

ENGINEERING ANTIRETROVIRAL ELUTING INTRAVAGINAL
RINGS FOR HUMAN IMMUNODEFICIENCY
VIRUS PREVENTION

by

Ryan Stevenson Teller

A dissertation submitted to the faculty of
The University of Utah
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Department of Bioengineering

The University of Utah

August 2015

Copyright © Ryan Stevenson Teller 2015

All Rights Reserved

The University of Utah Graduate School

STATEMENT OF DISSERTATION APPROVAL

The dissertation of Ryan Stevenson Teller
has been approved by the following supervisory committee members:

<u>Patrick F. Kiser</u>	, Chair	<u>02/06/2015</u> <small>Date Approved</small>
<u>David W. Grainger</u>	, Member	<u>02/06/2015</u>
<u>Robert W. Hitchcock</u>	, Member	<u>02/06/2015</u> <small>Date Approved</small>
<u>Vladimir Hlady</u>	, Member	<u>02/06/2015</u> <small>Date Approved</small>
<u>Margit Janat-Amsbury</u>	, Member	<u>02/06/2015</u> <small>Date Approved</small>
<u>Patrick A. Tresco</u>	, Member	<u>02/06/2015</u> <small>Date Approved</small>

and by Patrick A. Tresco, Chair/Dean of
the
Department/College/School of Bioengineering

and by David B. Kieda, Dean of The Graduate School.

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.

TABLE OF CONTENTS

ABSTRACT.....	iii
NOMENCLATURE.....	vii
ACKNOWLEDGEMENTS.....	x
Chapters	
1 INTRODUCTION.....	1
1.1 HIV/AIDS Pandemic, Therapy, and Pre-exposure Prophylaxis.....	1
1.2 Vaginal Drug Delivery.....	5
1.3 Vaginal and Oral Pre-Exposure Prophylaxis.....	7
1.4 Drug Release from IVRs.....	9
1.5 Mechanical Properties of IVRs.....	15
1.6 Modeling Drug Release from Hydrophilic Matrix Tablets.....	16
1.7 PK of Antiretrovirals Delivered from IVRs.....	18
1.8 Animal Models to Evaluate IVR Performance and Efficacy.....	20
1.9 Long-acting Injectables for HIV Therapy or Prevention.....	23
1.10 Dissertation Chapter Overview.....	25
1.11 References.....	31
2 INTRAVAGINAL RING ELUTING TENOFOVIR DISOPROXIL FUMARATE COMPLETELY PROTECTS MACAQUES FROM MULTIPLE VAGINAL SIMIAN-HIV CHALLENGES.....	49
2.1 Abstract.....	50
2.2 Introduction.....	50
2.3 Results and Discussion.....	51
2.4 Methods.....	54
2.5 Acknowledgements.....	55
2.7 References.....	55
2.8 Supporting Information.....	56
3 INTRAVAGINAL FLUX CONTROLLED PUMP FOR SUSTAINED	

	RELEASE OF MACROMOLECULES.....	57
	3.1 Abstract.....	58
	3.2 Introduction	58
	3.3 Materials and Methods.....	59
	3.4 Results	61
	3.5 Discussion.....	63
	3.6 Conclusions	66
	3.7 Acknowledgements.....	66
	3.8 References.....	66
	3.9 Supplemental Materials	68
4	CONTROLLING THE HYDRATION RATE OF A HYDROPHILIC MATRIX IN THE CORE OF AN INTRAVAGINAL RING DETERMINES ANTIRETROVIRAL RELEASE.....	76
	4.1 Abstract.....	77
	4.2 Introduction	77
	4.3 Materials and Methods.....	80
	4.4 Results	87
	4.5 Discussion.....	97
	4.6 Conclusions.....	101
	4.7 Acknowledgements.....	102
	4.8 References.....	103
5	VAGINAL PHARMACOKINETICS OF AN ANTIRETROVIRAL DELIVERED FROM AN INTRAVAGINAL RING CONTAINING DISSOLUTION CONTROLLED HYDROPHILIC MATRICES.....	112
	5.1 Abstract.....	113
	5.2 Introduction.....	113
	5.3 Materials and Methods.....	115
	5.4 Results.....	120
	5.5 Discussion.....	124
	5.6 Conclusions.....	130
	5.7 Acknowledgements.....	130
	5.8 References.....	131
6	CONCLUSIONS AND FUTURE DIRECTIONS.....	141
	6.1 Chapter Conclusions.....	142
	6.2 Discussion and Future Recommendations.....	145
	6.3 References.....	157
	VITA.....	161

NOMENCLATURE

A	Area
ABS	Acrylonitrile butadiene styrene
AIDS	Acquired immunodeficiency syndrome
ANOVA	Analysis of variance
APCI-ESI	Atmospheric pressure chemical ionization-electrospray ionization
AUC	Area under the curve
b	Constant that characterizes the water concentration dependence of the diffusion coefficient
C	Concentration
CAPRISA	Centre for the AIDS Programme of Research in South Africa
CDC	Centers for Disease Control and Prevention
CG	Carrageenan
C _{max}	Maximum observed concentration
C ₀	Initial drug concentration
CTMR	5-(and-6)-carboxytetramethylrhodamine
CVL	Cervicovaginal lavage
D	Diffusion coefficient
DESI	Desorption electrospray ionization
dm/dt	Drug release rate
DMPA	Depo-medroxyprogesterone

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

PEU	Polyether urethane
PK	Pharmacokinetics
PrEP	Pre-exposure prophylaxis
R ²	Coefficient of determination
RMSD	Root mean square deviation
SHIV	Simian-human immunodeficiency virus
SIV	Simian immunodeficiency virus
SMP	Shape-memory polyether urethane
STI	Sexually transmitted infection
TCID ₅₀	Tissue culture infectious dose 50
TDF	Tenofovir disoproxil fumarate
TFV	Tenofovir
TFV-DP	Tenofovir-diphosphate
T _g	Glass transition temperature
UNAIDS	The Joint Program United Nations Program on HIV and AIDS
UV-HPLC	Ultraviolet high performance liquid chromatography
ΔΠ	Osmotic pressure difference

ACKNOWLEDGEMENTS

I would first like to express my sincerest gratitude to my advisor, Dr. Patrick Kiser, for providing numerous hours of guidance over the years and providing a strong scientific training. As a mentor, you continuously challenged and motivated me to put forth my best effort. I would also like to thank my committee, Drs. Robert Hitchcock, David Grainger, Vladimir Hlady, Patrick Tresco, and Margit Janat-Amsbury, for providing constructive criticism and support throughout my research endeavors. I also wish to acknowledge all the current and past members of the Kiser lab with whom I have worked, particularly Drs. Rachna Rastogi, Todd Johnson, and Justin Clark for providing much appreciated support and input during my graduate career. I'd also like to thank all of the other individuals I have worked with at the University of Utah, Northwestern University, particularly Drs. Igal Szleifer and David Malaspina, and other institutions who have fostered a collaborative research environment. Finally, I would like to thank my friends and family; you have been the source of immense support and encouragement over the years.

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 *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

(49, 83). 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, $\mu\text{g/day}$ 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 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 .

$$\frac{dm}{dt} = K A h \Delta\Pi C \quad (\text{Eq. 1.1})$$

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 β_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 *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 *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 *in vivo* was attenuated compared to the *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 *in vivo* compared to *in vitro* release conditions, and also likely resulted in a nearly constant *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 is 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 an 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.

Publication: Smith JM, Rastogi R, Teller RS, Srinivasan P, Mesquita PMM, Nagaraja U, McNicholl JM, Hendry RM, Dinh CT, Martin A, Herold BC, and Kiser PK. 2013. *Intravaginal ring eluting tenofovir disoproxil fumarate completely protects macaques from multiple vaginal simian-HIV challenges*. PNAS: 110(4) 16145-16150.

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.

Publication: Teller RS, Rastogi R, Johnson TJ, Blair MJ, Hitchcock RW, and Kiser PK. 2014. *Intravaginal flux controlled pump for sustained release of macromolecules*. Pharm Res: 31(9):2344-53.

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 $\mu\text{g}/\text{day}$ 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, Szeleifer 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.

Under review.

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.

Publication: Teller RS, Rastogi R, Anderson M, Nagaraja U, Hattaway H, Hope TJ, and Kiser PK. 2015. *Vaginal pharmacokinetics of an antiretroviral delivered from an intravaginal ring containing dissolution controlled hydrophilic matrices*. Antimicrobial Agents and Chemotherapy. To be submitted.

1.10.4 Chapter 6 overview

Chapter 6 includes brief conclusions of the dissertations 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

1. **UNAIDS.** 2014. The Gap Report. Joint United Nations Programme on HIV/AIDS (UNAIDS).
2. **UNAIDS.** 2013. Report on the global AIDS epidemic. Joint United Nations Programme on HIV/AIDS (UNAIDS).
3. **Quinn TC, Wawer MJ, Sewankambo N, Serwadda D, Li C, Wabwire-Mangen F, Meehan MO, Lutalo T, Gray RH.** 2000. Viral load and heterosexual transmission of human immunodeficiency virus type 1. Rakai Project Study Group. *The New England journal of medicine* **342**:921-929.
4. **Cohen MS, Chen YQ, McCauley M, Gamble T, Hosseinipour MC, Kumarasamy N, Hakim JG, Kumwenda J, Grinsztejn B, Pilotto JH, Godbole SV, Mehendale S, Chariyalertsak S, Santos BR, Mayer KH, Hoffman IF, Eshleman SH, Piwowar-Manning E, Wang L, Makhema J, Mills LA, de Bruyn G, Sanne I, Eron J, Gallant J, Havlir D, Swindells S, Ribaud H, Elharrar V, Burns D, Taha TE, Nielsen-Saines K, Celentano D, Essex M, Fleming TR, Team HS.** 2011. Prevention of HIV-1 infection with early antiretroviral therapy. *The New England journal of medicine* **365**:493-505.
5. **Tanser F, Barnighausen T, Grapsa E, Zaidi J, Newell ML.** 2013. High coverage of ART associated with decline in risk of HIV acquisition in rural KwaZulu-Natal, South Africa. *Science* **339**:966-971.
6. **Torres RA, Lewis W.** 2014. Aging and HIV/AIDS: pathogenetic role of therapeutic side effects. *Laboratory investigation; a journal of technical methods and pathology* **94**:120-128.
7. **Al-Dakkak I, Patel S, McCann E, Gadkari A, Prajapati G, Maiese EM.** 2013. The impact of specific HIV treatment-related adverse events on adherence to antiretroviral therapy: a systematic review and meta-analysis. *AIDS Care* **25**:400-414.
8. **Rockstroh JK, Mohr R, Behrens G, Spengler U.** 2014. Liver fibrosis in HIV: which role does HIV itself, long-term drug toxicities and metabolic changes play? *Curr Opin HIV AIDS* **9**:365-370.
9. **Gardner EM, McLees MP, Steiner JF, Del Rio C, Burman WJ.** 2011. The spectrum of engagement in HIV care and its relevance to test-and-treat strategies for prevention of HIV infection. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **52**:793-800.
10. **UNAIDS.** 2014. 90–90–90 - An ambitious treatment target to help end the AIDS epidemic. Joint United Nations Programme on HIV/AIDS (UNAIDS).

11. **Bezemer D, de Wolf F, Boerlijst MC, van Sighem A, Hollingsworth TD, Prins M, Geskus RB, Gras L, Coutinho RA, Fraser C.** 2008. A resurgent HIV-1 epidemic among men who have sex with men in the era of potent antiretroviral therapy. *Aids* **22**:1071-1077.
12. **Phillips AN, Cambiano V, Nakagawa F, Brown AE, Lampe F, Rodger A, Miners A, Elford J, Hart G, Johnson AM, Lundgren J, Delpech VC.** 2013. Increased HIV incidence in men who have sex with men despite high levels of ART-induced viral suppression: analysis of an extensively documented epidemic. *PLoS One* **8**:e55312.
13. **Eaton JW, Johnson LF, Salomon JA, Barnighausen T, Bendavid E, Bershteyn A, Bloom DE, Cambiano V, Fraser C, Hontelez JA, Humair S, Klein DJ, Long EF, Phillips AN, Pretorius C, Stover J, Wenger EA, Williams BG, Hallett TB.** 2012. HIV treatment as prevention: systematic comparison of mathematical models of the potential impact of antiretroviral therapy on HIV incidence in South Africa. *PLoS medicine* **9**:e1001245.
14. **Cori A, Ayles H, Beyers N, Schaap A, Floyd S, Sabapathy K, Eaton JW, Hauck K, Smith P, Griffith S, Moore A, Donnell D, Vermund SH, Fidler S, Hayes R, Fraser C, Team HPS.** 2014. HPTN 071 (PopART): a cluster-randomized trial of the population impact of an HIV combination prevention intervention including universal testing and treatment: mathematical model. *PLoS One* **9**:e84511.
15. **Garnett GP, Becker S, Bertozzi S.** 2012. Treatment as prevention: translating efficacy trial results to population effectiveness. *Curr Opin HIV AIDS* **7**:157-163.
16. **Kretzschmar ME, Schim van der Loeff MF, Birrell PJ, De Angelis D, Coutinho RA.** 2013. Prospects of elimination of HIV with test-and-treat strategy. *Proc Natl Acad Sci U S A* **110**:15538-15543.
17. **Koff WC, Russell ND, Walport M, Feinberg MB, Shiver JW, Karim SA, Walker BD, McGlynn MG, Nweneka CV, Nabel GJ.** 2013. Accelerating the development of a safe and effective HIV vaccine: HIV vaccine case study for the Decade of Vaccines. *Vaccine* **31 Suppl 2**:B204-208.
18. **Lewis GK, DeVico AL, Gallo RC.** 2014. Antibody persistence and T-cell balance: Two key factors confronting HIV vaccine development. *Proc Natl Acad Sci U S A* **111**:15614-15621.
19. **Kent SJ, Reece JC, Petravic J, Martyushev A, Kramski M, De Rose R, Cooper DA, Kelleher AD, Emery S, Cameron PU, Lewin SR, Davenport MP.** 2013. The search for an HIV cure: tackling latent infection. *The Lancet. Infectious diseases* **13**:614-621.

20. **Fauci AS, Marston HD, Folkers GK.** 2014. An HIV cure: feasibility, discovery, and implementation. *Jama* **312**:335-336.
21. **Baeten JM, Grant R.** 2013. Use of Antiretrovirals for HIV Prevention: What Do We Know and What Don't We Know? *Curr Hiv-Aids Rep* **10**:142-151.
22. **Gengiah TN, Moosa A, Naidoo A, Mansoor LE.** 2014. Adherence challenges with drugs for pre-exposure prophylaxis to prevent HIV infection. *International journal of clinical pharmacy* **36**:70-85.
23. **Durevall D, Lindskog A.** 2014. Intimate partner violence and HIV in ten sub-Saharan African countries: what do the Demographic and Health Surveys tell us? *The Lancet Global Health*.
24. 2012. CDC. Vital signs: HIV infection, testing, and risk behaviors among youths - United States. *MMWR Morb Mortal Wkly Rep* **61**:971-976.
25. **Baral S, Sifakis F, Cleghorn F, Beyrer C.** 2007. Elevated risk for HIV infection among men who have sex with men in low- and middle-income countries 2000-2006: a systematic review. *PLoS medicine* **4**:e339.
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.
27. **Walensky RP, Park JE, Wood R, Freedberg KA, Scott CA, Bekker LG, Losina E, Mayer KH, Seage GR, 3rd, Paltiel AD.** 2012. The cost-effectiveness of pre-exposure prophylaxis for HIV infection in South African women. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **54**:1504-1513.
28. **Feldblum PJ, Adeiga A, Bakare R, Wevill S, Lendvay A, Obadaki F, Olayemi MO, Wang L, Nanda K, Rountree W.** 2008. SAVVY vaginal gel (C31G) for prevention of HIV infection: a randomized controlled trial in Nigeria. *PLoS One* **3**:e1474.
29. **Peterson L, Nanda K, Opoku BK, Ampofo WK, Owusu-Amoako M, Boakye AY, Rountree W, Troxler A, Dominik R, Roddy R, Dorflinger L.** 2007. SAVVY (C31G) gel for prevention of HIV infection in women: a Phase 3, double-blind, randomized, placebo-controlled trial in Ghana. *PLoS One* **2**:e1312.
30. **Van Damme L, Govinden R, Mirembe FM, Guedou F, Solomon S, Becker ML, Pradeep BS, Krishnan AK, Alary M, Pande B, Ramjee G, Deese J, Crucitti T, Taylor D, Group CSS.** 2008. Lack of effectiveness of cellulose sulfate gel for the prevention of vaginal HIV transmission. *The New England journal of medicine* **359**:463-472.

31. **Skoler-Karpoff S, Ramjee G, Ahmed K, Altini L, Plagianos MG, Friedland B, Govender S, De Kock A, Cassim N, Palanee T, Dozier G, Maguire R, Lahteenmaki P.** 2008. Efficacy of Carraguard for prevention of HIV infection in women in South Africa: a randomised, double-blind, placebo-controlled trial. *Lancet* **372**:1977-1987.
32. **McCormack S, Ramjee G, Kamali A, Rees H, Crook AM, Gafos M, Jentsch U, Pool R, Chisembele M, Kapiga S, Mutemwa R, Vallely A, Palanee T, Sookrajh Y, Lacey CJ, Darbyshire J, Grosskurth H, Profy A, Nunn A, Hayes R, Weber J.** 2010. PRO2000 vaginal gel for prevention of HIV-1 infection (Microbicides Development Programme 301): a phase 3, randomised, double-blind, parallel-group trial. *Lancet* **376**:1329-1337.
33. **Van Damme L, Ramjee G, Alary M, Vuylsteke B, Chandeying V, Rees H, Sirivongrangson P, Mukenge-Tshibaka L, Ettiegne-Traore V, Uaheowitchai C, Karim SS, Masse B, Perriens J, Laga M, Group COLS.** 2002. Effectiveness of COL-1492, a nonoxynol-9 vaginal gel, on HIV-1 transmission in female sex workers: a randomised controlled trial. *Lancet* **360**:971-977.
34. **Van Damme L, Corneli A, Ahmed K, Agot K, Lombaard J, Kapiga S, Malahleha M, Owino F, Manongi R, Onyango J, Temu L, Monedi MC, Mak'Oketch P, Makanda M, Reblin I, Makatu SE, Saylor L, Kiernan H, Kirkendale S, Wong C, Grant R, Kashuba A, Nanda K, Mandala J, Fransen K, Deese J, Crucitti T, Mastro TD, Taylor D, Grp F-PS.** 2012. Preexposure Prophylaxis for HIV Infection among African Women. *New Engl J Med* **367**:411-422.
35. **Grant RM, Lama JR, Anderson PL, McMahan V, Liu AY, Vargas L, Goicochea P, Casapia M, Guanira-Carranza JV, Ramirez-Cardich ME, Montoya-Herrera O, Fernandez T, Veloso VG, Buchbinder SP, Chariyalertsak S, Schechter M, Bekker LG, Mayer KH, Kallas EG, Amico KR, Mulligan K, Bushman LR, Hance RJ, Ganoza C, Defechereux P, Postle B, Wang FR, McConnell JJ, Zheng JH, Lee J, Rooney JF, Jaffe HS, Martinez AI, Burns DN, Glidden DV, Team iS.** 2010. Preexposure Chemoprophylaxis for HIV Prevention in Men Who Have Sex with Men. *New Engl J Med* **363**:2587-2599.
36. **Grant RM, Anderson PL, McMahan V, Liu A, Amico KR, Mehrotra M, Hosek S, Mosquera C, Casapia M, Montoya O, Buchbinder S, Veloso VG, Mayer K, Chariyalertsak S, Bekker LG, Kallas EG, Schechter M, Guanira J, Bushman L, Burns DN, Rooney JF, Glidden DV, iPrEx study t.** 2014. Uptake of pre-exposure prophylaxis, sexual practices, and HIV incidence in men and transgender women who have sex with men: a cohort study. *The Lancet. Infectious diseases* **14**:820-829.

37. **Baeten JM, Donnell D, Ndase P, Mugo NR, Campbell JD, Wangisi J, Tappero JW, Bukusi EA, Cohen CR, Katabira E, Ronald A, Tumwesigye E, Were E, Fife KH, Kiarie J, Farquhar C, John-Stewart G, Kakia A, Odoyo J, Mucunguzi A, Nakku-Joloba E, Twesigye R, Ngure K, Apaka C, Tamooch H, Gabona F, Mujugira A, Panteleeff D, Thomas KK, Kidoguchi L, Krows M, Revall J, Morrison S, Haugen H, Emmanuel-Ogier M, Ondrejcek L, Coombs RW, Frenkel L, Hendrix C, Bumpus NN, Bangsberg D, Haberer JE, Stevens WS, Lingappa JR, Celum C, Partners Pr EPST.** 2012. Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. *The New England journal of medicine* **367**:399-410.
38. **Abdool Karim Q, Abdool Karim SS, Frohlich JA, Grobler AC, Baxter C, Mansoor LE, Kharsany AB, Sibeko S, Mlisana KP, Omar Z, Gengiah TN, Maarschalk S, Arulappan N, Mlotshwa M, Morris L, Taylor D, Group CT.** 2010. Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. *Science* **329**:1168-1174.
39. **Baeten JM, Donnell D, Mugo NR, Ndase P, Thomas KK, Campbell JD, Wangisi J, Tappero JW, Bukusi EA, Cohen CR, Katabira E, Ronald A, Tumwesigye E, Were E, Fife KH, Kiarie J, Farquhar C, John-Stewart G, Kidoguchi L, Coombs RW, Hendrix C, Marzinke MA, Frenkel L, Haberer JE, Bangsberg D, Celum C, for the Partners Pr EPST.** 2014. Single-agent tenofovir versus combination emtricitabine plus tenofovir for pre-exposure prophylaxis for HIV-1 acquisition: an update of data from a randomised, double-blind, phase 3 trial. *The Lancet. Infectious diseases*.
40. **Kirby T, Thornber-Dunwell M.** 2014. Uptake of PrEP for HIV slow among MSM. *Lancet* **383**:399-400.
41. **Karris MY, Beekmann SE, Mehta SR, Anderson CM, Polgreen PM.** 2014. Are We Prepped for Preexposure Prophylaxis (PrEP)? Provider Opinions on the Real-World Use of PrEP in the United States and Canada. *Clinical Infectious Diseases* **58**:704-712.
42. **Sachdev DD, Stojanovski K, Liu AY, Buchbinder SP, Macalino GE.** 2014. Intentions to prescribe preexposure prophylaxis are associated with self-efficacy and normative beliefs. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **58**:1786-1787.
43. **Marrazzo JM, Ramjee G, Richardson BA, Gomez K, Mgodhi N, Nair G, Palanee T, Nakabiito C, van der Straten A, Noguchi L, Hendrix CW, Dai JY, Ganesh S, Mkhize B, Taljaard M, Parikh UM, Piper J, Masse B, Grossman C, Rooney J, Schwartz JL, Watts H, Marzinke MA, Hillier SL, McGowan IM, Chirenje ZM, Team VS.** 2015. Tenofovir-based

- preexposure prophylaxis for HIV infection among African women. *The New England journal of medicine* **372**:509-518.
44. **Amico KR, Mansoor LE, Corneli A, Torjesen K, van der Straten A.** 2013. Adherence support approaches in biomedical HIV prevention trials: experiences, insights and future directions from four multisite prevention trials. *AIDS Behav* **17**:2143-2155.
 45. **Marrazzo J, Ramjee G, Nair G, Palanee T, Mkhize B, Nakabiito C, Taljaard M, Piper J, Gomez Feliciano K.** 2013. Pre-exposure Prophylaxis for HIV in women: Daily Oral Tenofovir, Oral Tenofovir/Emtricitabine, or Vaginal Tenofovir Gel in the VOICE Study (MTN 003), CROI 2013, Atlanta, GA.
 46. **Mastro TD, Sista N, Abdool-Karim Q.** 2014. ARV-based HIV prevention for women - where we are in 2014. *Journal of the International AIDS Society* **17**:19154.
 47. **Hendrix CW, Chen BA, Guddera V, Hoesley C, Justman J, Nakabiito C, Salata R, Soto-Torres L, Patterson K, Minnis AM, Gandham S, Gomez K, Richardson BA, Bumpus NN.** 2013. MTN-001: randomized pharmacokinetic cross-over study comparing tenofovir vaginal gel and oral tablets in vaginal tissue and other compartments. *PLoS One* **8**:e55013.
 48. **Karim SSA, Kashuba ADM, Werner L, Karim QA.** 2011. Drug concentrations after topical and oral antiretroviral pre-exposure prophylaxis: implications for HIV prevention in women. *The Lancet* **378**:279-281.
 49. **Brache V, Faundes A.** 2010. Contraceptive vaginal rings: a review. *Contraception* **82**:418-427.
 50. **van der Straten A, Montgomery ET, Cheng H, Wegner L, Masenga G, von Mollendorf C, Bekker L, Ganesh S, Young K, Romano J, Nel A, Woodsong C.** 2012. High acceptability of a vaginal ring intended as a microbicide delivery method for HIV prevention in African women. *AIDS Behav* **16**:1775-1786.
 51. **Kiser PF, Johnson TJ, Clark JT.** 2012. State of the art in intravaginal ring technology for topical prophylaxis of HIV infection. *AIDS Rev* **14**:62-77.
 52. **Kruk ME, Schwalbe N.** 2006. The relation between intermittent dosing and adherence: preliminary insights. *Clin Ther* **28**:1989-1995.
 53. **Osterberg L, Blaschke T.** 2005. Adherence to medication. *The New England journal of medicine* **353**:487-497.

