four, three, and two, 50 mg pellets of 10 wt% IQP-0528 in HPC comparing results from experiments (symbols) and the model (solid lines).
CHAPTER 5

VAGINAL PHARMACOKINETICS OF AN ANTIRETROVIRAL DELIVERED FROM AN INTRAVAGINAL RING CONTAINING DISSOLUTION CONTROLLED HYDROPHILIC MATRICES

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5.1 Abstract

There is widespread agreement that inconsistent adherence has driven the unreliable effectiveness levels achieved in clinical trials evaluating antiretrovirals for HIV prevention. This has motivated the development of long-acting drug delivery systems that require less frequent user interventions in an attempt to achieve higher adherence levels. Intravaginal rings containing flux controlled pumps formulated with the antiretroviral drug IQP-0528, a potent non-nucleoside reverse transcriptase inhibitor, were evaluated in an ovine model. We measured high and consistent drug concentration in vaginal fluid over the month duration that resulted in near complete inhibition of viral replication in a cell-based assay. This device provides an adaptable platform for topical drug delivery and, due to its small size, can be readily combined with other intravaginal ring delivery systems for multiple women’s health applications.

5.2 Introduction

Recent clinical trials evaluating oral and vaginal pre-exposure prophylaxis (PrEP) have failed to consistently demonstrate protection from HIV acquisition, and low adherence remains the primary cause (1, 2). Independent markers for adherence in HIV prevention trials, i.e. antiretroviral concentration in plasma or vaginal fluid, typically indicate that the antiretroviral concentration correlates to protection (3-6). Moreover, multiple PrEP modalities have successfully protected nonhuman primates from mucosal SHIV infection depending on the relative timing between drug and viral dosing (7-12). Together, these data suggest topical
and oral administration of antiretroviral can prevent sexual transmission of HIV when a sufficient drug concentration is present when and where infection occurs. One approach to increase adherence to topical PrEP modalities is to increase the duration between dosing events, motivating the investigation of long-acting drug delivery systems like intravaginal rings (13-15).

Until recently, almost all published intravaginal ring designs necessitated diffusion of the drug molecule through the ring elastomer, thus limiting the type of drug molecules and release rates that were possible (16-18). In addition to the drug release properties, the ring stiffness and elastic recoil are innately linked to the ring dimensions, drug loading, and elastomer composition (19, 20). In order to decouple drug release from the elastomer chemistry, we designed a flux controlled pump (FCP), a device that consists of compressed pellets of drug and the water-soluble polymer contained within a hollow tubing core. An FCP has orifices in the elastomeric casing that simultaneously control the hydration rate of the hydrophilic matrix and resulting flux of a drug-containing semisolid gel from the device. (16) However, the major challenge to further development of this technology is its incorporation into an intravaginal ring for long-term retention in the female reproductive tract. If the elastomer utilized for the casing of the FCP is not sufficiently rigid, ring compression during insertion and use could result in luminal constriction and a burst of drug release. Concurrently, the overall ring compressibility and elastic recoil must be similar to commercially available intravaginal rings to achieve proper insertion, retention, and biocompatibility (21, 22).
This study presents the pharmacokinetic (PK) evaluation of a FCP intravaginal ring for the controlled delivery of IQP-0528, a non-nucleoside reverse transcriptase inhibitor with nanomolar activity against HIV-1, in an ovine model over a month duration. To measure drug distribution along the sheep vaginal canal, we utilized a multiswab to collect vaginal fluid at two locations in the sheep vagina with respect to the introitus (23). We then quantified the drug concentration and the antiviral activity of the material eluted from the swabs.

5.3 Materials and Methods

5.3.1 Fabrication of FCP vaginal rings

Compressed pellets, 4 mm in diameter and 51 ± 1 mg, consisting of 10 wt% IQP-0528 (ImQuest Biosciences, Frederick, MD) in hydroxypropyl cellulose (HPC GF, MW = 370 kDa; Klucel Pharm, Hercules, Wilmington, DE) were formed as previously described (16). Two types of Tecoflex aliphatic polyether thermoplastic polyurethanes (EG-65D and EG-85A; Lubrizol Advanced Materials; Wickliffe, OH) were formed into tubing by hot-melt extrusion with final dimensions of 0.7 mm wall thickness and 5.5 mm diameter (24). To fabricate the FCP, Tecoflex EG-65D tubing was cut to a length of 44 ± 0.5 mm, and one end was sealed by inductive tip-forming welding (PlasticWeld Systems, Inc., Newfane, NY) (25). The orifices were manually drilled, four compressed pellets were inserted into the open end of the device, and then the second end was sealed. Rings were made consisting of two FCPs and two placebo segments in an alternating manner with similar segments on opposing sides of the ring (Fig. 5.1a
and b). The placebo segments were made by cutting Tecoflex EG-85A tubing to a length of 63 ± 0.5 mm and sealing both ends by inductive tip-forming welding. The ends of the placebo and FCP segments were joined using a split-die induction welder (PlasticWeld Systems, Inc., Newfane, NY) to form a ring with an outer diameter of 55 mm (25). The rings were annealed into a circular shape by heating the rings to 65°C for 15 min followed by cooling the rings to room temperature in a custom-designed aluminum mold with ring sized cavities. The force to compress the rings 10% of the outer diameter was measured on an Instron 3342 uniaxial mechanical testing system as described previously (24).

5.3.2 In vitro drug release testing, drug content analysis, and pellet and device extractions

In parallel to the sheep PK studies, the in vitro drug release rate was measured from rings in triplicate in 25 mL of 25 mM acetate buffer pH 4.2 changed daily at 37°C and 80 rpm shaking. The release media on days 1, 2, 3, 5, 7, 10, 15, 20, 25, and 30 was collected and the drug was dissolved by the addition of 25 mL of methanol. Cumulative release was calculated by numerical integration of the release rate profile using a trapezoidal approximation. To determine the IQP-0528 loading in HPC pellets, pellets were placed in a 50 mL volumetric flask and dissolved overnight in methanol. Upon completion of in vitro and in vivo studies, the FCPs were cut out of the ring and then placed in a 50 mL centrifuge tube with approximately 10-15 mL of methanol and shaken overnight to dissolve the IQP-0528 and HPC. The methanol solution was transferred to a
50 mL volumetric flask and the FCP casing was rinsed at least 5 times with methanol. To confirm drug recovery, known amounts of drug, HPC, and Tecoflex EG-65 were dissolved in parallel. IQP-0528 content for extraction and in vitro release studies was measured by UV-HPLC using methods already described (26).

