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Coaxially electrospun fiber-based microbicides facilitate broadly tunable release of maraviroc

Cameron Ball¹, Shih-Feng Chou¹, Yonghou Jiang¹, and Kim A. Woodrow^{1,†}

¹Department of Bioengineering, University of Washington, 3720 15th Ave NE, Seattle WA, 98195-5061, USA

Abstract

Electrospun fibers show potential as a topical delivery system for vaginal microbicides. Previous reports have demonstrated delivery of anti-HIV and anti-STI (sexually transmitted infection) agents from fibers formulated using hydrophilic, hydrophobic, or pH-responsive polymers that result in rapid, prolonged, or stimuli-responsive release, respectively. However, coaxial electrospun fibers have yet to be evaluated as a highly tunable microbicide delivery vehicle. In this research, we explored the opportunities and limitations of a model coaxial electrospun fiber system to provide broad and tunable release rates for the HIV entry inhibitor maraviroc. Specifically, we prepared ethyl cellulose (EC)-shell and polyvinylpyrrolidone (PVP)-core fibers that were capable of releasing actives over a range of hours to several days. We further demonstrated simple and effective methods for combining core-shell fibers with rapid-release formulations to provide combined instantaneous and sustained maraviroc release. In addition, we investigated the effect of varying release media on maraviroc release from core-shell fibers, and found that release was strongly influenced by media surface tension and drug ionization. Finally, in vitro cell culture studies show that our fiber formulations were not cytotoxic and that electrospun maraviroc maintained similar antiviral activity compared to neat maraviroc.

Graphical abstract

Keywords

coaxial; core-shell; drug delivery; electrospinning; maraviroc; microbicide

[†]Corresponding author: Dr. Kim A. Woodrow, Foegle N410D, Department of Bioengineering, University of Washington, 3720 15th Ave NE, Seattle WA, 98195-5061, USA, 1-(206)-685-6831, woodrow@uw.edu.

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1. Introduction

In recent years, we and others have developed electrospun fibers as an alternative delivery platform to current anti-HIV microbicides [1–6]. Microbicides are agents that are applied vaginally or rectally to reduce the risk of sexually transmitted infections by interfering with potential pathogens [7]. Electrospinning is a process whereby polymer solutions are drawn into ultrafine fibers and assembled into nonwoven fabrics, which have diverse applications that include drug delivery. The goal of fiber-based anti-HIV microbicides is local delivery of anti-HIV actives to the vaginal or rectal mucosae in a format with a wide range of potential physicochemical configurations and payloads. For example, electrospun microbicides have been engineered for rapid [2,5], sustained [1,3,4], and even semen-responsive [6] release for a range of small molecule antiretrovirals and contraceptives.

The current diversity of fiber-based microbicides has been based primarily on simple matrix fibers produced by uniaxial electrospinning. In such systems, the polymer used to form the fibers typically dictates the release characteristics for any given drug. For example, we previously modified the release of specific actives from fiber-based microbicides by blending polymers with different aqueous solubility, chain mobility, or affinity for the loaded actives [1,4]. Other formulation attributes can also influence drug release such as drug loading and compatibility with the base polymer, drug ionization and solubility within the release medium, and excipient integration. While uniaxial fibers can afford considerable control over drug release, they may not be well suited to all applications. In particular, water-soluble actives often burst release from the surface of uniaxial fabrics meant for multi-day drug release. This is especially true of highly loaded fabrics, where much of the drug can be present on the fiber surface.

An alternative to simple uniaxial matrix fibers is coaxial electrospun core-shell fibers, which allow active agents to be loaded within a fiber core that is sheathed by a distinct, release-modulating polymer shell. Consequently, core-shell fibers promise to eliminate the burst release typically observed from the more common class of uniaxial electrospun materials. Tunable and sustained release from core-shell fibers has been achieved for the delivery of macromolecules (e.g., proteins and nucleic acids) [8–11], hydrophobic small molecules [10,12–14], and hydrophilic small molecules [15–21]. Current reports on coaxial electrospinning suggest that shell integrity and thickness, drug-polymer compatibility, shell wettability, and drug partitioning between core and shell regions may more reliably control drug release from core-shell fibers than from uniaxial fibers [22]. At present, there have been no attempts to apply coaxial electrospinning to formulations that may be useful as fiber-based microbicides.

