International Journal of Pharmaceutics 531 (2017) 118–133

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

# Multipurpose tenofovir disoproxil fumarate electrospun fibers for the prevention of HIV-1 and HSV-2 infections *in vitro*

Kevin M. Tyo[b,g], Hung R. Vuong[d], Danial A. Malik[b], Lee B. Sims[a], Houda Alatassi[e], Jinghua Duan[a,g], Walter H. Watson[b,f], Jill M. Steinbach-Rankins[a,b,c,g,*]

[a] Department of Bioengineering, Speed School of Engineering, University of Louisville, Louisville, KY, United States
[b] Department of Pharmacology and Toxicology, School of Medicine, University of Louisville, KY, United States
[c] Department of Microbiology and Immunology, School of Medicine, University of Louisville, KY, United States
[d] Department of Biochemistry, School of Medicine, University of Louisville, KY, United States
[e] Department of Pathology, University of Louisville, Louisville, KY, United States
[f] Department of Medicine, Division of Gastroenterology, Hepatology and Nutrition, School of Medicine, University of Louisville, KY, United States
[g] Center for Predictive Medicine, Louisville, KY, United States

ARTICLE INFO

*Article history:*
Received 2 June 2017
Received in revised form 27 July 2017
Accepted 2 August 2017
Available online 7 August 2017

*Keywords:*
Electrospun fiber
Microbicide
Tenofovir (TFV)
Tenofovir disoproxil fumarate (TDF)
Drug delivery fiber
Sexually transmitted infections (STIs)
HIV
HSV-2
Multipurpose prevention
MPT
Sustained release fiber
Antiretroviral (ARV)

ABSTRACT

Sexually transmitted infections affect hundreds of millions of people 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 h and 1 wk, respectively, based on the doses tested. *In vitro* cell culture and EpiVaginal tissue tests confirmed the safety of fibers in vaginal and cervical cells, highlighting the potential of PLGA and PLCL EFs as multipurpose next-generation drug delivery vehicles.



## 1. 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 (WHO (World Health Organization), 2013). Compounding these statistics, HSV-2 infection has been shown to significantly enhance HIV infection by as much as 2 to 7-fold (Freeman et al., 2006; Corey et al., 2004; Galvin and Cohen, 2004). Correspondingly, the challenges in HSV-2 prevention and treatment, combined with this high global incidence and propensity for co-infections, contribute to the need for multipurpose platforms that prevent both HSV-2 and HIV infections.

Despite the existence of multiple strategies to prevent and/or treat STIs, rates of infection among particular demographics remain high (Beyrer et al., 2012; Beyrer and Abdool Karim, 2013). Moreover, despite the numerous antivirals available to treat HIV and HSV-2 individually, to date there are no agents that completely prevent or cure these infections individually or together. In terms of prevention, pre-exposure prophylaxis (PrEP) has enabled high-risk individuals to prevent HIV-1 infection by taking oral medication daily. However, to date, only two compounds, Tenofovir Disoproxil Fumarate (TDF) and Emtricitabine (combined with TDF in Truvada), are approved for PrEP by the FDA (FDA, 2012).

Oral PrEP has demonstrated success in preventing HIV in clinical trials and is becoming increasingly acknowledged as a successful prevention platform (Baeten et al., 2012; Heneine and

* Corresponding author at: Department of Bioengineering, Speed School of Engineering, University of Louisville, Louisville, KY, United States.
*E-mail address:* jill.steinbach@louisville.edu (J.M. Steinbach-Rankins).

http://dx.doi.org/10.1016/j.ijpharm.2017.08.061
0378-5173/

Kashuba, 2012). By frequent administration of oral antiretroviral (ARV) compounds, such as TFV and Emtricitabine, prevention rates from 44 to 75% have been achieved in clinical trials (Thigpen et al., 2012; McMahon et al., 2014; Mastro et al., 2014). However, as exemplified in the VOICE (MTN-003) trial, frequent administration of oral tablets (or vaginal gels) and strict user adherence are critical to provide any meaningful protection (Marrazzo et al., 2015). 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 (Marrazzo et al., 2015). Thus new topical delivery 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. Traditionally, topical PrEP has been administered in the form of gels, but films, tablets, and intravaginal rings (IVRs) have also demonstrated promise in safety and efficacy trials. Antiviral gels have the potential to confer protection when frequently applied. 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% (Abdool Karim et al., 2010). However, similar to the oral tablet arm of the VOICE (MTN-003) trial, topical gels containing TFV required strict user adherence both prior to and after sexual intercourse to maintain effectiveness (Boonstra, 2015; Hankins and Dybul, 2013). This strict dosing resulted in suboptimal user adherence, leading to decreased protection against infection. In addition, some users experience discomfort or leakiness, which may have further reduced adherence to the prescribed dosing regimen. Intravaginal films have also demonstrated protection against STIs; however, the rapid release of the encapsulated agents within hours of administration (burst release) remains a major hurdle for long-term administration (Hankins and Dybul, 2013; Akil et al., 2015, 2014). In addition, challenges in self-administration of vaginal films, combined with reported irritation with long-term use has affected user adherence (Hankins and Dybul, 2013; Bunge et al., 2016). Similar to vaginal films, tablets exhibit rather transient activity and a lack of long-term release; however, they offer a cost-effective platform for antiviral delivery (Clark et al., 2014).