5.3.3 Multiswab design and fabrication

Multiswabs were fabricated on a lathe and mill from ½” acrylonitrile butadiene styrene rod (McMaster-Carr, Robbinsville, NJ). Orifices to hold the multiswab sponges were made with a square end mill to an outer diameter of 5 mm and depth of 4 mm. Pairs of such orifices on opposing sides were spaced longitudinally 2, 5, 8, and 11 cm from the handle end, representing the length from the introitus during application. Additional pairs, rotated 45° with respect to the first, were milled on opposing sides with a 0.5 mm offset towards the handle. The leading edge was rounded, and a handle was fashioned at a reduced diameter and approximately 5 cm long. (23)

5.3.4 Sheep pharmacokinetics studies

All animals were housed at the Center for Comparative Medicine at the University of Utah. All procedures were conducted under approved IACUC protocols in accordance with the standards incorporated in the Guide for the Care and Use of Laboratory Animals (National Research Council of the National Academies, 2010). IQP-0528 pharmacokinetics from FCP vaginal rings (N = 3)
was evaluated in adult, Columbian cross-bred ewes (*Ovis aries*) for 15 or 30 days (Table 5.1). Vaginal fluid was collected using a multiswab device (23), with swabs (5 mm in diameter cut from Ultracell® Nasal packs, Beaver Visitec International, Waltham, MA) inserted at 11 and 5 cm from the introitus with the 2 and 8 cm positions empty. For vaginal fluid collection, the multiswab was covered with a sheath of 12 cm long silicone tubing (1/2” inner diameter and 5/8” outer diameter platinum-cured silicone tubing; Cole-Parmer, Court Vernon Hills, IL). The tubing was lubricated with KY® Intrigue silicone lubricant (Johnson and Johnson, New Brunswick, NJ) for ease of insertion. Upon insertion, the sheath was removed and the multiswab remained in place for 2 min prior to removal. Vaginal fluid samples were collected at day 0 (baseline) and various days during the study: 1, 2, 3, 5, 7, 10, 15, 20, 25, and 30 days after ring insertion; and 1, 2, 3, and 5 days after ring removal (Fig. 5.1d).

5.3.5 Measuring the drug concentration in and antiviral activity of vaginal fluid

To measure the drug concentration in vaginal fluid, IQP-0528 was extracted from the swabs using methanol:water (4:1) as extractant solution and IQP-0532, a congener of IQP-0528 (27), as an internal standard used for recovery and quantitation measurements. Each swab was transferred to an Ultrafree® Centrifugal filter unit (Durapore®- PVDF, 0.65 μm, EMD Millipore, Billerica, MA) and 50 μL of an IQP-0532 standard in methanol was added. Two hundred μL of extractant solution was added, allowed to equilibrate for 10 min at
room temperature and then centrifuged at 16,000g for 10 min. The process was repeated with 100 µL of extractant solution. Then the complete extractant was transferred to a vial for analysis on an Agilent 1200 LC-6130 single quad MS fitted with an APCI-ESI multimode front end (Santa Clara, CA). The HPLC was equipped with a chilled autosampler (set to 10°C), a two-position 6-port valve column switch, and an isocratic and quaternary pump. The columns compartment was maintained at 40°C. The analyte and internal standard were eluted through the precolumn (Zorbax SB-C18 column, 2.1x30 mm, 3.5 µm; Agilent) onto the analytical column (Zorbax SB-C18 column, 2.1x100 mm, 3.5 µm; Agilent) with a 5 min gradient of 0.1% formic acid in water and 0.1% formic acid in 90:5:5 methanol:water:acetonitrile (0-5 min: 40-0% 0.1% formic acid in water) at 0.4 mL/min. After 5 min, the precolumn was removed from the circuit and back-flushed with 0.1% trifluoroacetic acid in 75:25 water:acetonitrile at 0.3 mL/min, and 0.1% formic acid in 90:5:5 methanol:water:acetonitrile was pumped through the analytical column at 0.4 mL/min. Positive secondary ions of IQP-0528 and IQP-0532 (341.2 and 343.4 m/z) were detected at approximately 9-10 min. The method was 20 min long. The lower limit of quantification (LLQ) for IQP-0528 calculated using the average mass of absorbed fluid for all samples was 0.1 µg/mL. Density of 1 g/mL was assumed. The area under the curve (AUC) was calculated by numerical integration of the drug concentration profile for each swab location using a trapezoidal approximation. To evaluate the antiviral activity, the material was eluted from the swab similarly except the extractant solution was 200 µL of complete DMEM repeated once. TZM-bl cells were plated
at 3 x 10⁴ per well, allowed to adhere overnight, and then exposed to approximately 10³ TCID₅₀ HIV-1BaL in the presence of a 1:5 dilution of the swab extracts. The plates were left in culture for 48 hrs at 37°C and then the media was removed by washing once with 100 µL of PBS. Cells were lysed in 20 µL luciferase cell culture lysis reagent and luciferase activity was measured using luciferase assay buffer (Promega, Fitchburg, WI) either immediately or stored at -20°C until the day of measurement. Data presented as means from two independent experiments and as the percent inhibition relative to cells in the present and absence of virus.

5.4 Results

The force required to compress the rings depended upon the axis of compression. The force to compress the rings 10% of the outer diameter was 1.4 ± 0.1 N (mean ± SD) when compressing the placebo portions and 3.8 ± 0.3 N when compressing the FCP portions. The force measured when compressing the placebo portion was in the range of NuvaRing® (0.7 N) and Estring® (1.6 N); however, along the axis of the FCPs, the rings were considerably stiffer than NuvaRing® and Estring®.