54. **Prescott GM, Matthews CM.** 2014. Long-acting reversible contraception: a review in special populations. *Pharmacotherapy* **34**:46-59.
55. **Winner B, Peipert JF, Zhao Q, Buckel C, Madden T, Allsworth JE, Secura GM.** 2012. Effectiveness of long-acting reversible contraception. *The New England journal of medicine* **366**:1998-2007.
56. **Woolfson AD, Malcolm RK, Gallagher R.** 2000. Drug delivery by the intravaginal route. *Critical reviews in therapeutic drug carrier systems* **17**:509-555.
57. **Alexander NJ, Baker E, Kaptein M, Karck U, Miller L, Zampaglione E.** 2004. Why consider vaginal drug administration? *Fertility and sterility* **82**:1-12.
58. **Friend DR, Clark JT, Kiser PF, Clark MR.** 2013. Multipurpose prevention technologies: Products in development. *Antiviral Res* **100**:S39-S47.
59. **Young Holt B, Romano J, Manning J, Hemmerling A, Shields W, Vyda L, Lusti-Narasimhan M.** 2014. Ensuring successful development and introduction of multipurpose prevention technologies through an innovative partnership approach. *BJOG: An International Journal of Obstetrics & Gynaecology* **121**:3-8.
60. **Nel AM, Mitchnick LB, Risha P, Muungo LTM, Norick PM.** 2011. Acceptability of Vaginal Film, Soft-Gel Capsule, and Tablet as Potential Microbicide Delivery Methods Among African Women. *J Womens Health* **20**:1207-1214.
61. **Visness CM, Ulin P, Pfannenschmidt S, Zekeng L.** 1998. Views of Cameroonian sex workers on a woman-controlled method of contraception and disease protection. *International journal of STD & AIDS* **9**:695-699.
62. **Akil A, Agashe H, Dezzutti CS, Moncla BJ, Hillier SL, Devlin B, Shi Y, Uranker K, Rohan LC.** 2014. Formulation and Characterization of Polymeric Films Containing Combinations of Antiretrovirals (ARVs) for HIV Prevention. *Pharm Res.*
63. **Ham AS, Rohan LC, Boczar A, Yang L, K WB, Buckheit RW, Jr.** 2012. Vaginal film drug delivery of the pyrimidinedione IQP-0528 for the prevention of HIV infection. *Pharm Res* **29**:1897-1907.
64. **Grammen C, Van den Mooter G, Appeltans B, Michiels J, Crucitti T, Arien KK, Augustyns K, Augustijns P, Brouwers J.** 2014. Development and characterization of a solid dispersion film for the vaginal application of the anti-HIV microbicide UAMC01398. *Int J Pharm* **475**:238-244.

65. **Pereira LE, Clark MR, Friend DR, Garber DA, McNicholl JM, Hendry RM, Doncel GF, Smith JM.** 2014. Pharmacokinetic and safety analyses of tenofovir and tenofovir-emtricitabine vaginal tablets in pigtailed macaques. *Antimicrob Agents Chemother* **58**:2665-2674.
66. **Ball C, Krogstad E, Chaowanachan T, Woodrow KA.** 2012. Drug-eluting fibers for HIV-1 inhibition and contraception. *PLoS One* **7**:e49792.
67. **Ball C, Woodrow KA.** 2014. Electrospun solid dispersions of Maraviroc for rapid intravaginal preexposure prophylaxis of HIV. *Antimicrob Agents Chemother* **58**:4855-4865.
68. **Woodrow KA, Cu Y, Booth CJ, Saucier-Sawyer JK, Wood MJ, Saltzman WM.** 2009. Intravaginal gene silencing using biodegradable polymer nanoparticles densely loaded with small-interfering RNA. *Nat Mater* **8**:526-533.
69. **Ham AS, Cost MR, Sassi AB, Dezzutti CS, Rohan LC.** 2009. Targeted delivery of PSC-RANTES for HIV-1 prevention using biodegradable nanoparticles. *Pharm Res* **26**:502-511.
70. **Ensign LM, Tang BC, Wang YY, Tse TA, Hoen T, Cone R, Hanes J.** 2012. Mucus-penetrating nanoparticles for vaginal drug delivery protect against herpes simplex virus. *Science translational medicine* **4**:138ra179.
71. **Schwartz JL, Rountree W, Kashuba AD, Brache V, Creinin MD, Poindexter A, Kearney BP.** 2011. A multi-compartment, single and multiple dose pharmacokinetic study of the vaginal candidate microbicide 1% tenofovir gel. *PLoS One* **6**:e25974.
72. **Rees H, Delany-Moretlwe S, Baron D, Lombard C, Gray G, Myer L, Panchia R, Schwartz J, Doncel G.** 2015. CROI 2015, Seattle, WA.
73. **Veazey RS, Ketas TJ, Dufour J, Moroney-Rasmussen T, Green LC, Klasse PJ, Moore JP.** 2010. Protection of rhesus macaques from vaginal infection by vaginally delivered maraviroc, an inhibitor of HIV-1 entry via the CCR5 co-receptor. *J Infect Dis* **202**:739-744.
74. **Dobard C, Sharma S, Martin A, Pau CP, Holder A, Kuklennyik Z, Lipscomb J, Hanson DL, Smith J, Novembre FJ, Garcia-Lerma JG, Heneine W.** 2012. Durable protection from vaginal simian-human immunodeficiency virus infection in macaques by tenofovir gel and its relationship to drug levels in tissue. *J Virol* **86**:718-725.
75. **Haase AT.** 2011. Early events in sexual transmission of HIV and SIV and opportunities for interventions. *Annu Rev Med* **62**:127-139.

76. **Dumond JB, Yeh RF, Patterson KB, Corbett AH, Jung BH, Rezk NL, Bridges AS, Stewart PW, Cohen MS, Kashuba AD.** 2007. Antiretroviral drug exposure in the female genital tract: implications for oral pre- and post-exposure prophylaxis. *Aids* **21**:1899-1907.
77. **Murnane PM, Celum C, Mugo N, Campbell JD, Donnell D, Bukusi E, Mujugira A, Tappero J, Kahle EM, Thomas KK, Baeten JM, Team PPS.** 2013. Efficacy of preexposure prophylaxis for HIV-1 prevention among high-risk heterosexuals: subgroup analyses from a randomized trial. *Aids* **27**:2155-2160.
78. **Garcia-Lerma JG, Cong ME, Mitchell J, Youngpairoj AS, Zheng Q, Masciotra S, Martin A, Kuklenyik Z, Holder A, Lipscomb J, Pau CP, Barr JR, Hanson DL, Otten R, Paxton L, Folks TM, Heneine W.** 2010. Intermittent prophylaxis with oral truvada protects macaques from rectal SHIV infection. *Science translational medicine* **2**:14ra14.
79. **Louissaint NA, Cao YJ, Skipper PL, Liberman RG, Tannenbaum SR, Nimmagadda S, Anderson JR, Everts S, Bakshi R, Fuchs EJ, Hendrix CW.** 2013. Single dose pharmacokinetics of oral tenofovir in plasma, peripheral blood mononuclear cells, colonic tissue, and vaginal tissue. *AIDS Res Hum Retroviruses* **29**:1443-1450.
80. **Patterson KB, Prince HA, Kraft E, Jenkins AJ, Shaheen NJ, Rooney JF, Cohen MS, Kashuba AD.** 2011. Penetration of tenofovir and emtricitabine in mucosal tissues: implications for prevention of HIV-1 transmission. *Science translational medicine* **3**:112re114.
81. **Anderson PL, Glidden DV, Liu A, Buchbinder S, Lama JR, Guanira JV, McMahan V, Bushman LR, Casapia M, Montoya-Herrera O, Veloso VG, Mayer KH, Chariyalertsak S, Schechter M, Bekker LG, Kallas EG, Grant RM, iPrEx Study T.** 2012. Emtricitabine-tenofovir concentrations and pre-exposure prophylaxis efficacy in men who have sex with men. *Science translational medicine* **4**:151ra125.
82. **Anderson PL, Glidden DV, Bushman LR, Heneine W, Garcia-Lerma JG.** 2014. Tenofovir diphosphate concentrations and prophylactic effect in a macaque model of rectal simian HIV transmission. *J Antimicrob Chemother* **69**:2470-2476.
83. **Dezarnaulds G, Fraser IS.** 2003. Vaginal ring delivery of hormone replacement therapy - a review. *Expert Opin Pharmaco* **4**:201-212.
84. **Novak A, de la Loge C, Abetz L, van der Meulen EA.** 2003. The combined contraceptive vaginal ring, NuvaRing: an international study of user acceptability. *Contraception* **67**:187-194.

85. **Chien YW, Lambert HJ, Lin TK.** 1975. Solution-solubility dependency of controlled release of drug from polymer matrix: mathematical analysis. *J Pharm Sci* **64**:1643-1647.
86. **Malcolm K, Woolfson D, Russell J, Tallon P, McAuley L, Craig D.** 2003. Influence of silicone elastomer solubility and diffusivity on the in vitro release of drugs from intravaginal rings. *J Control Release* **90**:217-225.
87. **Clark JT, Johnson TJ, Clark MR, Nebeker JS, Fabian J, Tuitupou AL, Ponnappalli S, Smith EM, Friend DR, Kiser PF.** 2012. Quantitative evaluation of a hydrophilic matrix intravaginal ring for the sustained delivery of tenofovir. *J Control Release* **163**:240-248.
88. **van Laarhoven JA, Krufft MA, Vromans H.** 2002. In vitro release properties of etonogestrel and ethinyl estradiol from a contraceptive vaginal ring. *Int J Pharm* **232**:163-173.
89. **Johnson TJ, Clark MR, Albright TH, Nebeker JS, Tuitupou AL, Clark JT, Fabian J, McCabe RT, Chandra N, Doncel GF, Friend DR, Kiser PF.** 2012. A 90-Day Tenofovir Reservoir Intravaginal Ring for Mucosal HIV Prophylaxis. *Antimicrob Agents Chemother* **56**:6272-6283.
90. **Clark JT, Clark MR, Shelke NB, Johnson TJ, Smith EM, Andreasen AK, Nebeker JS, Fabian J, Friend DR, Kiser PF.** 2014. Engineering a segmented dual-reservoir polyurethane intravaginal ring for simultaneous prevention of HIV transmission and unwanted pregnancy. *PLoS One* **9**:e88509.
91. **van Laarhoven JA, Krufft MA, Vromans H.** 2002. Effect of supersaturation and crystallization phenomena on the release properties of a controlled release device based on EVA copolymer. *J Control Release* **82**:309-317.
92. **Woolfson AD, Malcolm RK, Morrow RJ, Toner CF, McCullagh SD.** 2006. Intravaginal ring delivery of the reverse transcriptase inhibitor TMC 120 as an HIV microbicide. *Int J Pharm* **325**:82-89.
93. **Mishell DR, Jr., Talas M, Parlow AF, Moyer DL.** 1970. Contraception by means of a silastic vaginal ring impregnated with medroxyprogesterone acetate. *American journal of obstetrics and gynecology* **107**:100-107.
94. **Radomsky ML, Whaley KJ, Cone RA, Saltzman WM.** 1992. Controlled vaginal delivery of antibodies in the mouse. *Biology of Reproduction* **47**:133-140.
95. **Saltzman WM, Sherwood JK, Adams DR, Haller P.** 2000. Long-term vaginal antibody delivery: Delivery systems and biodistribution. *Biotechnology and Bioengineering* **67**:253-264.

96. **Langer R, Hsieh DST, Rhine W, Folkman J.** 1980. Control of release kinetics of macromolecules from polymers. *Journal of Membrane Science* **7**:333-350.
97. **Amsden BG, Cheng Y-L, Goosen MFA.** 1994. A mechanistic study of the release of osmotic agents from polymeric monoliths. *J. Controlled Release* **30**:45-56.
98. **Gupta KM, Pearce SM, Poursaid AE, Aliyar HA, Tresco PA, Mitchnik MA, Kiser PF.** 2008. Polyurethane intravaginal ring for controlled delivery of dapivirine, a nonnucleoside reverse transcriptase inhibitor of HIV-1. *Journal of Pharmaceutical Sciences* **97**:4228-4239.
99. **Johnson TJ, Gupta KM, Fabian J, Albright TH, Kiser PF.** 2010. Segmented polyurethane intravaginal rings for the sustained combined delivery of antiretroviral agents dapivirine and tenofovir. *Eur J Pharm Sci* **39**:203-212.
100. **Smith JM, Rastogi R, Teller RS, Srinivasan P, Mesquita PM, Nagaraja U, McNicholl JM, Hendry RM, Dinh CT, Martin A, Herold BC, Kiser PF.** 2013. Intravaginal ring eluting tenofovir disoproxil fumarate completely protects macaques from multiple vaginal simian-HIV challenges. *Proc Natl Acad Sci U S A* **110**:16145-16150.
101. **Morrow RJ, Woolfson AD, Donnelly L, Curran R, Andrews G, Katinger D, Malcolm RK.** 2011. Sustained release of proteins from a modified vaginal ring device. *Eur J Pharm Biopharm* **77**:3-10.
102. **Baum MM, Butkyavichene I, Gilman J, Kennedy S, Kopin E, Malone AM, Nguyen C, Smith TJ, Friend DR, Clark MR, Moss JA.** 2012. An intravaginal ring for the simultaneous delivery of multiple drugs. *Journal of Pharmaceutical Sciences* **101**:2833-2843.
103. **Teller RS, Rastogi R, Johnson TJ, Blair MJ, Hitchcock RW, Kiser PF.** 2014. Intravaginal flux controlled pump for sustained release of macromolecules. *Pharm Res* **31**:2344-2353.
104. **Rastogi R, Teller RS, Mesquita PM, Herold BC, Kiser PF.** 2013. Osmotic pump tablets for delivery of antiretrovirals to the vaginal mucosa. *Antiviral Res* **100**:255-258.
105. **Moss JA, Malone AM, Smith TJ, Butkyavichene I, Cortez C, Gilman J, Kennedy S, Kopin E, Nguyen C, Sinha P, Hendry RM, Guenther P, Holder A, Martin A, McNicholl J, Mitchell J, Pau CP, Srinivasan P, Smith JM, Baum MM.** 2012. Safety and pharmacokinetics of intravaginal rings delivering tenofovir in pig-tailed macaques. *Antimicrob Agents Chemother* **56**:5952-5960.

106. **Theeuwes F.** 1975. Elementary osmotic pump. *Journal of Pharmaceutical Sciences* **64**:1987-1991.
107. **Amkraut A, Eckenhoff JB, Nichols K.** 1989. Osmotic delivery of peptides and macromolecules. *Advanced Drug Delivery Reviews* **4**:255-276.
108. **Pendergrass PB, Reeves CA, Belovicz MW, Molter DJ, White JH.** 1996. The shape and dimensions of the human vagina as seen in three-dimensional vinyl polysiloxane casts. *Gynecologic and obstetric investigation* **42**:178-182.
109. **Pendergrass PB, Reeves CA, Belovicz MW, Molter DJ, White JH.** 2000. Comparison of vaginal shapes in Afro-American, caucasian and hispanic women as seen with vinyl polysiloxane casting. *Gynecologic and obstetric investigation* **50**:54-59.
110. **Pendergrass PB, Belovicz MW, Reeves CA.** 2003. Surface area of the human vagina as measured from vinyl polysiloxane casts. *Gynecologic and obstetric investigation* **55**:110-113.
111. **Koetsawang S, Ji G, Krishna U, Cuadros A, Dhall GI, Wyss R, Rodriquez la Puente J, Andrade AT, Khan T, Kononova ES, et al.** 1990. Microdose intravaginal levonorgestrel contraception: a multicentre clinical trial. II. Expulsions and removals. World Health Organization. Task Force on Long-Acting Systemic Agents for Fertility Regulation. *Contraception* **41**:125-141.
112. **Roumen FJ, Dieben TO.** 1999. Clinical acceptability of an ethylene-vinyl-acetate nonmedicated vaginal ring. *Contraception* **59**:59-62.
113. **Bounds W, Szarewski A, Lowe D, Guillebaud J.** 1993. Preliminary report of unexpected local reactions to a progestogen-releasing contraceptive vaginal ring. *Eur J Obstet Gynecol Reprod Biol* **48**:123-125.
114. **Weisberg E, Fraser IS, Baker J, Archer D, Landgren BM, Killick S, Soutter P, Krause T, d'Arcangues C.** 2000. A randomized comparison of the effects on vaginal and cervical epithelium of a placebo vaginal ring with non-use of a ring. *Contraception* **62**:83-89.
115. **Fraser IS, Lacarra M, Mishell DR, Alvarez F, Brache V, Lahteenmaki P, Elomaa K, Weisberg E, Nash HA.** 2000. Vaginal epithelial surface appearances in women using vaginal rings for contraception. *Contraception* **61**:131-138.
116. **Clark MR, Johnson TJ, McCabe RT, Clark JT, Tuitupou A, Elgendy H, Friend DR, Kiser PF.** 2012. A hot-melt extruded intravaginal ring for the

- sustained delivery of the antiretroviral microbicide UC781. *Journal of Pharmaceutical Sciences* **101**:576-587.
117. **Fetherston SM, Geer L, Veazey RS, Goldman L, Murphy DJ, Ketas TJ, Klasse PJ, Blois S, La Colla P, Moore JP, Malcolm RK.** 2013. Partial protection against multiple RT-SHIV162P3 vaginal challenge of rhesus macaques by a silicone elastomer vaginal ring releasing the NNRTI MC1220. *J Antimicrob Chemother* **68**:394-403.
 118. **Fetherston SM, Boyd P, McCoy CF, McBride MC, Edwards KL, Ampofo S, Malcolm RK.** 2013. A silicone elastomer vaginal ring for HIV prevention containing two microbicides with different mechanisms of action. *Eur J Pharm Sci* **48**:406-415.
 119. **Chien YW, Lambert HJ.** 1974. Controlled drug release from polymeric delivery devices. II. Differentiation between partition-controlled and matrix-controlled drug release mechanisms. *J Pharm Sci* **63**:515-519.
 120. **Chien YW, Lambert HJ, Grant DE.** 1974. Controlled drug release from polymeric devices. I. Technique for rapid in vitro release studies. *Journal of Pharmaceutical Sciences* **63**:365-369.
 121. **Chien YW, Mares SE, Berg J.** 1975. Controlled drug release from polymeric delivery devices. III. In vitro in vivo correlation for intravaginal release of ethynodiol diacetate from silicone devices in rabbits. *Journal of Pharmaceutical Sciences* **64**:1776-1781.
 122. **Helbling IM, Ibarra JCD, Luna JA.** 2014. The Optimization of an Intravaginal Ring Releasing Progesterone Using a Mathematical Model. *Pharm Res* **31**:795-808.
 123. **Helbling IM, Luna JA, Cabrera MI.** 2011. Mathematical modeling of drug delivery from torus-shaped single-layer devices. *J Control Release* **149**:258-263.
 124. **Siepmann J, Peppas NA.** 2001. Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). *Advanced Drug Delivery Reviews* **48**:139-157.
 125. **Maderuelo C, Zarzuelo A, Lanao JM.** 2011. Critical factors in the release of drugs from sustained release hydrophilic matrices. *Journal of Controlled Release* **154**:2-19.
 126. **Siepmann J, Kranz H, Bodmeier R, Peppas NA.** 1999. HPMC-matrices for controlled drug delivery: a new model combining diffusion, swelling, and dissolution mechanisms and predicting the release kinetics. *Pharm Res* **16**:1748-1756.

127. **Siepmann J, Podual K, Sriwongjanya M, Peppas NA, Bodmeier R.** 1999. A new model describing the swelling and drug release kinetics from hydroxypropyl methylcellulose tablets. *J Pharm Sci* **88**:65-72.
128. **Kaunisto E, Abrahmsen-Alami S, Borgquist P, Larsson A, Nilsson B, Axelsson A.** 2010. A mechanistic modelling approach to polymer dissolution using magnetic resonance microimaging. *J Control Release* **147**:232-241.
129. **Zhu XX, Wang F, Nivaggioli T, Winnik MA, Macdonald PM.** 1993. Poly(methyl methacrylate) film dissolution and solvent diffusion coefficients: correlations determined using laser interferometry-fluorescence quenching and pulsed-gradient spin-echo NMR spectroscopy. *Macromolecules* **26**:6397-6402.
130. **Matsukawa S, Ando I.** 1996. A study of self-diffusion of molecules in polymer gel by pulsed-gradient spin-echo H-1 NMR. *Macromolecules* **29**:7136-7140.
131. **Wu N, Wang LS, Tan DC, Moochhala SM, Yang YY.** 2005. Mathematical modeling and in vitro study of controlled drug release via a highly swellable and dissoluble polymer matrix: polyethylene oxide with high molecular weights. *J Control Release* **102**:569-581.
132. **Crank J.** 1975. *The Mathematics of Diffusion*, 2nd ed. Clarendon Press, Oxford.
133. **Fujita H.** 1961. Diffusion in polymer-diluent systems, p. 1-47, *Fortschritte Der Hochpolymeren-Forschung*, vol. 3/1. Springer Berlin Heidelberg.
134. **Gao P, Fagerness PE.** 1995. Diffusion in HPMC gels. I. Determination of drug and water diffusivity by pulsed-field-gradient spin-echo NMR. *Pharm Res* **12**:955-964.
135. **Ferrero C, Massuelle D, Jeannerat D, Doelker E.** 2008. Towards elucidation of the drug release mechanism from compressed hydrophilic matrices made of cellulose ethers. I. Pulse-field-gradient spin-echo NMR study of sodium salicylate diffusivity in swollen hydrogels with respect to polymer matrix physical structure. *Journal of Controlled Release* **128**:71-79.
136. **Alvarez-Lorenzo C, Gomez-Amoza JL, Martinez-Pacheco R, Souto C, Concheiro A.** 1999. Microviscosity of hydroxypropylcellulose gels as a basis for prediction of drug diffusion rates. *Int J Pharm* **180**:91-103.
137. **Mustafa MB, Tipton DL, Barkley MD, Russo PS, Blum FD.** 1993. Dye Diffusion in Isotropic and Liquid-Crystalline Aqueous (Hydroxypropyl)Cellulose. *Macromolecules* **26**:370-378.

138. **Bu Z, Russo PS.** 1994. Diffusion of Dextran in Aqueous (Hydroxypropyl)Cellulose. *Macromolecules* **27**:1187-1194.
139. **Romano J, Variano B, Coplan P, Van Roey J, Douville K, Rosenberg Z, Temmerman M, Verstraelen H, Van Bortel L, Weyers S, Mitchnick M.** 2009. Safety and availability of dapivirine (TMC120) delivered from an intravaginal ring. *AIDS Res Hum Retroviruses* **25**:483-488.
140. **Nel A, Smythe S, Young K, Malcolm K, McCoy C, Rosenberg Z, Romano J.** 2009. Safety and pharmacokinetics of dapivirine delivery from matrix and reservoir intravaginal rings to HIV-negative women. *J Acquir Immune Defic Syndr* **51**:416-423.
141. **Nel AM, Haazen W, Nuttal JP, Romano J, Mesquita PMM, Herold BC, Rosenberg ZF.** 2014. Pharmacokinetics and Safety Assessment of Anti-HIV Dapivirine Vaginal Microbicide Rings with Multiple Dosing. *Journal of AIDS & Clinical Research* **05**.
142. **Johnson TJ, Srinivasan P, Albright TH, Watson-Buckheit K, Rabe L, Martin A, Pau CP, Hendry RM, Otten R, McNicholl J, Buckheit R, Jr., Smith J, Kiser PF.** 2012. Safe and sustained vaginal delivery of pyrimidinedione HIV-1 inhibitors from polyurethane intravaginal rings. *Antimicrob Agents Chemother* **56**:1291-1299.
143. **Stieh DJ, Maric D, Kelley ZL, Anderson MR, Hattaway HZ, Beilfuss BA, Rothwangl KB, Veazey RS, Hope TJ.** 2014. Vaginal Challenge with an SIV-Based Dual Reporter System Reveals That Infection Can Occur throughout the Upper and Lower Female Reproductive Tract. *PLoS pathogens* **10**:e1004440.
144. **Carias AM, McCoombe S, McRaven M, Anderson M, Galloway N, Vandergrift N, Fought AJ, Lurain J, Duplantis M, Veazey RS, Hope TJ.** 2013. Defining the Interaction of HIV-1 with the Mucosal Barriers of the Female Reproductive Tract. *J Virol* **87**:11388-11400.
145. **Eckstein P, Jackson MC, Millman N, Sobrero AJ.** 1969. Comparison of vaginal tolerance tests of spermicidal preparations in rabbits and monkeys. *J Reprod Fertil* **20**:85-93.
146. **Doncel GF, Chandra N, Fichorova RN.** 2004. Preclinical assessment of the proinflammatory potential of microbicide candidates. *J Acquir Immune Defic Syndr* **37 Suppl 3**:S174-180.
147. **Trifonova RT, Bajpai M, Pasicznyk JM, Chandra N, Doncel GF, Fichorova RN.** 2007. Biomarkers of leukocyte traffic and activation in the vaginal mucosa. *Biomarkers* **12**:608-622.

