Multipurpose tenofovir disoproxil fumarate electrospun fibers for the prevention of HIV-1 and HSV-2 infections.

Kevin Tyo
MULTIPURPOSE TENOFOVIR DISOPROXIL FUMARATE ELECTROSPUN FIBERS FOR THE PREVENTION OF HIV-1 AND HSV-2 INFECTIONS

By

Kevin Tyo
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School of Medicine
University of Louisville
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DEDICATION

To my Grandmother Teruko Reynolds
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I would like to acknowledge and give thanks to my mentor Dr. Jill Steinbach-Rankins. She has continuously offered support and guidance to both me and my scientific research. Despite my occasional failures, she has never wavered in her support. Additionally, I would like to acknowledge the additional support given from my fellow lab members as well as colleagues in the Department of Pharmacology and Toxicology.
Sexually transmitted infections affect hundreds of millions of worldwide. Both human immunodeficiency virus (HIV-1 and -2) and herpes simplex virus-2 (HSV-2) remain incurable, urging the development of new prevention strategies. While current prophylactic technologies are dependent on strict user adherence to achieve efficacy, there is a dearth of delivery vehicles that provide discreet and convenient administration, combined with prolonged-delivery of active agents. To address these needs, we created electrospun fibers (EFs) comprised of FDA-approved polymers, poly(lactic-co-glycolic acid) (PLGA) and poly(DL-lactide-co-ε-caprolactone) (PLCL), to provide sustained-release and in vitro protection against HIV-1 and HSV-2. PLGA and PLCL EFs, incorporating the antiretroviral, tenofovir disoproxil fumarate (TDF), exhibited sustained-release for up to 4 weeks, and provided complete in vitro protection against HSV-2 and HIV-1 for 24 hr and 2 wk, respectively. In vitro tests confirmed the safety of these fibers in vaginal and cervical cells, highlighting the potential of polymeric EFs as multipurpose next-generation drug delivery vehicles.
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BACKGROUND AND INTRODUCTION

Sexually transmitted infections (STIs) are a global health challenge, with over one million new cases of STIs reported daily. Over 530 and 36 million people globally are infected by herpes simplex virus type-2 (HSV-2) and human immunodeficiency virus (HIV), respectively [1]. Compounding these statistics, HSV-2 infection has been shown to significantly enhance HIV infection by as much as 2 to 7-fold [2, 3]. Furthermore, co-infected individuals harbor higher concentrations of both viruses relative to individuals with only one infection [3]. Despite the existence of multiple strategies to prevent STIs, no cure exists for these diseases, and rates of infection among particular demographics remain high [4, 5].

The first known incidence of HIV infection originated in the late 1950s in what is today known as the Democratic Republic of the Congo [6]. The general consensus of scientists is that HIV originated from cross-species contamination of simian immunodeficiency viruses (SIVs), which normally infect primates. The first known cases of the current HIV pandemic in the United States date back to 1981, where previously healthy homosexual men began suffering and dying from opportunistic infections [7]. At first this emerging disease, designated Acquired Immune Deficiency Syndrome (AIDS) was widely believed to only affect homosexuals. However, in 1984 it was discovered that AIDS is caused by the HIV, which is transmitted by hetero- and homosexual intercourse, blood-to-blood
transmission, or mucosal exposure [8]. Today, over 36 million people worldwide carry the virus, with women in the developing world bearing the highest burden of infection (Figure 1).

Currently, there two types of HIV known: HIV-1 and HIV-2, with HIV-1 being more prevalent due to higher virulence [9]. Considering genetic variability, HIV-1 can be further divided into three groups: major (M), outlier (O), and new (N) [10]. Currently, HIV-1 group M is the most prominent group and can be further divided by subtypes (clades). These clades, designated A-K, vary in incidence depending on location. In North America, clade B predominates, whereas in Africa clade C is the most common form of infection [11]. This sheer genetic variability of HIV, more thoroughly discussed in [12, 13], contributes to the challenges in curing HIV.

Despite the plethora of HIV subtypes, all viral particles share the same general structure. At its genetic and structural levels, each HIV particle is comprised of two single stranded RNAs enclosed within a capsule comprised of p24 proteins [14]. Proteins such as reverse transcriptase and integrase are also enveloped within the capsule, which contains glycoproteins 120 (gp120) and 41 (gp41). These two glycoproteins comprise the viral envelope (Env) protein and are essential for cellular infection.
Figure 1. A heat map depicting the prevalence of HIV infection. The developing world is afflicted by higher rates of infection. Additionally, women in these areas are more susceptible to infection. Image taken from the World Health Organization’s website [15].
HIV utilizes gp120 and gp41 to infect cells expressing the CD4 receptor as well as chemokine receptor 5 (CCR5) or chemokine receptor type 4 (CXCR4) [16]. Once inside the body, HIV selectively targets CD4 T-helper cells, dendritic cells, macrophages, as well as Langerhans cells. Broadly, the envelope glycoproteins bind to the CD4 receptor [17] causing a conformational change in the virus envelope. This conformation triggers additional binding to either CCR5 or CXCR4 co-receptors [18, 19], leading to viral fusion with the cell. Once inside, the viral proteins and RNA appropriate the cellular machinery to rapidly begin producing additional viral particles.

Like AIDS, genital herpes is also caused by viral infection. This infection is caused by HSV, of which there are two types. HSV-2 generally results in genital infection with corresponding symptoms; whereas HSV-1 results in oral manifestations. More rarely, HSV-1 may infect the genital area. Currently, over 530 million people worldwide live with HSV-2 infection [20]. This high number of infections is attributed to the virus's ability to infect surface epithelial cells, while producing often asymptomatic and latent infections within neuronal cells.

At its core, each HSV-2 virion is comprised of a capsid that contains double stranded DNA [21]. Unlike HIV, the capsid only encodes viral DNA; the host’s cellular machinery provides the means for producing additional viral particles. The (capsule) is comprised of proteins that form an icosahedral shape, surrounded by a lipid bilayer that forms the viral envelope. The lipid bilayer contains a variety of surface glycoproteins, five of which are essential for binding and entry to host cells: gB, gC, gD, gH, and gL [22, 23].
While HSV-2 initially infects epithelial cells, post-infection the virus anteroretrograde transports to infect neuronal ganglia, providing a reservoir for latent infection. Entry into a cell is facilitated by viral glycoproteins gB and gC, which non-specifically bind to heparan sulfate [24], a proteoglycan found on the surface of host cells. These attachment points induce viral “surfing” to the cell surface where gD facilitates receptor-mediated cell binding and internalization [21]. After this binding event, gD interacts with a receptor and changes confirmation to mediate fusion of the viral envelope with the cell membrane. The conformational change is stabilized by both gB and the gH-gL dimer, which play a critical role in viral entry. After entry, the capsule is transferred into the cellular nucleus, where viral DNA is transcribed by the cellular machinery.
CURRENT HIV AND HSV-2 THERAPEUTICS

Since isolating HIV in 1983, there has been a monumental effort worldwide to prevent, treat, and cure infections. Zidovudine (or azidothymidine, AZT), a dideoxynucleoside reverse transcriptase inhibitor (NRTI), was the first drug approved by the Food and Drug Administration (FDA) to treat HIV in 1987 [25]. However, low tolerance, antiviral resistance, and incomplete replication inhibition hampered the success of Zidovudine as a stand-alone drug. It is currently used in combinational highly active antiretroviral therapy (HAART) against HIV. Since the introduction of AZT, dozens of new therapeutics have been introduced to combat the AIDS pandemic.

Today, there are a variety of drugs that have since been developed and are widely available to treat HIV. These drugs are categorized based on their mechanism of action. Currently there are six types of anti-HIV agents: nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside/nucleotide reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), fusion inhibitors, co-receptor inhibitors, and integrase inhibitors [26]. Nucleotide and nucleoside reverse transcriptase inhibitors (such as AZT) are compounds that directly interact with the binding site of the viral reverse transcriptase enzyme to inhibit virus replication. Non-nucleotide/nucleoside reverse transcriptase inhibitors also work to inhibit reverse transcriptase
but do not act on the enzyme’s catalytic binding site. Protease inhibitors, similar to the previous classes, act against HIV after the virus has entered the cell. PIs inhibit HIV protease to prevent the formation of mature viral proteins. In contrast, fusion inhibitors act to prevent HIV entry into the cell, by binding to and inactivating Env proteins such as gp41. Another method to prevent viral entry is the use of co-receptor inhibitors (such as Maraviroc), which prevent gp120 and CD4 interaction. Finally, integrase inhibitors, much like reverse transcriptase and protease inhibitors, work after viral infection and prevent the virus from integrating its genetic material into the host cell DNA.

This diversity of HIV therapeutics is a result of attempts to combat HIV’s highly mutagenic nature. Often, the administration of one compound only temporarily hinders HIV infection, and mutation restores HIV replication competence, leading to AIDS [27]. With this in mind, Highly Active Antiretroviral Therapy (HAART) was introduced. With HAART, a regimen of at least three drugs from a minimum of two different classes of drugs is administered to treat HIV-1. Typically, several RTIs and at least one PI are administered [28, 29]. The vast majority of these medications is taken orally to help increase user adherence. Additionally, the different classes of agents help reduce toxicity, relative to using drugs of the same class. With the implementation of HAART, HIV is suppressed, increasing patient life expectancy by years.

In contrast to the variety of agents available to treat HIV, only a limited number of therapeutics are available to treat HSV-2 [30]. The top three current therapeutics are: Acyclovir, Valaciclovir, and Famciclovir. Acyclovir, a guanosine
derivative, acts to specifically inhibit viral DNA polymerase activity. The drug was one of the earliest therapeutics available to combat HSV-2. However, due to the relatively low bioavailability of Acyclovir, the prodrug Valaciclovir was developed. Valaciclovir possesses a bioavailability three to five times that of Acyclovir, thereby enhancing oral delivery and efficacy [31]. The last drug, Famciclovir, is a prodrug of Penciclovir, and possesses a similar mechanism of action as Acyclovir but can be administered topically [32]. It is important to note that there are currently no FDA-approved therapeutics that prevent or cure HSV. Furthermore, latent infection, combined with frequent asymptomatic and irregular recurrences add to the challenge of curing this disease.