The first two PK studies evaluated FCPs containing three, 1.5 mm and four, 2.3 mm in diameter orifices for 15 days (Table 5.1). Both of these device configurations achieved high and consistent IQP-0528 concentrations in vaginal fluid with an average [range] of 180 [8-920] and 310 [8-1,800] µg/mL from days 5-15 with the FCPs with three, 1.5 mm and four, 2.3 mm orifices, respectively
(Fig. 5.2a and b). The FCPs with four, 2.3 mm orifices achieved higher drug exposure in the vaginal fluid compared to the three, 1.5 mm orifice devices with an average [range] AUC<sub>0-15</sub> of 4,200 [1,200-7,300] µg*d/mL for the four, 2.3 mm FCPs, and 2,300 [470-5,700] µg*d/mL for the three, 1.5 mm FCPs (p = 0.003; paired t-test for means). A lag time to reach a consistent drug concentration in vaginal fluid was observed with both devices as seen by the low and more variable drug concentration in vaginal fluid in the first 3 days with a number of samples below the LLQ. Notably, FCPs with three, 1.5 mm orifices exhibited lower and more variable drug levels compared to the four, 2.3 mm orifice design during the lag period (days 1-3). We observed comparable drug concentrations at each time point at the two locations tested, 11 and 5 cm from the introitus, in both of the 15-day studies (p = 0.29-0.66 for three, 1.5 mm orifice FCPs and p = 0.082-0.99 for four, 2.3 mm orifice FCPs for days 5-15; paired t-test for means at each time point). To study the drug washout kinetics, we measured the drug concentration in vaginal fluid after ring removal. In the study of the three, 1.5 mm orifice FCPs, all samples on day 15+3 (3 days after ring removal) were below the LLQ (data not shown). However, in the study of the four, 2.3 mm orifice FCPs, variable drug concentrations in vaginal fluid were observed on days 15+1, +2, and +3 (1, 2, and 3 days after ring removal) (Fig. 5.2a and b).

Overall, the FCP rings exhibited a reduced release rate in vivo compared to in vitro release conditions (Fig. 5.3a). The average in vitro release rate and corresponding cumulative release calculated from residual drug extraction over 15 days from rings containing two FCPs was 1.4 ± 0.08 mg/day (mean ± SD) and
54 ± 3.2% for the three, 1.5 mm orifice design, and 2.5 ± 0.08 mg/day and 94 ± 3.5% for the four, 2.3 mm orifice FCP intravaginal rings (Fig. 5.3a and c). We observed a 57% and 29% reduction in release in vivo for the three, 1.5 mm and four, 2.3 mm orifice FCP rings, respectively. This corresponded to average and cumulative in vivo drug release of 0.6 ± 0.1 mg/day and 23 ± 4.9% for the three, 1.5 mm orifice FCPs, and 1.8 ± 0.2 mg/day and 67 ± 7.6% for the four, 2.3 mm orifice FCPs (Fig. 5.3a). Devices with three, 2.3 mm orifices did not achieve controlled 30-day release in vitro with a significant drop in the release rate after ~75% of the total load was released, as seen from the cumulative release plateauing on day 20 (Fig. 5.3c). Therefore, with ~67% cumulative release after 15 days in vivo, the four, 2.3 mm orifice design would likely not result in 30-day controlled release. In summary, the four, 2.3 mm orifice design exhibited a higher overall release in 15 days, and less of a lag time to reach the higher drug concentration in the vaginal fluid compared to the three, 1.5 mm orifice device.

The two 15-day PK studies enabled us to select an orifice configuration that could result in 30-day controlled release in sheep. In the third ovine PK study, we evaluated an FCP design with an intermediate orifice area, FCPs with three, 2.3 mm orifices (13 mm² compared to 5.3 mm² for three, 1.5, and 17 mm² for four, 2.3 mm; Table 5.1). We observed variable drug concentration in vaginal fluid on the first 2 days with a limited number of samples below the LLQ and measured high drug concentration in vaginal fluid from day 3 to 30 with an average [range] of 270 [4.5-1,200] μg/mL (Fig. 5.2c). From days 3-30, the drug concentration at the 11 and 5 cm from the introitus was statistically
undistinguishable ($p = 0.067-0.90$, paired t-test for means at each time point) on all days except day 15 where a higher concentration was measured at the 5 cm location compared to the 11 cm location ($p = 0.025$). The three, 2.3 mm design did not achieve sustained and controlled drug release \textit{in vitro} for 30 days (Fig. 5.3c). \textit{In vivo} this design exhibited an average drug release of $1.0 \pm 0.21$ mg/day (mean ± SD) corresponding to $75 \pm 16\%$ cumulative release (Fig. 5.3b). This together with the high and consistent drug concentration in vaginal fluid measured up to day 30 suggests the drug release was sustained over 30 days. We measured variable drug levels [range: 0.018 – 660 µg/mL] after ring removal on days 30+1, +2, +3, and +5 (1, 2, 3, and 5 days after ring removal) in vaginal fluid with multiple samples below the LLQ (Fig. 5.2c).

Fluid eluted from the swabs collected while the rings were in place in the 30-day study exhibited significantly greater antiviral activity on both days 10 (90 ± 35%; mean ± SD; $p = 0.0025$, Mann-Whitney test) and 15 (91 ± 47%; $p = 0.012$) compared to the baseline activity (16 ± 36%; Fig. 5.4). Only two samples tested that were collected during ring use demonstrated low antiviral activity, whereas the remaining swabs yielded a viral inhibition of $107 \pm 15\%$ (N = 12). The two samples showing negligible antiviral activity absorbed 1-2 mg of vaginal fluid, suggesting that an insufficient amount of drug containing vaginal fluid was absorbed to demonstrate activity in this assay. No correlation between the antiviral activity and IQP-0528 concentration measured in adjacent swabs was observed (Spearman correlation, $p = 0.39$). This likely occurred since the antiviral activity of the swabs collected during ring use approached 100% and therefore
was outside of the dynamic range of the assay.

5.5 Discussion

The non-nucleoside reverse transcriptase inhibitor IQP-0528 is a promising antiretroviral for HIV prevention due to its nanomolar activity, high therapeutic index, and chemical stability (27, 28), and has been formulated for vaginal delivery in a number of different dosage forms including gels (28, 29), a vaginal film (30), an osmotic pump tablet (23), and an intravaginal ring (26). Additionally, a gel formulation of IQP-0528 has an approved IND, which will accelerate development of future long-acting dosage forms. The drug release rate from the previous intravaginal ring was limited by the solubility of the drug in the vaginal fluid; moreover, the authors observed a concentration gradient of the drug in vaginal fluid along the vaginal canal (26). We engineered a new drug delivery system where the drug release rate is controlled by the device geometry, the chemistry of the water-soluble polymer contained within, and the drug loading (16). In this system, drug release is not a function of the solubility or diffusivity of the drug in the ring elastomer. Drug release from this device occurs by hydration, swelling, and dissolution of the hydrophilic matrix at the orifice interface (Fig. 5.1c). In the case of a hydrophobic drug like IQP-0528, the pump releases a semisolid gel containing microparticles of the drug substance. Upon release, the water-soluble polymer likely enhances the distribution of the drug along the vaginal canal. In this report, we evaluated the performance of FCP containing rings in sheep and measured the concentration of IQP-0528 in vaginal fluid, and
the antiviral activity of the vaginal fluid collected during ring use. The anatomical similarities of the sheep and human vaginal tracts enable testing of human-sized devices without altering the dimensions or design (24, 31). However, the device performance in sheep and humans could differ due to anatomical and biological disparities; therefore, long-term device retention and drug release in this ovine model may not directly translate to overall performance in humans.