148. **Jacques M, Olson ME, Crichlow AM, Osborne AD, Costerton JW.** 1986. The normal microflora of the female rabbit's genital tract. *Canadian journal of veterinary research* **50**:272-274.
149. **Castle PE, Hoen TE, Whaley KJ, Cone RA.** 1998. Contraceptive testing of vaginal agents in rabbits. *Contraception* **58**:51-60.
150. **Clark MR, Kiser PF, Loxley A, McConville C, Malcolm RK, Friend DR.** 2011. Pharmacokinetics of UC781-loaded intravaginal ring segments in rabbits: a comparison of polymer matrices. *Drug Delivery and Translational Research* **1**:238-246.
151. **Vincent KL, Bourne N, Bell BA, Vargas G, Tan A, Cowan D, Stanberry LR, Rosenthal SL, Motamedi M.** 2009. High resolution imaging of epithelial injury in the sheep cervicovaginal tract: a promising model for testing safety of candidate microbicides. *Sexually transmitted diseases* **36**:312-318.
152. **Vargas G, Patrikeev I, Wei J, Bell B, Vincent K, Bourne N, Motamedi M.** 2012. Quantitative assessment of microbicide-induced injury in the ovine vaginal epithelium using confocal microendoscopy. *BMC infectious diseases* **12**:48.
153. **Moss JA, Baum MM, Malone AM, Kennedy S, Kopin E, Nguyen C, Gilman J, Butkyavichene I, Willis RA, Vincent KL, Motamedi M, Smith TJ.** 2012. Tenofovir and tenofovir disoproxil fumarate pharmacokinetics from intravaginal rings. *Aids* **26**:707-710.
154. **Moss JA, Malone AM, Smith TJ, Kennedy S, Kopin E, Nguyen C, Gilman J, Butkyavichene I, Vincent KL, Motamedi M, Friend DR, Clark MR, Baum MM.** 2012. Simultaneous delivery of tenofovir and acyclovir via an intravaginal ring. *Antimicrob Agents Chemother* **56**:875-882.
155. **Veazey RS, Shattock RJ, Klasse PJ, Moore JP.** 2012. Animal models for microbicide studies. *Current HIV research* **10**:79-87.
156. **Otten RA, Adams DR, Kim CN, Jackson E, Pullium JK, Lee K, Grohskopf LA, Monsour M, Butera S, Folks TM.** 2005. Multiple vaginal exposures to low doses of R5 simian-human immunodeficiency virus: strategy to study HIV preclinical interventions in nonhuman primates. *J Infect Dis* **191**:164-173.
157. **Parikh UM, Dobard C, Sharma S, Cong ME, Jia H, Martin A, Pau CP, Hanson DL, Guenthner P, Smith J, Kersh E, Garcia-Lerma JG, Novembre FJ, Otten R, Folks T, Heneine W.** 2009. Complete protection from repeated vaginal simian-human immunodeficiency virus exposures in macaques by a topical gel containing tenofovir alone or with emtricitabine. *J Virol* **83**:10358-10365.

158. **Veazey RS, Shattock RJ, Pope M, Kirijan JC, Jones J, Hu Q, Ketas T, Marx PA, Klasse PJ, Burton DR, Moore JP.** 2003. Prevention of virus transmission to macaque monkeys by a vaginally applied monoclonal antibody to HIV-1 gp120. *Nat Med* **9**:343-346.
159. **Garcia-Lerma JG, Otten RA, Qari SH, Jackson E, Cong ME, Masciotra S, Luo W, Kim C, Adams DR, Monsour M, Lipscomb J, Johnson JA, Delinsky D, Schinazi RF, Janssen R, Folks TM, Heneine W.** 2008. Prevention of rectal SHIV transmission in macaques by daily or intermittent prophylaxis with emtricitabine and tenofovir. *PLoS medicine* **5**:e28.
160. **Radzio J, Aung W, Holder A, Martin A, Sweeney E, Mitchell J, Bachman S, Pau CP, Heneine W, Garcia-Lerma JG.** 2012. Prevention of vaginal SHIV transmission in macaques by a coitally-dependent Truvada regimen. *PLoS One* **7**:e50632.
161. **Andrews CD, Spreen WR, Mohri H, Moss L, Ford S, Gettie A, Russell-Lodrigue K, Bohm RP, Cheng-Mayer C, Hong Z, Markowitz M, Ho DD.** 2014. Long-acting integrase inhibitor protects macaques from intrarectal simian/human immunodeficiency virus. *Science* **343**:1151-1154.
162. **Singer R, Mawson P, Derby N, Rodriguez A, Kizima L, Menon R, Goldman D, Kenney J, Aravantinou M, Seidor S, Gettie A, Blanchard J, Piatak M, Lifson JD, Fernandez-Romero JA, Robbiani M, Zydowsky TM.** 2012. An Intravaginal Ring That Releases the NNRTI MIV-150 Reduces SHIV Transmission in Macaques. *Science translational medicine* **4**.
163. **Aravantinou M, Singer R, Derby N, Calenda G, Mawson P, Abraham CJ, Menon R, Seidor S, Goldman D, Kenney J, Villegas G, Gettie A, Blanchard J, Lifson JD, Piatak M, Jr., Fernandez-Romero JA, Zydowsky TM, Teleshova N, Robbiani M.** 2012. The nonnucleoside reverse transcription inhibitor MIV-160 delivered from an intravaginal ring, but not from a carrageenan gel, protects against simian/human immunodeficiency virus-RT Infection. *AIDS Res Hum Retroviruses* **28**:1467-1475.
164. **Smith JM, Srinivasan P, Teller RS, Lo Y, Dinh CT, Kiser PF, Herold BC.** 2015. Tenofovir Disoproxil Fumarate Intravaginal Ring Protects High-Dose Depot Medroxyprogesterone Acetate-Treated Macaques From Multiple SHIV Exposures. *J Acquir Immune Defic Syndr* **68**:1-5.
165. **van 't Klooster G, Hoeben E, Borghys H, Looszova A, Bouche MP, van Velsen F, Baert L.** 2010. Pharmacokinetics and Disposition of Rilpivirine (TMC278) Nanosuspension as a Long-Acting Injectable Antiretroviral Formulation. *Antimicrob Agents Chemother* **54**:2042-2050.

166. **Jackson AG, Else LJ, Mesquita PM, Egan D, Back DJ, Karolia Z, Ringner-Nackter L, Higgs CJ, Herold BC, Gazzard BG, Boffito M.** 2014. A compartmental pharmacokinetic evaluation of long-acting rilpivirine in HIV-negative volunteers for pre-exposure prophylaxis. *Clinical pharmacology and therapeutics* **96**:314-323.
167. **Spreen W, Ford SL, Chen S, Wilfret D, Margolis D, Gould E, Piscitelli S.** 2014. GSK1265744 Pharmacokinetics in Plasma and Tissue Following Single-Dose Long-Acting (LA) Injectable Administration in Healthy Subjects. *J Acquir Immune Defic Syndr* **67**:481-486.
168. **Spreen W, Williams P, Margolis D, Ford SL, Crauwels H, Lou Y, Gould E, Stevens M, Piscitelli S.** 2014. Pharmacokinetics, Safety, and Tolerability With Repeat Doses of GSK1265744 and Rilpivirine (TMC278) Long-Acting Nanosuspensions in Healthy Adults. *JAIDS Journal of Acquired Immune Deficiency Syndromes* **67**:487-492
410.1097/QAI.0000000000000365.
169. **Andrews CD, Yueh YL, Spreen WR, St Bernard L, Boente-Carrera M, Rodriguez K, Gettie A, Russell-Lodrigue K, Blanchard J, Ford S, Mohri H, Cheng-Mayer C, Hong Z, Ho DD, Markowitz M.** 2015. A long-acting integrase inhibitor protects female macaques from repeated high-dose intravaginal SHIV challenge. *Science translational medicine* **7**:270ra274.
170. **Radzio J, Spreen W, Yueh YL, Mitchell J, Jenkins L, Garcia-Lerma JG, Heneine W.** 2015. The long-acting integrase inhibitor GSK744 protects macaques from repeated intravaginal SHIV challenge. *Science translational medicine* **7**:270ra275.
171. **Spreen WR, Margolis DA, Pottage JC, Jr.** 2013. Long-acting injectable antiretrovirals for HIV treatment and prevention. *Curr Opin HIV AIDS* **8**:565-571.

CHAPTER 2

INTRAVAGINAL RING ELUTING TENOFOVIR DISOPROXIL FUMARATE COMPLETELY PROTECTS MACAQUES FROM MULTIPLE VAGINAL SIMIAN-HIV CHALLENGES

James M. Smith, Rachna Rastogi, Ryan S. Teller, Priya Srinivasan, Pedro M.M. Mesquita, Umadevi Nagaraja, Janet M. McNicholl, R. Michael Hendry, Chuong T. Dinh, Amy Martin, Betsy C. Herold, and Patrick F. Kiser

Proceedings of the National Academy of Sciences 2013; 110(40): 16145-16150

Reprinted with permission from National Academy of Sciences

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

James M. Smith^{a,1}, Rachna Rastogi^{b,1}, Ryan S. Teller^b, Priya Srinivasan^a, Pedro M. M. Mesquita^c, Umadevi Nagaraja^b, Janet M. McNicholl^a, R. Michael Hendry^a, Chuong T. Dinh^a, Amy Martin^a, Betsy C. Herold^c, and Patrick F. Kiser^{b,4,2}

^aLaboratory Branch, Division of HIV/AIDS Prevention, National Center for HIV/AIDS, Viral Hepatitis, Sexually Transmitted Disease, and Tuberculosis Prevention, Coordinating Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30333; ^bDepartment of Bioengineering, University of Utah, Salt Lake City, UT 84112; ^cDepartment of Pediatrics and Microbiology-Immunology, Albert Einstein College of Medicine, Bronx, NY 10461; and ^dDepartment of Biomedical Engineering, Northwestern University, Evanston, IL 60208

Edited by Malcolm A. Martin, National Institute of Allergy and Infectious Diseases, Bethesda, MD, and approved August 19, 2013 (received for review June 25, 2013)

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 1.1×10^4 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.

controlled drug delivery | PrEP | nonhuman primate | pharmacokinetics

The prodrug tenofovir disoproxil fumarate (TDF) and its hydrolysis 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 pharmaceutical ingredient in the first vaginal gel to show partial HIV protection (2). Both molecules have a long safety record and have been widely used in humans with minimal toxicity. The 1% (wt/wt) 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 exposure 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 system [species, use of depo-medroxyprogesterone (DMPA), viral dose, and strain], the pharmacologic properties of the drugs tested,

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 month. The ring protected pigtailed macaques from weekly vaginal simian–human immunodeficiency virus challenges for 4 mo. The sterilizing performance of this drug delivery system supports the concept that an intravaginal ring delivering TDF could be an effective tool for prevention of HIV sexual transmission in women.

Author contributions: J.M.S., R.R., J.M.M., R.M.H., B.C.H., and P.F.K. designed research; R.R., R.S.T., P.S., P.M.M.M., U.N., C.T.D., and A.M. performed research; J.M.S., R.R., R.S.T., P.S., P.M.M.M., A.M., and P.F.K. analyzed data; and J.M.S., R.R., B.C.H., and P.F.K. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

¹J.M.S. and R.R. contributed equally to this work.

²To whom correspondence should be addressed. E-mail: patrick.kiser@northwestern.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1311355110/-DCSupplemental.

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 hydrolytically unstable TDF at therapeutically relevant rates is a challenge. Although TDF is more hydrophobic 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 tubes (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/TFV 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 in the female reproductive tract and lymph nodes. Finally, we tested the ability of the IVR to provide continuous protection against infection by repeated weekly vaginal 50 tissue culture infectious dose (TCID₅₀) SHIV162p3 challenges over multiple monthly IVR changes coupled to ex vivo viral inhibition in cervicovaginal lavage (CVL) samples.

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 wt% TDF showed a 20-fold reduction in drug release rates in vitro from day 1 to day 28 (Fig. 1*C*). A similar effect was seen in vivo in rhesus macaques ($n = 3$) in which more than a 2-log reduction was noted in mean TDF vaginal fluid concentrations from 1.5×10^5 ng/mL (range 2.3×10^4 to 3.3×10^5) on day 3 to 6.3×10^2 ng/mL (range 0 to 2.2×10^3) on day 21 (Fig. 2*A*) and no detectable drug on day 28 (13). The chemical stability of TDF in this device was also unacceptable. Although high levels of TFV were still detected in vaginal fluid at day 28, TDF levels were undetectable.

Given the poor performance of the TDF matrix device, we investigated reservoir IVR designs composed of HPEU tubing with a drug-bearing core sealed by induction-melt welding into a torus (19). The tubing wall acts as rate-controlling membrane 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 long drug-release lag time of more than 20 d (Fig. 2*B*). 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*). A reservoir IVR designed to deliver a TDF formulation with NaCl (14 wt%) as an osmotic excipient was selected by scanning a series of osmoattractants in vitro (Fig. S1*A*). Fig. 1*B* shows human and macaque reservoir IVRs with outer diameters of 55 and 25 mm, respectively. This IVR design delivers in vitro 0.4–4 mg/day TDF over 28 d, with an average release rate of 2.3 ± 0.3 mg/d [macaque reservoir IVR (mean \pm SD; $n = 3$); Fig. 1*C*]. This HPEU-reservoir IVR design eliminated the need for exposing TDF to elevated temperatures required for creation of thermoplastic matrix IVRs. Functionally, we found that the maximum rate of NaCl release preceded the large increase in TDF release (Fig. 2*C*), implying that core hydration was rate-limiting in achieving sufficient drug release. Finally, we found that we could load the wall of IVRs with TDF after ring fabrication by exposing the loaded device to elevated temperature, which decreased the lag time of drug elution (Fig. 2*D*), potentially providing a loading dose of TDF. We were able to obtain release rates of milligram per day quantities of TDF in vitro from the reservoir device and thereby achieve drug levels in vaginal fluid at all time points, exceeding the macaque (4) and clinical correlate of protection (24) of $\sim 1,000$ ng/mL of TFV in vaginal fluid. This was not the case with the inadequate release observed over time from the matrix TDF IVR (Fig. 2*A*) (13). Although the in vitro release rate from the reservoir design was not constant over 28 d, the IVRs generated reproducible TFV levels (Fig. 3*A*) over the first 15 d when the in vitro release rate changed the most, potentially implying early saturation of the system with drug or differences between the in vitro and in vivo TDF release rates.

Drug PK Study in Pigtailed Macaques. We investigated the TDF reservoir IVRs in pigtailed macaques ($n = 6$) in a “28 + 2” day study (28 d of IVR exposure and 2 d after removal) for drug

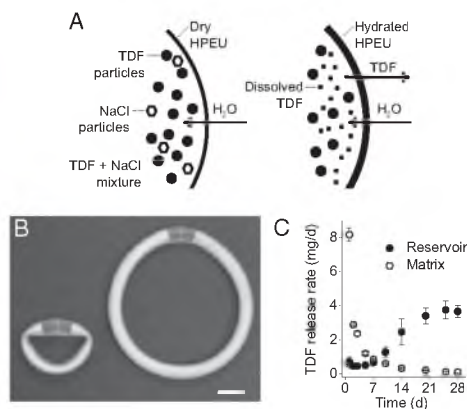


Fig. 1. TDF IVR design and in vitro release. (*A*) Schema depicting mechanism of drug release from TDF reservoir IVR. Vaginal fluid hydrates the swellable HPEU tubing (*Left*) and water is driven into the osmotically active drug–NaCl core along a gradient, resulting in TDF dissolution and elution from the IVR (*Right*). NaCl aids in establishing soluble drug in the core and achieving equilibrium drug release (Fig. 2*B*). (*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 \pm SD. The SDs of some of the collected data are too small to be visualized.

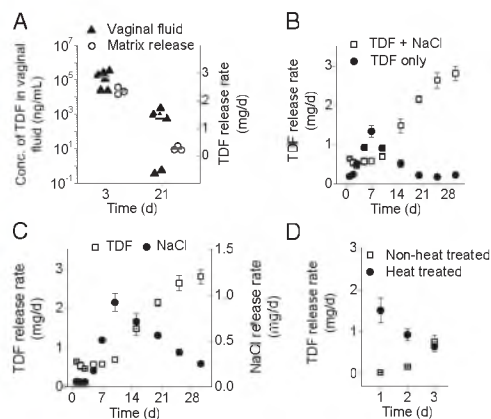


Fig. 2. Matrix release and design evaluation of reservoir TDF IVR. (A) Comparison of TDF released from matrix PEU IVR in vitro on days 3 and 21 to drug concentrations in vaginal fluid of Chinese rhesus macaques ($n = 3$) in a 28 + 3-d study. Each data point represents a single sample and the bar corresponds to the mean for that dataset. (B) In vitro TDF release rate from HPEU reservoir IVR filled with TDF and TDF-NaCl formulation under simulated vaginal conditions ($n = 3$). Data represented as mean \pm SD. (C) In vitro TDF and NaCl release rates from HPEU reservoir IVR under simulated vaginal conditions ($n = 3$). Data represented as mean \pm SD. (D) Comparison of initial in vitro TDF release (up to day 3) from heat-treated and control-unheated TDF IVR ($n = 3$). Heat treatment of the TDF reservoir IVR at 65 °C for 5 d increased TDF release in the first 2 d. Data represented as mean \pm SD.

distribution and concentration in vaginal fluid and tissue (Fig. 3A and B and Fig. S2). The TDF IVR provided high TFV (Fig. 3A, Upper) and TDF (Fig. 3A, Lower) mean vaginal fluid concentrations of 7.2×10^4 ng/mL (range 7.1×10^3 to 3.5×10^5) and 1.0×10^2 ng/mL (range 5 to 6.1×10^3), respectively. The former consistently exceeded the TFV concentration of 1,000 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 from days 3 to 28 and levels seemed similar proximally and distally (Fig. 3A). TFV levels also appeared stable over time in proximal and distal tissues (Fig. 3B), whereas TDF levels were more variable (Fig. S2). This suggests that despite the variable drug release in vitro, the IVRs exhibited a minimal lag time to reach high antiretroviral concentrations in the macaque vaginal vault.

Because TDF hydrolytically converts into TFV, we found variable levels of TDF in swabs and there was no quantifiable TDF detected in vaginal swabs 2 d after ring removal. However, mean TFV levels in swabs were 4.4×10^3 ng/mL (range 2.2×10^2 to 6.9×10^4) and in tissue were 2.9×10^3 ng/g (range 5.4×10^2 to 2.5×10^4) 2 d after ring removal (Fig. 3A and B), exceeding the TFV levels detected after TFV gel application in clinical studies (2, 25). In tissues, the TFV concentrations exceeded the in vitro IC_{50} by ~ 80 times. These high and sustained vaginal fluid and tissue levels of TFV likely reflect the diffusion of luminal pro-drug through the vaginal tissue and its hydrolysis to TFV (8). We do not know the instantaneous in vivo TDF release rate in the macaque vagina. However, as determined by the amount of recovered TDF from the IVRs after use in this PK study, the time-averaged TDF release rate was similar under both in vitro and vivo conditions (Fig. S1B). It has been difficult for others in practice to obtain and sustain these levels of drug in vivo; our

IVR delivered ~ 50 -fold more TDF in vivo compared with the one other TDF IVR in the literature (26). These data and the prolonged half-life of intracellular TFV-DP strongly suggest that this drug-device combination would pharmacologically tolerate removal of the device for hours without significant diminution of drug levels. Furthermore, the high TFV levels in vaginal fluid and undetectable TDF levels 2 d after ring removal suggest the presence of a tissue and/or cellular reservoir of drug that is continuously exchanging with vaginal fluid.

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 1.4×10^3 fmol/ 10^6 cells (4). Mean TFV-DP levels after 14 d of IVR application were highest in vaginal and cervical lymphocytes, 3.3×10^3 fmol/ 10^6 cells (range 1.5×10^3 to 7.5×10^3) and 1.7×10^3 fmol/ 10^6 cells (range 8.4×10^2 to 3.2×10^3), respectively. The mean of the intracellular levels exceeds the TFV-DP IC_{50} of 1.4×10^3 fmol/ 10^6 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 1.3×10^2) and 81 fmol/ 10^6 cells (range 17 to 1.3×10^2), 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.

Repeat SHIV Challenge Study. Based on achieving the TFV concentrations in vivo described previously, we initiated a weekly challenge study in sexually mature, normal cycling pigtailed macaques (12). Six TDF IVR-treated macaques received weekly 50 TCID₅₀ SHIV162p3 vaginal inoculations starting 6 d after IVR insertion (Fig. 4A). Control macaques ($n = 6$ real time and $n = 6$ historical controls) were challenged similarly, of which 11/12 became infected after a median of four exposures to infection, assuming a 7-d eclipse period from time of infection to detection of viral RNA in plasma; peak viral RNA levels were $3.4 \times 10^6 \pm 1.9 \times 10^7$ copies/mL, median \pm SD (Fig. 4B and C). In contrast, all TDF IVR-treated macaques (6/6) remained SHIV viral RNA-negative and -seronegative after 16 weekly exposures spanning 4 mo involving monthly IVR changes. A nonparametric log-rank test was used to compare survival probabilities ($P = 0.0007$, Fig. 4B). Differences in infection probabilities between control and treated animals were statistically significant (Fisher's exact test, $P < 0.0001$). The median survival time among control animals was four exposures, 95% CL (2, 10). All TDF treated macaques remained uninfected after 4 additional weeks of follow-up with the IVRs in place. The infection probability per exposure among control animals was 0.162, 95% CL (0.084–0.271); the infection probability among treated animals was 0.0, 95% CL (0.0–0.038). Estimated efficacy was 100%, 95% CL (80.31–100).

The complete protection observed in the TDF IVR-treated macaques is consistent with the high TFV levels in vaginal fluid samples taken at the time of each ring change [Fig. 5A; 1.8×10^5 ng/mL (mean, range 1.1×10^4 to 6.6×10^5)]. With each monthly IVR change, TDF and TFV levels in vaginal fluid samples remained high (Fig. 5A). Plasma TDF levels were below

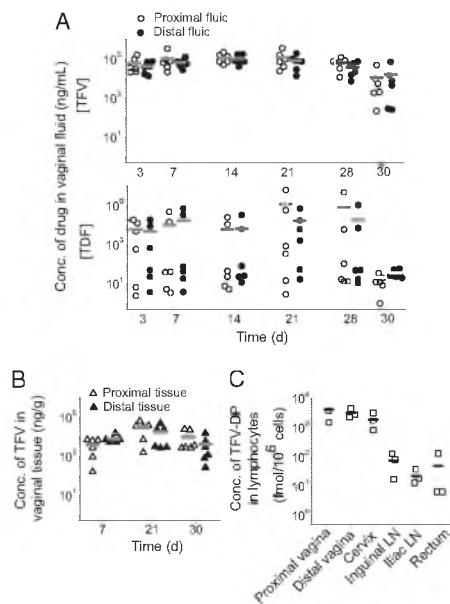


Fig. 3. Drug PK in pigtailed (28 + 2-d) and rhesus (14-d) macaques. Each data point represents a single sample and the bar corresponds to the mean for that dataset. (A) TFV (Upper) and TDF (Lower) concentrations in pigtailed macaque vaginal fluid with 28-d TDF IVR administration ($n = 6$). Samples were collected proximal (open symbols) and distal (closed symbols) to IVR placement for the indicated time points. (B) TFV concentrations in vaginal biopsies from 28-d TDF IVR administration. Samples were collected proximal (open symbols) and distal (closed symbols) to IVR placement for the indicated time points. (C) TFV-DP levels in lymphocytes isolated from the indicated tissues of rhesus macaques after 14-d IVR administration ($n = 3$).

