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Evaluation of poly(lactic-co-glycolic acid) and poly(DL-lactide-co-ε-caprolactone) electrospun fibers for the treatment of HSV-2 infection

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ABSTRACT

More diverse multipurpose prevention technologies are urgently needed to provide localized, topical pre-exposure prophylaxis against sexually transmitted infections (STIs). In this work, we established the foundation for a multipurpose platform, in the form of polymeric electrospun fibers (EFs), to physicochemically treat herpes simplex virus 2 (HSV-2) infection. To initiate this study, we fabricated different formulations of poly(lactic-co-glycolic acid) (PLGA) and poly(DL-lactide-co-ε-caprolactone) (PLCL) EFs that encapsulate Acyclovir (ACV), to treat HSV-2 infection *in vitro*. Our goals were to assess the release and efficacy differences provided by these two different biodegradable polymers, and to determine how differing concentrations of ACV affected fiber efficacy against HSV-2 infection and the safety of each platform *in vitro*. Each formulation of PLGA and PLCL EFs exhibited high encapsulation efficiency of ACV, sustained-delivery of ACV through one month, and *in vitro* biocompatibility at the highest doses of EFs tested. Additionally, all EF formulations provided complete and efficacious protection against HSV-2 infection *in vitro*, regardless of the timeframe of collected fiber eluates tested. This work demonstrates the potential for PLGA and PLCL EFs as delivery platforms against HSV-2, and indicates that these delivery vehicles may be expanded upon to provide protection against other sexually transmitted infections.

1. Introduction

A multipurpose, safe, and effective microbicide has the potential to prevent millions of sexually transmitted infections (STIs) globally. While STIs as a whole affect approximately 340 million new people each year, herpes simplex virus 2 (HSV-2) is particularly pervasive, affecting 536 million people worldwide, and causing an estimated 23 million new infections each year [1–6]. Furthermore, infection with HSV-2 increases the propensity of co-infection with other STIs, such as human immunodeficiency virus (HIV) by 2 to 6-fold [7–12]. Yet, despite the crucial need to develop prophylactic agents and therapeutics, vaccines and oral antivirals have only been moderately successful in preventing STIs and curing them post-infection. Microbicides, in comparison, have the potential to offer a promising alternative to prevent and treat STIs, while imparting female-controlled protection against a diversity of infections.

Next-generation microbicides seek to incorporate multipurpose prevention technologies that can prevent and treat a diversity of STIs, including HSV-2 and HIV, while also providing prolonged release, specificity, simultaneous delivery of antivirals with biologics, and safety and durability in the female reproductive tract. While recent clinical trials have demonstrated that frequent application of oral anti(retro)virals or topical vaginal microbicides can reduce STI transmission, these trials have underscored the need for new approaches to improve long-term efficacy and user adherence [13–28]. Yet, despite their potential, the scarcity of multipurpose technologies that offer long-term (>1 month) administration to the unique microenvironment of the female reproductive tract has hindered microbicide success.

Thus far, many of the microbicide delivery vehicles in preclinical or clinical trials consist of gels and intravaginal rings (IVRs) [19,20,23,29–35]. Vaginal gels may be prone to user adherence challenges, due to their viscous "leaky" formulations, relative to more solid delivery platforms such as IVRs. Due to the discomfort associated with vaginal

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leakage, gels have been shown to be less acceptable among users [22,36–43]. While IVRs by comparison offer a more discreet option to provide prolonged protection, until recently, IVRs were challenged with providing the release of both hydrophilic and hydrophobic molecules from one delivery vehicle [27,44–49]. Moreover, the high temperature processing conditions often associated with IVR fabrication may prove challenging for the incorporation of biological agents. These challenges in combination with the need to increase user adherence, highlight the need for new microbicide delivery platforms. To address these needs, electrospun fibers (EFs) have emerged as a delivery platform to offer early- and sustained-delivery of antiviral agents to the female reproductive tract [50–56].

While EFs have been successfully used in many other delivery applications [57–59]; EFs have only recently been explored for microbicide delivery [35,50,52–54,56,60–62]. Due to their ability to incorporate a diversity of agents with different temporal release characteristics, EFs have the potential to provide prolonged delivery systems for topical active agent delivery to the female reproductive tract. To demonstrate tunable delivery, to date, EF formulations have primarily focused on HIV prevention. Ball et al. developed poly(L-lactic acid)/polyethylene oxide (PLLA/PEO) and polyvinylpyrrolidone (PVP) or PEO fibers for the delivery of anti-HIV and contraceptive agents [53,55]; whereas Blakney et al. assessed different multiscale geometries for delivery candidates for HIV prevention and unintended pregnancy [56], and Krogstad et al. evaluated the potential to scale-up manufacturing of polyvinyl alcohol (PVA) fibers [51]. Most recently poly(lactic-co-glycolic acid) (PLGA) and polycaprolactone (PCL) fiber blends were investigated for the delivery of Tenofovir against HIV infection [61]. To exemplify stimuli-responsive tunability, Huang et al. have taken a different approach to evaluate cellulose acetate phthalate fibers for semen-induced release of TDF [50] and shave utilized polystyrene fibers to entrap HIV [54]. While many of these EF platforms have focused on HIV inhibition, their effect on HSV-2 inhibition has been less thoroughly explored.