Correspondingly, the challenges in HSV-2 prevention and treatment, combined with its high global incidence and propensity for co-infections, contribute to the challenges in preventing and curing HIV. In addition to increasing the risk of HIV co-infections [33], the asymptomatic nature of HSV infection contributes to the lack of awareness of infection, and subsequent co-infections [34]. Even for asymptomatic cases of HSV-2 infection or latent activation, viral loads are present with increased inflammatory and immune cell infiltrates. In more severe cases of infection and reactivation, the genital lesions caused by HSV-2 significantly increase the likelihood of HIV transmission via epithelial tears [35]. With these challenges, multipurpose platforms that prevent both HSV-2 and HIV infections are critically needed.
ORAL AND TOPICAL PrEP

Despite the numerous antivirals available to treat HIV and HSV-2, to date there are no agents that completely prevent or cure these infections. In terms of prevention, pre-exposure prophylaxis (PrEP) has enabled high-risk individuals to prevent HIV-1 infection by taking oral medication each day. However, to date, only two compounds Tenofovir (TFV) and Emtricitabine (combined with TFV in Truvada), are approved for PrEP by the FDA [36].

Oral PrEP has demonstrated success in preventing HIV in clinical trials and is becoming increasingly acknowledged as a successful prevention platform [37, 38]. By frequent administration of oral antiretroviral (ARV) compounds, such as TFV and Emtricitabine, prevention rates of up to 62% have been achieved in clinical trials [39, 40]. Clinical trials such as the Centers for Disease Control and Prevention CDC 4243 and CDC 4940, and the International AIDS Vaccine Initiative IAVI E001 and IAVI E002, demonstrate the potential effectiveness of oral PREP, by reducing HIV infection by up to 75% [41]. However, to achieve these levels of protection, frequent administration and strict user adherence are critical. This was validated in the VOICE (MTN-003) clinical trial, in which oral PrEP (and topical microbicide gels) failed to provide any meaningful protection due to a lack of user adherence [42]. In addition to user adherence, additional challenges of oral PrEP, based on the administration of ARVs, include renal and bone toxicity; associated decreases in condom use; the development of antiviral
resistance; and reduction of drug concentration via first pass metabolism [42].

Thus new strategies are urgently needed to provide safe, effective, and long-term protection against multiple STIs.

Given the disadvantages of oral PrEP, several topical PrEP strategies have been developed that provide localized protection to overcome these limitations. Topical PrEP eliminates first-pass hepatic clearance, while providing localized doses that result in more efficacious delivery of active agents to the local infection site [43]. These localized doses also have the effect of potentially reducing drug toxicity. Additionally, by acting at the site of infection, topical PrEP improves the ability of active agents to exert their effect upon incoming virus. The potential of topical PrEP was demonstrated in the CAPRISA-004 clinical study, where a topical gel containing TFV was used to effectively reduce HIV transmission by 39% [44].

While traditionally topical PrEP has been administered in the form of gels, topical PrEP delivery vehicles also include films, tablets, and intravaginal rings (IVRs). Antiviral gels have the potential to confer adequate protection when frequently applied. However in the MTN-003 (Voice) clinical trials, gels containing TFV required strict user adherence in administration both prior to and after sexual intercourse to maintain effectiveness [43, 45]. The strict dosing needed to maintain protection resulted in suboptimal user adherence which led to decreased protection against infection. In addition, some users experience discomfort or leakiness, which may further reduce user acceptance and adherence. Intravaginal films have also demonstrated protection against STIs;
however, burst release of incorporated agents is a frequent concern [45]. Indeed, the rapid and complete release of the encapsulated agents within hours of administration remains a major hurdle of this technology for long-term administration [46, 47]. User adherence is another common concern of films, with reports of difficulty of administration as well as localized irritation after prolonged contact [45, 48]. Vaginal tablets, another topical technology, offer a cost effective platform to rapidly release antivirals. However, tablets suffer from the same concerns as films, exhibiting transient activity and a lack of long-term release [49].

In comparison to the previous technologies, intravaginal rings (IVRs) provide the gold standard for sustained-release and have demonstrated long-term protection against STIs for up to 4 months [50, 51]. IVRs have been utilized for over a decade, particularly for hormonal contraceptive delivery. The translation of IVRs into HIV PrEP has been recently demonstrated in the ASPIRE (MTN-020) clinical trial, where HIV infection was reduced by 27% for up to one month with Dapivirine encapsulated IVRs [52]. However, concerns remain regarding the lack of complete protection provided in clinical trials, and their ability to incorporate less stable agents, such as biologics, due to the high processing temperatures utilized during the manufacturing process [53]. Lastly, user adherence of IVRs remains another major concern. In the ASPIRE trial, women most vulnerable to infection (ages 18-21) were less likely to adhere to IVR application [52], suggesting that alternative dosage forms may prove
beneficial to better address women’s’ preferences and needs for protection; improve user adherence; and achieve increased efficacy.
ELECTROSPUN FIBERS

Relative to the existing technologies of gels, films, and IVRs, electrospun fibers (EFs) provide a promising alternative for prolonged and localized agent delivery, with the potential to protect against multiple STIs. Some of the advantages of electrospun fibers include high loading efficiency to increase local concentration, enhanced agent stability relative to administration of free agent, and tunable sustained-release imparted by material choice. Biodegradable polymers, such as poly(lactic-co-glycolic acid (PLGA) and poly(caprolactone) (PCL) (Figure 2), are approved by the U.S. Food and Drug Administration (FDA) for therapeutic use, indicating their proven biocompatibility and potential for translation [54]. Furthermore, due to the variety of polymers available, virtually any compound can be incorporated, and delivery durations can be altered to suit the specific delivery requirements of a topical delivery vehicle [55, 56]. Additionally, EFs have the potential to simultaneously deliver co-incorporated compounds including biologics such as proteins and oligonucleotides, as well as traditional antiviral drugs [57-59]. These attributes make polymeric EFs an attractive platform for multipurpose drug delivery against STIs.

Over the past decade, researchers have begun to incorporate antiviral agents into polymeric EFs to prevent HIV infection. One of the first studies to utilize electrospun fibers to combat HIV, developed pH-responsive fibers that
encapsulated cellulose acetate phthalate (CAP) [60]. The affinity of CAP for the HIV surface protein gp120 directly neutralizes HIV. While CAP EFs exhibited long-term stability in low pH environments characteristic of the female reproductive tract, the EFs quickly degraded with the introduction of semen, to release active CAP and neutralize HIV particles. Later research by the same group utilized polystyrene and polypropylene fibers to bind to and inhibit HIV [61]. In this latter study, fibers were surface-modified with poly(allylamine hydrochloride) or dextran sulfate sodium to confer a positive or negative charge to the fibers. Both surface-modified EFs inhibited HIV infection with higher efficacy than unmodified fibers alone.

In addition to pH-sensitive and surface-modified fibers, researchers have utilized EFs to provide tunable release of one or more incorporated active agents for HIV-1 prevention [43-47]. The utilization of polymer blends has enabled fibers to be finely tailored to release multiple drugs. Blends of polyethylene oxide (PEO) and poly(L-lactic acid) (PLLA) were synthesized to encapsulate the antivirals Maraviroc (entry inhibitor) and AZT [62]. By altering ratios of these polymers, drug release was tailored to effectively deliver both compounds for up to several weeks. In another study, PLGA and PCL fibers were loaded with various concentrations of the ARV Tenofovir (TFV) [63]. These fibers demonstrated sustained-release of TFV for 30 days as well as efficacy against HIV infection in vitro.
Figure 2. Chemical structure of PLGA and PCL. Both PLGA and PCL are biodegradable polymers widely used in drug delivery research. (A) PLGA is comprised of repeating units of glycolic or lactic acid. The addition of lactic acid confers greater hydrophobicity to the polymer, resulting in lower degradation rate in water. In contrast (B) poly(caprolactone) is highly hydrophobic and does not appreciably degrade in water [64]. Lactic acid monomers may be introduced to increase the flexibility and hydrophilicity of PCL, resulting in PLCL (poly(DL-lactide-co-ε-caprolactone)). In our work, this modified derivative of PCL was compared with PLGA for the delivery of the ARV drug, Tenofovir disoproxil fumarate (TDF).
Similarly, but less extensively for HSV-2, PCL matrices were fabricated using a heat-based injection molding technique to incorporate increasing concentrations of Acyclovir (10, 15, and 20% w/v), a potent antiviral used for treatment against HSV-2 [65]. Encapsulated ACV exhibited release up to 30 days and retained comparable 50% inhibitory concentration (IC50) to free drug. Further work in this field involved the use of TDF-encapsulated IVRs to protect against HIV and HSV-2 in vitro and in vivo. Beyond demonstrating protection against HIV and HSV-2 in vitro, these IVRs also prevented HIV infection in macaques for up to 4 months (with monthly IVR changes) [50, 51].