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 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 collected from these two macaques over the course of 28 + 1 d (28-d IVR exposure and 1 d after removal) displayed high antiviral activity against HIV-1 in vitro (range 73–100%) even after a 1:10 dilution (Fig. 5B and C). Importantly, and consistent with the persistence of TFV in vaginal fluid, CVL collected 1 d following removal of the IVR inhibited HIV infection by 86% (Fig. 5B). The anti-HIV activity correlated with both TDF and TFV levels in the CVL (Spearman PK/pharmacodynamic (PD) correlation; TDF: $r = 0.57$, $P = 0.04$; TFV: $r = 0.61$, $P = 0.02$, Fig. 5C).

Potential Behavioral, Pharmacological, and Biological Implications. The choice of TDF over the less potent TFV, combined with the HPEU reservoir IVR delivery system, may overcome several of the behavioral and biological limitations observed to date with vaginal gels and other drug-IVR combinations studied preclinically and clinically. The major reason for embarking on the more

complex drug delivery technology for topical antiretrovirals is to facilitate PrEP use, increase adherence, and thereby improve clinical outcomes. Many studies have shown a general increase in adherence as device duration increases (27, 28). Thus, it is reasonable to assume that it will be easier for women to adhere to long-duration IVR delivery systems compared with daily, episodic, or coitally dependent gels. This is supported by the fact that IVRs have seen excellent product demand and commercial success as a form of birth control in high-income countries and are gaining acceptance in low-income countries (7, 29).

Pharmacologically, sustained drug delivery from reservoir-type devices should provide tissue drug concentrations that are consistently above the level required to protect immune cells resident in and trafficking through the mucosa and submucosa of the genital tract over the time course of mucosal exposure to virus. Furthermore, behaviorally we need to expect and plan for the fact that women will likely periodically remove IVRs either during sex or around the time of menses. This TDF IVR may meet all of these requirements, whereas other drug-ring combination devices have limitations. First, TDF requires significantly lower doses than TFV because of its increased potency, resulting from its capability to more efficiently permeate cell membranes. This property combined with the long TFV-DP intracellular half-life (14) and corresponding low tissue elimination rate may allow for sustained activity for many hours (possibly days) following ring removal. With this IVR, we observed an approximate 0.5-log drop in TFV concentration in vaginal fluid 2 d after ring removal (Fig. 3a). This differs from a NNRTI such as dapivirine or MIV-150, in which the drug can more freely diffuse in and out of cells and can display, in the case of dapivirine matrix IVRs, approximately a 3-log drop in drug concentration from maximum levels to 2 d following IVR removal in humans (16, 22). It is therefore possible that NNRTI matrix rings that

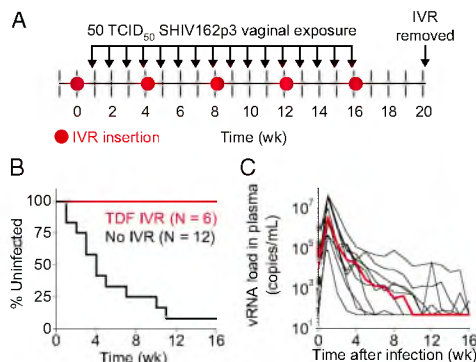


Fig. 4. TDF IVR protects macaques from repeated vaginal viral challenge. (A) Six TDF IVR-treated cycling female macaques received weekly 50 TCID₅₀ SHIV162p3 inoculations starting 6 d after the first IVR insertion. Control macaques ($n = 6$ real time and $n = 6$ historical controls) were challenged similarly. The ring was replaced as shown in red (every 28 d starting 2 d after the fourth virus exposure). Macaques were monitored weekly (until week 20) for presence of SHIV by RT-PCR and confirmed by Western blot. Macaques were defined as infected and exposures discontinued if vRNA was detected in plasma for 2 consecutive weeks. (B) Kaplan-Meier plot showing time to infection for TDF IVR ($n = 6$; red) and control ($n = 6$ real time and 6 historical naive; black) groups (nonparametric log-rank test; $P = 0.0007$). The median number of exposures to infection in the untreated group was four. (C) Plasma viral load kinetics in infected macaques aligned at peak. The red line is the median for all infected macaques (11/12). vRNA, viral RNA.

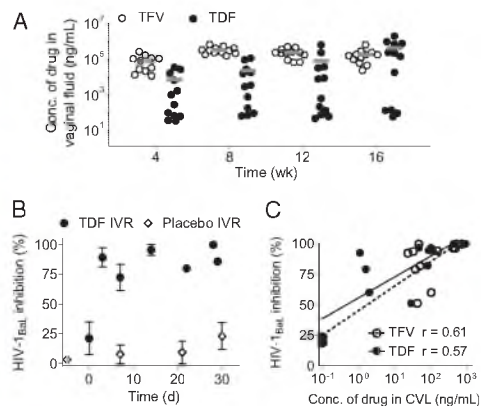


Fig. 5. Drug PK from efficacy study and PK/PD correlation. (A) Monthly TFV (open symbols) and TDF (closed symbols) concentrations in vaginal fluid of pigtailed macaques in the efficacy study with four TDF IVR changes ($n = 6$ macaques). Each data point represents a single sample (proximal or distal to IVR placement) and the bar corresponds to the mean for that dataset ($n = 12$; two samples per animal). (B) To monitor drug PK/PD during the efficacy challenge study, two macaques were treated with TDF IVR (closed symbol) or placebo IVR control (open symbol) and CVL samples were collected at the indicated times in the absence of viral challenge. CVL samples (1:10 dilution) were assayed for drug levels and ability to inhibit HIV-1_{6aa} infection in TZM-bl cells. Results are presented as percentage inhibition of infection relative to control wells; each data point represents the average of two experiments conducted in triplicate ($n = 2$ macaques, mean \pm SEM). (C) Correlation of CVL (diluted 1:10) antiviral activity against HIV-1_{6aa} infection in TZM-bl cells to TDF and TFV concentrations (Spearman PK/PD correlation; TDF, $r = 0.57$; TFV, $r = 0.61$). Samples with TDF or TFV levels below the LLOQ were attributed the value of 0.1 ng/mL so that data could be plotted on a log scale.

deliver compounds that are not retained inside target cells may be more prone to fail in women who remove the device for a sustained time, particularly late in the release curve when release rates are dropping along with tissue levels (16, 22).

HIV sexual transmission occurs in a more complex environment of sexual intercourse, semen, contraceptive hormones, co-infections, and other variables not explored in these studies. Chemoprevention strategies have to be effective in the context of mucosal inflammation prevalent in many women. Sex (30, 31) and intercurrent sexually transmitted infections (32) are associated with an inflammatory environment that may recruit and maintain new target cells in the mucosa and possibly alter drug PK. The drugs and delivery systems used must protect in the context of these factors. Indeed, this effect may have contributed to the observation in Centre for the AIDS Programme of Research in South Africa (CAPRISA) 004 that immune activation was associated with HIV acquisition, even among women using TFV gel (33). Another clear and related advantage of TDF over TFV is the potential for providing protection against HSV-2 acquisition and outbreaks because TDF is \sim 100-fold more potent against HSV-2 (13). In principle, the increased potency of TDF over TFV could allow for more effective protection in the more stringent context of human sexual HIV transmission.

We designed this ring to exceed drug levels in vaginal fluid and tissue that correlated with protection in CAPRISA 004 (24). However, it is possible that lower levels of TDF, because of its greater potency (13), may be protective (24). Future dose escalation/descalation studies are needed to identify the minimal protective TDF dose via vaginal route. It is also important to

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 co-infections 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 trial outcomes, rigorous 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 (AdvanSource 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 inductive tip-forming welder (PlasticWeld Inc.) (19). The open tube was filled with TDF only or with a mixture of TDF (Gilead Sciences) with NaCl [US Pharmacopeia (USP) grade, Spectrum Chemicals] or sodium acetate (anhydrous, USP grade, Spectrum Chemicals) in differing ratios (Fig. S1A). 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 rod. 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 (LPS Industries) and were placed at 65°C for 5 d to load the wall of the IVR with TDF. To fabricate matrix TDF IVRs, TDF-loaded HPEU, ATPU-1 (DSM Biomedical) segments was extruded as described previously (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 2003DOBMONC (PK) and 2004SMIMONC (terminal 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 rhesus macaques of Chinese origin ($n = 3$; $t = 28 + 3$ d). Macaque-sized reservoir IVRs were administered to female pigtailed macaques (TDF IVR, $n = 6$ and placebo $n = 2$, $t = 28 + 2$ d) and female rhesus macaques of Indian origin (TDF IVR, $n = 3$, $t = 14$ d). The latter rhesus macaques were infected with SHIV162p3 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 ketamine. In the pigtailed macaque PK study, IVRs were inserted at day 0 and removed at day 28 and evaluated for residual drug content. Samples were taken at days -7 , 0, 3, 7, 14, 21, 28, and 30. For the terminal 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, 38). TDF and TFV levels in vaginal fluid collected using Weck-Cels (Beaver Visited), CVL (wash of genital tract with 5 mL PBS), and vaginal tissue (days 7, 21, and 30) were determined using liquid chromatography (LC-MS/MS as described (21, 38). Procedures involving euthanasia and evaluation of intracellular TFV-DP were performed as described previously (4).

TDF, TFV, and TFV-DP levels were measured in blood, vaginal fluid, CVL, tissue and lymphocytes by LC-MS/MS methods as described previously (38, 39). The LLOQ for TDF was 1 ng/mL (tissue and blood) and 0.5 ng/mL (vaginal fluid), LLOQ for TFV was 5 ng/mL, and LLOQ for intracellular TFV-DP was 10 ng/mL, which is equivalent to $\sim 13 \text{ fmol}/10^6$ 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 swab 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 TCID₅₀ SHIV162P3 in six TDF-treated and 12 untreated controls (six real time and six historical controls) (39). The first virus exposure was started 6 d after IVR insertion; thereafter, the macaques were inoculated on a weekly basis. The first TDF IVR was replaced on day 30; subsequent IVR changes were done every 28 d, which corresponded to 2 d after the fourth, eighth, and 12th viral inoculation resulting in a total of four

IVR changes in the study period (Fig. 4A). Vaginal swabs for determining drug concentration were collected with every IVR change. Infection status was monitored by RT-PCR and confirmed by serology (ZeptoMetrix) (3, 4). The detection limit of the assay was 50 copies/mL. Positive macaques were defined as having two consecutive positive PCR results above detection limit. Macaques were monitored for 28 d after the last viral inoculation. The antiviral activity of CVL samples diluted 1:10 in PBS was assessed using HIV-1_{ELISA} in the TZM-bl assay as previously detailed (41).

Statistical Methods. Fisher's exact test was used to compare the treated and control groups for number of infections per total number of virus exposures. A nonparametric log-rank test was used to compare survival probability curves. Spearman rank-order correlation coefficients were calculated to assess associations between antiviral activity of CVL and drug levels.

ACKNOWLEDGMENTS. We thank David Garber, James Mitchell, Frank Deyoungs, Sharon Ellis, and Leecesia Jenkins for all animal procedures; Chou-Pong Pao for analysis of drug levels; and Gerardo Garcia-Lerma and Jessica Radzio for providing the historical macaque control data. We acknowledge Gilead Sciences for providing tenofovir disoproxil fumarate. This work 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.

- Holmes D (2012) FDA paves the way for pre-exposure HIV prophylaxis. *Lancet* 380(9839):325.
- Abdool Karim Q, et al.; CAPRISA 004 Trial Group (2010) Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. *Science* 329(5996):1168–1174.
- Parikh UM, et al. (2009) Complete protection from repeated vaginal simian-human immunodeficiency virus exposures in macaques by a topical gel containing tenofovir alone or with emtricitabine. *J Virol* 83(20):10358–10365.
- Dobard C, et al. (2012) Durable protection from vaginal simian-human immunodeficiency virus infection in macaques by tenofovir gel and its relationship to drug levels in tissue. *J Virol* 86(2):718–725.
- Hendrix CW, et al. (2013) MTN-001: Randomized pharmacokinetic cross-over study comparing tenofovir vaginal gel and oral tablets in vaginal tissue and other compartments. *PLoS ONE* 8(1):e55013.
- Amico KR, Mansoor LE, Cornelli A, Torjesen K, van der Straten A (2013) Adherence support approaches in biomedical HIV prevention trials: Experiences, insights and future directions from four multisite prevention trials. *AIDS Behav* 17(6):2143–2155.
- Montgomery ET, et al. (2012) Vaginal ring adherence in sub-Saharan Africa: Expulsion, removal, and perfect use. *AIDS Behav* 16(7):1787–1798.
- Kiser PF, Johnson TJ, Clark JT (2012) State of the art in intravaginal ring technology for topical prophylaxis of HIV infection. *AIDS Rev* 14(1):62–77.
- Singer R, et al. (2012) An intravaginal ring that releases the NNRTI MIV-150 reduces SHIV transmission in macaques. *Sci Transl Med* 4(150):150a123.
- Aravantinou M, et al. (2012) The nonnucleoside reverse transcription inhibitor MIV-160 delivered from an intravaginal ring, but not from a carrageenan gel, protects against simian-human immunodeficiency virus-RT infection. *AIDS Res Hum Retroviruses* 28(11):1467–1475.
- Fetherston SM, et al. (2013) Partial protection against multiple RT-SHIV162P3 vaginal challenge of rhesus macaques by a silicone elastomer vaginal ring releasing the NNRTI MC1220. *J Antimicrob Chemother* 68(2):394–403.
- Otten RA, et al. (2005) Multiple vaginal exposures to low doses of R5 simian-human immunodeficiency virus: Strategy to study HIV preclinical interventions in nonhuman primates. *J Infect Dis* 191(2):164–173.
- Mesquita PM, et al. (2012) Intravaginal ring delivery of tenofovir disoproxil fumarate for prevention of HIV and herpes simplex virus infection. *J Antimicrob Chemother* 67(7):1730–1738.
- Robbins BL, Srinivas RV, Kim C, Bischofberger N, Fridland A (1998) Anti-human immunodeficiency virus activity and cellular metabolism of a potential prodrug of the acyclic nucleoside phosphonate 9-R-(2-phosphonomethoxypropyl)adenine (PMPA), Bis(sopropyl)methylphosphonate (PMPA). *Antimicrob Agents Chemother* 42(3):612–617.
- Chen J, et al. (2012) Biphasic elimination of tenofovir diphosphate and nonlinear pharmacokinetics of zidovudine triphosphate in a microdosing study. *J Acquir Immune Defic Syndr* 61(5):593–599.
- Mesquita P, Kay M, Herold B (2013) Differential intracellular retention of drugs: A tool for rational design of pre-exposure prophylaxis combinations. *20th Conference on Retroviruses and Opportunistic Infections* (CROI Foundation, Atlanta), p 987.
- Yuan LC, Dahl TC, Olyai R (2001) Degradation kinetics of oscarboxylomethyl prodrugs of phosphonates in solution. *Pharm Res* 18(2):234–237.
- Fardis M, Olyai R (2007) Case study: Tenofovir disoproxil fumarate: An oral prodrug of tenofovir prodrugs. *Biotechnology: Pharmaceutical Aspects*, eds Stella VJ, et al. (Springer, New York), pp 1347–1357.
- Johnson TJ, et al. (2012) A 90-day tenofovir reservoir intravaginal ring for mucosal HIV prophylaxis. *Antimicrob Agents Chemother* 56(12):6272–6283.
- Johnson TJ, Gupta KM, Fabian J, Albright TH, Kiser PF (2010) Segmented polyurethane intravaginal rings for the sustained combined delivery of antiretroviral agents dapivirine and tenofovir. *Eur J Pharm Sci* 39(4):203–212.
- Johnson TJ, et al. (2012) Safe and sustained vaginal delivery of pyrimidinedione HIV-1 inhibitors from polyurethane intravaginal rings. *Antimicrob Agents Chemother* 56(3):1291–1299.
- Neil A, et al. (2009) Safety and pharmacokinetics of dapivirine delivery from matrix and reservoir intravaginal rings to HIV-negative women. *J Acquir Immune Defic Syndr* 51(4):416–423.
- Gupta KM, et al. (2008) Polyurethane intravaginal ring for controlled delivery of dapivirine, a nonnucleoside reverse transcriptase inhibitor of HIV-1. *J Pharm Sci* 97(10):4228–4239.
- Karim SS, Kashuba AD, Werner L, Karim QA (2011) Drug concentrations after topical and oral antiretroviral pre-exposure prophylaxis: Implications for HIV prevention in women. *Lancet* 378(9787):279–281.
- Schwartz JL, et al. (2011) A multi-compartment, single and multiple dose pharmacokinetic study of the vaginal candidate microbicide 1% tenofovir gel. *PLoS ONE* 6(10):e25974.
- Moss JA, et al. (2012) Tenofovir and tenofovir disoproxil fumarate pharmacokinetics from intravaginal rings. *AIDS* 26(6):707–710.
- Osterberg L, Blaschke T (2005) Adherence to medication. *N Engl J Med* 353(5):487–497.
- Kruk ME, Schwabbe N (2006) The relation between intermittent dosing and adherence: Preliminary insights. *Clin Ther* 28(12):1989–1995.
- van der Straten A, et al. (2012) High acceptability of a vaginal ring intended as a microbicide delivery method for HIV prevention in African women. *AIDS Behav* 16(7):1775–1786.
- Sharkey DJ, Macpherson AM, Tremellen KP, Robertson SA (2007) Seminal plasma differentially regulates inflammatory cytokine gene expression in human cervical and vaginal epithelial cells. *Mol Hum Reprod* 13(7):491–501.
- Sharkey DJ, Tremellen KP, Jasper MJ, Gemzell-Danielsson K, Robertson SA (2012) Seminal fluid induces leukocyte recruitment and cytokine and chemokine mRNA expression in the human cervix after coitus. *J Immunol* 188(5):2445–2454.
- Rebbapragada A, Kaul R (2007) More than their sum in your parts: The mechanisms that underpin the mutually advantageous relationship between HIV and sexually transmitted infections. *Drug Discov Today Dis Mech* 4(4):237–246.
- Valley-Omar Z, et al. (2012) CAPRISA 004 tenofovir microbicide trial: No impact of tenofovir gel on the HIV transmission bottleneck. *J Infect Dis* 206(1):35–40.
- Chakraborty H, et al. (2001) Viral burden in genital secretions determines male-to-female sexual transmission of HIV-1: A probabilistic empiric model. *AIDS* 15(5):621–627.
- Henning T, et al. (2011) Development of a pigtailed macaque model of sexually transmitted infection/HIV coinfection using Chlamydia trachomatis, Trichomonas vaginalis, and SHIV(SF162P3). *J Med Primatol* 40(4):214–223.
- Radzio J, et al. (2012) Prevention of vaginal SHIV transmission in macaques by a co-italy-dependent Truvada regimen. *PLoS ONE* 7(12):e50632.
- Promadej-Lanier N, et al. (2009) Development and evaluation of a vaginal ring device for sustained delivery of HIV microbicides to non-human primates. *J Med Primatol* 38(4):263–271.
- Moss JA, et al. (2012) Safety and pharmacokinetics of intravaginal rings delivering tenofovir in pig-tailed macaques. *Antimicrob Agents Chemother* 56(11):5952–5960.
- Kukienyik Z, et al. (2009) Effect of mobile phase pH and organic content on LC-MS analysis of nucleoside and nucleotide HIV reverse transcriptase inhibitors. *J Chromatogr Sci* 47(3):265–272.
- Kukienyik Z, et al. (2009) On-line coupling of anion exchange and ion-pair chromatography for measurement of intracellular triphosphate metabolites of reverse transcriptase inhibitors. *J Chromatogr B Analyt Technol Biomed Life Sci* 877(29):3659–3666.
- Keller MJ, et al. (2010) Postcoital bioavailability and antiviral activity of 0.5% PRO 2000 gel: implications for future microbicide clinical trials. *PLoS ONE* 5(1):e8781.

Supporting Information

Smith et al. 10.1073/pnas.1311355110

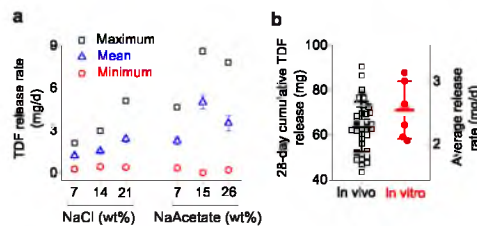


Fig. S1. (A) Maximum, mean, and minimum in vitro tenofovir disoproxil fumarate (TDF) release rates over 30 d from hydrophilic polyether urethane reservoir intravaginal rings (IVRs) filled with TDF and NaCl or NaAc formulations. TDF mass per IVR was kept constant and the NaCl and NaAc amounts varied (TDF:NaCl: 93:7, 86:14, and 79:21; TDF:NaAc: 93:7, 85:15, and 74:26; w:w, $n = 3$). Data represented as mean \pm 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.

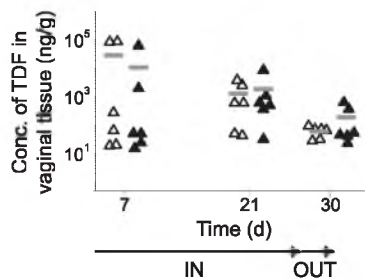


Fig. S2. TDF concentrations in pigtailed macaque vaginal tissue (28 + 2-d pharmacokinetic study). TDF concentrations in pigtailed macaque tissue 28-d TDF IVR administration ($n = 6$). Samples were collected proximal (open symbols) and distal (closed symbols) to IVR placement for the indicated time points. 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

Ryan S. Teller, Rachna Rastogi, Todd J. Johnson, Michael J. Blair, Robert W.

Hitchcock, Patrick F. Kiser

Pharmaceutical Research 2014; 31: 2344-2353

Reprinted with permission from Springer International Publishing

Intravaginal Flux Controlled Pump for Sustained Release of Macromolecules

Ryan S. Teller · Rachna Rastogi · Todd J. Johnson · Michael J. Blair · Robert W. Hitchcock · Patrick F. Kiser

Received: 19 September 2013 / Accepted: 8 February 2014 / Published online: 2 May 2014
© Springer Science+Business Media New York 2014

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-swellaible polymer containing the drug substance. We performed testing both *in vitro* and in rabbits. To ensure vaginal 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 $24 \pm 2 \mu\text{g/day}$ (mean \pm SD) and range of 16 to $42 \mu\text{g/day}$. We observed a slightly lower average release rate *in vivo* of $20 \pm 0.6 \mu\text{g/day}$ (mean \pm 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

Electronic supplementary material The online version of this article (doi:10.1007/s11095-014-1331-5) contains supplementary material, which is available to authorized users.

R. S. Teller · R. Rastogi · T. J. Johnson · M. J. Blair · R. W. Hitchcock · P. F. Kiser (✉)
Department of Bioengineering, University of Utah, 20 S 2030 E, Salt Lake City, Utah 84112, USA
e-mail: patrick.kiser@northwestern.edu

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 (2–5,8), 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 elastomers challenging, and their activity depends on complex intra and inter-molecular structures. Pioneering work by Saltzman (9,10) and Malcolm (11) addressed the need for new solid dosage forms eluting macromolecules to the vaginal mucosa. This work displayed first-order release kinetics proportional to $t^{1/2}$, characterized by an initial burst followed by a continuous reduction in release rate. (9–11) 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 (30–50 wt.%) loading of drug and/or hydrophilic 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 hydrated contents. Model compounds, fluorescently labeled dextran and a model protein (MP) insulin, were selected based on similar molecular weights as candidate antiviral macromolecules (2–5). We investigated several swelling polymers all previously utilized in vaginal formulations (6,21,22), exhibiting a range of swelling rates and swelling extents. We controlled the release rate and duration by altering the identity of the swelling polymers and orifice diameter. As part of this report, we also describe the design of a shape-memory polyether urethane (SMP) retainer device as a non-surgical approach for long-term evaluation of solid dosage forms for PK and safety in the female rabbit reproductive tract. Lastly, we evaluated the FCP design in a 30-day *in vivo* PK study using our SMP vaginal device retainer.