Of these existing delivery technologies, intravaginal rings (IVRs) provide the current "gold standard" to prolong the release of active agents for 3 to 4 months. While IVRs have been utilized for hormonal contraceptive delivery for over a decade, the translation of IVRs to HIV PrEP has been recently demonstrated in the ASPIRE (MTN-020) and IPM clinical trials (Baeten et al., 2016; Nel et al., 2009). Additionally, IVRs have demonstrated the ability to provide sustained-release of multiple ARVs such as Tenofovir (TFV) and Acyclovir, providing *in vitro* protection against HSV-2 and HIV, as well as protecting against HSV-2 *in vivo* (Moss et al., 2012). However, concerns remain regarding the lack of complete protection provided in clinical trials, and their ability to incorporate less stable agents, such as proteins and oligonucleotides, due to the high processing temperatures utilized during the manufacturing process (Ho, 2013). Lastly, similar to the above technologies, user adherence of IVRs, particularly in young age groups (18–25 yr), remains another major concern (Baeten et al., 2016). These results suggest that the development of alternative dosage forms may improve user adherence and achieve increased efficacy, by providing options that are more amenable to female preferences

As a relatively new microbicide delivery technology, electrospun fibers (EFs) may provide a promising alternative for prolonged and localized agent delivery, with the potential to protect against multiple STIs. Some of the advantages of EFs include the ability to highly incorporate a diversity of active agents including drugs and biologics (Steinbach, 2015; Grooms et al., 2016), to tailor sustained-release by selecting different polymeric materials, and to maintain agent stability during the course of delivery (Pillay et al., 2013; Gunatillake and Adhikari, 2003). Biodegradable polymers, such as poly(lactic-co-glycolic acid (PLGA) and poly(caprolactone) (PCL), are approved by the U.S. Food and Drug Administration (FDA) for therapeutic use, indicating their proven biocompatibility and potential for translation (Makadia and Siegel, 2011). Together, these attributes have recently established polymeric EFs as an attractive platform for localized 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) (Huang et al., 2012). 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 surface-modified polystyrene and polypropylene fibers to bind to and inhibit HIV with higher efficacy than unmodified fibers alone (Huang et al., 2014).

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 (Carson et al., 2016; Ball et al., 2012). Polymer blends of polyethylene oxide (PEO) and poly(L-lactic acid) (PLLA) were synthesized to encapsulate and tailor the release of the antivirals Maraviroc (entry inhibitor) and AZT for up to several weeks (Ball et al., 2012). In another study, PLGA and PCL fibers were loaded with various concentrations of the antiretroviral TFV (Carson et al., 2016). These fibers demonstrated sustained-release of TFV for 30 days, as well as efficacy against HIV infection *in vitro*.

Similarly, but less extensively for HSV-2, sustained-release delivery vehicles have been recently developed. In one study, PCL molded matrices were fabricated using a heat-based injection molding technique to incorporate increasing concentrations of Acyclovir (10, 15, and 20% w/v) into IVR-like dosage forms (Asvadi et al., 2013). The encapsulated ACV exhibited release up to 30 days and retained comparable 50% inhibitory concentration (IC50) to free drug. In another study, ACV was incorporated into EFs. Release eluate collected up to 28 days post-release provided sustained protection against HSV-2 infection *in vitro* (Aniagyei et al., 2017). Other work that demonstrated protection against both HSV-2 and HIV-1 *in vitro* and *in vivo*, examined the incorporation of TDF, a newer and more potent prodrug of TFV, into IVRs. These IVRs demonstrated promise in multipurpose prevention and sustained-release due to the combined efficacy of TDF against both HIV and HSV-2; its increased oral and topical lipophilicity and cell permeability; and improved HIV-1 IC50 (by 160-fold) relative to TFV (Mesquita et al., 2012). In subsequent studies, these TDF IVRs prevented HIV infection in macaques for up to 4 months, with monthly IVR changes (Mesquita et al., 2012; Smith et al., 2013).

Building upon this previous research, the goal of our work was to develop PLGA and poly(DL-lactide-co-ε-caprolactone) (PLCL) EFs containing TDF to demonstrate safe and efficacious inhibition of both HIV-1 and HSV-2 infections *in vitro*. TDF was selected as a model ARV to demonstrate proof-of-concept of our delivery vehicles, as at the time of this study, it was a next-generation, more lipophilic form of TFV, that had demonstrated strong protection after sustained-release from IVRs. Here we fabricated both PLGA and PLCL EFs to evaluate and compare two different biodegradable polymers known to impart the sustained-release of active agents. We synthesized 3 different formulations for each