Building upon this previous research, the goal of our work was to develop PLGA and PLCL EFs containing three concentrations of the ARV prodrug TDF to safely inhibit HIV-1 and HSV-2 infections in vitro (Figure 3). TDF was selected as a model ARV, to demonstrate proof-of-concept of our delivery vehicles, due to its potent efficacy against both HIV and HSV-2; its increased oral and topical lipophilicity and cell permeability; and enhanced IC50 (by 160-fold) relative to its prodrug counterpart, TFV [50]. Here we fabricated both PLGA and PLCL EFs to evaluate and compare two different biodegradable polymers known to impart sustained-release of active agents. In this work we synthesized 3 different formulations for each polymer, PLGA and PLCL, and we characterized the loading and sustained-release of TDF from EFs. We subsequently assessed the efficacy of fiber release eluates against HSV-2 and HIV-1 infection in vitro for up
to 4 weeks. We additionally demonstrated the biocompatibility of these EFs in vaginal keratinocytes, ectocervical cells, and endocervical cells.
Figure 3. Structure of Tenofovir disoproxil fumarate (TDF). TDF, a prodrug of Tenofovir (TVF), was developed to enhance oral bioavailability and efficacy. Both TDF and TFV are classified as potent NRTIs; however, TDF has an over 160-fold lower IC50 compared to TFV in vivo [50]. Newer derivatives of TFV such as Tenofovir alafenamide (TAF) are currently being researched to impart even greater efficacy.
MATERIALS AND METHODS

Materials

Poly(lactic-co-glycolic acid) (PLGA 50:50, 0.55-0.75 dL/g, 31-57k MW) and poly(DL-lactide-co-ε-caprolactone) (PLCL 80:20, 0.75 dL/g, 37k MW) were both purchased from Lactel Absorbable Polymers (Cupertino, CA). Solvents 1, 1, 1, 3, 3, 3—hexafluoro-2-propanol (HFIP) and trifluoroethanol (TFE) were obtained from Fisher Scientific (Pittsburgh, PA). TDF was purchased as Viread® (Tenofovir disoproxil fumarate, Gilead Sciences Inc., Foster City, CA) tablets from the University of Louisville Pharmacy. Other chemicals, including dimethyl sulfoxide (DMSO) and Thiazolyl Blue Tetrazolium Bromide (MTT) were purchased from Sigma Aldrich (St Louis, MO). Fetal bovine serum (FBS), antibiotics (penicillin/streptomycin and gentamicin), Minimum Essential Medium (MEM), Keratinocyte Serum-free Medium (KSFM), and Dulbecco’s Modified Eagle Medium (DMEM) were purchased from VWR and Thermofisher. Simulated vaginal fluid (SVF) was prepared in house using a previously established protocol [66].

Cell Lines and Virus

TZM-bl cells were obtained from the NIH AIDS Research and Reference Reagent Program (ARRRP). These cells are a genetically engineered HeLa cell clone that expresses CD4, CXCR4, and CCR5 and contain Tat-responsive
reporter genes for firefly luciferase (Luc) and Escherichia coli β-galactosidase under regulatory control of an HIV-1 long terminal repeat [67, 68]. TZM-bl cells were maintained in Gibco Dulbecco’s modified Eagle’s medium (DMEM; Invitrogen) containing 10% heat-inactivated FBS, 25 nM HEPES, and 50 µg/mL gentamicin, in a vented T-75 culture flask. Env-pseudotype HIV was kindly provided by Dr. Nobuyuki Matoba from the University of Louisville. To conduct HSV-2 plaque assays, African green monkey kidney (Vero E6) cells and HSV-2 (4674) were kindly provided by Dr. Kenneth Palmer from the University of Louisville. Cells were maintained in MEM supplemented with 10% FBS, penicillin (100 µg/mL), and streptomycin (100 µg/mL).

To assess cytotoxicity, Endocervical, End1/E6E7 (End1); ectocervical, Ect1/E6E7 (Ect1); and vaginal keratinocyte, VK2/E6E7 (VK2) cell lines were used (courtesy of Dr. Kenneth Palmer). These cell lines were selected because they are representative of the cell types in the female reproductive tract that would be exposed to the topical EFs. End1, Ect1, and VK2 cells were maintained in KSFM supplemented with bovine pituitary extract (50 µg/mL), epidermal growth factor (0.1 ng/mL), penicillin (100 µg/mL), and streptomycin (100 µg/mL). The media was further supplemented with calcium chloride (CaCl₂) to a final concentration of 0.4 mM. During cell trypsinization for plating and cell count, cells were neutralized using DMEM/F12 (Gibco) with 10% FBS, and 1% Penicillin/Streptomycin.

**Synthesis of Electrospun Fibers**
PLGA and PLCL EFs were prepared with different solvents and TDF concentrations spanning (1-20% wt drug/wt polymer (w/w)). Blank fibers containing no TDF were prepared as negative controls. For blank EFs, 15-20% PLGA w/w and 12-20% PLCL w/w were dissolved in either 3 mL TFE or HFIP solvent overnight while shaking at 37 °C. The following day, 2 mL of PLGA or PLCL solution was aspirated into, and electrospun from a 3 mL plastic syringe on a custom built device housed in an air-filtered Plexiglas chamber. All formulations were electrospun with a flow rate of 2.0 mL/hr and an applied voltage of 20 kV. EFs were collected on a rotating 4 mm outer-diameter stainless steel mandrel, located 20 cm from the blunt needle tip. Sample flow rate was monitored by an infusion pump (Fisher Scientific, Pittsburgh, PA) and the voltage was applied using a high voltage power supply (Spellman CZE 1000R). For fibers incorporating TDF, 1, 10, or 20% w/w TDF was dissolved in 1.2 mL solvent overnight. The next day the TDF solution was added to 1.8 mL polymer solution prior to electrospinning. After electrospinning, fibers were removed from the mandrel and dried overnight in a desiccator cabinet.

Electrospun Fiber Size and Morphology

The impact of various parameters including: solvent choice, polymer composition, and TDF concentration on fiber size and morphology were evaluated using scanning electron microscopy (SEM). Desiccated EFs were placed on carbon tape, sputter coated with gold, and imaged using SEM (Supra 35 SEM Zeiss). SEM images were acquired at magnifications ranging from
1,000-5,000x to enable clear visualization of the fiber microstructure. The average fiber diameter was determined by analyzing SEM images in NIH ImageJ, and drawing line elements across a minimum of 50 fibers per image. Statistical significance between fiber diameters was determined using the Tukey post hoc t-test (p < 0.05).

**Fiber Characterization: Loading, Encapsulation Efficiency, and Controlled Release**

Incorporated TDF was quantified by dissolving 5 mg of PLGA and PLCL EFs in 1 mL of (1 M) NaOH overnight. TDF in the supernatant was measured using UV absorption spectroscopy at 260 nm (Synergy HT Biotek) in UV transparent 96-well plates. Quantification was performed using a TDF standard diluted in NaOH, with blank fibers as background correction. TDF values determined from absorbance measurements were compared with the quantity of TDF added prior to electrospinning to obtain percent encapsulation efficiency (EE), where EE = [(Mass of TDF Incorporated)/(Mass of TDF Initially Added)] x 100. Unless otherwise noted, all samples were analyzed in triplicate.

Controlled release experiments were performed to assess the release of TDF from EFs. Triplicate 10 mg fiber pieces were cut and suspended in 1 mL of simulated vaginal fluid (SVF) to represent intravaginal conditions *in vitro*. Samples were incubated at 37 °C and constantly shaken. The complete volume of SVF was removed and replaced with fresh SVF at time points: 1, 2, 4, 6, 24, 48, 72 hr, and 1, 2, 3, and 4 wk. Statistical significance of both loading and
release profiles between fiber formulations were determined by one-way ANOVA with the Bonferroni post hoc t-test (p < 0.05)

**In Vitro Efficacy of PLGA and PLCL Fibers against HIV-1 infection**

HIV pseudovirus assays were used to assess the efficacy of TDF released from EFs against HIV infection *in vitro*. TZM-bl cells were infected with Env-pseudotype HIV, kindly provided by both Dr. Nobuyuki Matoba (University of Louisville) and the NIH AIDS Research and Reference Reagent Program (ARRRP). To produce and propagate HIV Env-pseudovirus, HEK293T/17 cells were transfected with two plasmids, one containing an Env-defective HIV genome and a plasmid solely expressing Env. Transfection was facilitated with the use of FuGENE (Promega). HEK293T cells were allowed to incubate for 48 hr, after which viral particles were collected and titered using the 50% Tissue Culture Infectious Doses assay (TCID<sub>50</sub>). Viral particles were stored at -80 °C until use [69].

To determine the *in vitro* efficacy of PLGA and PLCL TDF EFs against Env-pseudotype HIV infection, TZM-bl cells were seeded in 96-well plates at 100,000 cells/well in 100 μL of DMEM. Fifty microliters of fiber eluate media (DMEM 10% FBS) collected from time points: 1 and 24 hr; week 1; week 2 (release from days 7-14), week 3 (release from days 14-21), and week 4 (release from days 21-28) were diluted by a maximum of 5 orders of magnitude from collected eluate (1:100,000 maximum dilution). Eluate dilutions were added to cells in triplicate, and 50 μL of diluted virus stock (1:8) was subsequently added
to each well. The administered virus dose resulted in relative luminescence units (RLU) of at least twenty times that of background (untreated/uninfected cells), yielding an average maximum of 100,000 RLUs in our experiments. Three controls were used in the experiment: untreated/uninfected cells, untreated/infected cells, and blank fiber eluate-treated/infected cells. For wells containing untreated/uninfected and untreated/infected cell controls, 100 µL DMEM was added to the wells; for infected cells with blank fiber eluate, 50 µL DMEM was added to 50 µL blank fiber eluate. After infection, plates were incubated 48 hr at 37 °C. After incubation, 100 µL of media was removed from each well and replaced with 100 µL of Bright Glo Reagent (Promega). Cells were incubated at room temperature for another 5 minutes and the luminescence of each well was read. The amount of virus inhibition was determined by normalizing the RLUs of infected treated cells to untreated/infected cells. Additionally, all RLU values were corrected by subtracting the RLU of untreated/uninfected cells. IC50 values were determined using GraphPad 6.0 sigmoidal regression analysis. Unless otherwise noted, all experiments were run with three or more replicates per treatment group. Statistical significance between the IC50s was determined using one-way ANOVA with the Bonferroni post hoc t-test.