MATERIALS AND METHODS

Preparation of Compressed Pellets

Model compounds, MP, 5-(and-6)-carboxytetramethylrhodamine (CTMR) labelled insulin (see supplemental

materials) or 10 kDa rhodamine B dextran (Sigma Aldrich, St. Louis, MO), at 1 wt.% were mixed with water-swelling polymers; LF, JF and GF hydroxypropyl cellulose (HPC: Klucel Pharm, Mw 95, 140 and 370 kDa respectively; Hercules, Wilmington, DE), hydroxyethyl cellulose (HEC: Natrasol™ 250 Hx; Ashland Chemicals, Covington, KY), or carrageenan (CG: Gelcarin® PC 379; FMC BioPolymer, Philadelphia, PA); using a homogenizer (Qiagen TissueLyser II, Valencia, CA) for 9 min at 30 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).

Fabrication of Rabbit Vaginal Device Retainer

Barium sulfate at 35 wt.% (USP grade, Spectrum Chemicals, Gardena, CA) was compounded with SMP category MM-3520 with glass transition temperature (T_g) 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 Babyplast 6/10P 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 FCP 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 taking into account the SMP vaginal retainer. The inner diameter was chosen to enclose a 100 mg pellet tightly in the chamber. Orifices were manually drilled, and the diameter of the orifices was measured using a stereomicroscope. Digitized

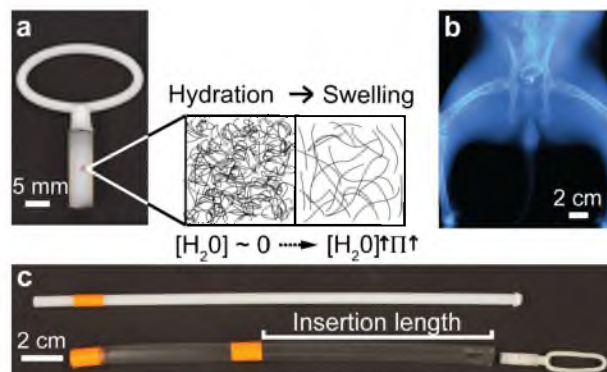


Fig. 1 FCP-SMP retainer device. **a** Photograph showing the FCP-SMP retainer device with two 1.5 mm diameter orifices, containing 1 wt.% MP in GF-HPC with a schematic depicting polymer swelling and chain relaxation. **b** X-ray of a device retained in a rabbit. The SMP retainer device contained 35 wt.% 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-swellaible polymers (CG, IIEC or LF, JF or GF HPC) made with a $\frac{1}{4}$ " 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, IIEC 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^{1/2} graph. (26)

In Vitro Release of Rhodamine B Dextran and MP

Release studies were performed in 25 mM acetate 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 compound into 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 the end 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 set 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 an 18 cm long Tygon® tubing (3606; ID 1/4", OD 5/16"; Cole Parmer, Vernon Hills, Illinois) and a plunger with a flatten end. We inserted the catheter 10 cm beyond the introitus (28) and used the plunger to expel to 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 intramuscular 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 \pm 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 $1.8 \pm 0.1 \text{ s}^{-1/2} >$ HEC $1.4 \pm 0.09 \text{ s}^{-1/2} >$ GF HPC $0.38 \pm 0.02 \text{ s}^{-1/2}$; $p < 0.01$; Fig. 2a). With the CG formulation, $390 \pm 31 \mu\text{g}$ of MP was observed on day 1 in comparison to $110 \pm 39 \mu\text{g}$ and $41 \pm 6.6 \mu\text{g}$ from HEC and GF HPC containing FCPs respectively ($p < 0.01$) resulting in nearly 100% release from CG FCPs in 5 days (CG $(100 \pm 4.0\%) >$ HEC $(55 \pm 4.9\%) >$ GF HPC $(19 \pm 3.1\%)$; $p < 0.01$; Fig. 3a). The average MP release [$t=5$ days] was CG $210 \pm 8.0 \mu\text{g/day}$, HEC $110 \pm 10 \mu\text{g/day}$, and GF HPC $38 \pm 6.2 \mu\text{g/day}$ ($p < 0.01$; 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 $6.8 \pm 1.5 \text{ s}^{-1/2} >$ JF $3.3 \pm 1.1 \text{ s}^{-1/2} >$ LF $2.1 \pm 1.4 \text{ s}^{-1/2}$; $p < 0.05$; 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 $45 \pm 16\%$ released by day 5; in comparison, moderate and high molecular weight HPCs (JF and GF respectively) resulted in $27 \pm 1.4\%$ and $19 \pm 3.1\%$ released in the same duration ($p < 0.05$; Fig. 3c). The average MP release [$t=10$ days] was $69 \pm 18 \mu\text{g/day}$ LF, $51 \pm 11 \mu\text{g/day}$ JF and $41 \pm 4.2 \mu\text{g/day}$ GF ($p=0.07$;

Fig. 3d). Due to the high initial release rate for the LF and JF formulations, the release dropped significantly after day 10 (data not shown).

Effect of Orifice Size on *In Vitro* Release

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 $51 \pm 2.0 \mu\text{g/day}$ respectively ($p < 0.01$; Fig. 4b). Interestingly, a linear relationship between total orifice area and average release was observed ($R^2=0.976$; 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 ($R^2 > 0.98$).

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 $24 \pm 2.1 \mu\text{g/day}$ and 79% cumulative release with a maximum and minimum daily release rates of $42 \pm 8.2 \mu\text{g/day}$ on day 3 and $16 \pm 1.8 \mu\text{g/day}$ on day 20 respectively (Fig. 5). The average release rate of rhodamine B dextran was statistically comparable to MP [$t=10$ days] with $35 \pm 7.0 \mu\text{g/day}$ and $41 \pm 4.2 \mu\text{g/day}$ of rhodamine B dextran and MP respectively ($p=0.2$, Fig. 5 and Fig. S2a and b). A power law curve fit of the cumulative release profiles for both model compounds was performed (29), with exponent values of 0.99, 0.83 (rhodamine B dextran, $R^2=0.99$ over 10 and 30 days respectively), and 0.99 (MP over 10 days, $R^2=0.99$) validating 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 $35 \pm 7.0 \mu\text{g/day}$ from days 1–10, $20 \pm 1.8 \mu\text{g/day}$ from days 10–30, and $24 \pm 2.1 \mu\text{g/day}$ 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 $40 \pm 4.1\%$, $63 \pm 5.9\%$ and $78 \pm 1.9\%$ on days 10, 20 and 30. This was comparable to the cumulative percent release of rhodamine B dextran of $39 \pm 7.8\%$, $54 \pm 7.4\%$ and $79 \pm 6.9\%$ on days 10, 20 and 30 ($p=0.58$, 0.11 and 0.93 for days 10, 20 and 30 respectively). The cumulative percent release of rhodamine B dextran calculated from the

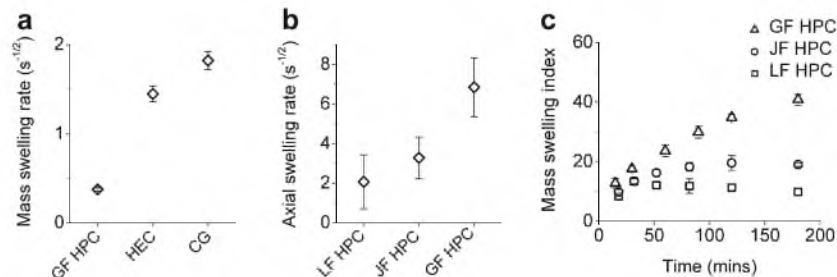


Fig. 2 Swelling properties of hydrophilic polymers. **a** Swelling rate of GF HPC, HEC and CG determined by mass. **b** Swelling rate of LF, JF and GF HPC determined by axial dimensions. **c** Mass swelling indices of LF, JF and GF HPC demonstrating that LF asymptotes the quickest because of dissolution 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 $r=0.90$, $p=0.002$; Fig. S2c).

Furthermore, we performed *in vitro* release on the rhodamine B dextran containing FCPs in two release medias 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. 5). 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 LF, JF and GF HPC as the swelling polymers.

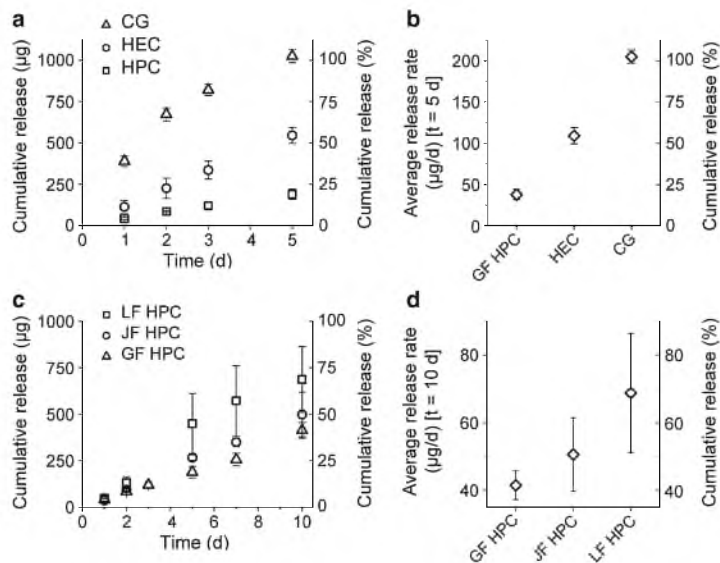
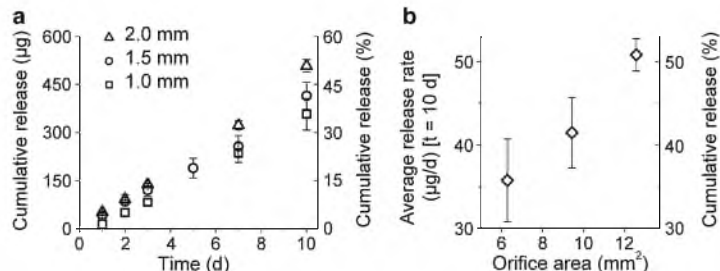


Fig. 4 *In vitro* MP release. **a** Cumulative *in vitro* release of MP from FCPs as a function of orifice size: 1.0, 1.5 and 2.0 mm. **b** *In vitro* average release rate and cumulative release compared to orifice area. A linear correlation between orifice area and average release [t = 10 days] was observed ($R^2 = 0.976$).



In Vivo Rabbit Study

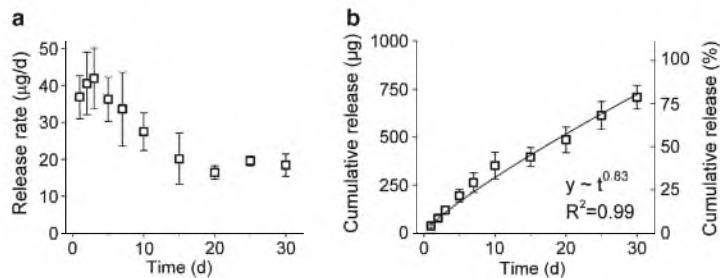
In a 30-day study we tested the safety and PK of rhodamine B dextran loaded FCP-SMP retainer devices in adult female New Zealand White rabbits (Fig. 1). Drug release calculated by residual polymer extraction after 10, 20 and 30 days *in vivo* application was lower compared to *in vitro* samples. We noted a marked difference for all time points ($p=0.020$, 0.002 and <0.0001 for 10, 20 and 30-day arms; Fig. 7a). 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* µg/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 µg/day, $p=0.98$). No irritation or immune cell infiltration was observed in tissue sections from treated animals in comparison to the naïve 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 elementary osmotic pump 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 polyelectrolytes 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. 5 *In vitro* release of rhodamine B dextran in GF HPC. **a** *In vitro* release rate and **(b)** cumulative release of rhodamine B dextran over 30 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. FCPs contained 1 wt.% rhodamine B dextran in GF HPC with two 1.5 mm diameter orifices.



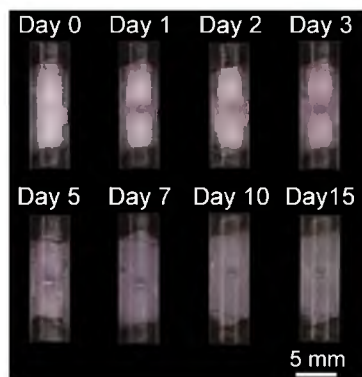


Fig. 6 Mechanism of release from the FCP Photographs showing acrylic MP loaded FCP depicting polymer hydration and release during the initial 15 days. The devices had two 1.5 mm diameter orifices and contained a GF HPC pellet with 1 wt.% MP. Water enters in through the orifices due to a concentration gradient resulting in polymer hydration, expansion and release.

the core pellet. A 100 mg pellet of GF IIPC with 1 wt.% drug enclosed in the FCP resulted in an average release rate of 24 $\mu\text{g}/\text{day}$ over 30 days *in vitro* (Fig. 5).

Our observations support that polymer hydration and swelling rate are critical variables in controlling the drug release rate. We observed dramatic differences in *in vitro* 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 antiprogesterins for cervical ripening. GF IIPC 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 vitro* 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 (JF) molecular weight IIPC displayed higher initial release that decreased beyond day 10, compared to GF (Fig. 3c and d). Since the lower molecular weight LF and JF HPCs swelled less and dissolved more readily (Fig. 2) 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 vitro* studies with the extended high radius of gyration rhodamine B dextran molecule and compact MP molecule demonstrated release rate was comparable and was

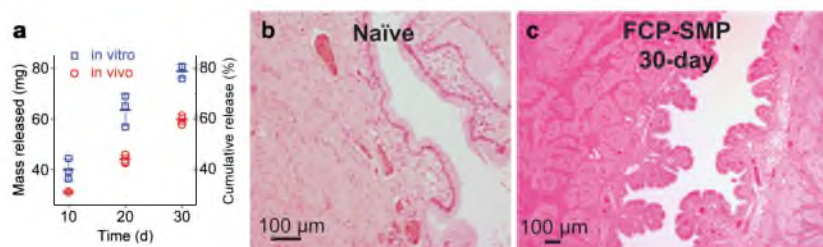


Fig. 7 *In vivo* rabbit study. **a** Mass and percent of polymer pellet released under *in vivo* and *in vitro* conditions determined by residual content. Representative H&E histology sections of vaginal tissue from **(b)** Naïve and **(c)** FCP-SMP device treated animals (30 days). No significant immune 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 predominate 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 suture the device to the vaginal wall to ensure retention (23–25). While peritonitis and inflammation were not reported, it is unknown if the trauma and resulting healing response influences drug release and PK. Therefore, 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 T_g, 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 T_g between room and body temperature facilitated easy insertion through the catheter. We observed attenuated release *in vivo* compared to *in vitro* conditions with a 16% 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 devices, 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 (30–50 wt.%) in hydrophobic 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 vitro* release of BSA, with a daily release profile approximately proportional to $t^{1/2}$ 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

drug release from the FCP and this can lead to macromolecular degradation reactions. Moreover the drug could interact or bind to the swellable polymer and this could reduce the overall drug release. Therefore, the activity and physical stability of the macromolecule released from this system needs to be assessed.

CONCLUSIONS

This study presents the use of 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 demonstrate tens of micrograms release per day of model macromolecular compounds, MP and rhodamine B dextran, from a rabbit-sized device. We observed a small decrease in drug release rate *in vivo* compared to *in vitro*. Further, this study describes an improved *in vivo* methodology for testing vaginal solid dosage forms in the rabbit model that does not require surgery by using an ellipsoidal device for vaginal retention. These results support further development of the FCP for sustained intravaginal delivery of macromolecules. Utilizing the FCP technology in combination with other drug delivery schemes adds to our ability to create drug delivery devices to improve women's health.

ACKNOWLEDGMENTS

The work was supported by grants funded by National Institutes of Health Grant U19AI076980 and CONRAD under a cooperative agreement with USAID (GPO-A-00-08-00005-00). The contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH or USAID. The authors would like to thank Drs. Shweta Ugaonkar and Namdev Shelke, and Anthony Tuitupou for their assistance with the animal studies.

REFERENCES

- Kiser PF, Johnson TJ, Clark JT. State of the art in intravaginal ring technology for topical prophylaxis of HIV infection. *AIDS Rev.* 2012;14(1):62–77.
- Tsai CC, Emau P, Jiang Y, Agy MB, Shattock RJ, Schmidt A, *et al.* Cyanovirin-N inhibits AIDS virus infections in vaginal transmission models. *AIDS Res Hum Retrovir.* 2004;20(1):11–8.
- Veazey RS, Ling B, Green LC, Ribka EP, Lifson JD, Piatak Jr M, *et al.* Topically applied recombinant chemokine analogues fully protect macaques from vaginal simian-human immunodeficiency virus challenge. *J Infect Dis.* 2009;199(10):1525–7.
- O'Keefe BR, Vojdani F, Buffa V, Shattock RJ, Montefiori DC, Bakke J, *et al.* Scaleable manufacture of HIV-1 entry inhibitor griffithsin and validation of its safety and efficacy as a topical microbicide component. *Proc Natl Acad Sci U S A.* 2009;106(15):6099–104.
- Welch BD, Francis JN, Redman JS, Paul S, Weinstock MT, Reeves JD, *et al.* Design of a potent D-peptide HIV-1 entry inhibitor with a strong barrier to resistance. *J Virol.* 2010;84(21):11235–44.
- Marais D, Gawarecki D, Allan B, Ahmed K, Altini L, Cassim N, *et al.* The effectiveness of Carraguard, a vaginal microbicide, in protecting women against high-risk human papillomavirus infection. *Antivir Ther.* 2011;16(8):1219–26.
- Curran RM, Donnelly L, Morrow RJ, Fraser C, Andrews G, Cranage M, *et al.* Vaginal delivery of the recombinant HIV-1 clade-C trimeric gp140 envelope protein CN54gp140 within novel rheologically structured vehicles elicits specific immune responses. *Vaccine.* 2009;27(48):6791–8.
- Conn PM, Crowley Jr WF. Gonadotropin-releasing hormone and its analogs. *Annu Rev Med.* 1994;45:391–405.
- Radomsky ML, Whaley KJ, Cone RA, Saltzman WM. Controlled vaginal delivery of antibodies in the mouse. *Biol Reprod.* 1992;47(1):133–40.
- Saltzman WM, Sherwood JK, Adams DR, Haller P. Long-term vaginal antibody delivery: delivery systems and Biodistribution. *Biotechnol Bioeng.* 2000;67(3):253–64.
- Morrow RJ, Woolfson AD, Donnelly L, Curran R, Andrews G, Katinger D, *et al.* Sustained release of proteins from a modified vaginal ring device. *Eur J Pharm Biopharm.* 2011;77(1):3–10.
- Buckheit Jr RW, Roberson JL, Lackman-Smith C, Wyatt JR, Vickers TA, Ecker DJ. Potent and specific inhibition of HIV envelope-mediated cell fusion and virus binding by G quartet-forming oligonucleotide (ISIS 5320). *AIDS Res Hum Retrovir.* 1994;10(11):1497–506.
- Woodrow KA, Cu Y, Booth CJ, Saucier-Sawyer JK, Wood MJ, Saltzman WM. Intravaginal gene silencing using biodegradable polymer nanoparticles densely loaded with small-interfering RNA. *Nat Mater.* 2009;8(6):526–33.
- Mahalingam A, Geonotti AR, Balzarini J, Kiser PF. Activity and safety of synthetic lectins based on benzoboroxole-functionalized polymers for inhibition of HIV entry. *Mol Pharm.* 2011;8(6):2465–75.
- Amico KR, Mansoor LE, Corneil A, Torjesen K, van der Straten A. Adherence support approaches in biomedical HIV prevention trials: experiences, insights and future directions from four multisite prevention trials. *AIDS Behav.* 2013;17:2143–55.
- Kruk MF, Schwalbe N. The relation between intermittent dosing and adherence: preliminary insights. *Clin Ther.* 2006;28(12):1989–95.
- Novak A, de la Loge C, Abetz L, van der Meulen EA. The combined contraceptive vaginal ring, NuvaRing: an international study of user acceptability. *Contraception.* 2003;67(3):187–94.
- van der Straten A, Montgomery ET, Cheng H, Wegner L, Masenga G, von Mollendorf C, *et al.* High acceptability of a vaginal ring intended as a microbicide delivery method for HIV prevention in African women. *AIDS Behav.* 2012;16(7):1775–86.
- Langer R, Hsieh DST, Rhine W, Folkman J. Control of release kinetics of macromolecules from polymers. *J Membr Sci.* 1980;7(3):333–50.
- Amsden BG, Cheng Y-L, Goosen MFA. A mechanistic study of the release of osmotic agents from polymeric monoliths. *J Control Release.* 1994;30:45–56.
- Tien D, Schnaare RL, Kang F, Cohl G, McCormick TJ, Moench TR, *et al.* In vitro and in vivo characterization of a potential universal placebo designed for use in vaginal microbicide clinical trials. *AIDS Res Hum Retrovir.* 2005;21(10):845–53.
- Ham AS, Ugaonkar SR, Shi L, Buckheit KW, Lakouagna H, Nagaraja U, *et al.* Development of a combination microbicide gel

- formulation containing IQP-0528 and tenofovir for the prevention of HIV infection. *J Pharm Sci.* 2012;101(4):1423–35.
23. Chien YW, Mares SE, Berg J. Controlled drug release from polymeric delivery devices. III. In vitro in vivo correlation for intravaginal release of ethynodiol diacetate from silicone devices in rabbits. *J Pharm Sci.* 1975;64(11):1776–81.
 24. Clark MR, Kiser PF, Loxley A, McConville C, Malcolm RK, Friend DR. Pharmacokinetics of UC781-loaded intravaginal ring segments in rabbits: a comparison of polymer matrices. *Drug Deliv Transl Res.* 2011;1(3):238–46.
 25. Clark MR, Johnson TJ, McCabe RT, Clark JT, Tuitupou A, Elgendy H, *et al.* A hot-melt extruded intravaginal ring for the sustained delivery of the antiretroviral microbicide UC781. *J Pharm Sci.* 2011;101(2):576–87.
 26. Talukdar MM, Kinget R. Swelling and drug release behaviour of xanthan gum matrix tablets. *Int J Pharm.* 1995;120(1):63–72.
 27. Owen DH, Katz DF. A vaginal fluid simulant. *Contraception.* 1999;59(2):91–5.
 28. Castle PE, Hoen TE, Whaley KJ, Cone RA. Contraceptive testing of vaginal agents in rabbits. *Contraception.* 1998;58(1):51–60.
 29. Siepmann J, Peppas NA. Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). *Adv Drug Deliv Rev.* 2001;48(2–3):139–57.
 30. Jacques M, Olson ME, Crichlow AM, Osborne AD, Costerton JW. The normal microflora of the female rabbit's genital tract. *Can J Vet Res.* 1986;50(2):272–4.
 31. Theeuwes F. Elementary osmotic pump. *J Pharm Sci.* 1975;64(12):1987–91.
 32. Horkay F, Tasaki I, Bassar PJ. Osmotic swelling of polyacrylate hydrogels in physiological salt solutions. *Biomacromolecules.* 2000;1(1):84–90.
 33. Rastogi R, Teller RS, Mesquita PM, Herold BC, Kiser PF. Osmotic pump tablets for delivery of antiretrovirals to the vaginal mucosa. *Antiviral Res.* 2013;100(1):255–8.
 34. Eckstein P, Jackson MC, Millman N, Sobrero AJ. Comparison of vaginal tolerance tests of spermicidal preparations in rabbits and monkeys. *J Reprod Fertil.* 1969;20(1):85–93.
 35. Doncel GF, Chandra N, Fichorova RN. Preclinical assessment of the proinflammatory potential of microbicide candidates. *J Acquir Immune Defic Syndr.* 2004;37 Suppl 3:S174–80.
 36. Trifonova RT, Bajpai M, Pasichnyk JM, Chandra N, Doncel GF, Fichorova RN. Biomarkers of leukocyte traffic and activation in the vaginal mucosa. *Biomarkers.* 2007;12(6):608–22.
 37. Doncel GF, Clark MR. Preclinical evaluation of anti-HIV microbicide products: new models and biomarkers. *Antiviral Res.* 2010;88 Suppl 1:S10–8.
 38. Harrison PF, Hemmerling A, Romano J, Whaley KJ, Young Holt B. Developing multipurpose reproductive health technologies: an integrated strategy. *AIDS Res Treat.* 2013;2013:790154.
 39. Johnson TJ, Clark MR, Albright TH, Nebeker JS, Tuitupou AL, Clark JT, *et al.* A 90-day Tenofovir reservoir intravaginal ring for mucosal HIV prophylaxis. *Antimicrob Agents Chemother.* 2012;56(12):6272–83.
 40. Johnson TJ, Gupta KM, Fabian J, Albright TH, Kiser PF. Segmented polyurethane intravaginal rings for the sustained combined delivery of antiretroviral agents dapivirine and Tenofovir. *Eur J Pharm Sci.* 2010;39(4):203–12.
 41. Woolfson AD, Malcolm RK, Morrow RJ, Toner CF, McCullagh SD. Intravaginal ring delivery of the reverse transcriptase inhibitor TMC 120 as an HIV microbicide. *Int J Pharm.* 2006;325(1–2):82–9.