It is known from animal studies that vaginal transmission of HIV can occur along the female reproductive tract (32) and therefore, the antiretrovirals should be well distributed in the vaginal tract to prevent early transmission events. However, the drug release rate necessary to completely inhibit HIV transmission in the vaginal mucosa is unknown and will depend in part on the drug activity, distribution, and elimination. The ongoing clinical studies of the dapivirine matrix intravaginal ring, depending on effectiveness, may clarify the required dose to achieve an adequate concentration in vaginal fluid and tissue to prevent sexual HIV transmission in women. For instance, dapivirine-releasing matrix rings achieved drug concentrations $10^3$-$10^4$ times the \textit{in vitro} IC$_{50}$ in vaginal fluid of women (33-35). Despite slight differences between the IQP-0528 concentrations in vaginal fluid across the three FCP designs tested, the mean drug concentrations after the initial lag were at least $10^5$ times the \textit{in vitro} IC$_{50}$ (27) suggesting a potentially sufficient drug concentration to prevent HIV-1 infection (Fig. 5.2). We measured variable antiviral activity in the baseline samples, and endogenous antiviral activity of cervicovaginal fluid is commonly observed in humans (36, 37). The swabs collected on day 10 and 15 of the 30-day study...
exhibited significantly higher antiviral activity compared to the baseline samples (Fig. 5.4). Furthermore, the assay represents a 24-2,000x dilution of the vaginal fluid depending on the amount of fluid absorbed, confirming the high concentration of drug present in the vaginal fluid during ring use and its potential to be effective in preventing HIV transmission.

Previous evaluations of intravaginal rings eluting hydrophobic drugs, IQP-0528 in macaques and dapivirine in women, revealed higher drug concentration in vaginal fluid proximal to the ring compared to distal locations (26, 33-35). However, in all three PK studies of the FCP rings, we measured similar drug concentrations in vaginal fluid at the two locations tested, 11 and 5 cm from the introitus (Fig. 5.2). The previous IQP-0528 intravaginal ring was a matrix design made from polyether urethane and due to higher solubility and diffusivity of the drug in this elastomer, the authors were able to achieve high average drug release rates in macaques of approximately 1 mg/d scaled to a human sized ring over a month duration (26). However, despite this high drug flux from the previous design, the FCP resulted in a more consistent drug concentration in vaginal fluid with respect to time and location in the vaginal canal. We propose the drug containing gel released from the FCP ring was well distributed in the vaginal canal from the native forces present in the vagina, predominately normal forces leading to squeezing flow, and gravity (38). However, the dissolution of the drug within the vaginal canal and the diffusion of the drug into vaginal tissue warrants further study. The rate of drug dissolution in the vaginal fluid may be limited by the solubility of the drug and therefore limit drug diffusion into the
vaginal tissue. Additionally, this device delivers small quantities of gel in a sustained fashion, approximately 10 mg of gel over a day, compared to a 4 g bolus of a typical vaginal gel (39). Thus, a gel-eluting FCP ring may retain the superior drug distribution of a vaginal gel while not possessing negative stereotypical gel qualities, such as messiness and inconvenience of frequent application (2, 3).

We identified an initial lag time to reach a high and consistent drug concentration in vaginal fluid (Fig. 5.2) consistent with our in vitro release experiments (16). As a consequence, the first few days of ring use could present a potential window of susceptibility to infection. This lag time could be exaggerated in vivo due to the time required for the crystalline drug microparticles to distribute and dissolve in the vaginal fluid. In the 30-day study, drug was measured in the vaginal fluid up to 5 days after ring removal, suggesting a reservoir of drug in tissue that could diminish the potential window of susceptibility in the first few days during sequential ring use. We provide evidence that increasing the orifice area results in an increased drug release and drug concentration in vaginal fluid, and a shorter lag time (Fig. 5.2a, 5.2b, and 5.3). With the higher release rate and drug concentration in vaginal fluid, we hypothesize the four, 2.3 mm design achieved a higher drug concentration in vaginal tissue than the three, 1.5 mm design. This is further validated by the higher drug concentration in vaginal fluid observed during washout in the three days after ring removal with the four, 2.3 mm compared to the three, 1.5 mm orifice FCPs. This could have resulted from higher IQP-0528 concentrations in
the vaginal tissue that then acted as a drug reservoir upon ring removal. After the ring is removed, drug present in the vaginal tissue can diffuse not only towards the bloodstream or lymph system but also back through the vaginal mucosa and into the canal if a concentration gradient of the drug exists in both directions.

We hypothesized an intermediate orifice area compared to the designs evaluated in the two 15-day PK studies would exhibit a reduced lag time compared to the three, 1.5 mm design and result in a decreased release rate compared to the four, 2.3 mm design to achieve controlled release over 30 days. In the 30-day PK study, intravaginal rings with three, 2.3 mm diameter orifices in each FCP produced a brief lag time to reach high and consistent drug concentration in vaginal fluid through 30 days of ring use. Moreover, variable drug concentrations in vaginal fluid were observed after ring removal in the 30-day study (Fig. 5.2c), supporting the idea that high drug concentration in tissue was achieved. We measured a reduced release rate in vivo compared to in vitro release conditions which was comparable to the reduction observed with a similar device tested in rabbits (16). For this type of system, in vitro release conditions with complete, daily replacement of the release media do not accurately represent the vaginal mucosal environment. However, these data can be used to estimate the in vivo performance from in vitro release experiments. The reduced in vivo release rate could arise due to various complexities of the biological system including a limited amount of vaginal fluid to drive polymer hydration and release, a higher osmolarity of vaginal fluid compared to release media, and vaginal fluid dynamics and physiology controlling vaginal fluid
clearance and production.