This study aimed to investigate the potential and limitations of coaxial electrospinning for microbicide development with broadly tunable core-shell fibers for rapid or sustained release of the anti-HIV drug maraviroc. We hypothesized that modulating the core-shell structure of coaxial fabrics could tune the rate of maraviroc release. We chose to use maraviroc because of our prior expertise formulating maraviroc-loaded uniaxial fibers for rapid release applications. Furthermore, we have found that sustained maraviroc release from uniaxial blends of poly(lactic-co-glycolic) acid and polycaprolactone has proven to be less tunable

than that of other water-soluble antiretroviral drugs [4], and we desired an alternative sustained release strategy for maraviroc. In this study, we electrospun various core-shell fibers comprised of a polyvinylpyrrolidone (PVP) core and an ethyl cellulose (EC) shell. We chose EC as the shell material because it is hydrophobic, inexpensive, and commonly used in sustained release pharmaceuticals. In contrast hydroxyethylcellulose, which is commonly used in water-soluble microbicide gels, EC is hydrophobic and swells minimally in water. Furthermore, unpublished work in our lab with EC has shown that the polymer has good compatibility with maraviroc. PVP was chosen as the core polymer because it also shows good compatibility with maraviroc, and rapid-release uniaxial PVP fibers have already been well characterized [2], which provides an opportunity to investigate the effects of a hydrophobic shell on release behavior.

2. Materials and methods

2.1 Formulation and electrospinning

Maraviroc was purchased through the University of Washington's Investigative Drug Services facility followed by purification and recrystallization from Selzentry® (ViiV Healthcare) [2]. Metronidazole, EC (22 cP at 5% in toluene/ethanol (80:20) and 48% ethoxyl content), PVP ($M_w \sim 1,300$ kDa), and 2,2,2-trifluoroethanol (>99%) were purchased from Sigma Aldrich (St. Louis, MO). 100% ethanol (USP grade) was purchased from the University of Washington's Biochemistry supplies store. Glycerol was purchased from ThermoFisher Scientific (Waltham, MA). All fibers were electrospun from a total solution volume of 500 μ L per run and collected onto a stationary collector. See supplementary for electrospinning procedures.

2.2 SEM imaging for fiber morphology

Fibers morphology was examined by a Sirion SEM (Nanotechnology User Facility, University of Washington) as described previously [1,2]. PVP core fibers were cut with a fresh scalpel and set into distilled water containing 0.1% Liquinox for 5 minutes followed by drying to image the core-shell structure. These hollowed-out fibers were used to estimate the ratio of shell thickness to outer diameter (δ/R_O). Comparisons between δ/R_O of fibers spun from different Q_S/Q_C ratios were made using ANOVA (Prism, GraphPad).

2.3 Verification of core-shell structure by chemical surface analysis

XPS was performed using a Surface Science Instruments S-Probe at the University of Washington's NESAC/BIO surface analysis recharge center. Care was taken to prepare samples with no surface contamination. Freshly electrospun materials were collected onto aluminum foil and immediately lyophilized. Samples were analyzed in duplicate and illuminated with low intensity electrons to reduce charging of the insulated materials. Peak assignment and integration were performed using XPS analysis software (CasaXPS).

2.4 In vitro release testing and drug loading measurement

1.9 cm diameter fiber disc samples were placed into 25 mL of pre-warmed 37°C release media and kept at 37°C in a rotary incubator at 200 rpm (VWR). At predetermined time points out to 120 h, 50 μ L samples were removed, placed into HPLC vials, and stored at

–80°C prior to analysis. Release media included pH 4.0 10 mM citrate (sodium) buffer with a total ionic strength of 154 mM (henceforth called citrate buffer), citrate buffer with 0.1% vol/vol Tween 20, vaginal fluid simulant [23], and complete DMEM media (Gibco Life Technologies). See supplementary for release curve fitting procedures [24].

To measure drug loading, 5 mg of fiber sample was dissolved in 1 mL of ethanol followed by adding 9 mL of citrate buffer to precipitate EC. After 24 h, samples were filtered through 0.22 μ m filters to remove particulates and run through HPLC. Spiked controls containing 100% to 50% EC and 0% to 50% PVP were prepared in triplicate to validate the measurements. See supplementary for HPLC procedures [2].