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'-Discuccinimidyl 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 NaHCO_3 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.

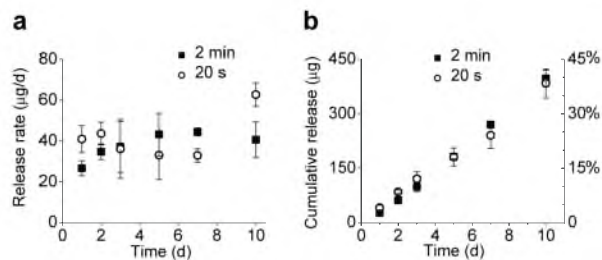


Figure S1. (a) *In vitro* release rate and (b) cumulative release of MP from FCPs comparing different compression times for pellet fabrication: 20 s and 2 min.

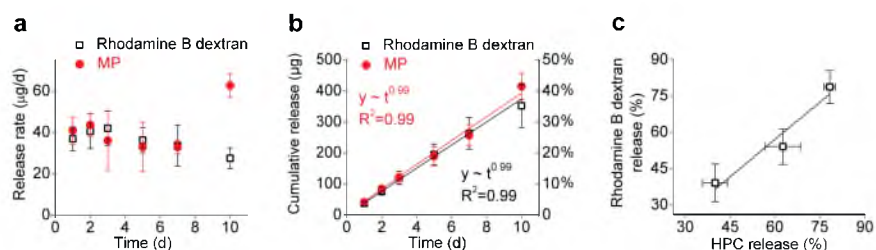


Figure S2. (a) *In vitro* release rate and (b) cumulative release of MP and rhodamine B dextran over 10 days. The line on the cumulative release graph (b) represents a power law curve fit of the data with the associated exponent and R^2 values. (c) Cumulative rhodamine B dextran release calculated from *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).














Study #	Device	#	# Lost	# Retrieved (t =14 days)	
				Uterovaginal	Urovaginal
I		3	1		
		3	0		
II		12	4	 x5 	 
III		8	0	 x8	

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.

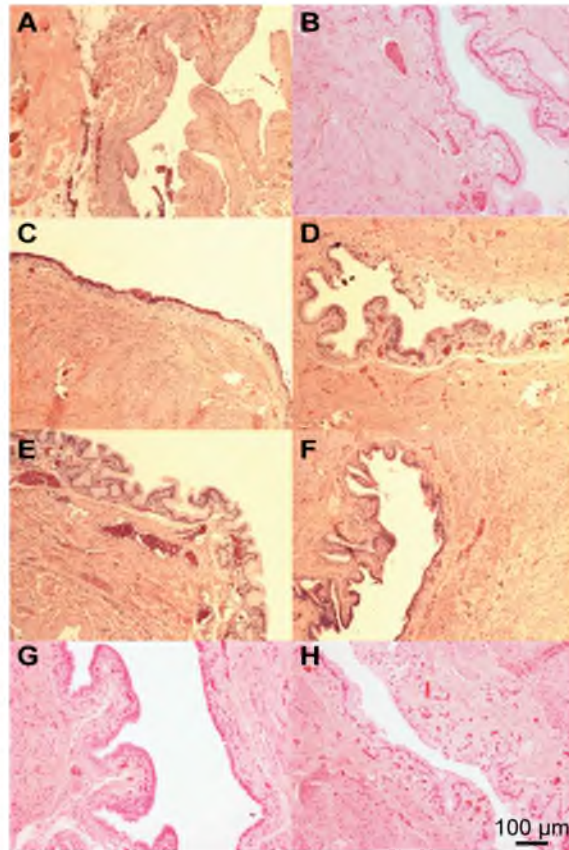


Figure S4. Representative images of H&E stained vaginal sections from the different study groups: (A, B) naïve, (C, D) T-device, (E, F) O-device and (G, H) oval-SMP device. Epithelial disruption noted for panel D.



Figure S5. Time dependent x-ray of barium sulphate loaded oval retainer devices attached to a drug delivery segment during a 14 day study.

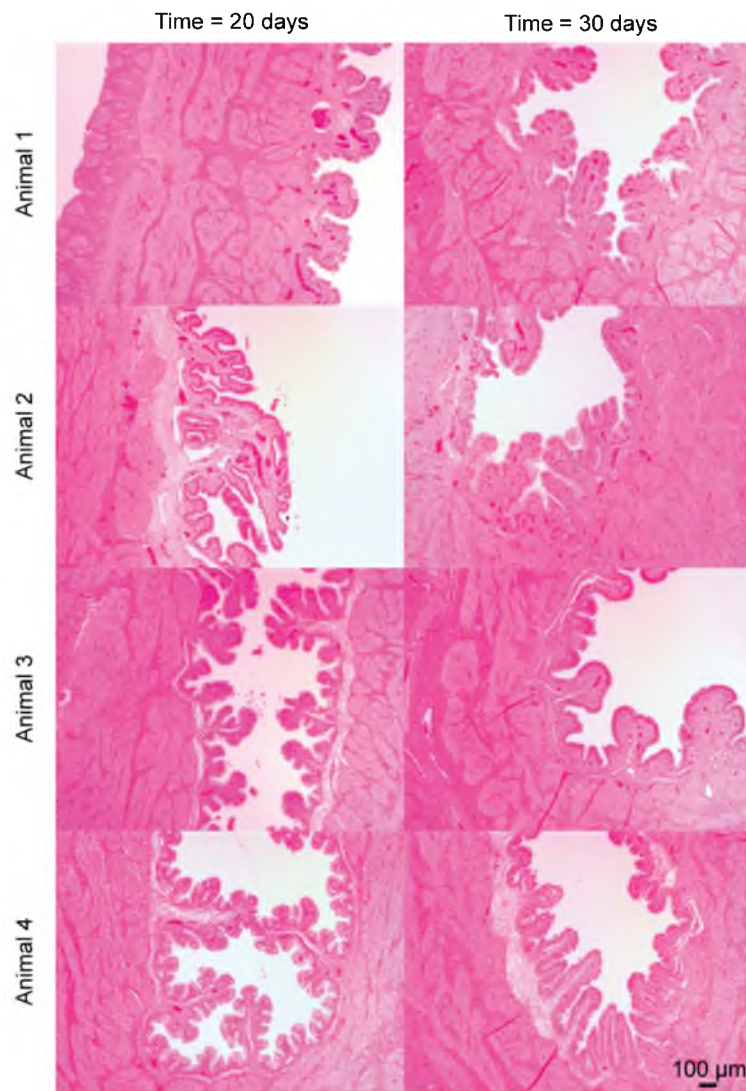


Figure S6. Representative images of H&E stained vaginal sections from the 20 and 30 day endpoints evaluating the FCP-oval SMP retainer device.

CHAPTER 4

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

Ryan S. Teller, David C. Malaspina, Rachna Rastogi, Justin T. Clark, Igal
Szeifer, and Patrick F. Kiser

Manuscript submitted to The Journal of Controlled Release

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 $\mu\text{g}/\text{day}$ 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 *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 μ L 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 μ m 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_o is the initial drug concentration in the HPC solution, and A is the exposed area. All data are presented as the mean \pm SD.

$$M_t = 2 C_o A \sqrt{Dt/\pi} \quad (\text{Eq. 4.1})$$

4.3.5 Drug content analysis

IQP-0528 (25), TDF (26), and TFV (12) concentration for extraction and *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 μ m; 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):

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

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):

$$D_k = D_{k,eq} \exp\left(-\beta_k \left(1 - \frac{C_{water}}{C_{water, eq}}\right)\right) \quad (\text{Eq. 4.3})$$

where C_k are the concentrations of each species (k), D_k are diffusion coefficients, and β_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 $D_{water, eq}$.

$e_q = 5.6 \times 10^{-10} \text{ m}^2/\text{s}$. 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 ~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 $\mu\text{g}/\text{d}$, respectively. This corresponded to peak drug release rates of 900 ± 38 , 670 ± 29 , 480 ± 110 , and 224 ± 40 $\mu\text{g}/\text{d}$ 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 $\mu\text{g}/\text{d}$, and calculated cumulative drug releases of 93 ± 1.8 , 75 ± 1.8 , 54 ± 2.3 , and $30 \pm 2.9\%$ 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 $99 \pm 6.8\%$ 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 ($R^2 = 0.992,$

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 ($y = a \cdot t^b$ 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 $\pm 1.7\%$ 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 $\pm 6.9\%$ 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 $\mu\text{g}/\text{d}$, respectively. The 30-day cumulative release was 68 ± 2.0 , 77 ± 3.3 , 74 ± 3.6 , 81 ± 0.93 , and $72 \pm 1.9\%$ 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 ± 0.06 , 2.7 ± 0.08 , 2.6 ± 0.04 , 3.0 ± 0.20 , 3.1 ± 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 $\sim 100,000x$ 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 $\mu\text{g/mL}$ 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 $\pm 2.0\%$ 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 $\pm 3.7\%$ 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 $\pm 1.3\%$ 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 $\pm 21.3\%$ 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 $60 \pm 1.9\%$, 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 $\pm 4.5\%$, 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 $\mu\text{g/d}$ 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_{HPC} and β_{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-swallowable 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

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

4.8 References

1. **UNAIDS.** 2013. Report on the global AIDS epidemic. Joint United Nations Programme on HIV/AIDS (UNAIDS).
2. **Amico KR, Mansoor LE, Corneli A, Torjesen K, van der Straten A.** 2013. Adherence support approaches in biomedical HIV prevention trials: experiences, insights and future directions from four multisite prevention trials. *AIDS Behav* **17**:2143-2155.
3. **Baeten JM, Grant R.** 2013. Use of Antiretrovirals for HIV Prevention: What Do We Know and What Don't We Know? *Curr Hiv-Aids Rep* **10**:142-151.
4. **Abdool Karim Q, Abdool Karim SS, Frohlich JA, Grobler AC, Baxter C, Mansoor LE, Kharsany AB, Sibeko S, Mlisana KP, Omar Z, Gengiah TN, Maarschalk S, Arulappan N, Mlotshwa M, Morris L, Taylor D, Group CT.** 2010. Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. *Science* **329**:1168-1174.
5. **Marrazzo J, Ramjee G, Nair G, Palanee T, Mkhize B, Nakabiito C, Taljaard M, Piper J, Gomez Feliciano K, Chirenje M.** 2013. Pre-exposure Prophylaxis for HIV in Women: Daily Oral Tenofovir, Oral Tenofovir/Emtricitabine, or Vaginal Tenofovir Gel in the VOICE Study (MTN 003) (Paper 26LB), 20th Conference on Retroviruses and Opportunistic Infections, Atlanta, Georgia.
6. **Karim SSA, Kashuba ADM, Werner L, Karim QA.** 2011. Drug concentrations after topical and oral antiretroviral pre-exposure prophylaxis: implications for HIV prevention in women. *The Lancet* **378**:279-281.
7. **Carias AM, McCoombe S, McRaven M, Anderson M, Galloway N, Vandergrift N, Fought AJ, Lurain J, Duplantis M, Veazey RS, Hope TJ.** 2013. Defining the Interaction of HIV-1 with the Mucosal Barriers of the Female Reproductive Tract. *J Virol* **87**:11388-11400.
8. **Kiser PF, Johnson TJ, Clark JT.** 2012. State of the art in intravaginal ring technology for topical prophylaxis of HIV infection. *AIDS Rev* **14**:62-77.
9. **Brache V, Faundes A.** 2010. Contraceptive vaginal rings: a review. *Contraception* **82**:418-427.
10. **Smith DJ, Wakasiaka S, Hoang TD, Bwayo JJ, Del Rio C, Priddy FH.** 2008. An evaluation of intravaginal rings as a potential HIV prevention device in urban Kenya: behaviors and attitudes that might influence

- uptake within a high-risk population. *J Womens Health (Larchmt)* **17**:1025-1034.
11. **van der Straten A, Montgomery ET, Cheng H, Wegner L, Masenga G, von Mollendorf C, Bekker L, Ganesh S, Young K, Romano J, Nel A, Woodsong C.** 2012. High acceptability of a vaginal ring intended as a microbicide delivery method for HIV prevention in African women. *AIDS Behav* **16**:1775-1786.
 12. **Johnson TJ, Clark MR, Albright TH, Nebeker JS, Tuitupou AL, Clark JT, Fabian J, McCabe RT, Chandra N, Doncel GF, Friend DR, Kiser PF.** 2012. A 90-Day Tenofovir Reservoir Intravaginal Ring for Mucosal HIV Prophylaxis. *Antimicrob Agents Chemother* **56**:6272-6283.
 13. **Chien YW, Lambert HJ, Grant DE.** 1974. Controlled drug release from polymeric devices. I. Technique for rapid in vitro release studies. *Journal of Pharmaceutical Sciences* **63**:365-369.
 14. **Malcolm K, Woolfson D, Russell J, Tallon P, McAuley L, Craig D.** 2003. Influence of silicone elastomer solubility and diffusivity on the in vitro release of drugs from intravaginal rings. *J Control Release* **90**:217-225.
 15. **Morrow RJ, Woolfson AD, Donnelly L, Curran R, Andrews G, Katinger D, Malcolm RK.** 2011. Sustained release of proteins from a modified vaginal ring device. *Eur J Pharm Biopharm* **77**:3-10.
 16. **Moss JA, Malone AM, Smith TJ, Kennedy S, Kopin E, Nguyen C, Gilman J, Butkyavichene I, Vincent KL, Motamedi M, Friend DR, Clark MR, Baum MM.** 2012. Simultaneous delivery of tenofovir and acyclovir via an intravaginal ring. *Antimicrob Agents Chemother* **56**:875-882.
 17. **Rastogi R, Teller RS, Mesquita PM, Herold BC, Kiser PF.** 2013. Osmotic pump tablets for delivery of antiretrovirals to the vaginal mucosa. *Antiviral Res* **100**:255-258.
 18. **Baum MM, Butkyavichene I, Gilman J, Kennedy S, Kopin E, Malone AM, Nguyen C, Smith TJ, Friend DR, Clark MR, Moss JA.** 2012. An intravaginal ring for the simultaneous delivery of multiple drugs. *Journal of Pharmaceutical Sciences* **101**:2833-2843.
 19. **Moss JA, Malone AM, Smith TJ, Kennedy S, Nguyen C, Vincent KL, Motamedi M, Baum MM.** 2013. Pharmacokinetics of a multipurpose pod-intravaginal ring simultaneously delivering five drugs in an ovine model. *Antimicrob Agents Chemother* **57**:3994-3997.
 20. **Moss JA, Srinivasan P, Smith TJ, Butkyavichene I, Lopez G, Brooks AA, Martin A, Dinh CT, Smith JM, Baum MM.** 2014. Pharmacokinetics and preliminary safety study of pod-intravaginal rings delivering

- antiretroviral combinations for HIV prophylaxis in a macaque model. *Antimicrob Agents Chemother* **58**:5125-5135.
21. **Theeuwes F.** 1975. Elementary osmotic pump. *Journal of Pharmaceutical Sciences* **64**:1987-1991.
 22. **Amkraut A, Eckenhoff JB, Nichols K.** 1989. Osmotic delivery of peptides and macromolecules. *Advanced Drug Delivery Reviews* **4**:255-276.
 23. **Teller RS, Rastogi R, Johnson TJ, Blair MJ, Hitchcock RW, Kiser PF.** 2014. Intravaginal flux controlled pump for sustained release of macromolecules. *Pharm Res* **31**:2344-2353.
 24. **Crank J.** 1975. *The Mathematics of Diffusion*, 2nd ed. Clarendon Press, Oxford.
 25. **Johnson TJ, Srinivasan P, Albright TH, Watson-Buckheit K, Rabe L, Martin A, Pau CP, Hendry RM, Otten R, McNicholl J, Buckheit R, Jr., Smith J, Kiser PF.** 2012. Safe and sustained vaginal delivery of pyrimidinedione HIV-1 inhibitors from polyurethane intravaginal rings. *Antimicrob Agents Chemother* **56**:1291-1299.
 26. **Mesquita PM, Rastogi R, Segarra TJ, Teller RS, Torres NM, Huber AM, Kiser PF, Herold BC.** 2012. Intravaginal ring delivery of tenofovir disoproxil fumarate for prevention of HIV and herpes simplex virus infection. *J Antimicrob Chemother* **67**:1730-1738.
 27. **Fujita H.** 1961. Diffusion in polymer-diluent systems, p. 1-47, *Fortschritte Der Hochpolymeren-Forschung*, vol. 3/1. Springer Berlin Heidelberg.
 28. **Siepmann J, Kranz H, Bodmeier R, Peppas NA.** 1999. HPMC-matrices for controlled drug delivery: a new model combining diffusion, swelling, and dissolution mechanisms and predicting the release kinetics. *Pharm Res* **16**:1748-1756.
 29. **Siepmann J, Podual K, Sriwongjanya M, Peppas NA, Bodmeier R.** 1999. A new model describing the swelling and drug release kinetics from hydroxypropyl methylcellulose tablets. *J Pharm Sci* **88**:65-72.
 30. **Kaunisto E, Abrahmsen-Alami S, Borgquist P, Larsson A, Nilsson B, Axelsson A.** 2010. A mechanistic modelling approach to polymer dissolution using magnetic resonance microimaging. *J Control Release* **147**:232-241.
 31. **Zhu XX, Wang F, Nivaggioli T, Winnik MA, Macdonald PM.** 1993. Poly(methyl methacrylate) film dissolution and solvent diffusion coefficients: correlations determined using laser interferometry-

- fluorescence quenching and pulsed-gradient spin-echo NMR spectroscopy. *Macromolecules* **26**:6397-6402.
32. **Matsukawa S, Ando I.** 1996. A study of self-diffusion of molecules in polymer gel by pulsed-gradient spin-echo H-1 NMR. *Macromolecules* **29**:7136-7140.
 33. **Wu N, Wang LS, Tan DC, Moochhala SM, Yang YY.** 2005. Mathematical modeling and in vitro study of controlled drug release via a highly swellable and dissoluble polymer matrix: polyethylene oxide with high molecular weights. *J Control Release* **102**:569-581.
 34. **Young Holt B, Romano J, Manning J, Hemmerling A, Shields W, Vyda L, Lusti-Narasimhan M.** 2014. Ensuring successful development and introduction of multipurpose prevention technologies through an innovative partnership approach. *BJOG: An International Journal of Obstetrics & Gynaecology* **121**:3-8.
 35. **Johnson TJ, Gupta KM, Fabian J, Albright TH, Kiser PF.** 2010. Segmented polyurethane intravaginal rings for the sustained combined delivery of antiretroviral agents dapivirine and tenofovir. *Eur J Pharm Sci* **39**:203-212.
 36. **Clark JT, Clark MR, Shelke NB, Johnson TJ, Smith EM, Andreasen AK, Nebeker JS, Fabian J, Friend DR, Kiser PF.** 2014. Engineering a segmented dual-reservoir polyurethane intravaginal ring for simultaneous prevention of HIV transmission and unwanted pregnancy. *PLoS One* **9**:e88509.
 37. **Pirrone V, Thakkar N, Jacobson JM, Wigdahl B, Krebs FC.** 2011. Combinatorial approaches to the prevention and treatment of HIV-1 infection. *Antimicrob Agents Chemother* **55**:1831-1842.
 38. **Mesquita PM, Srinivasan P, Johnson TJ, Rastogi R, Evans-Strickfaden T, Kay MS, Buckheit KW, Buckheit RW, Jr., Smith JM, Kiser PF, Herold BC.** 2013. Novel preclinical models of topical PrEP pharmacodynamics provide rationale for combination of drugs with complementary properties. *Retrovirology* **10**:113.

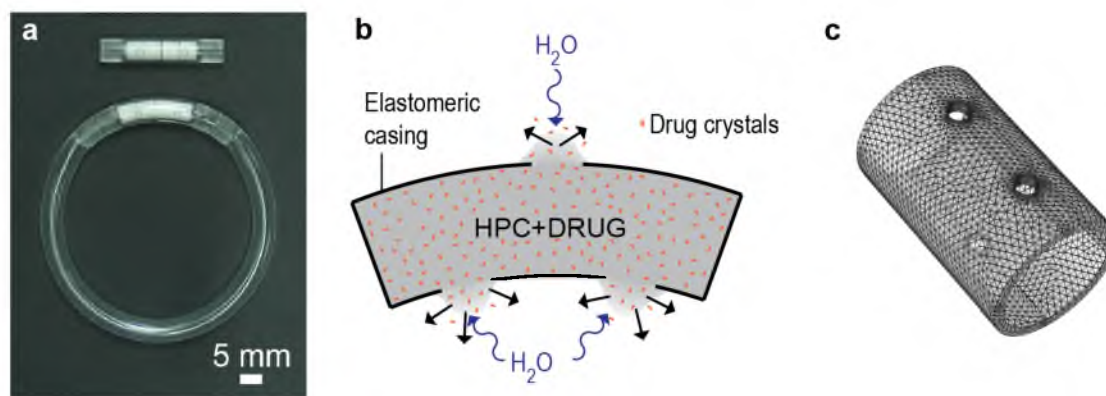


Figure 4.1. Photograph and schematics of the flux controlled pump-IVR. (a) The photograph depicts an FCP (top), and an FCP integrated into an IVR (bottom). The FCP contains compressed pellets of drug and hydrophilic polymer within a sealed polymer tube with orifices. (b) Schematic of drug (orange dots) 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).