**In Vitro Efficacy of PLGA and PLCL Fibers against HSV-2 Infection**

HSV-2 plaque assays were conducted to test the efficacy of TDF EFs against HSV-2 infection *in vitro*. Fibers were incubated in 10 mL complete plating
media (1% FBS MEM) for 1 and 24 hr. Additional fiber eluates were collected at week 1; week 2 (release from days 7-14); week 3 (release from days 14-21); and week 4 (release from days 21-28) to assess the ability of PLGA and PLCL TDF EFs to provide prolonged delivery and corresponding HSV-2 protection. The antiviral activity of PLGA and PLCL TDF EF eluates was determined using HSV-2 (4674) plaque assays in Vero E6 cells. Vero E6 cells were seeded at 600,000 cells/well and grown to near confluence for 24 hr in a 6-well flat bottom plate. After 24 hr, the media was removed and cells were simultaneously administered 2 mL of fiber eluate serial dilutions from the above collected time points and 3,000 PFU of HSV-2 per well. Free TDF was used as a positive control for HSV-2 inhibition, in parallel with untreated/uninfected cells; whereas untreated/infected cells were used as a positive control of cell infection and death. After 48 hr, cells were fixed with methanol for 10 min, stained with 0.1% crystal violet for 30 min, and washed with DI water. Plaques were counted, and plaque numbers from experimental groups were normalized relative to the number of plaques in untreated/infected cells (~280-300 plaques). Samples were analyzed in triplicate, and GraphPad was used to determine the IC50 values of the TDF EF formulations. Statistical analysis was performed by comparing the average percent inhibition of HSV-2 using one-way ANOVA with the Bonferroni post hoc t-test.

*In Vitro* Cytotoxicity of PLGA and PLCL Fibers
Vaginal epithelial (VK2/E6E7), ectocervical (Ect1/E6E7), and endocervical (End1/E6E7) cells were incubated with TDF EFs in KSFM to assess the *in vitro* biocompatibility of TDF fibers. Cells were plated at a density of 300,000 cells/well in 12-well plates and incubated in triplicate with 1 mg fiber pieces placed in transwell inserts (1 mg/mL final concentration). No treatment (media alone) and 10% DMSO were used as positive and negative controls of cell viability, respectively. After 24, 48, and 72 hr incubation, 10 mL of MTT reagent was added to the cells, cells were lysed, and absorbance was read at 570 nm the following day. PLGA and PLCL EF-treated cell absorbance values were normalized to untreated cell absorbance to obtain percent viability.
RESULTS

Electrospun Fiber Size and Morphology

Fiber morphology was evaluated using SEM, and ImageJ software was used to assess fiber diameters. Figure 4 shows blank PLGA and PLCL fibers fabricated using either HFIP or TFE solvent. For fibers electrospun in HFIP, 15% w/w PLGA or 12% w/w PLCL provided well-defined fiber morphologies. However, with TFE, both polymers required an increase in concentration to 20% (w/w) to produce well-delineated microstructures. The average diameters were 2.0 ± 0.8 and 1.7 ± 0.4 µm for 15% PLGA and 12% PLCL fibers made with HFIP, and 1.9 ± 0.9 and 1.9 ± 0.8 µm for 20% PLGA and 20% PLCL fibers made with TFE. Once well-delineated fibers were established, the effect of TDF incorporation on PLGA and PLCL EF morphologies electrospun with HFIP was evaluated (Figure 5).

The morphologies and diameters of TDF EF formulations are shown in Figures 5 and 6, respectively. Prior to TDF incorporation, the average diameters of all blank fiber formulations were similar, ranging from 1.7 to 2.0 µm, with no statistical significance observed between formulations (Figure 6A). In comparison, fibers incorporating TDF showed a random distribution of fiber diameters for PLGA; whereas for PLCL, a decreased trend in fiber diameter was observed. The average fiber diameters for HFIP 15% PLGA 1%, 10%, and 20% TDF were 1.1 ± 0.3, 0.7 ± 0.2, and 1.1 ± 0.4 µm. For HFIP 12%
PLCL 1%, 10%, 20% TDF fibers, the resulting fiber diameters were 1.1 ± 0.5, 0.9 ± 0.3, 0.7 ± 0.2 µm, with PLCL fibers demonstrating a decrease in fiber diameter between blank (no TDF) and TDF-incorporated formulations. Additionally, TDF incorporated fibers electrospun with TFE solvent, displayed a similar decrease in diameters to 1.2 ± 0.4 µm and 0.6 ± 0.2 µm, for PLGA and PLCL respectively. All TDF fiber diameters had statistically different diameters relative to blank PLGA and PLCL EFs. However, no statistical significance was observed between the 1, 10, and 20% TDF fiber formulations as a function of TDF incorporation. Additionally, there was no statistical difference in diameter between TDF incorporated PLGA and PLCL fibers. Thus any addition of TDF resulted in decreased fiber diameter relative to blank fibers; whereas additional TDF incorporation had no significant effect on fiber diameter.
Figure 4. SEM images of blank PLGA and PLCL fibers electrospun using different solvents. (A) 15% w/w PLGA in HFIP; (B) 20% w/w PLGA in TFE; (C) 12% w/w PLCL in HFIP; and (D) 20% w/w PLCL in TFE. Scale bars represent 10 µm.
Figure 5. SEM images of PLGA and PLCL fibers prepared with increasing concentrations of TDF (1%, 10%, 20% w/w), using HFIP as solvent. (A) Blank PLGA, (B) 1% TDF, (C) 10% TDF, and (D) 20% TDF PLGA fibers; (E) Blank PLCL, (F) 1% TDF, (G) 10% TDF, and (H) 20% TDF PLCL fibers. Scale bars represent 10 µm.
Figure 6. Average diameters of electrospun fibers measured from SEM images, using ImageJ. (A) Diameters of blank PLGA and PLCL fibers electrospun with either HFIP or TFE solvents. Diameters ranged from 1.7 to 2.0 µm. No statistical significance was observed between fiber diameters prepared with either HFIP or TFE. (B) Diameters of TDF-incorporated EFs were significantly smaller than blank fiber counterparts, ranging from 0.7 to 1.2 µm (TFE data not shown). While the PLGA TDF fiber diameters seemed randomly distributed, PLCL TDF fibers demonstrated a trend of decreased diameter with increasing TDF concentration. While statistical significance was observed between blank fibers and all TDF fiber formulations, no statistical difference in diameters was observed between the TDF EF formulations.
Fiber Characterization: Loading, Encapsulation Efficiency, and Controlled Release

To determine the loading of TDF in PLGA and PLCL EFs, fibers and unincorporated TDF were weighed and dissolved overnight in (1M) NaOH. Serial dilutions of TDF in NaOH were used to create a standard curve (Figure 7). The linear range of the standard curve extended from 1 mg/mL to 488 ng/mL of TDF. All concentrations of TDF (1, 10, and 20% w/w) fibers and standards were measured via absorbance at 260 nm. Table 1 summarizes the total loading (µg TDF/mg fiber) and encapsulation efficiency (EE) achieved for the various fiber formulations. Overall, we observed that fibers electrospun with HFIP resulted in high EEs spanning 43-75%. The 10% TDF PLGA and PLCL fibers electrospun with HFIP solvent were compared to 10% TDF PLGA and PLCL fibers electrospun with TFE, to assess the loading difference based on electrospinning solvent choice. Whereas the 10% TDF PLGA and PLCL EFs electrospun with HFIP showed a high encapsulation efficiency of 69% for each formulation, the TFE EFs incorporated only 41-44% TDF. Within the HFIP electrospun fibers, the 1% TDF PLGA and PLCL fibers incorporated less than half of the added TDF, showing the least incorporation of all formulations, while the 10% TDF fibers demonstrated consistently high EE around 69%. The 20% TDF fibers showed some variability in EE based on polymer type, with PLGA incorporating 56% TDF and PLCL incorporating 76%. Since the electrospun fibers made with HFIP showed 30% higher encapsulation than TFE fibers (for the 10% formulation), in addition to the high polymer concentration required to fabricate well-delineated
fibers with TFE (20% for PLGA and PLCL vs. 12 and 15% for PLGA and PLCL, respectively), HFIP was selected to electrospin subsequent formulations.

After determining the PLGA and PLCL fiber loading, we assessed the release of TDF from PLGA and PLCL EFs in SVF for up to 4 weeks. Standard solutions of TDF in SVF were prepared to assess TDF release in SVF eluates (Figure 8). Measuring absorbance at 260 nm, the standard curve ranged from 488 ng/mL to 1mg/mL of TDF. Figure 9 demonstrates increased TDF release per mass of fiber, with increased incorporation of TDF for both PLGA and PLCL. All fiber formulations experienced a burst release spanning 0-60% for each formulation after 48 hr of release. Based on total percent loading, the 10% and 20% PLCL EFs showed greater burst release of 3% and 21%, respectively at 1 hr, compared to the 10% and 20% PLGA fibers (Figure 9B). However, after 24 hr incubation, both 10% and 20% PLGA and PLCL EFs released nearly equivalent amounts of TDF per total loading. Similarly, cumulative release of TDF per mass of fiber showed that 10% and 20% PLCL fibers released more TDF (35 and 39 µg/mg) than corresponding PLGA fibers at 1 hr. After 24 hours, the differences in TDF released between PLCL and PLGA fibers decreased to 29 and 7 µg/mg for the 10% and 20% formulations, respectively.

For prolonged release after 4 weeks, PLGA fibers exhibited higher cumulative release of incorporated TDF per mass of fiber, compared to PLCL fibers, with the exception of the 10% TDF EFs (Figure 9A). On a percent loading basis however, PLGA fibers consistently released more TDF than PLCL fibers for the 1%, 10%, and 20% TDF formulations. PLGA fibers released 25, 70, and 86%
of their cargo; whereas PLCL fibers released, 52, and 73% of their cargo for the 10% and 20% formulations after 4 wk. TDF release was not detected from the 1% PLCL formation. Furthermore, we observed an increase in release at 21 days from all PLGA fibers, which can be attributed to polymer matrix degradation under acidic conditions. From these experiments we observed that PLGA fibers have the potential for increased sustained-release over a 4 week period based on percent loading, relative to PLCL fibers which seemed to plateau after initial burst release. However, on a mass-by-mass basis, PLGA fibers were only slightly improved for release relative to PLCL fibers, with the exception of the 10% TDF PLGA formulation.
Table 1.
Quantification of TDF fiber loading and encapsulation efficiency.