Incorporating an FCP as a segment of an intravaginal ring presented a number of design challenges. Initially the FCP casing was made of a softer polymer and we observed a significant burst release caused by constriction of the FCP lumen associated with ring compression previously observed in women (40). Therefore, we utilized a harder polymer for the FCP casing, resulting in a segment that did not result in lumen collapse even during complete ring compression necessary for insertion. However, an intravaginal ring must also be compliant since a ring that is too stiff can cause tissue damage and discomfort (41). To conserve ring compliance similar to commercially available rings, the less stiff, non-pump segment(s) must occupy a large percentage of the ring. In the present study, the non-pump segments were placebo; however, we envision integrating this design with other intravaginal ring technologies (17, 19, 24, 25, 31) to create multipurpose technology for not only the prevention of HIV but also other sexually transmitted infections or unwanted pregnancy in an effort to improve user demand and adherence (42). Incorporating a single FCP into a ring will result in a softer ring compared to the intravaginal rings with two FCPs evaluated here. The nonsymmetric mechanical properties could present an issue for product acceptability, and this would require clinical studies to assess. However, a segmented ring with a single stiff segment of comparable length is currently in a clinical study and the results of which will begin to answer questions associated with mechanically nonsymmetric, segmented vaginal rings (25). In this study, we did not investigate device safety. However, the
components of the ring have all been previously examined in vaginal formulations (26, 29) and no adverse effects are expected from this drug-device combination.

5.6 Conclusions

In summary, we measured millimolar concentrations of IQP-0528, a nanomolar active reverse transcriptase inhibitor, in vaginal fluid. We observed a reduced drug release rate \textit{in vivo} compared to \textit{in vitro} conditions, and provide evidence that we are able to overcome this reduction by increasing the orifice area. A more detailed PK evaluation of this FCP ring is warranted to measure the drug concentration and distribution in vaginal tissue where the initial HIV transmission events occur. The FCP design only occupies a small portion of the ring, allowing for the incorporation of other intravaginal ring technologies for the delivery of agents for other women’s health applications.

5.7 Acknowledgements

The work was supported by National Institutes of Health grants U19 AI076980 and U19 AI103461.
5.8 References


Table 5.1. Summary of the different FCP designs

<table>
<thead>
<tr>
<th>Number of orifices</th>
<th>Orifice diameter (mm)</th>
<th>Orifice area (mm²)</th>
<th>Study duration (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Three</td>
<td>1.5</td>
<td>5.3</td>
<td>15</td>
</tr>
<tr>
<td>Four</td>
<td>2.3</td>
<td>17</td>
<td>15</td>
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<tr>
<td>Three</td>
<td>2.3</td>
<td>13</td>
<td>30</td>
</tr>
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</table>
Figure 5.1. Photographs and schematic of flux controlled pumps, and an outline of the sheep PK studies. (a) Photographs of a FCP vaginal ring with FCP segments on the top and bottom of the ring and placebo segments on the left and right, and (b) a close up of a single FCP segment contained within the ring. Each FCP has three, 2.3 mm orifices. Both scale bars are 5 mm. (c) Illustration of a flux controlled pump with water entry through the orifices which leads to polymer hydration, swelling and dissolution from the device, and resulting release of the drug-loaded semisolid gel. (d) Diagram of the sheep PK study including ring insertion and removal, and time points of vaginal fluid sampling, marked with vertical lines, to measure drug concentration and antiviral activity of fluid eluted from the swabs.
Figure 5.2. Drug concentration in vaginal fluid over 15 and 30 days of ring use at two locations, 11 and 5 cm from the introitus after administration of FCP containing intravaginal rings with (a) three, 1.5 mm or (b) four, 2.3 mm diameter orifices in each FCP over 15 days, and (c) three, 2.3 mm diameter orifices in each FCP over 30 days. Symbols represent individual samples and the bars represent the means; 1-2 swabs per distance per time point were analyzed. Samples with IQP-0528 concentration below LLQ were plotted as 1/10th of the calculated sample LLQ. The horizontal line at the top of the grey area represents the average LLQ for the experiment.
Figure 5.3. Comparison of the *in vivo* and *in vitro* IQP-0528 release over 15 and 30-days of ring use. IQP-0528 cumulative percent release and the average release rate for rings containing two FCPs comparing *in vitro* (blue) and *in vivo* (red) conditions after (a) 15 days and (b) 30 days of study. (b) FCPs with three, 2.3 mm orifices did not achieve 30-day release under *in vitro* release conditions and therefore were not included. Data represented with the symbols as individual FCPs, bars as means, and error bars as SD. (c) *In vitro* IQP-0528 cumulative release from rings containing two FCPs over 15 days for the three, 1.5 and four, 2.3 mm orifice FCPs and 30 days for the three, 2.3 mm FCPs. Data represented as the mean ± SD.
Figure 5.4. Antiviral activity of vaginal fluid collected in the 30-day PK study. The ability of the vaginal fluid eluted from swabs to inhibit HIV-1\textsubscript{Bal} infection of TZM-bl cells from the 30-day PK study evaluating FCPs with three, 2.3 mm orifices. Symbols represent individual swabs, and bars indicate the means.
CHAPTER 6

CONCLUSIONS AND FUTURE DIRECTIONS

The aim of the work presented in this manuscript was to expand the range of achievable drug release rates and the types of molecules delivered from an intravaginal ring (IVR) by utilizing reservoir designs composed of polyether urethane tubing formed into an IVR and designing new technologies where the mechanism of drug release does not depend on diffusion of the drug molecule through the IVR elastomer. IVRs were initially designed to deliver hormones as a contraceptive or hormone replacement therapy. As such, traditional IVR technologies effectively deliver relatively small amounts of small hydrophobic molecules. However, the ongoing high prevalence of new HIV infections, particularly in young women in sub-Saharan Africa, has resulted in a resurgence of research and development of IVR delivery systems over the last decade. Despite these new technologies, vaginal sustained delivery of macromolecular agents, and micro- and nanoparticles for women’s health applications is still difficult to achieve since in both cases, it is essentially impossible to achieve diffusive release through the IVR elastomer while maintaining the required ring stiffness. To increase the diversity of the drugs that can be delivered from IVRs and to obtain a better control of the drug release, we created a new drug delivery
technology where the mechanism of drug release is controlled by the hydration rate of the hydrophilic matrix contained within the IVR and not the diffusivity of the drug in IVR the elastomer. Orifices in the casing control the hydration rate of the compressed pellets composed of a mixture of micronized drug and swelling polymer that are contained in the hollow core of the IVR, and flux of the drug-containing gel that is forced through the orifice by polymer swelling and diffusion. This system provides controlled and sustained drug release of any stable molecule.