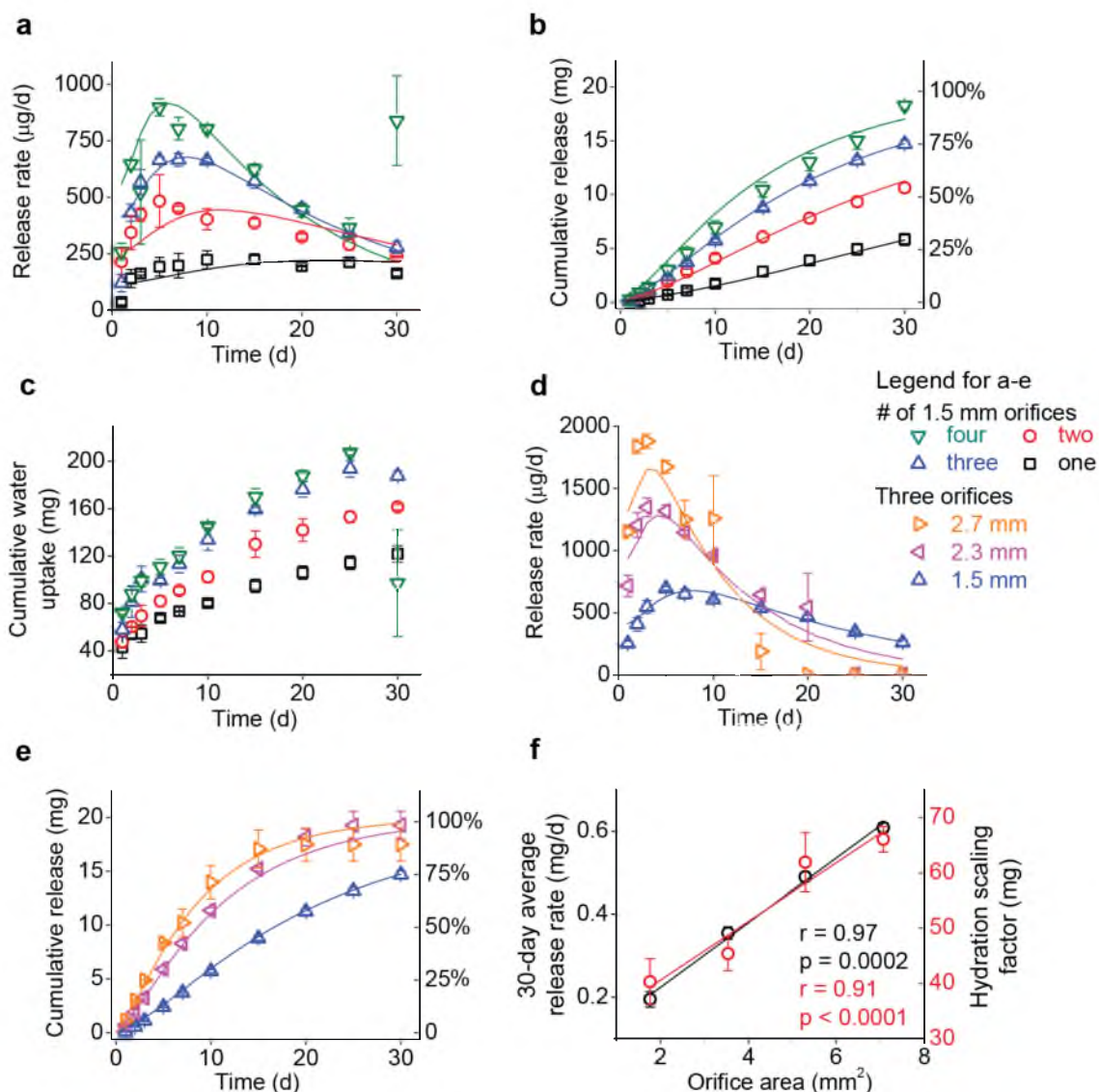
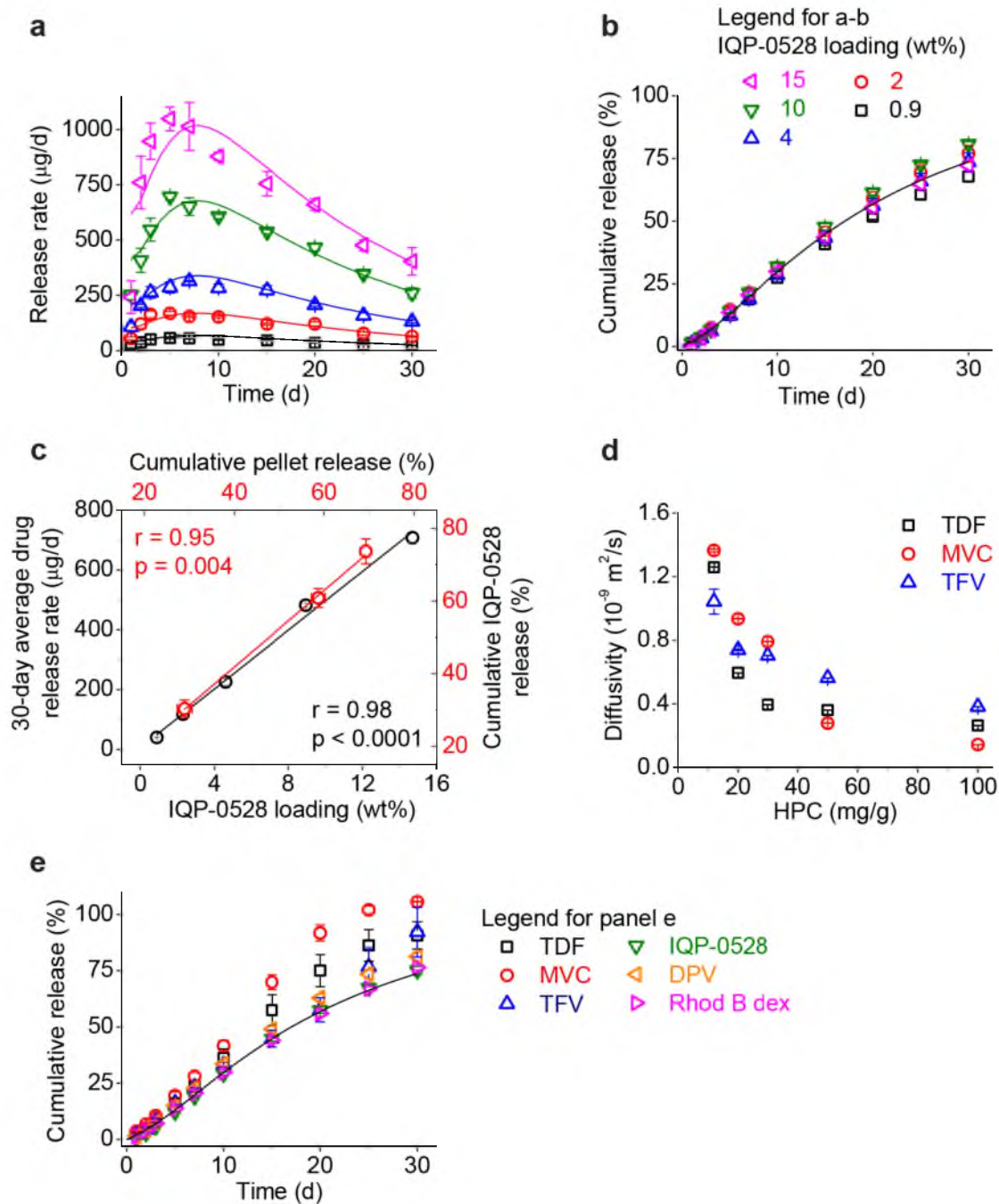


Figure 4.2. *In vitro* release of IQP-0528 comparing different orifice configurations. (a and d) IQP-0528 release rate and (b and e) cumulative release from FCPs with four, three, two, and one, 1.5 mm diameter orifices and three, 2.7, 2.3, and 1.5 mm diameter orifices containing four, 50 mg pellets of 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 $\mu\text{g}/\text{d}$ (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.



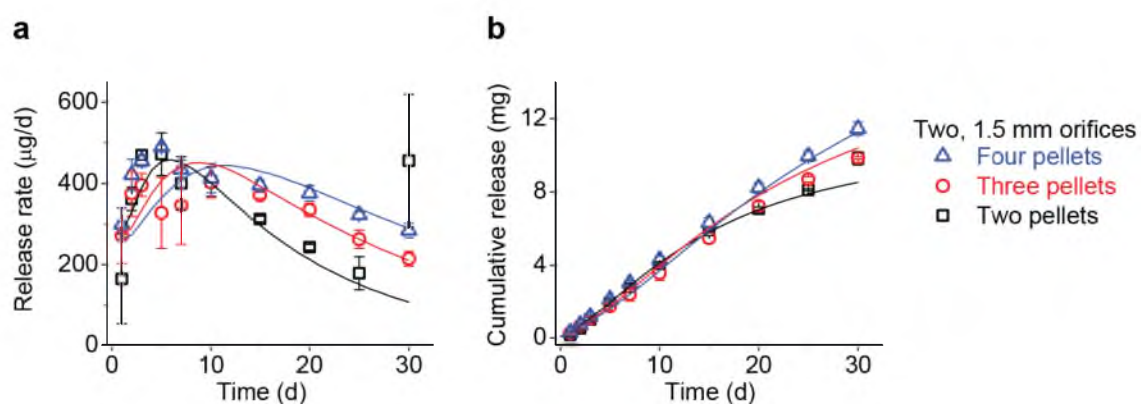


Figure 4.4. *In vitro* release of IQP-0528 comparing four, three, and two pellets per FCP. (a) IQP-0528 release rate and (b) cumulative amount release from FCPs with two, 1.5 mm orifices and four, three, and two, 50 mg pellets of 10 wt% IQP-0528 in HPC comparing results from experiments (symbols) and the model (solid lines).

CHAPTER 5

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

Ryan S. Teller, Rachna Rastogi, Meegan Anderson, Umadevi Nagaraja, Holly
Hattaway, Thomas J. Hope, and Patrick F. Kiser

Manuscript to be submitted to Antimicrobial Agents and Chemotherapy

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, Billierica, 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 μ L 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 μ m; Agilent) onto the analytical column (Zorbax SB-C18 column, 2.1x100 mm, 3.5 μ m; 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 μ g/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 μ L of complete DMEM repeated once. TZM-bl cells were plated

at 3×10^4 per well, allowed to adhere overnight, and then exposed to approximately 10^3 TCID₅₀ HIV-1_{BaL} 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 \pm 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 Estrin® (1.6 N); however, along the axis of the FCPs, the rings were considerably stiffer than NuvaRing® and Estrin®.

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_{0-15} of 4,200 [1,200-7,300] $\mu\text{g}\cdot\text{d}/\text{mL}$ for the four, 2.3 mm FCPs, and 2,300 [470-5,700] $\mu\text{g}\cdot\text{d}/\text{mL}$ 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 \pm 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 *in vitro* for 30 days (Fig. 5.3c). *In vivo* this design exhibited an average drug release of 1.0 ± 0.21 mg/day (mean \pm 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 $\mu\text{g/mL}$] 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 ($90 \pm 35\%$; mean \pm SD; $p = 0.0025$, Mann-Whitney test) and 15 ($91 \pm 47\%$; $p = 0.012$) compared to the baseline activity ($16 \pm 36\%$; 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 *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 *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 *in vivo* compared to *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

1. **Baeten JM, Grant R.** 2013. Use of Antiretrovirals for HIV Prevention: What Do We Know and What Don't We Know? *Curr Hiv-Aids Rep* **10**:142-151.
2. **Amico KR, Mansoor LE, Corneli A, Torjesen K, van der Straten A.** 2013. Adherence support approaches in biomedical HIV prevention trials: experiences, insights and future directions from four multisite prevention trials. *AIDS Behav* **17**:2143-2155.
3. **Karim SSA, Kashuba ADM, Werner L, Karim QA.** 2011. Drug concentrations after topical and oral antiretroviral pre-exposure prophylaxis: implications for HIV prevention in women. *The Lancet* **378**:279-281.
4. **Grant RM, Lama JR, Anderson PL, McMahan V, Liu AY, Vargas L, Goicochea P, Casapia M, Guanira-Carranza JV, Ramirez-Cardich ME, Montoya-Herrera O, Fernandez T, Veloso VG, Buchbinder SP, Chariyalertsak S, Schechter M, Bekker LG, Mayer KH, Kallas EG, Amico KR, Mulligan K, Bushman LR, Hance RJ, Ganoza C, Defechereux P, Postle B, Wang FR, McConnell JJ, Zheng JH, Lee J, Rooney JF, Jaffe HS, Martinez AI, Burns DN, Glidden DV, Team iS.** 2010. Preexposure Chemoprophylaxis for HIV Prevention in Men Who Have Sex with Men. *New Engl J Med* **363**:2587-2599.
5. **Van Damme L, Corneli A, Ahmed K, Agot K, Lombaard J, Kapiga S, Malahleha M, Owino F, Manongi R, Onyango J, Temu L, Monedi MC, Mak'Oketch P, Makanda M, Reblin I, Makatu SE, Saylor L, Kiernan H, Kirkendale S, Wong C, Grant R, Kashuba A, Nanda K, Mandala J, Fransen K, Deese J, Crucitti T, Mastro TD, Taylor D, Grp F-PS.** 2012. Preexposure Prophylaxis for HIV Infection among African Women. *New Engl J Med* **367**:411-422.
6. **Anderson PL, Glidden DV, Liu A, Buchbinder S, Lama JR, Guanira JV, McMahan V, Bushman LR, Casapia M, Montoya-Herrera O, Veloso VG, Mayer KH, Chariyalertsak S, Schechter M, Bekker LG, Kallas EG, Grant RM, iPrEx Study T.** 2012. Emtricitabine-tenofovir concentrations and pre-exposure prophylaxis efficacy in men who have sex with men. *Science translational medicine* **4**:151ra125.
7. **Garcia-Lerma JG, Otten RA, Qari SH, Jackson E, Cong ME, Masciotra S, Luo W, Kim C, Adams DR, Monsour M, Lipscomb J, Johnson JA, Delinsky D, Schinazi RF, Janssen R, Folks TM, Heneine W.** 2008. Prevention of rectal SHIV transmission in macaques by daily or intermittent prophylaxis with emtricitabine and tenofovir. *PLoS medicine* **5**:e28.

8. **Veazey RS, Ketas TJ, Dufour J, Moroney-Rasmussen T, Green LC, Klasse PJ, Moore JP.** 2010. Protection of rhesus macaques from vaginal infection by vaginally delivered maraviroc, an inhibitor of HIV-1 entry via the CCR5 co-receptor. *J Infect Dis* **202**:739-744.
9. **Radzio J, Aung W, Holder A, Martin A, Sweeney E, Mitchell J, Bachman S, Pau CP, Heneine W, Garcia-Lerma JG.** 2012. Prevention of vaginal SHIV transmission in macaques by a coitally-dependent Truvada regimen. *PLoS One* **7**:e50632.
10. **Dobard C, Sharma S, Martin A, Pau CP, Holder A, Kuklenyik Z, Lipscomb J, Hanson DL, Smith J, Novembre FJ, Garcia-Lerma JG, Heneine W.** 2012. Durable protection from vaginal simian-human immunodeficiency virus infection in macaques by tenofovir gel and its relationship to drug levels in tissue. *J Virol* **86**:718-725.
11. **Smith JM, Rastogi R, Teller RS, Srinivasan P, Mesquita PM, Nagaraja U, McNicholl JM, Hendry RM, Dinh CT, Martin A, Herold BC, Kiser PF.** 2013. Intravaginal ring eluting tenofovir disoproxil fumarate completely protects macaques from multiple vaginal simian-HIV challenges. *Proc Natl Acad Sci U S A* **110**:16145-16150.
12. **Andrews CD, Spreen WR, Mohri H, Moss L, Ford S, Gettie A, Russell-Lodrigue K, Bohm RP, Cheng-Mayer C, Hong Z, Markowitz M, Ho DD.** 2014. Long-acting integrase inhibitor protects macaques from intrarectal simian/human immunodeficiency virus. *Science* **343**:1151-1154.
13. **Brache V, Faundes A.** 2010. Contraceptive vaginal rings: a review. *Contraception* **82**:418-427.
14. **van der Straten A, Montgomery ET, Cheng H, Wegner L, Masenga G, von Mollendorf C, Bekker L, Ganesh S, Young K, Romano J, Nel A, Woodsong C.** 2012. High acceptability of a vaginal ring intended as a microbicide delivery method for HIV prevention in African women. *AIDS Behav* **16**:1775-1786.
15. **Kiser PF, Johnson TJ, Clark JT.** 2012. State of the art in intravaginal ring technology for topical prophylaxis of HIV infection. *AIDS Rev* **14**:62-77.
16. **Teller RS, Rastogi R, Johnson TJ, Blair MJ, Hitchcock RW, Kiser PF.** 2014. Intravaginal flux controlled pump for sustained release of macromolecules. *Pharm Res* **31**:2344-2353.
17. **Morrow RJ, Woolfson AD, Donnelly L, Curran R, Andrews G, Katinger D, Malcolm RK.** 2011. Sustained release of proteins from a modified vaginal ring device. *Eur J Pharm Biopharm* **77**:3-10.

18. **Baum MM, Butkyavichene I, Gilman J, Kennedy S, Kopin E, Malone AM, Nguyen C, Smith TJ, Friend DR, Clark MR, Moss JA.** 2012. An intravaginal ring for the simultaneous delivery of multiple drugs. *Journal of Pharmaceutical Sciences* **101**:2833-2843.
19. **Johnson TJ, Gupta KM, Fabian J, Albright TH, Kiser PF.** 2010. Segmented polyurethane intravaginal rings for the sustained combined delivery of antiretroviral agents dapivirine and tenofovir. *Eur J Pharm Sci* **39**:203-212.
20. **Clark JT, Johnson TJ, Clark MR, Nebeker JS, Fabian J, Tuitupou AL, Ponnappalli S, Smith EM, Friend DR, Kiser PF.** 2012. Quantitative evaluation of a hydrophilic matrix intravaginal ring for the sustained delivery of tenofovir. *J Control Release* **163**:240-248.
21. **Koetsawang S, Ji G, Krishna U, Cuadros A, Dhall GI, Wyss R, Rodriguez la Puente J, Andrade AT, Khan T, Kononova ES, et al.** 1990. Microdose intravaginal levonorgestrel contraception: a multicentre clinical trial. II. Expulsions and removals. World Health Organization. Task Force on Long-Acting Systemic Agents for Fertility Regulation. *Contraception* **41**:125-141.
22. **Weisberg E, Fraser IS, Baker J, Archer D, Landgren BM, Killick S, Soutter P, Krause T, d'Arcangues C.** 2000. A randomized comparison of the effects on vaginal and cervical epithelium of a placebo vaginal ring with non-use of a ring. *Contraception* **62**:83-89.
23. **Rastogi R, Teller RS, Mesquita PM, Herold BC, Kiser PF.** 2013. Osmotic pump tablets for delivery of antiretrovirals to the vaginal mucosa. *Antiviral Res* **100**:255-258.
24. **Johnson TJ, Clark MR, Albright TH, Nebeker JS, Tuitupou AL, Clark JT, Fabian J, McCabe RT, Chandra N, Doncel GF, Friend DR, Kiser PF.** 2012. A 90-Day Tenofovir Reservoir Intravaginal Ring for Mucosal HIV Prophylaxis. *Antimicrob Agents Chemother* **56**:6272-6283.
25. **Clark JT, Clark MR, Shelke NB, Johnson TJ, Smith EM, Andreasen AK, Nebeker JS, Fabian J, Friend DR, Kiser PF.** 2014. Engineering a segmented dual-reservoir polyurethane intravaginal ring for simultaneous prevention of HIV transmission and unwanted pregnancy. *PLoS One* **9**:e88509.
26. **Johnson TJ, Srinivasan P, Albright TH, Watson-Buckheit K, Rabe L, Martin A, Pau CP, Hendry RM, Otten R, McNicholl J, Buckheit R, Jr., Smith J, Kiser PF.** 2012. Safe and sustained vaginal delivery of pyrimidinedione HIV-1 inhibitors from polyurethane intravaginal rings. *Antimicrob Agents Chemother* **56**:1291-1299.

27. **Watson Buckheit K, Yang L, Buckheit RW, Jr.** 2011. Development of dual-acting pyrimidinediones as novel and highly potent topical anti-HIV microbicides. *Antimicrob Agents Chemother* **55**:5243-5254.
28. **Mahalingam A, Simmons AP, Ugaonkar SR, Watson KM, Dezzutti CS, Rohan LC, Buckheit RW, Jr., Kiser PF.** 2011. Vaginal microbicide gel for delivery of IQP-0528, a pyrimidinedione analog with a dual mechanism of action against HIV-1. *Antimicrob Agents Chemother* **55**:1650-1660.
29. **Ham AS, Ugaonkar SR, Shi L, Buckheit KW, Lakouagna H, Nagaraja U, Gwozdz G, Goldman L, Kiser PF, Buckheit RW, Jr.** 2012. Development of a combination microbicide gel formulation containing IQP-0528 and tenofovir for the prevention of HIV infection. *Journal of Pharmaceutical Sciences* **101**:1423-1435.
30. **Ham AS, Rohan LC, Boczar A, Yang L, K WB, Buckheit RW, Jr.** 2012. Vaginal film drug delivery of the pyrimidinedione IQP-0528 for the prevention of HIV infection. *Pharm Res* **29**:1897-1907.
31. **Moss JA, Malone AM, Smith TJ, Kennedy S, Kopin E, Nguyen C, Gilman J, Butkyavichene I, Vincent KL, Motamedi M, Friend DR, Clark MR, Baum MM.** 2012. Simultaneous delivery of tenofovir and acyclovir via an intravaginal ring. *Antimicrob Agents Chemother* **56**:875-882.
32. **Stieh DJ, Maric D, Kelley ZL, Anderson MR, Hattaway HZ, Beilfuss BA, Rothwangl KB, Veazey RS, Hope TJ.** 2014. Vaginal Challenge with an SIV-Based Dual Reporter System Reveals That Infection Can Occur throughout the Upper and Lower Female Reproductive Tract. *PLoS pathogens* **10**:e1004440.
33. **Nel A, Smythe S, Young K, Malcolm K, McCoy C, Rosenberg Z, Romano J.** 2009. Safety and pharmacokinetics of dapivirine delivery from matrix and reservoir intravaginal rings to HIV-negative women. *J Acquir Immune Defic Syndr* **51**:416-423.
34. **Romano J, Variano B, Coplan P, Van Roey J, Douville K, Rosenberg Z, Temmerman M, Verstraelen H, Van Bortel L, Weyers S, Mitchnick M.** 2009. Safety and availability of dapivirine (TMC120) delivered from an intravaginal ring. *AIDS Res Hum Retroviruses* **25**:483-488.
35. **Nel AM, Haazen W, Nuttal JP, Romano J, Mesquita PMM, Herold BC, Rosenberg ZF.** 2014. Pharmacokinetics and Safety Assessment of Anti-HIV Dapivirine Vaginal Microbicide Rings with Multiple Dosing. *Journal of AIDS & Clinical Research* **05**.
36. **Venkataraman N, Cole AL, Svoboda P, Pohl J, Cole AM.** 2005. Cationic polypeptides are required for anti-HIV-1 activity of human vaginal fluid. *Journal of immunology* **175**:7560-7567.

37. **Keller MJ, Madan RP, Torres NM, Fazzari MJ, Cho S, Kalyoussef S, Shust G, Mesquita PM, Louissaint N, Chen J, Cohen HW, Diament EC, Lee AC, Soto-Torres L, Hendrix CW, Herold BC.** 2011. A randomized trial to assess anti-HIV activity in female genital tract secretions and soluble mucosal immunity following application of 1% tenofovir gel. *PLoS One* **6**:e16475.
38. **Kieweg SL, Katz DF.** 2006. Squeezing flows of vaginal gel formulations relevant to microbicide drug delivery. *J Biomech Eng* **128**:540-553.
39. **Abdool Karim Q, Abdool Karim SS, Frohlich JA, Grobler AC, Baxter C, Mansoor LE, Kharsany AB, Sibeko S, Mlisana KP, Omar Z, Gengiah TN, Maarschalk S, Arulappan N, Mlotshwa M, Morris L, Taylor D, Group CT.** 2010. Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. *Science* **329**:1168-1174.
40. **Barnhart KT, Timbers K, Pretorius ES, Lin K, Shaunik A.** 2005. In vivo assessment of NuvaRing placement. *Contraception* **72**:196-199.
41. **Bounds W, Szarewski A, Lowe D, Guillebaud J.** 1993. Preliminary report of unexpected local reactions to a progestogen-releasing contraceptive vaginal ring. *Eur J Obstet Gynecol Reprod Biol* **48**:123-125.
42. **Young Holt B, Romano J, Manning J, Hemmerling A, Shields W, Vyda L, Lusti-Narasimhan M.** 2014. Ensuring successful development and introduction of multipurpose prevention technologies through an innovative partnership approach. *BJOG: An International Journal of Obstetrics & Gynaecology* **121**:3-8.

Table 5.1. Summary of the different FCP designs

Number of orifices	Orifice diameter (mm)	Orifice area (mm ²)	Study duration (d)
Three	1.5	5.3	15
Four	2.3	17	15
Three	2.3	13	30

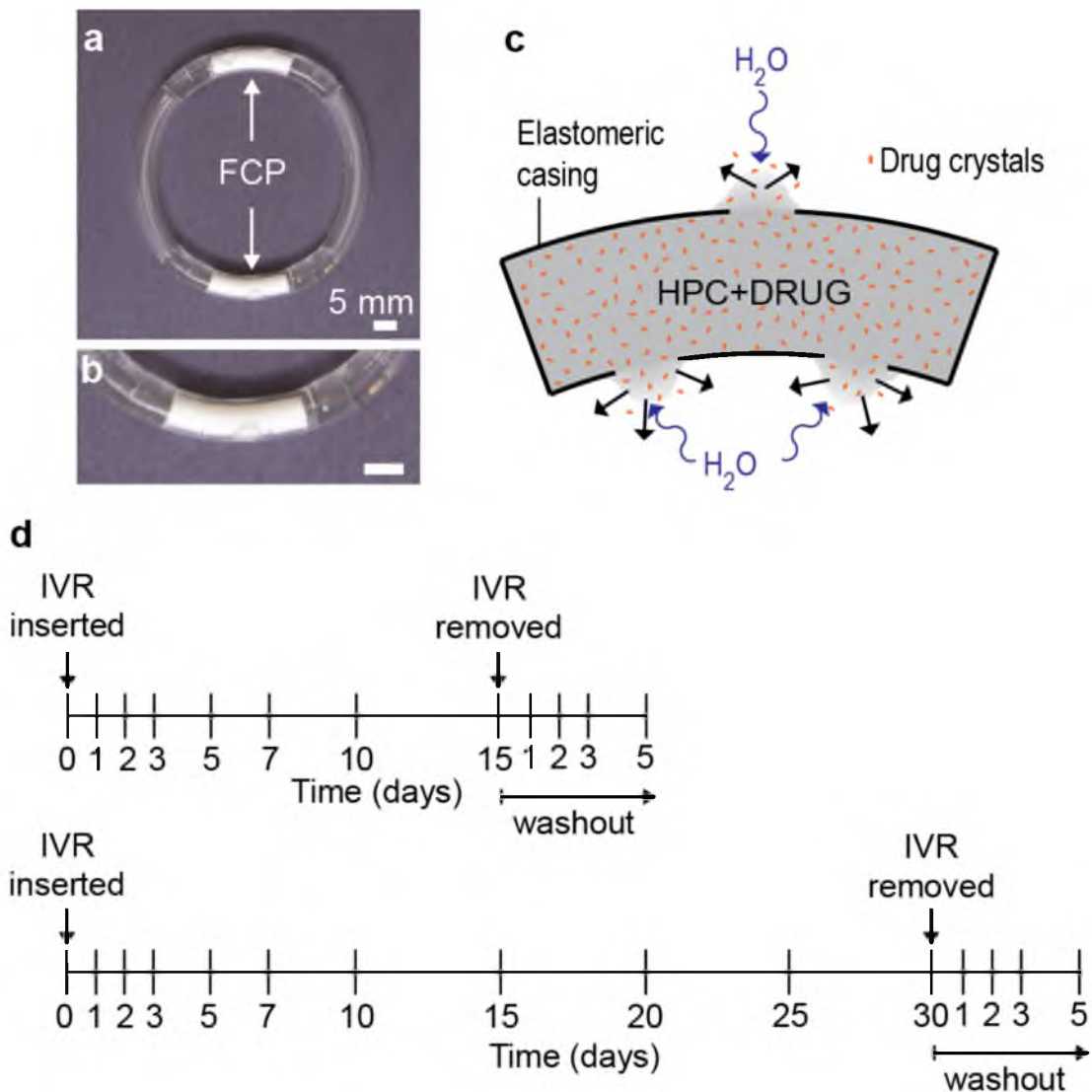


Figure 5.1. Photographs and schematic of flux controlled pumps, and an outline of the sheep PK studies. (a) Photographs of a FCP vaginal ring with FCP segments on the top and bottom of the ring and placebo segments on the left and right, and (b) a close up of a single FCP segment contained within the 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.