<table>
<thead>
<tr>
<th>Fiber Formulation</th>
<th>Loading TDF/Fiber (µg/mg)</th>
<th>Encapsulation Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HFIP 15% PLGA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1% TDF</td>
<td>10 ± 0.3</td>
<td>45 ± 1.6</td>
</tr>
<tr>
<td>10% TDF</td>
<td>88 ± 3.9</td>
<td>69 ± 2.5</td>
</tr>
<tr>
<td>20% TDF</td>
<td>162 ± 1.8</td>
<td>56 ± 0.6</td>
</tr>
<tr>
<td><strong>HFIP 12% PLCL</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1% TDF</td>
<td>7 ± 0.1</td>
<td>43 ± 0.6</td>
</tr>
<tr>
<td>10% TDF</td>
<td>87 ± 1.8</td>
<td>69 ± 1.4</td>
</tr>
<tr>
<td>20% TDF</td>
<td>171 ± 3.3</td>
<td>75 ± 1.4</td>
</tr>
<tr>
<td><strong>TFE 20% PLGA 10% TDF</strong></td>
<td>91 ± 6.5</td>
<td>44 ± 2.5</td>
</tr>
<tr>
<td><strong>TFE 20% PLCL 10% TDF</strong></td>
<td>98 ± 10.8</td>
<td>41 ± 3.7</td>
</tr>
</tbody>
</table>

PLGA and PLCL fibers electrospun with HFIP demonstrated higher loading and encapsulation efficiencies, relative to PLGA and PLCL fibers electrospun with TFE. Furthermore, linear increases in encapsulation efficiency were observed based on the amount of TDF added to PLGA and PLCL formulations.
Figure 7. The standard curve used to quantify TDF loading and encapsulation efficiency. Fibers and TDF standard were dissolved overnight in (1M) NaOH. Samples and TDF standard absorbance were read at 260 nm. The initial stock concentration of free TDF was 1000 µg/mL. Serial dilutions of 1:2 were performed in UV 96-well plates. The limit of detection determined from this standard curve was 0.488 µg/mL TDF, and the linear range extended from 0.488 to 1000 µg/mL TDF.
Figure 8. The standard curve used to quantify TDF sustained-release. The free TDF standard was diluted in simulated vaginal fluid (SVF) to quantify fiber release eluates in SVF. Samples and TDF standard absorbance were read at 260 nm. The initial stock concentration of free TDF was 2000 µg/mL; however, the linear range of this standard curve extended from 0.488 to 1000 µg/mL TDF.
Figure 9. Sustained-release profiles of 1, 10, and 20% TDF PLGA and PLCL fibers in SVF. (A) Cumulative release of TDF per milligram of fiber (µg TDF/mg fiber) over one month. (B) Cumulative release of TDF as percent total loading (%) over one month. While PLCL fibers showed a higher burst release after 1 hr, overall PLGA fibers exhibited greater sustained-release over the course of 4 wk, relative to PLCL fibers. In agreement with our previous work release of TDF from PLGA fibers in the acidic environment of SVF increased at 21 days [70].
In Vitro Efficacy of PLGA and PLCL Fibers against HIV-1 infection

Short-Term Efficacy

To assess the antiviral activity of TDF PLGA and PLCL fibers, HIV inhibition assays were performed using fiber eluates collected at different controlled-release time points. Both short- and long-term release samples were collected to assess efficacy after different time points. For short-term (1 and 24 hr) assessment of antiviral activity, 10 mg fibers were incubated in 1 mL DMEM for 1 or 24 hr. A histogram of the HIV inhibition after administration of the 1 hr (Figure 10A) or 24 hr fiber release eluates (Figure 10B) is shown in Figure 10 and the corresponding IC50s are shown in Table 2. All EFs with 10 or 20% TDF completely inhibited viral infection in TZM-bl cells down to a 1:100 dilution.

For dilutions of 1 and 24 hr eluates exceeding 1:100, viral inhibition was more pronounced after administration of the 24 hr eluates, relative to 1 hr eluates. The increased efficacy observed with 24 hr eluates can be attributed to the higher amount of TDF released within 24 hr. For both time points, the PLCL fibers seemed to impart greater protection against in vitro infection. This was quantified by calculating the IC50 values for all PLGA and PLCL TDF fibers (Table 2).

Overall, the antiviral activities of these eluate dilutions demonstrate that the amount of TDF in the fiber correlates well with increased viral inhibition. For the 1% TDF PLGA and PLCL fibers, 1 hr undiluted eluates decreased infection to 27% and 10%, relative to untreated/infected controls at 100% infection. However, subsequent dilutions of the 1% TDF 1 hr eluates yielded decreased protection
against HIV-1 infection. In contrast, after 24 hr release, the 1% TDF PLGA and PLCL fibers completely inhibited virus infection, with subsequent decreases in virus inhibition corresponding with increased eluate dilution. Full infection resulted after administration of the 1% PLGA and PLCL fibers at a dilution of 1:10, and 1:1000, respectively for the 1 hr eluates; and 1:1000 and 1:100 for the PLGA and PLCL 24 hr eluates.

For the 10% TDF formulations, both PLGA and PLCL fibers exhibited complete protection against HIV down to 1:100 and 1:1000 eluate dilutions, respectively after 1 hr. After administration of the 24 hr eluates, complete protection was observed even after a 1:1000 dilution of each formulation. Subsequent dilutions of PLGA eluates for 1 hr and 24 hr time points exhibited an increase in infectivity (decrease in prevention) to 31% and 13% for 1:1000, and complete infectivity for 1:10,000 dilutions, respectively. For PLCL, complete protection was achieved with the 1:1000 eluate dilutions at both 1 and 24 hr time points. Additionally, these fibers showed efficacy even at eluate dilutions of 1:10,000, with 48% and 69% infectivity at 1 and 24 hr. The corresponding IC50s for 10% TDF PLGA and PLCL EFs were 33 and 49 ng/mL after 1 hr, and 55 and 43 ng/mL after 24 hr (Table 2).

As expected, the 20% TDF fibers exhibited the highest efficacy against HIV infection per mass of fiber tested. Similar to the 10% TDF PLGA and PLCL EFs, 20% TDF fibers completely inhibited infection after a 1:1000 dilution, and exhibited partial efficacy (58-100%) between 1:10,000 and 1:100,000 dilutions.
The corresponding IC50s for 20% TDF PLGA and PLCL EFs were 34 and 29 ng/mL after 1 hr release, and 63 and 30 ng/mL after 24 hr release (Table 2).

Overall, the IC50s of TDF PLCL fibers were lower (more efficacious) than the IC50s of PLGA TDF fibers for the 10% and 20% formulations at 1 and 24 hr. The only exception was the 1 hr 10% TDF PLGA EF release eluate, which demonstrated higher efficacy relative to PLCL EFs. In addition, the IC50s of all TDF fiber eluates were compared to the IC50s of free TDF exposed to media for similar durations. As shown in Table 2, all fibers demonstrated equivalent or lower IC50s relative to free TDF (90 and 65 ng/mL at 1 and 24 hr respectively). Although increased TDF incorporation correlated to higher protection against HIV infection, after accounting for TDF concentration, all TDF fibers proved similarly efficacious. There was no statistical difference between the IC50s of the different polymer formulations at 1 and 24 hr time points of release. Additionally, no statistical significance was found between the same fiber formulations at different time points. Finally, there was no statistically significant difference between fiber and free TDF IC50s, suggesting that either could be used to provide short-term protection for 1 or 24 hr.
Figure 10. Results from *in vitro* HIV-1 infection assays. PLGA and PLCL fiber eluates inhibit HIV infection after 1 and 24 hr (A and B), and 1 and 2 wk of release (C). Dilutions of release eluate from 10 mg/mL fiber concentrations at different time points were normalized to untreated/infected cell control RLUs to assess percent HIV inhibition *in vitro*. Figures show the percent of cells infected, after incubation with: (A) 1 hr release eluate; (B) 24 hr release eluate; and (C) 1
and 2 wk release eluate from 20% TDF PLGA and PLCL fibers only, compared to (D) blank fiber eluates at each time point, and (E) free TDF (200 µg/mL) eluate for 1 and 2 wk. After 3 and 4 wk incubation, minimal protection against HIV-1 was shown for all fiber eluates (F). The 20% TDF PLGA fibers eluates showed no protection even for undiluted eluates. In contrast, undiluted 20% TDF PLCL eluates inhibited infection by 55%; however, no protection was demonstrated at 1:10 dilution.
Table 2.

PLGA and PLCL fiber IC50s (against HIV-1) after administration of the 1 and 24 hr eluates.

<table>
<thead>
<tr>
<th>Fiber Formulation</th>
<th>IC₅₀ at 1 Hr (ng/mL)</th>
<th>IC₅₀ at 24 Hr (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLGA 10% TDF</td>
<td>33 (27 to 40)</td>
<td>55 (46 to 67)</td>
</tr>
<tr>
<td>PLGA 20% TDF</td>
<td>34 (27 to 51)</td>
<td>63 (43 to 89)</td>
</tr>
<tr>
<td>PLCL 10% TDF</td>
<td>49 (39 to 60)</td>
<td>43 (20 to 90)</td>
</tr>
<tr>
<td>PLCL 20% TDF</td>
<td>29 (19 to 46)</td>
<td>30 (18 to 45)</td>
</tr>
<tr>
<td>Free TDF</td>
<td>91 (82 to 100)</td>
<td>65 (18 to 120)</td>
</tr>
</tbody>
</table>

The IC50s of TDF fiber eluate are similar or less than free TDF (91 and 65 ng/mL). However, no statistical significance between formulations at the same time point, within the same formulation at different time points, or between formulations and free TDF at the same time point were observed (p > 0.05). Confidence intervals of IC50s are shown in parentheses.
Long-Term Efficacy

To assess the long-term efficacy of the fibers against HIV infection \textit{in vitro}, eluates were collected from 10 mg of 20% TDF PLGA and PLCL fibers after incubation in 1 mL DMEM for 1, 2, 3, and 4 wk. Twenty percent TDF fibers were selected due to their greater efficacy and applicability to future dosing potential \textit{in vivo}. A histogram of HIV inhibition after administration of the 1 and 2 wk fiber release eluates is shown in Figure 10C and the corresponding IC50s are quantified in Table 3. After 1 and 2 wk, both PLGA and PLCL EFs completely inhibited HIV infection with no dilution. However, only the 1 wk eluates completely inhibited HIV infection, even after a 1:100 dilution. Two week eluates demonstrated weaker activity, showing only marginal protection (14% and 27%) at 1:10 dilutions of PLGA and PLCL fiber eluates, respectively. The corresponding IC50s were 60 and 350 ng/mL for 1 wk PLGA and PLCL eluates and 18,530 and 2900 ng/mL (18.5 and 2.9 μg/mL) for 2 wk PLGA and PLCL eluates. Free TDF controls showed a similar decrease in inhibition, relative to their IC50s after 1 and 24 hr exposure to media (91 and 65 ng/mL), to 34,050 and 37,250 ng/mL (34 and 37.2 μg/mL) at 1 and 2 wk, suggesting the degradation of free TDF after prolonged exposure to media (Figure 10E).