In summary, this work was motivated by the need for new technologies to prevent the male to female sexual transmission of HIV, specifically IVRs for the topical delivery of antiretrovirals for improved adherence and drug pharmacokinetics (PK) compared to frequently administered oral tablets or vaginal gels. Included below are brief conclusions from each chapter, followed by recommendations for future research specifically in vaginal drug delivery with an IVR and more generally in HIV PrEP.

6.1 Chapter Conclusions

6.1.1 Chapter 2 conclusions

In Chapter 2, we described the PK and pharmacodynamics of the prodrug tenofovir disoproxil fumarate (TDF) delivered from an IVR in macaques. Major conclusions from this chapter are:

1) High mg/day release rates of the hydrolytically unstable prodrug TDF can be achieved by controlling the chemistry and dimensions of the polyether
urethane tubing and formulation loaded inside of the IVR.

2) The IVR resulted in drug concentration in the surrounding tissue that were similar or exceeded previous levels associated with protection in this animal model.

3) The IVR completely protected macaques from multiple vaginal viral exposures.

6.1.2 Chapter 3 conclusions

In Chapter 3, we introduced the flux controlled pump (FCP) for the controlled topical delivery of macromolecules to the vaginal mucosa. We evaluated this new vaginal drug delivery system in rabbits to study the \textit{in vivo} device performance with the aid of an oval vaginal retainer. Major conclusions from this chapter are:

1) Drug release depends on the hydration rate and release of the hydrophilic polymer contained within the core of the device, and not the solubility or diffusivity of the model macromolecules within the hydrophilic polymer.

2) The orifice size and the polymer chemistry of the hydrophilic pellet contained within the device controls the hydration rate of pellets contained in the device and thereby the drug release rate.

3) An oval holder made of a shape memory polymer enables nonsurgical intravaginal insertion and retention of solid dosage forms in rabbits.
6.1.3 Chapter 4 conclusions

In Chapter 4, we further characterized the FCP to gain a better understanding of the mechanism of drug release for small molecule drugs in addition to macromolecules, and developed a model of drug release to enable quantitative predictions of the effect design changes have on the drug release profile. Major conclusions from this chapter are:

1) The dominate mechanism of drug release is not dependent on the drug solubility or diffusivity in the IVR elastomer.

2) The mechanism of drug release is predominately and in some cases completely controlled by the hydration and dissolution of the water-soluble polymer released from the device and not the solubility or diffusivity of the drug in that polymer.

3) The model accurately predicts the drug release rate when drug diffusion through the hydrated polymer matrix contained within the pump does not contribute to drug release, as was the case for hydrophobic small molecule drugs and macromolecular model compounds.

6.1.4 Chapter 5 conclusions

In Chapter 5, we evaluated the drug release and PK of FCP containing IVRs in sheep. We utilized a multiswab device to collect spatially registered vaginal fluid samples along the vaginal canal. Major conclusions from this chapter are:

1) The drug concentration was homogeneous along the vaginal canal,
suggesting the semisolid gel released from the FCP containing the drug aids in the distribution along the tract.

2) The FCP displayed a reduced release rate in sheep compared to \textit{in vitro} release conditions and this reduction can be overcome by increasing the orifice size.

3) The concentration of drug measured in the vaginal fluid was $\sim 10^5$ times the \textit{in vitro} EC$_{50}$ which corresponded to nearly complete inhibition of viral replication in a cell-based assay.

6.2 Discussion and Future Recommendations

The human-sized version of the TDF IVR described in Chapter 2 has completed a Phase 1 safety and PK clinical study. Placebo and drug containing IVRs were used over a 14-day duration. This TDF IVR design completely protected macaques from multiple vaginal viral exposures; however, the required dose in humans is unknown. PK data from clinical studies in humans, and PK and pharmacodynamics data from macaque studies evaluating a tenofovir vaginal gel suggest that the TDF release rate should be sufficient. However, there are a number of differences, comparing the more potent prodrug TDF to tenofovir, and sustained delivery from an IVR compared to a bolus release with a vaginal gel. Despite these differences, the tenofovir concentration in vaginal fluid and tissue achieved with the IVR were similar and maintained for a longer duration than that achieved with the 1% tenofovir gel. With the IVR in addition to the tenofovir concentration in vaginal tissue and fluid, there is also TDF present. Together, this
supports the idea that the TDF IVR delivers sufficient drug to inhibit viral transmission in the vaginal mucosa. Further clinical studies are needed to determine the TDF dose delivered from an IVR necessary to prevent HIV transmission at the vaginal mucosa. However, the current TDF IVR design can be altered to increase or decrease the drug release rate.

A concern with tubing reservoir IVRs made of hydrophilic polyether urethane is the possibility of bacterial growth in the lumen of the device from bacteria either crossing the tubing wall or being introduced into the ring during manufacturing. This bacterial growth could alter the local microflora necessary for proper vaginal health and immunity and present safety concerns. We found that nanoparticles and quantum dots cannot diffuse across the wall of the polyether urethane tubing, suggesting that the pore size of the tubing used in this IVR is sufficiently small to not allow bacteria to enter the device after fabrication. Additionally, to reduce the lag time to reach high drug release rates, the formulation undergoes a heat-treatment step of 65°C for 5 days which effectively pasteurized the device to further limit the bacterial load inside of the IVR. As part of macaque and clinical studies of the TDF IVR, the fluid within the IVR was sampled and cultured. To date, no bacteria have been found inside of the ring after use, whereas bacteria was found on the surface of the IVR and was comparable to bacteria found in the vagina.

There are still a number of unanswered questions regarding the performance and acceptability of FCPs for vaginal drug delivery that require additional preclinical evaluation. A potential safety issue of vaginal drug delivery systems is adverse alterations of the native vaginal flora. This is particularly
relevant for vaginal products that can absorb and/or release bacteria and could potentially select for pathogenic flora. Unlike the TDF IVR described in Chapter 2, the FCP are designed with multiple orifices for water influx and drug loaded gel efflux that are millimeters in diameter. The FCP has not been evaluated in an animal model with a comparable vaginal flora to women such as pigtailed macaques, and it is unknown if this system would alter the microflora and present safety concerns. PK and safety studies performed in pigtailed macaques where the vaginal microflora is monitored prior to and during IVR use would begin to address this concern. Moreover, Chapters 3 and 5 demonstrated that in vitro release conditions did not accurately represent the vaginal environment since a reduced release rate was observed in rabbits and sheep. This could be due in part to the relatively limited amount of vaginal fluid available to hydrate the internal contents of the device to drive drug release and/or a higher osmolarity of vaginal fluid compared to the release media. Since the amount of fluid present depends on a number of factors including presence of semen, atrophic vaginitis associated with menopause, or secretions due to sex or menses; variable amounts of vaginal fluid could drive variable drug release. However, at least for IVRs delivering hormones as a contraceptive or hormone replacement, this has not been established as an issue. The effect of increased amounts of fluid present in the vagina on the in vivo drug release rate could be measured in an animal model by measuring the drug remaining in the IVR after use with animals that are vaginally instilled with saline daily compared to a nontreated control. If the FCP yields consistent release rates in vivo needs to be evaluated in a diverse population of women not abstaining from
vaginal intercourse.