In our previous work, we developed a safe and effective microbicide, using poly(lactic-co-glycolic acid) (PLGA) nanoparticles that encapsulate siRNA targeting host receptors, to significantly increase survival in a murine model after a lethal dose of HSV-2 infection [63]. Our goal here was to expand our materials expertise into electrospun fibers, utilizing similar biodegradable polymers that might complement nanoparticle delivery, as a more durable platform to remain in and prolong delivery to the female reproductive tract. Building the foundation for this platform, our aim was to utilize our expertise in biodegradable polymers and HSV-2 infection, to develop and compare the feasibility of two FDA-approved biodegradable polymers, PLGA and poly(DL-lactide-co-ε-caprolactone) (PLCL), electrospun into fibers, to provide early- and sustained-delivery of a model HSV-2 antiviral, ACV.

We selected PLGA and PLCL, as model polymers, as they are known for their biocompatibility across a variety of applications in different vehicular forms and long-term durability in *in vivo* applications including stents and tissue engineering [64–68]. Furthermore, both PLGA and PLCL have outstanding sustained-delivery properties, demonstrating release in both microbicide and other delivery applications from months to years [35,61,64,68–70]. PLGA can be copolymerized to optimize both the mechanical fiber properties and release profiles of encapsulated materials, while PLCL has demonstrated more favorable intermediate release compared to the rapid degradation of poly(lactic acid) (PLA) or prolonged release of PCL [71]. Due to these attributes and similar biocompatibility [72], we selected these platforms for comparison of ACV incorporation and fiber functionality.

Our goal in this work was to develop, test, and compare two different biodegradable EF platforms that address the needs of short- and long-term microbicide delivery by: achieving early short-term release accompanied by prolonged sustained-release; ensuring high encapsulant loading; exhibiting biocompatibility; and providing complete *in vitro* efficacy against HSV-2 infection. We were specifically interested in characterizing the release profiles from these fibers, establishing their functionality against HSV-2 infection, and determining their safety *in vitro*. Here we demonstrate that PLGA and PLCL EFs highly encapsulate and provide early burst- and long-term sustained-release of ACV for up to one month. We determined that all formulation eluates completely inhibited HSV-2 infection after a variety of release times *in vitro*. Additionally, as a secondary mode of barrier protection, both PLGA and PLCL EFs physically decreased virus penetration *in vitro* for up to 72 h. To our knowledge this is the first study to compare the feasibility of two different biodegradable electrospun fiber platforms — PLGA and PLCL EFs — to offer early- and sustained-delivery of ACV, and subsequent physicochemical protection against HSV-2 transmission *in vitro*.

2. Materials and methods

2.1. Materials

Poly(DL-lactide-co-ε-caprolactone) 80:20 (PLCL, 0.75 dL/g, 37 kDa MW), obtained from Lakeshore Biomaterials (Birmingham, AL), was kindly provided by Dr. Stuart Williams III. Carboxylic acid terminated 50:50 poly(DL-lactide-co-glycolide) (PLGA, 0.55–0.75 dL/g, 31–57 kDa MW) was purchased from Lactel Absorbable Polymers (Cupertino, CA). Acyclovir (ACV), polyvinyl alcohol (PVA), dichloromethane (DCM), chloroform (CF), *N*,*N*-dimethylformamide (DMF), and dimethyl sulfoxide (DMSO) were obtained from Sigma Aldrich (St Louis, MO). 1,1,1,3,3,3—hexafluoro-2-propanol (HFIP) and trifluoroethanol (TFE) were obtained from Fisher Scientific (Pittsburgh, PA). African green monkey kidney (Vero E6) cells, human cervical carcinoma (HeLa) cells, and HSV-2 (4674) were kindly provided by Dr. Kenneth Palmer (University of Louisville). Fetal bovine serum (FBS), antibiotics (penicillin/streptomycin) and Minimum Essential Medium (MEM) were purchased from VWR. Simulated vaginal fluid (SVF) was prepared as described in [73].

2.2. Synthesis of electrospun fibers

PLGA and PLCL EFs were prepared and electrospun with different solvents and compositions spanning (8–30% wt drug/wt polymer (w/w)) to establish a baseline blank EF (no drug) formulation. For blank polymer EFs, solutions of 10–30% PLGA w/w and 8–12% PLCL w/w were prepared in various solvents (CF:DMF (3:1 and 9:1 vol%), TFE, or HFIP) and allowed to solubilize overnight on a shaker at room temperature. Three milliliters of each polymer solution were aspirated into, and spun from a 3 mL plastic syringe on a custom built device housed in an air-filtered Plexiglas chamber. Flow rates spanning (0.5–3.0 mL/h) were optimized over a range of voltages (15–27 kV) and the resulting fiber mat was collected on a rotating 4 or 26 mm outer diameter stainless steel mandrel, located 25 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). Final optimized formulations of fibers were spun at 27 kV at 2.0 mL/h for both PLGA and PLCL formulations. For ACV incorporation, fibers were prepared with 1, 10, and 20% w/w ACV (to polymer) dissolved in polymer solution overnight. After electrospinning, fibers were removed from the mandrel and dried overnight in a desiccator cabinet.

2.3. Electrospun fiber solution conductivity and viscometry

The impact of solvent choice, polymer composition and ACV concentration on conductivity and viscosity were assessed. To assess conductivity, solvent alone (HFIP, TFE, 3:1 CF:DMF, or 9:1 CF:DMF); solvent plus polymer (HFIP plus 15% PLGA or 12% PLCL); or HFIP plus PLGA or PLCL and 20% ACV were prepared in 3 mL aliquots the night before testing. Conductivity measurements were performed using a NanoBrook ZetaPALS zeta-potential analyzer (Brookhaven Instruments), courtesy