Undiluted samples from weeks 3 and 4 showed minimal protection against HIV at the doses tested (Figure 10F).

Although PLGA showed higher efficacy (60 ng/mL), relative to PLCL (350 ng/mL) for 1 wk eluates, the IC50s between PLGA and PLCL were not
statistically significant. However, the IC50s of both PLGA and PLCL fibers were statistically significant compared to free TDF ($p < 0.05$). Furthermore, all IC50s of the 1 wk eluates were statistically significant ($p < 0.05$) relative to the 1 hr, 24 hr, and 2 wk time points. For 2 wk eluates, all samples exhibited IC50s that were statistically different from each other. While TDF PLGA EFs demonstrated a marked increase in IC50 after 2 wk compared to PLCL with IC50s of 18,530 ng/mL and 2,900 ng/mL (18.5 and 2.9 ng/mL) respectively, both PLGA and PLCL EFs proved more efficacious at 1 and 2 wk than administration of free TDF.
Table 3.

PLGA and PLCL fiber IC50s (against HIV-1) after administration of long-term 1 and 2 wk release eluates.

<table>
<thead>
<tr>
<th>Fiber Formulation</th>
<th>IC50 at 1 or 2 Wk (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLGA 1 Wk</td>
<td>60 (28 to 126)</td>
</tr>
<tr>
<td>PLGA 2 Wk</td>
<td>18530 (6609 to 51000)</td>
</tr>
<tr>
<td>PLCL 1 Wk</td>
<td>350 (260 to 460)</td>
</tr>
<tr>
<td>PLCL 2 Wk</td>
<td>2900 (1600 to 5300)</td>
</tr>
<tr>
<td>TDF 1 Wk</td>
<td>34050 (29710 to 39750)</td>
</tr>
<tr>
<td>TDF 2 Wk</td>
<td>37250 (33790 to 41070)</td>
</tr>
</tbody>
</table>

As exposure time to media increased, the efficacy of TDF fibers decreased. However, both PLGA and PLCL fiber eluates were more efficacious than free TDF after 1 and 2 wk exposure to media. Confidence intervals of IC50s are shown in parentheses.
**In Vitro Efficacy of PLGA and PLCL Fibers against HSV-2 Infection**

To evaluate the potential of these fibers to inhibit HSV-2 infection, the antiviral efficacy of TDF EFs was also assessed in HSV-2 plaque assays. Due to higher concentrations required for future *in vivo* work, only 20% TDF PLGA and PLCL fibers were evaluated. Similar to the HIV infection assay, eluate from 5 mg/mL fibers at 1 and 24 hr was tested and shown to completely inhibit viral plaque formation. Figure 11 illustrates the results of serial dilutions (1:1.5) of 1 and 24 hr eluates. Both PLGA and PLCL fiber eluates completely inhibited HSV-2 infection, and exhibited decreased protection with increased dilution. Eluates from 24 hr showed greater efficacy against HSV-2 infection due to the increased amount of released TDF.

The IC50s of incorporated TDF from both PLGA and PLCL fibers were assessed using these dilutions. These results (Figure 12 and Table 4) show that the IC50s of PLGA and PLCL 1 and 24 hr eluates were comparable to the free TDF IC50 of 16 µg/mL. While the plaque assays showed enhanced protection across dilutions provided by PLGA EFs after 1 and 24 hr, after accounting for fiber loading, there was no significant difference in efficacy between most of the fiber IC50s and free TDF. The only statistical differences were found with PLGA 1 and 24 hr eluates exhibiting higher efficacy against HSV-2 relative to PLCL 1 hr eluates.

Fiber eluates were also collected to assess HSV-2 protection after 1, 2, and 3 wk. For these time points and fiber concentration (5 mg/mL) tested, no
virus inhibition was observed (data not shown). Similarly, free TDF controls showed a dramatic decrease in efficacy against HSV-2 infection by 1 wk.
Figure 11. Results from *in vitro* HSV-2 infection assays. Plaque assays were conducted to assess the efficacy of 20% TDF PLGA and PLCL fiber eluates (5 mg/mL) against HSV-2 infection *in vitro*. Plaques were counted and normalized to untreated/uninfected samples. Results demonstrate HSV-2 efficacy attained with: (A) 1 hr and (B) 24 hr fiber eluate dilutions. TDF fibers were effective against HSV-2 infection at 1 and 24 hr; however, at the concentration tested, proved ineffective for all other eluate time points (data not shown).
**Figure 12.** IC50 curve of HSV-2 plaque assay inhibition. The log of TDF fiber eluate concentration is plotted against plaque numbers that have been normalized to untreated/infected samples. Both PLGA and PLCL TDF fiber eluates from 1 and 24 hr show similar IC50s to free TDF (~16 µg/mL).
Table 4.

PLGA and PLCL fiber IC50s (against HSV-2) after administration of the 1 and 24 hr eluates

<table>
<thead>
<tr>
<th>Fiber Formulation</th>
<th>IC50 (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLGA 20% TDF (1 hr)</td>
<td>14.78 (13.25 to 16.49)</td>
</tr>
<tr>
<td>PLGA 20% TDF (24 hr)</td>
<td>14.95 (13.55 to 16.55)</td>
</tr>
<tr>
<td>PLCL 20% TDF (1 hr)</td>
<td>14.93 (14.42 to 15.47)</td>
</tr>
<tr>
<td>PLCL 20% TDF (24 hr)</td>
<td>15.35 (13.86 to 16.99)</td>
</tr>
<tr>
<td>Free TDF</td>
<td>15.73 (14.0 to 17.68)</td>
</tr>
</tbody>
</table>

Plaque assays were performed to assess the antiviral activity of 20% TDF fiber eluates against HSV-2 infection. Fiber eluates from 1 and 24 hr showed similar activity, relative to free TDF. However, 1, 2, 3, and 4 wk eluates were ineffective with the dose administered, against HSV-2 infection (data not shown).

Confidence intervals of IC50s are shown in parentheses.
Figure 13. Representative pictures from HSV-2 plaque assays using undiluted fiber eluates. Undiluted eluates from the 24 hr release of 5 mg/mL fibers were incubated with Vero cells and HSV-2 for 48 hr. Eluates from fibers with increasing percent TDF exhibited greater protection against HSV-2 infection. 20% TDF PLGA and PLCL fibers completely inhibited HSV-2 plaque formation. Control wells with untreated/uninfected cells and TDF-treated (400 μg/mL TDF-infected cells similarly showed no plaque formation. Conversely, the positive control of HSV-2 infection – administration of eluates from blank fibers – showed the highest number of plaques.
In Vitro Cytotoxicity of PLGA and PLCL Fibers

Fiber cytotoxicity was assessed in VK2, Ect1, and End1 cells, using the MTT assay. As seen in Figure 14, high cell viability was maintained after administration of TDF fibers for 24, 48, and 72 hr. All cells demonstrated greater than 93, 91, and 96% cell viability at 24, 48, and 72 hr respectively, for all formulations tested. Ten percent DMSO was used as a positive control for cell death.
Figure 14. Cytotoxicity assessed via MTT assay. Vaginal epithelial cell lines: (A) Ect1 E6/E7, (B) VK2 E6/E7, (C) and End1 E6/E7, were incubated with blank or TDF PLGA or PLCL fibers (10 mg/mL) in 12-well plates. Viability was assessed at 24, 48, and 72 hr. All cells demonstrated greater than 93, 91, and 96% cell viability at 24, 48, and 72 hr respectively, for all formulations tested.
DISCUSSION

There is an urgent need for new topical PrEP technologies that can confer the sustained-release of active agents, while providing discreet and convenient protection against STIs. The emerging technology of biodegradable polymeric electrospun fibers offers the potential to fill this unique role. In these studies, we evaluated two electrospun fiber delivery platforms, comprised of the polymers, PLGA or PLCL, for their ability to prolong the release of TDF in a potential non-coital delivery vehicle, and to protect against both HIV-1 and HSV-2 infections in vitro. Here TDF served as a model antiretroviral drug, as it is only one of two agents approved by the FDA to prevent HIV infection. Furthermore, TDF has demonstrated antiviral activity against both HIV-1 and HSV-2 in vivo, establishing its versatility as a multipurpose active agent. The goal of this work was to develop and characterize polymeric electrospun fibers to safely and efficaciously provide protection against both HIV-1 and HSV-2 in vitro, as a multipurpose prevention platform. For the doses tested in our studies, TDF PLGA and PLCL fibers demonstrated enhanced protection, relative to free TDF, against HIV-1 and HSV-2 infections for up to 2 wks.

The first goal of this study was to determine the formulation of TDF PLGA and PLCL fibers that resulted in the most cohesive and well-defined fiber macro- and microstructures. During the fabrication of blank PLGA and PLCL fibers, several solvents were assessed. We observed that using HFIP and
TFE solvents yielded more reproducible PLGA and PLCL fiber morphologies, relative to dichloromethane, chloroform, and dimethylformamide (Figure 4). These solvents also enabled the incorporation of high weight percent polymer to solvent, which is critical to incorporating high concentrations of active agents in polymers. Using PLGA and PLCL fibers electrospun with HFIP as our baseline platforms, we sought to evaluate the effect of TDF incorporation on fiber diameter (Figures 5 and 6).