There is a compelling interest in designing multipurpose prevention technologies (MPTs) that possess the ability to prevent HIV transmission and/or other sexually transmitted infections potentially combined with a contraceptive as an approach to improve user demand and adherence (1, 2). There are a number of possible approaches to design MPTs. For example, the condom is effective as a contraceptive and inhibits transmission of disease. The two main drug delivery approaches to designing MPTs is to either deliver multiple drugs (3-5), or deliver a drug that is active against multiple infectious agents (6). The first MPT IVRs in clinical studies deliver tenofovir or the tenofovir prodrug TDF to prevent HIV and HSV transmission, and tenofovir combined with levonorgestrel as a contraceptive (3, 6). Particularly in the case of delivering multiple drugs with disparate chemical properties and delivery requirements, multisegment IVRs are often necessary where separate segments are designed for the different drugs to achieve the desired release rate of drugs (3, 7, 8). The TDF IVR described in Chapter 2 could be combined with the FCP described in Chapters 3-5 to enable to deliver of multiple drugs with diverse chemical and delivery requirements. The FCP was designed as a segment of an IVR occupying approximately a quarter of the ring with the remainder containing a different design, and as such, incorporating the FCP into the TDF IVR only necessitate a small reduction in the length of the TDF segments, resulting in a marginal reduction in the drug release rate. Moreover, the TDF design could be altered slightly to compensate for the reduced length. For example, a slight reduction in the tubing wall thickness or utilizing a polyether urethane with a
higher equilibrium water uptake would increase the drug release rate. The FCP technology could also be utilized to simultaneously deliver more than one drug. This could be useful to deliver different drugs for multiple indications or multiple drugs active against HIV in an attempt to increases effectiveness and reduce the emergence of drug resistant virus. The acceptability of a segmented IVR with asymmetric mechanical properties due to a single soft and hard segment like an FCP is unknown. However, an IVR with similar mechanical properties and segmented design (3) is currently in Phase 1 clinical study and may begin to address questions of acceptability of IVRs exhibiting asymmetric stiffness.

Chapter 5 describes the in vivo evaluation of IVRs containing FCPs in sheep, and presented the drug concentration in vaginal fluid but not tissue. Additional studies are required to determine the drug concentration in vaginal tissue achieved with the FCP. Since vaginal fluid is easier to collect and can be collected more frequently, it is often used as a measure of the drug release kinetics and reveals more detail of the kinetics of drug release particularly early. However, the drug concentration in vaginal tissue where the initial transmission events occur is more important for drugs that are active intracellularly. Future studies need to evaluate the drug concentration where inhibition of HIV transmission by a reverse transcriptase inhibitor occurs, i.e. vaginal and/or cervical tissue. Additionally, under the in vitro release conditions investigated, the FCP releases a semisolid gel containing drug microparticles that must dissolve for the drug to partition and diffuse into the surrounding tissue. In the sheep PK study, we did not differentiate between dissolved and undissolved drug released into the vaginal fluid. With the high,
mg/day release rates of IQP-0528 that possesses micromolar water solubility, it is possible that drug dissolution was rate limiting and therefore, insoluble drug was present in the vaginal tract of the sheep. Measuring the drug concentration in the vaginal tissue would begin to address this question. A direct measure of the relative fraction of dissolved and undissolved drug in the vaginal fluid would be to aspirate fluid from the vaginal fornix, separate out the undissolved drug by centrifugation, and measure the volume and drug concentration of the two fractions.

Further improvements to the model of drug release from FCPs described in Chapter 4 would enable predictions of drug release for hydrophilic small molecule drugs. That takes into account the kinetics of the hydration and diffusion of the water-soluble polymer within the FCP. Higher drug release rates for hydrophilic drugs were observed experimentally compared to the model predictions. This suggests that in the case of more hydrophilic small molecule drugs, in addition to drug release from the extrusion of the water-soluble polymer, drug release also occurred from the drug diffusing through the swollen polymer matrix within the casing. Diffusive drug release could be included into the model by adding an additional component to the model. The concentration dependent diffusivity of the drug in the swollen polymer matrix as a function of the polymer concentration could either be determined by fitting to experimental drug release from the device, or measured using nuclear magnetic resonance (9, 10) or Franz cell diffusion (11) techniques. Chapter 4 presented the diffusivity of the hydrophilic drugs tenofovir, TDF, and maraviroc as a function of the water-soluble polymer concentration determined using Franz cells. Issues could arise by simply adding the additional
component to the model of the drug diffusion since the drug dissolution kinetics inside the FCP will likely impact the drug concentration inside the device. The drug dissolution rate and the drug concentration will depend on the drug particle size, the water concentration, and the chemistry of the drug.

Topical pre-exposure prophylaxis (PrEP) is designed to chemically inhibit HIV transmission in the tissue where it occurs. Simply, sufficient drug must be present in the genital mucosa when and where HIV infection takes place. Non-human primate animal models have guided clinical testing of oral and topical PrEP, and often parity between the effective drug concentrations is found (12, 13). However, there is still much unknown including the kinetics and extend that drug distributes throughout the genital tract after systemic and topical antiretroviral administration, and what drug concentration is required in what tissue to prevent the sexual transmission of HIV. Drug PK after topical administration of antiretrovirals is commonly measured in the vaginal fluid, vaginal and cervical tissue, and plasma. For nonterminal animal studies, the vaginal and cervical tissue is collected with a pinch biopsy. Typically, the tissue is homogenized prior to measuring the drug concentration and therefore represents an average concentration. A concentration gradient of the drug is hypothesized to exist within the epithelium with the highest drug concentration near the vaginal lumen that diminishes further into the tissue. Also many antiretrovirals are active intracellularly and therefore intracellular drug concentration, particularly in immune cells that carry the infection to the lymph system, is the most relevant.