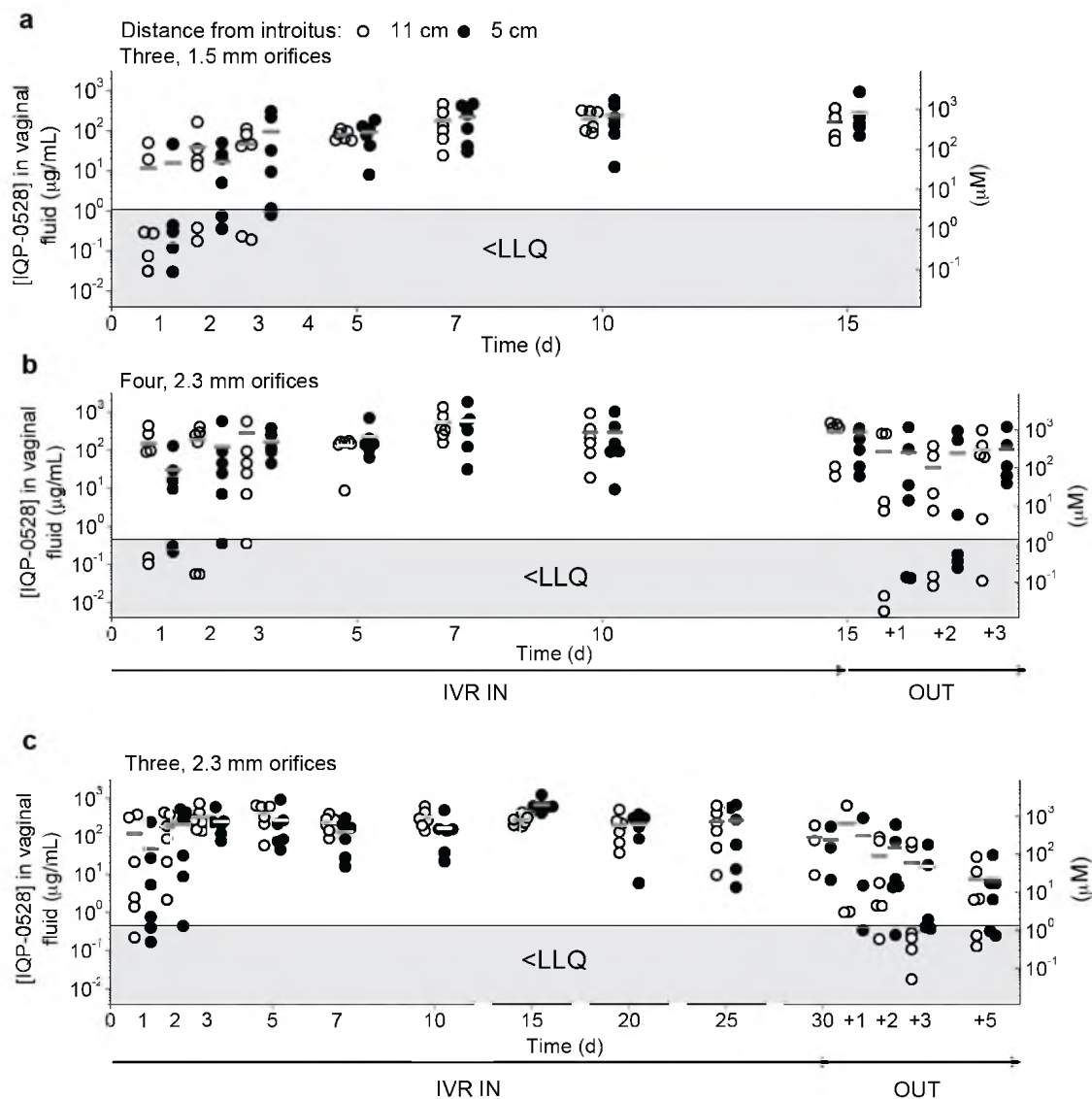


Figure 5.2. Drug concentration in vaginal fluid over 15 and 30 days of ring use at two locations, 11 and 5 cm from the introitus after administration of FCP containing intravaginal rings with (a) three, 1.5 mm or (b) four, 2.3 mm diameter orifices in each FCP over 15 days, and (c) three, 2.3 mm diameter 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 $1/10^{\text{th}}$ of the calculated sample LLQ. The horizontal line at the top of the grey area represents the average LLQ for the experiment.

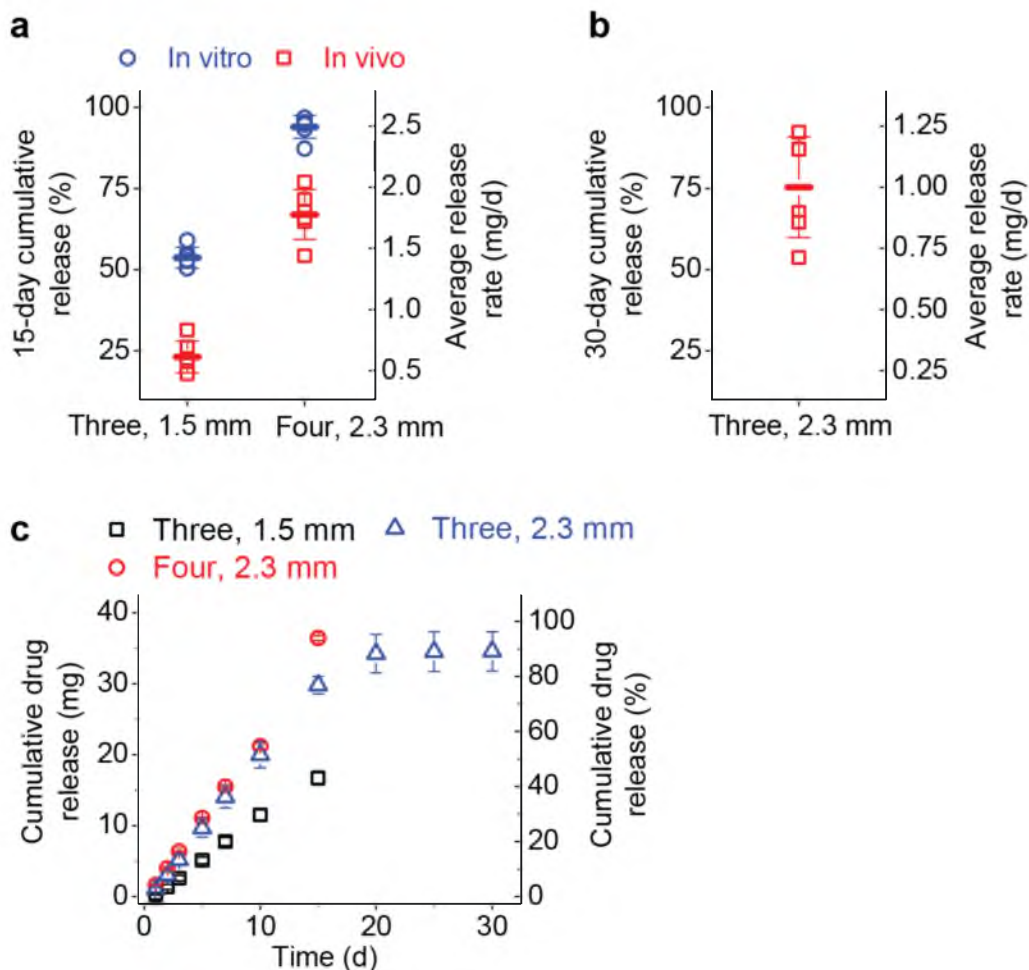


Figure 5.3. Comparison of the *in vivo* and *in vitro* IQP-0528 release over 15 and 30-days of ring use. IQP-0528 cumulative percent release and the average release rate for rings containing two FCPs comparing *in vitro* (blue) and *in vivo* (red) conditions after (a) 15 days and (b) 30 days of study. (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 \pm SD.

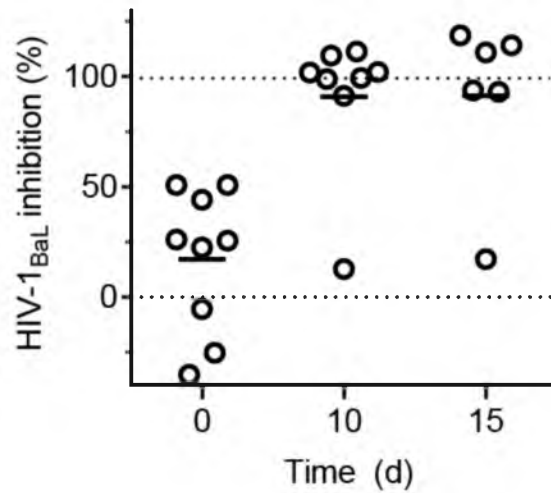


Figure 5.4. Antiviral activity of vaginal fluid collected in the 30-day PK study. The ability of the vaginal fluid eluted from swabs to inhibit HIV-1_{BaL} infection of TZM-bl cells from the 30-day PK study evaluating FCPs with three, 2.3 mm orifices. Symbols represent individual swabs, and bars indicate 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 *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 *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 *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 increase 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 extent 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

1. **Friend DR, Clark JT, Kiser PF, Clark MR.** 2013. Multipurpose prevention technologies: Products in development. *Antiviral Res* **100**:S39-S47.
2. **Young Holt B, Romano J, Manning J, Hemmerling A, Shields W, Vyda L, Lusti-Narasimhan M.** 2014. Ensuring successful development and introduction of multipurpose prevention technologies through an innovative partnership approach. *BJOG: An International Journal of Obstetrics & Gynaecology* **121**:3-8.
3. **Clark JT, Clark MR, Shelke NB, Johnson TJ, Smith EM, Andreasen AK, Nebeker JS, Fabian J, Friend DR, Kiser PF.** 2014. Engineering a segmented dual-reservoir polyurethane intravaginal ring for simultaneous prevention of HIV transmission and unwanted pregnancy. *PLoS One* **9**:e88509.
4. **Moss JA, Malone AM, Smith TJ, Kennedy S, Kopin E, Nguyen C, Gilman J, Butkyavichene I, Vincent KL, Motamedi M, Friend DR, Clark MR, Baum MM.** 2012. Simultaneous delivery of tenofovir and acyclovir via an intravaginal ring. *Antimicrob Agents Chemother* **56**:875-882.
5. **Moss JA, Malone AM, Smith TJ, Kennedy S, Nguyen C, Vincent KL, Motamedi M, Baum MM.** 2013. Pharmacokinetics of a multipurpose pod-intravaginal ring simultaneously delivering five drugs in an ovine model. *Antimicrob Agents Chemother* **57**:3994-3997.
6. **Smith JM, Rastogi R, Teller RS, Srinivasan P, Mesquita PM, Nagaraja U, McNicholl JM, Hendry RM, Dinh CT, Martin A, Herold BC, Kiser PF.** 2013. Intravaginal ring eluting tenofovir disoproxil fumarate completely protects macaques from multiple vaginal simian-HIV challenges. *Proc Natl Acad Sci U S A* **110**:16145-16150.
7. **Johnson TJ, Gupta KM, Fabian J, Albright TH, Kiser PF.** 2010. Segmented polyurethane intravaginal rings for the sustained combined delivery of antiretroviral agents dapivirine and tenofovir. *Eur J Pharm Sci* **39**:203-212.
8. **Baum MM, Butkyavichene I, Gilman J, Kennedy S, Kopin E, Malone AM, Nguyen C, Smith TJ, Friend DR, Clark MR, Moss JA.** 2012. An intravaginal ring for the simultaneous delivery of multiple drugs. *Journal of Pharmaceutical Sciences* **101**:2833-2843.
9. **Gao P, Fagerness PE.** 1995. Diffusion in HPMC gels. I. Determination of drug and water diffusivity by pulsed-field-gradient spin-echo NMR. *Pharm Res* **12**:955-964.

10. **Ferrero C, Massuelle D, Jeannerat D, Doelker E.** 2008. Towards elucidation of the drug release mechanism from compressed hydrophilic matrices made of cellulose ethers. I. Pulse-field-gradient spin-echo NMR study of sodium salicylate diffusivity in swollen hydrogels with respect to polymer matrix physical structure. *Journal of Controlled Release* **128**:71-79.
11. **Alvarez-Lorenzo C, Gomez-Amoza JL, Martinez-Pacheco R, Souto C, Concheiro A.** 1999. Microviscosity of hydroxypropylcellulose gels as a basis for prediction of drug diffusion rates. *Int J Pharm* **180**:91-103.
12. **Anderson PL, Glidden DV, Liu A, Buchbinder S, Lama JR, Guanira JV, McMahan V, Bushman LR, Casapia M, Montoya-Herrera O, Veloso VG, Mayer KH, Chariyalertsak S, Schechter M, Bekker LG, Kallas EG, Grant RM, iPrEx Study T.** 2012. Emtricitabine-tenofovir concentrations and pre-exposure prophylaxis efficacy in men who have sex with men. *Science translational medicine* **4**:151ra125.
13. **Anderson PL, Glidden DV, Bushman LR, Heneine W, Garcia-Lerma JG.** 2014. Tenofovir diphosphate concentrations and prophylactic effect in a macaque model of rectal simian HIV transmission. *J Antimicrob Chemother* **69**:2470-2476.
14. **Thompson CG, Cohen MS, Kashuba AD.** 2013. Antiretroviral pharmacology in mucosal tissues. *J Acquir Immune Defic Syndr* **63 Suppl 2**:S240-247.
15. **Bokhart MT, Rosen E, Thompson C, Sykes C, Kashuba AD, Muddiman DC.** 2014. Quantitative mass spectrometry imaging of emtricitabine in cervical tissue model using infrared matrix-assisted laser desorption electrospray ionization. *Analytical and bioanalytical chemistry*.
16. **Schwartz SA, Reyzer ML, Caprioli RM.** 2003. Direct tissue analysis using matrix-assisted laser desorption/ionization mass spectrometry: practical aspects of sample preparation. *J Mass Spectrom* **38**:699-708.
17. **Wiseman JM, Ifa DR, Song QY, Cooks RG.** 2006. Tissue imaging at atmospheric pressure using desorption electrospray ionization (DESI) mass spectrometry. *Angew Chem Int Edit* **45**:7188-7192.
18. **Brache V, Faundes A.** 2010. Contraceptive vaginal rings: a review. *Contraception* **82**:418-427.
19. **Dezarnaulds G, Fraser IS.** 2003. Vaginal ring delivery of hormone replacement therapy - a review. *Expert Opin Pharmaco* **4**:201-212.
20. **Montgomery ET, van der Straten A, Cheng H, Wegner L, Masenga G, von Mollendorf C, Bekker L, Ganesh S, Young K, Romano J, Nel A,**

- Woodsong C.** 2012. Vaginal ring adherence in sub-Saharan Africa: expulsion, removal, and perfect use. *AIDS Behav* **16**:1787-1798.
21. **van der Straten A, Stadler J, Luecke E, Laborde N, Hartmann M, Montgomery ET, Team V-CS.** 2014. Perspectives on use of oral and vaginal antiretrovirals for HIV prevention: the VOICE-C qualitative study in Johannesburg, South Africa. *Journal of the International AIDS Society* **17**:19146.
 22. **Hendrix CW, Chen BA, Guddera V, Hoesley C, Justman J, Nakabiito C, Salata R, Soto-Torres L, Patterson K, Minnis AM, Gandham S, Gomez K, Richardson BA, Bumpus NN.** 2013. MTN-001: randomized pharmacokinetic cross-over study comparing tenofovir vaginal gel and oral tablets in vaginal tissue and other compartments. *PLoS One* **8**:e55013.
 23. **Garcia-Lerma JG, Cong ME, Mitchell J, Youngpairoj AS, Zheng Q, Masciotra S, Martin A, Kuklenyik Z, Holder A, Lipscomb J, Pau CP, Barr JR, Hanson DL, Otten R, Paxton L, Folks TM, Heneine W.** 2010. Intermittent prophylaxis with oral truvada protects macaques from rectal SHIV infection. *Science translational medicine* **2**:14ra14.
 24. **Louissaint NA, Cao YJ, Skipper PL, Liberman RG, Tannenbaum SR, Nimmagadda S, Anderson JR, Everts S, Bakshi R, Fuchs EJ, Hendrix CW.** 2013. Single dose pharmacokinetics of oral tenofovir in plasma, peripheral blood mononuclear cells, colonic tissue, and vaginal tissue. *AIDS Res Hum Retroviruses* **29**:1443-1450.
 25. **Patterson KB, Prince HA, Kraft E, Jenkins AJ, Shaheen NJ, Rooney JF, Cohen MS, Kashuba AD.** 2011. Penetration of tenofovir and emtricitabine in mucosal tissues: implications for prevention of HIV-1 transmission. *Science translational medicine* **3**:112re114.
 26. **Veselinovic M, Yang KH, LeCureux J, Sykes C, Remling-Mulder L, Kashuba AD, Akkina R.** 2014. HIV pre-exposure prophylaxis: mucosal tissue drug distribution of RT inhibitor Tenofovir and entry inhibitor Maraviroc in a humanized mouse model. *Virology* **464-465**:253-263.
 27. **Spren W, Ford SL, Chen S, Wilfret D, Margolis D, Gould E, Piscitelli S.** 2014. GSK1265744 Pharmacokinetics in Plasma and Tissue Following Single-Dose Long-Acting (LA) Injectable Administration in Healthy Subjects. *J Acquir Immune Defic Syndr* **67**:481-486.
 28. **Jackson AG, Else LJ, Mesquita PM, Egan D, Back DJ, Karolia Z, Ringner-Nackter L, Higgs CJ, Herold BC, Gazzard BG, Boffito M.** 2014. A compartmental pharmacokinetic evaluation of long-acting rilpivirine in HIV-negative volunteers for pre-exposure prophylaxis. *Clinical pharmacology and therapeutics* **96**:314-323.

29. **Patterson KB, Prince HA, Stevens T, Shaheen NJ, Dellon ES, Madanick RD, Jennings S, Cohen MS, Kashuba AD.** 2013. Differential penetration of raltegravir throughout gastrointestinal tissue: implications for eradication and cure. *Aids* **27**:1413-1419.
30. **Li Q, Estes JD, Schlievert PM, Duan L, Brosnahan AJ, Southern PJ, Reilly CS, Peterson ML, Schultz-Darken N, Brunner KG, Nephew KR, Pambuccian S, Lifson JD, Carlis JV, Haase AT.** 2009. Glycerol monolaurate prevents mucosal SIV transmission. *Nature* **458**:1034-1038.
31. **Veazey RS, Klasse PJ, Schader SM, Hu Q, Ketas TJ, Lu M, Marx PA, Dufour J, Colonna RJ, Shattock RJ, Springer MS, Moore JP.** 2005. Protection of macaques from vaginal SHIV challenge by vaginally delivered inhibitors of virus-cell fusion. *Nature* **438**:99-102.
32. **Herrera C, Cranage M, McGowan I, Anton P, Shattock RJ.** 2009. Reverse transcriptase inhibitors as potential colorectal microbicides. *Antimicrob Agents Chemother* **53**:1797-1807.
33. **Pirrone V, Thakkar N, Jacobson JM, Wigdahl B, Krebs FC.** 2011. Combinatorial approaches to the prevention and treatment of HIV-1 infection. *Antimicrob Agents Chemother* **55**:1831-1842.
34. **Baeten JM, Donnell D, Mugo NR, Ndase P, Thomas KK, Campbell JD, Wangisi J, Tappero JW, Bukusi EA, Cohen CR, Katabira E, Ronald A, Tumwesigye E, Were E, Fife KH, Kiarie J, Farquhar C, John-Stewart G, Kidoguchi L, Coombs RW, Hendrix C, Marzinke MA, Frenkel L, Haberer JE, Bangsberg D, Celum C, for the Partners Pr EPST.** 2014. Single-agent tenofovir versus combination emtricitabine plus tenofovir for pre-exposure prophylaxis for HIV-1 acquisition: an update of data from a randomised, double-blind, phase 3 trial. *The Lancet. Infectious diseases*.
35. **Baeten JM, Haberer JE, Liu AY, Sista N.** 2013. Preexposure prophylaxis for HIV prevention: where have we been and where are we going? *J Acquir Immune Defic Syndr* **63 Suppl 2**:S122-129.
36. **Abdool Karim Q, Abdool Karim SS, Frohlich JA, Grobler AC, Baxter C, Mansoor LE, Kharsany AB, Sibeko S, Mlisana KP, Omar Z, Gengiah TN, Maarschalk S, Arulappan N, Mlotshwa M, Morris L, Taylor D, Group CT.** 2010. Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. *Science* **329**:1168-1174.

VITA

Ryan Stevenson Teller

Education

University of Michigan, B.S.E. in Biomedical
Engineering 2009

University of Utah, Ph.D. in Bioengineering 2015

Publications

1. **Mesquita PM, Rastogi R, Segarra TJ, Teller RS, Torres NM, Huber AM, Kiser PF, Herold BC.** 2012. Intravaginal ring delivery of tenofovir disoproxil fumarate for prevention of HIV and herpes simplex virus infection. *J Antimicrob Chemother* **67**:1730-1738.
2. **Rastogi R, Teller RS, Mesquita PM, Herold BC, Kiser PF.** 2013. Osmotic pump tablets for delivery of antiretrovirals to the vaginal mucosa. *Antiviral Res* **100**:255-258.
3. **Smith JM, Rastogi R, Teller RS, Srinivasan P, Mesquita PM, Nagaraja U, McNicholl JM, Hendry RM, Dinh CT, Martin A, Herold BC, Kiser PF.** 2013. Intravaginal ring eluting tenofovir disoproxil fumarate completely protects macaques from multiple vaginal simian-HIV challenges. *Proc Natl Acad Sci U S A* **110**:16145-16150.
4. **Nixon B, Jandl T, Teller RS, Taneva E, Wang Y, Nagaraja U, Kiser PF, Herold BC.** 2014. Vaginally Delivered Tenofovir Disoproxil Fumarate Provides Greater Protection than Tenofovir against Genital Herpes in a Murine Model of Efficacy and Safety. *Antimicrob Agents Chemother* **58**:1153-1160.

5. **Teller RS, Rastogi R, Johnson TJ, Blair MJ, Hitchcock RW, Kiser PF.** 2014. Intravaginal flux controlled pump for sustained release of macromolecules. *Pharm Res* **31**:2344-2353.
6. **Srinivasan P, Dinh C, Zhang J, Pau C-P, McNicholl JM, Lo Y, Herold BC, Teller R, Kiser P, Smith JM.** 2014. Pharmacokinetic evaluation of tenofovir disoproxil fumarate released from an intravaginal ring in pigtailed macaques after 6 months of continuous use. *J Med Primatol* **43**:364-369.
7. **Smith JM, Srinivasan P, Teller RS, Lo Y, Dinh CT, Kiser PF, Herold BC.** 2015. Tenofovir Disoproxil Fumarate Intravaginal Ring Protects High-Dose Depot Medroxyprogesterone Acetate-Treated Macaques From Multiple SHIV Exposures. *J Acquir Immune Defic Syndr* **68**:1-5.