Fiber diameters play a critical role in the release properties of active agents incorporated in polymeric vehicles. Previous research has shown that decreasing fiber diameter has been correlated to the enhanced release of active agents. This is attributed to the increased surface-to-volume ratio, and decreased distance necessary for encapsulate diffusion [71, 72]. In our studies, the incorporation of TDF resulted in decreased fiber diameters relative to blank fibers (Figure 6). The diameters of blank fibers ranged from 1.7 to 2.0 µm, whereas TDF fiber diameters ranged from 1.1 to 0.7 µm. In fact, even for the lowest concentration (1%) TDF fibers tested, we observed a 50% decrease in fiber diameter. This decrease in fiber diameter may be attributed to the charge of the active agent, and/or polymer instability resulting from these charge effects. Correspondingly, this instability in the polymer solution may result in the polymer traveling longer distance/duration during the electrospinning process prior to reaching the mandrel, promoting elongation and decreased fiber diameter [73, 74]. Thus, incorporated active agents can affect the microstructural morphologies and diameters of electrospun fibers.
However, the incorporation of antiviral or biological agents has been shown to affect fiber diameters in other ways. In previous studies, Tenofovir (TFV), a compound less hydrophilic than the fumaric salt form of TFV, TDF (TFV solubility = 1.87 mg/mL; TDF salt form (Viread, used in this study) = 13.4 mg/mL; Tenofovir disoproxil: 0.71 mg/mL [75, 76]), was shown to slightly increase fiber diameter, though the differences were not statistically significant [77]. In another study, an increase in fiber diameter was seen with increased bovine serum albumin incorporation. In this study, the increased diameter was attributed to an increase in viscosity of the solvent solution prior to electrospinning [78]. In other studies, the incorporation of antivirals resulted in the opposite effect on fiber diameter. Incorporation of TFV in polyvinyl alcohol polymers resulted in slightly smaller diameters, which was attributed to the increased instability described above [79]. Finally, some experiments show no change in fiber diameter after active agent incorporation. Fibers comprised of the pH-responsive CAP polymer, incorporating TDF, showed no change in fiber diameter compared to blank fiber counterparts [60]. The compilation of these studies highlights that a variety of parameters including solvent choice, polymer selection, solvent-polymer interaction, active agent characteristics, and solution viscosity can all contribute to the differing microstructural properties of electrospun fibers.

After obtaining well-defined and reproducible EFs, we next assessed the loading of TDF in PLGA and PLCL fibers as a function of solvent type (HFIP vs. TFE) used in the electrospinning process. From these loading studies (Table 1), we observed that fibers electrospun with HFIP showed higher encapsulation
efficiencies (25% more on average), relative to fibers electrospun with TFE. Furthermore, variations in polymer concentration were needed to obtain well-defined fiber microstructure and high loading efficiency, based on solvent type (15 and 12% w/w for PLGA and PLCL in HFIP; 20% PLGA and PLCL in TFE), which may be attributed to solvent used during the electrospinning process.

Despite both HFIP and TFE sharing many characteristics such as high polarity and similar molecular structure, there are several key differences that may impact fiber properties. First, TFE has a higher dielectric constant [26.1 (F/m)] compared to TFE [16.7 (F/m)] [80]. This increased charge capacity may confer additional instability to TFE solvents during electrospinning, requiring more polymer to produce well-defined fiber morphology [81]. Additionally, this increase in dielectric constant may result in decreased TDF incorporation, causing TDF to localize on or near the fiber surface. HFIP also possesses a much lower boiling point (58.2°C) relative to TFE (73.6°C). Solvents with lower boiling points tend to produce more stable fiber morphology due to complete evaporation during electrospinning; whereas less volatile (higher boiling point) solvents may not fully evaporate from the polymer, causing beaded morphologies [82]. These undesirable properties, in addition to the higher polymer concentrations required to produce TFE fibers with well-defined fiber microstructures and lower loading efficiencies, prompted us to fabricate all subsequent formulations with HFIP.

Controlled release studies using TDF EFs yielded several interesting results (Figure 9). First, as expected with most polymeric delivery vehicles, all
PLGA and PLCL fibers demonstrated a burst release of TDF during the first 24 hr. The exception was 1% TDF fibers, which released amounts below our limit of detection. On average, 0-60% of total incorporated TDF was released by EFs within the first 2 days. Overall, the 10% and 20% TDF PLCL fibers showed a higher burst release at 1 hr, based on total percent loaded, compared to corresponding 10% and 20% TDF PLGA fibers. However, after 24 hr, PLGA and PLCL formulations released roughly the same percent of total TDF loaded. Based on TDF released per mass polymer, both the 10% and 20% PLCL fiber released more TDF at 1 and 24 hours compared with PLGA EFs. As observed in our studies, burst release in nanoscale delivery systems is a common phenomenon due to high concentrations of drug localizing on the polymer surface [83]. In particular, surface localization may be exacerbated during the electrospinning process, due to charge effects between the incorporated drug, polymers and solvent. Solution instability during electrospinning due to these charge effects, as well as hydrophilic interactions between the solvent and drug can also result in agent localization near the fiber surface [74, 84]. Because of the increased hydrophobicity of PLCL, a higher percentage of TDF may have accumulated on the fiber surface, allowing for higher burst release within the first 1 hr.

In contrast, we observed that all PLGA fibers released higher percentages of TDF compared to PLCL fibers, after long-term incubation (4 wk) in SVF. On average, PLGA released 20% more of the total incorporated TDF compared to PLCL after 4 wk. We attribute this increased long-term TDF release to PLGA
being more hydrophilic than PLCL. The increased hydrophilicity of PLGA allows for enhanced aqueous solution penetration into the fiber, resulting in increased diffusion of TDF from the fiber into the surrounding eluant [56, 85]. We attribute this enhanced permeability to water, to the higher amount of TDF released from PLGA fibers after the initial burst release phase.

In addition to the burst and diffusion release phases, we also noted enhanced release of TDF from all PLGA and the 20% PLCL fibers around 21 days, with PLGA exhibiting the greatest enhancement in release. On average, the 20% TDF PLGA and PLCL fibers released 7.2% and 4.8% of TDF between the 2 and 3 wk time points, compared to only 3.7% and 0.6% released between 1 and 2 wk respectively. This enhanced release correlates with the second stage of polymer degradation, when matrix degradation enables the release of additional encapsulated drug.

Other studies using similar polymers yielded equivalent controlled release results. In one recent study, TFV was incorporated in PLGA and PCL polymers and polymer blends, and controlled release was evaluated for 10 days [63]. Similar to our work, PLGA demonstrated greater overall release of drug while showing an initial lower burst release. In contrast, PCL released all incorporated TFV after 24 hours, while PLGA released only ~20% of incorporated drug during this time. Similar burst release of TDF was also observed with 20:80 PCL:PLGA fibers, a trend that differed from the prolonged release observed from polymer blend incorporation of TFV [63]. Furthermore, several formulations of PCL/PLGA blends were fabricated, demonstrating decreased burst release of TFV with
increasing PLGA concentration [63]. We expect that similar blends may prove suitable to tailor the release of TDF, despite its increased lipophilicity.

In another study, the antiviral compounds, MVC (Maraviroc) and AZT, were encapsulated in PCL, polyethylene oxide (PEO), and poly-L-lactic acid (PLLA) polymer blends. Sustained-release from 70:30 PEO/PLLA blends showed almost complete release of hydrophilic compounds after 1 hr, due to the extreme hydrophilicity and quick degradation of PEO. In contrast, 30:70 PEO:PLLA blends exhibited lower burst release and higher sustained-release relative to the more hydrophilic 70:30 PEO/PLLA blends. Additionally, the moderately hydrophobic 30:70 PEO/PLLA fibers demonstrated better release profiles relative to pure PCL fibers, which released around 95% of incorporated drugs after 1 hr. This more efficacious release profile was attributed to the intermediate hydrophobicity and crystallinity of PLLA compared to PEO and PCL [62]. The hydrophilicity of PEO confers quick degradation in aqueous solutions, resulting in burst release; whereas PLCL is highly hydrophobic, causing incorporated compounds to localize on the fiber surface, also guaranteeing high burst release. The results from these studies are in agreement with our observations that polymers comprised of lactic and glycolic acid, relative to the more hydrophobic PLCL, exhibit less burst release of moderately hydrophilic compounds.

Last, we compared TDF release, based on the percent TDF incorporated (1, 10, or 20%) in the fiber. While we expected that 20% TDF fibers would release more TDF than 1 or 10% TDF EFs, based on equal polymer mass, as a percent of total loading, we observed that 20% TDF PLGA and PLCL fibers
released a higher proportion of the total incorporated TDF (86% and 74% respectively) over 4 wk, relative to 10% TDF PLGA and PLCL EFs (70% and 52%). This phenomenon may be partially due to the decreased fiber diameters resulting from higher TDF concentrations, as exhibited by PLCL EFs. Here the slightly smaller diameter fibers associated with increased TDF concentration may enhance release due to a higher fiber surface area. Another possibility may be due to the distribution of TDF within the polymer fibers. Due to the differences in hydrophobicity between PLGA and PLCL, combined with the increased concentration of hydrophilic/lipophilic TDF, higher proportions of the drug may localize to the fiber surface. Thus, increased TDF concentrations per mass of fiber, may also contribute to higher burst release, corresponding with our observations.