Therefore, an active segment of HIV prevention research is to measure the drug
distribution in mucosal tissue both as a function of depth into the tissue, and cell
type. A number of investigators are utilizing different mass spectroscopy
techniques to map the drug distribution in mucosal tissue (14, 15). These mass
spectroscopy imaging techniques include matrix-assisted laser
desorption/ionization (MALDI) (16) and desorption electrospray ionization (DESI)
(17). Analyzing multiple tissue sections by mass spectroscopy to determine the
drug content, and histology to determine the cell morphology enables the
researcher to overlay the drug concentration profile over an image of the tissue
section.

Despite decades of IVR acceptability research, most for hormone eluting
IVRs but more recently for HIV prevention, it is still not known what the preferred
duration for an IVR for HIV chemoprophylaxis is balancing the potential cost
saving advantage of increasing the device duration, and the complexity of the
ring design in the context of the desires and preferences of target populations.
Contraceptive and hormone replacement IVRs currently available have durations
ranging from three weeks to one year (18, 19), suggesting durations longer than
one month could be acceptable. Many in the HIV prevention field have advocated
for longer duration IVRs as a potential strategy to amortize cost. Potential
disadvantages of IVRs that are designed to last longer than a month are
predominately associated with menstruation. Menses was the leading
circumstance for voluntary removal and involuntary expulsion in a recent
acceptability study of IVRs in African women (20), and it is unknown if an IVR
replaced monthly would be preferred over a longer duration IVR. Moreover,
further education is required for sub-Saharan African populations to conceptualize using antiretrovirals for HIV prevention, and overcome the stigma associated with using antiretrovirals for HIV treatment (21). Further clinical studies in relevant populations are required where women use IVRs to begin to understand these and other questions including the preferred ring dimensions, stiffness, and color. A main conclusion of all of the recent clinical studies of HIV prevention agents has been that adherence and acceptability are fundamentally essential to designing effective HIV prevention modalities. Acceptability and adherence are particularly important for IVRs for HIV prevention since one of the main drivers for IVR development over other vaginal delivery systems is to improve adherence over dosage forms that require more frequent user interventions. If the dapivirine IVR is found to be effective in protecting women from HIV infection, programs to further educate potential users would be required since IVRs for contraception and hormone replacement therapy are not widely used in sub-Saharan Africa where these type of prevention modalities would have the greatest effect.

It is unknown how systemic administration of antiretrovirals prevents HIV transmission, whether preventing the initial transmission events in the local tissue or preventing the establishment of a systemic infection, and if systemic or topical delivery of antiretrovirals will result in better effectiveness. Different antiretrovirals display differential concentrations at the portal of virus entry compared to plasma drug concentration when administered systemically, suggesting that the relative contribution of preventing local and systemic infection will be different depending
on the drug. For example, tenofovir derivatives are known to concentrate within HIV target cells in certain mucosal compartments, and TDF concentrates in the rectal, but not in the vaginal compartment after oral dosing (22-26). Upon parenteral administration, the concentration of cabotegravir is significantly lower at the rectal and vaginal mucosa relative to the blood (27) whereas rilpivirine concentration in plasma and vaginal tissue and fluid was similar (28). Oral administration of raltegravir confers higher drug concentration in gastrointestinal tissue compared to blood plasma, presenting a potential advantage to prevent rectal HIV transmission (29). The differential drug distribution that is specific to the antiretroviral and route of administration has critical implications relating to the use of systemic PrEP to prevent sexual transmission of HIV since there is mounting evidence that systemic PrEP can allow local viral replication at the mucosal site of infection if drug concentrations are inadequate. This localized infection could result in a systemic infection (30) if the drug concentration drops further, for example, for missed doses or a discontinuation of the dosing regimen. This could potentially fuel the development of drug resistant virus. A number of groups are developing improved parenteral antiretroviral formulations compared to the injectables of rilpivirine and cabotegravir currently in clinical development. These questions regarding how systemic and topical delivery of antiretrovirals inhibits HIV transmission are important as both topical and parenteral delivery systems are being evaluated in the clinic.

As proven with HIV therapy, delivering multiple drugs with different mechanisms of action for HIV prevention may result in a reduced incidence of drug-
resistant virus and more efficient protection (31-33); however, this hypothesis has not yet been confirmed for HIV PrEP. The most data are available for oral PrEP of either TDF alone or with emtricitabine; both drugs are nucleoside reverse transcriptase inhibitors. The variable adherence levels observed in different clinical trials make comparison between trials difficult. To date, there has only been a single trial evaluating both oral TDF alone and with emtricitabine, and no significant difference in effectiveness was observed in preventing HIV infection, suggesting that the difference, if any exists, is slight (34). Particularly since the effectiveness of oral PrEP in at-risk populations has been directly correlated to adherence, an important next step to maximize the public health impact is to effectively rollout oral PrEP to promote high adherence (35). Moreover, the lack of tenofovir resistant virus present in women who were infected during clinical studies of a tenofovir vaginal gel suggests that delivering multiple antiretroviral may not be necessary (36). The lack of drug resistance might also reflect low adherence and/or how quickly the drug is cleared after a single dose that might not be conducive for the selection of drug-resistant virus. The results of the Phase 3 studies of the dapivirine IVR are expected in 2016 and will likely shed light on the required dose and resulting drug concentration in vaginal fluid and tissue to prevent mucosal HIV infection, and importantly, if topical delivery of a single antiretroviral can effectively prevent infection.

The development of new IVR technologies for HIV prevention has led to a number of promising candidates for the delivery of a wide variety of drugs including small molecule, biologics, and synthetic macromolecules, and these
delivery systems can be utilized for other women’s health applications. In summary, this dissertation describes the design and evaluation of 1) the PK and efficacy of a reservoir IVR for the vaginal delivery of TDF in macaques and 2) a new vaginal drug delivery device where the mechanism of drug release is predominately controlled by the hydration and diffusion of the water-soluble polymer contained within and not the drug diffusion through the polymers of the ring. The rate of new HIV infections has been in decline over the last decade; however, the number of new HIV infections remains persistently high, particularly for young women in sub-Saharan Africa and young men who have sex with men. This suggests that in addition to expansion of individual’s knowledge of their HIV status, treatment as prevention, and oral PrEP, new HIV prevention modalities are needed to control the pandemic.
6.3 References


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