After characterizing these fibers, TDF EFs were evaluated for their potential to protect against HIV and HSV-2 infections in vitro (Figures 10-13). In these studies, TDF EF eluates of up to 2 wk, conferred protection against HIV, particularly for the 10 and 20% formulations (Figure 10); whereas HSV-2 inhibition was only achieved using the 1 or 24 hr release eluate (Figure 11). One factor that we believe contributes to this lack of efficacy associated with longer release times is the difference in TDF potency against HSV-2 and HIV-1. While TDF is efficacious against both HSV-2 and HIV-1, TDF is much less efficacious for HSV-2 (IC50 = 16 µg/mL) relative to HIV (0.06 µg/mL). Based on the release profiles of the 20% TDF PLGA and PLCL polymers, we expect that we would need approximately 15-20 mg fiber to provide 1 mg of TDF release (over one
month), and corresponding efficacy after 2 wk release. To account for this and in concurrent work, correspondingly increased amounts of TDF fibers will be used to assess HSV-2 efficacy after longer durations of fiber release. These estimates are within the dosing we envision for in vivo studies, in which similar studies have delivered a range of 0.2 to 0.7 mg/mL TDF per day within the murine reproductive tract to prevent HIV/HSV-2 infections [50, 51].

In addition to the increased TDF concentration needed to prevent HSV-2 relative to HIV-1 infections, the duration of fiber exposure to eluate likely impacts the potency of TDF released from the fibers. This is clearly observed in our efficacy studies where the administration of 1 and 24 hr TDF fiber eluates demonstrated similar efficacy to free TDF; whereas, after 1 wk of release, TDF fiber eluates exhibited greater efficacy against HIV-1, relative to free TDF (Tables 2 and 3). Moreover, after 2 wk, the IC50s of both TDF eluates and free TDF increased; with the potency of free TDF more dramatically affected (Table 3).

One factor validating the improved IC50s of TDF fibers, relative to free TDF with respect to time, is that TDF is known to crystallize and degrade in aqueous environments both in vitro and in vivo [50, 85-87]. While, increasing the stability of active agents is a benefit of utilizing delivery platforms such as fibers, even TDF fibers exhibited decreased efficacy against HIV-1 after 1 to 2 wk in eluate (Table 3). Relative to each other, PLGA fibers showed a more rapid decrease in efficacy relative to PLCL fibers. This may be attributed to an increase in polymer hydrophilicity with corresponding release of TDF from PLGA fibers (Figure 9), leading to longer drug exposure in the aqueous environment.
Additionally, long-term incubation of PLGA releases lactic and glycolic acid, resulting in a localized decrease in pH. Correspondingly, acidic conditions are known to enhance TDF degradation and may contribute to the decreased efficacy of TDF incorporated in PLGA fibers [87]. Thus, the longer an incorporated drug remains within the polymer under physiological pH, the longer it will retain efficacy. For future work this suggests that utilization of a different polymer or polymer blends may more optimally maintain active agent activity for durations exceeding 1 wk. Finally, the safety of both TDF PLGA and PLCL fibers was assessed after administration to vaginal and cervical cells. Our studies demonstrate that all cell lines had greater than 91% viability after fiber administration from 1 to 3 days (Figure 14). This is in agreement with our expectations; given that both polymers and TDF are FDA-approved (PLCL is a derivative of the FDA-approved polymer PCL). Based on these in vitro results, we expect to see similar safety profiles from fibers in tissue mimic and in vivo testing. This high biocompatibility, sustained-release, and demonstrated efficacy of PLGA and PLCL fibers warrant their investigation in immunological, pharmacokinetic/dynamic (PK/PD), and efficacy studies in vivo.

In parallel with advancing these formulations for in vivo study, our laboratory currently seeks to enhance the efficacy of our electrospun fibers against multiple STIs. Other polymer formulations are currently being evaluated to enhance sustained-release properties of antivirals and biological agents. The use of different solvents (or combination of) might confer enhanced loading of drugs of interest. However, more importantly, different polymers or polymer
blends might reduce the initial burst release of incorporated products while simultaneously optimizing long-term release. Furthermore, the development of multilayered and coaxially-spun fibers may also provide a platform suited for the delivery of multiple compounds with sustained-release profiles. Finally, surface modification of fibers or nanoparticle incorporation might offer alternative avenues to enhance or complement the prophylactic potential of these fibers.

Another potential route to complement the efficacy of our fibers is the incorporation of active agents with other mechanisms of action. The incorporation of novel non-ARV agents may avert some of the effects of toxicity and antiviral resistance recognized as impediments to long-term ARV use. One promising biologic that may fulfill the needs of safety and potency against multiple STIs is the antiviral lectin, griffithsin (GRFT), which has previously demonstrated efficacy against both HIV-1 and HSV-2. Furthermore, GRFT can be covalently bound to the fiber surface, acting as an adhesive scaffold to bind to and inactivate virus, in addition to its sustained-release potential. While more challenging, the incorporation of short interfering RNAs (siRNAs) may provide genetically-targeted mechanisms to inhibit viral and/or host targets, such as CCR5, to further enhance protection, while minimizing off-target effects. The incorporation of GRFT and other biologics in EFs highlights the seemingly limitless potential of EFs as novel topical delivery vehicles for microbicide applications.
SUMMARY AND CONCLUSIONS

There is an urgent need to develop new and alternative sustained-release technologies to prevent HIV-1 and HSV-2 infections. To address these needs, we fabricated PLGA and PLCL electrospun fibers, and compared the loading and sustained-release properties of these fibers, using TDF as a model antiviral. Both PLGA and PLCL fibers exhibited sustained-release for up to 4 weeks and provided complete protection against both HIV-1 and HSV-2 infections in vitro. Both short- (1 and 24 hr) and long-term (1 and 2 wk) protection were achieved against HIV-1; whereas short-term protection (due to fiber dosing) was achieved against HSV-2 in vitro. Additionally, TDF fibers demonstrated significantly enhanced efficacy against HIV-1, relative to free TDF, after long-term release of 1 and 2 wk. Lastly, vaginal and cervical cells exposed to TDF PLGA and PLCL fibers showed high viability, after up to 3 days administration, demonstrating their safety in vitro.

Comparing the attributes of PLGA and PLCL TDF EFs, PLGA initially appears to be a more promising candidate compared to PLCL, based on its improved sustained-release profile. PLGA possesses a lower burst release as well as increased long-term release of TDF relative to PLCL. However, upon further investigation, PLCL EFs maintained the stability of TDF for longer durations in vitro. However, as both formulations demonstrated efficacy against
HIV and HSV-2 *in vitro*, future testing may reveal both fiber formulations to be equally efficacious *in vivo*.

This work serves as the foundation for our future work. Using the information obtained here, we seek to further enhance the efficacy and delivery duration of small molecule antivirals and biologics from EFs by utilizing a variety of encapsulants and polymer blends. Additionally, future *in vivo* work with our TDF fibers is planned to demonstrate fiber efficacy and safety in a murine model of infection. We predict that these, or similar electrospun fibers will confer long lasting and sustained protection against both HIV and HSV-2 infections.
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CURRICULUM VITAE

Kevin M. Tyo
Graduate Research Assistant,
University of Louisville
Department of Pharmacology and Toxicology
Louisville, KY 40292

Phone: (502) 424-8625
Email: kmyo01@louisville.edu

Education:

University of Louisville, Department of Pharmacology and Toxicology
M.S and Ph.D. in Pharmacology and Toxicology 08/2014 - Present
Project: Griffithsin incorporated electrospun polymeric fibers as long-term drug delivery vehicles.
Dissertation Advisor: Dr. Jill Steinbach-Rankins

Virginia Tech, Department of Biochemistry
B.S in Biochemistry 08/2005 – 05/2010

Research and Professional Experience:

Graduate Research Assistant 10/2014 – Present
University of Louisville, Department of Pharmacology and Toxicology
My project involves the fabrication of electrospun fibers and polymeric nanoparticles as drug and gene delivery vehicles to provide more efficacious prophylaxis/treatments for STIs. We envision fibers and nanoparticles that will confer long-term, safe, and discreet protection for women against multiple STIs including HIV and HSV-2. By incorporating biologics such as the novel and potent antiviral lectin Griffithsin (GRFT) into our delivery platforms, we hypothesize that we may create drug delivery vehicles that will prevent viral infection for up to 1 month.

Associate Scientist 04/2012 – 05/2014
Arista Laboratories, Gas Chromatography Department, Henrico, VA
My primary duties included extraction and analysis of target compounds by employing GC instrumentation and performing daily and preventative maintenance on instruments. This was done under GLP compliant conditions.
Often, I worked with difficult biological materials such as tobacco smoke and plant fibers. Additionally, I assisted management on R&D projects and collaborative studies. This involved the development of new methods as well as optimization of established procedures.

Laboratory Technician 03/2011 – 04/2012
Schneider Laboratories Global Incorporated, Richmond, VA
During my tenure at Schneider Laboratories Global Incorporated, I assisted the company in establishing certification in drinking water analysis. Prior to my arrival, the company focused solely on industrial and waste water analysis. I worked with a small team to write new standard operating procedures, validate methods, and perform quality assurance tests. Within a year, the laboratory was accredited in analyzing organic contaminants in drinking water and was awarded several lucrative contracts.

Undergraduate Research 05/2009 – 08/2009
Virginia Tech, Department of Biochemistry, Blacksburg, VA
In this laboratory, work was performed on tuberculosis proteins. New antibiotic resistant strains of tuberculosis bacteria have emerged and new therapy targets are urgently required to treat this deadly pathogen. Our work involved isolating and purifying proteins of interest to elucidate their structure. I was instrumental in training new students on laboratory techniques and safety.

Undergraduate Research 08/2007 – 08/2008
Virginia Tech, Department of Biology, Blacksburg, VA
My first research experience was in performing the extraction and isolation of proteins derived from Shigella pseudomonas. During this position, I learned many new skills, including new laboratory techniques such as cell culture and protein extraction; In addition, I developed personal and professional skills such as laboratory notebook record keeping and proper usage of personal protection equipment.

Professional Memberships:

Biomedical Engineering Society 2015 – Present

Posters and Presentations:

Tyo, Kevin, Jill M. Steinbach. “Electrospun Nanofibers as a Novel Drug Delivery System for the Prevention of STIs”, Research Louisville; Louisville, KY; October 27, 2015.