2.5 In vitro cytotoxicity and anti-HIV activity of maraviroc

Fiber eluates were taken from drug loaded fibers following 120 h release into cDMEM. Cytotoxicity of fiber eluates was evaluated in TZM-bL cells. Briefly, cells were cultured in 96 well plates (10,000 cells/well) in the absence or presence of various concentrations of fiber eluates for 72 h. Cell viability was assessed using CellTiter-Blue® Cell Viability Assay following manufacturer's recommended procedures.

Antiviral activity of fiber eluates was assessed based on a reduction in luciferase reporter gene expression after infection of TZM-bl cells with HIV-1 BaL as reported previously [1,2]. Briefly, TZM-bl cells (1×10^4 cells/well) were incubated with various concentrations of fiber eluates at 37°C for 1h prior to virus exposure. Then cell free HIV-1 BaL (200TCID₅₀) was added to the cultures and incubated for 48 h. Untreated wells were used as control. The Promega™ Luciferase Assay System was used to determine luciferase expression. Antiviral activity was expressed as an IC₅₀ value, which is the sample concentration giving 50% of relative luminescence units (RLUs) compared with those of virus control after subtraction of background RLUs.

2.6 Construction of layered electrospun fiber composites

Composite materials were created using either a solvent weld or mechanical pressure to join materials, and 6 composites of each type were prepared. For solvent welding technique, the cutting edge of a 3/4" metal die (Grainger) was dipped into a shallow bath of ethanol and then placed atop the stacked fibers to seal the fabrics together at their edges. For mechanical pressure technique, one large and one small square were cut out from fiber mats using scissors. The EC/PVP fibers were placed on top of the PVP fibers. The protruding PVP fiber edges were folded onto the EC/PVP fibers and firmly pressed for a few seconds using the edge of a blunt plastic ruler.

3. Results

3.1 Core-shell fiber electrospinning and physicochemical properties

We produced coaxial fiber geometries with tunable drug loading, polymer composition, and shell thickness using a custom-built coaxial nozzle and the electrospinning setup diagrammed in Figure 1A. By varying the core drug loading and flow rate ratio of the shell and core solution (Q_S/Q_C), we obtained 16 distinct core-shell fabrics with a hydrophobic EC shell

surrounding a hydrophilic PVP core loaded with maraviroc (Table S1). For comparison, we also prepared 8 different core-shell fabrics using hydrophobic EC as both the shell and core and 5 uniaxial EC-maraviroc fabrics (Table S1). In figures and tables, we denote the formulations by the shell and core polymers, followed by a value for the drug loading in the core polymer (%wt drug/wt polymer), and finally by the flow rate ratio of the shell to core polymer (Q_S/Q_C). For example, EC/PVP-100 4.0 fabric has an EC shell, PVP core, 100% wt/wt drug/PVP, and $Q_S/Q_C = 4.0$. In all cases, coaxial electrospinning produced smooth and uniform fibers of approximately 1 μm in diameter (Figure 1B,C). Viewed in cross-section, fabrics appeared highly porous and uniform across their thickness (Figure 1D). Final core-shell fabric drug loading ranged from 4 wt% to 39 wt% depending upon initial core loading and flow rate ratio, and we observed no drug lost during electrospinning (Table S1). The mechanical properties of representative core-shell fabrics are presented in supplementary materials (Table S2). In general, fiber mechanical properties depended on both the core polymer type and electrospinning flow rate ratio. For example, average modulus and tensile strength of the coaxial fibers were significantly different between equivalent formulations containing either EC or PVP cores and between equivalent core materials with different Q_S/Q_C ($P < 0.05$), respectively. Overall, coaxial fibers with PVP-cores were less elastic and weaker than equivalent fibers with EC-cores.

We confirmed the core-shell structure using a combination of bulk and surface analysis methods. Fiber cross-sections that were then hollowed out by immersion in water to solubilize the PVP core allowed direct observation of EC/PVP fibers' core-shell structures (Fig. 1E–G). As expected, the ratio of the fibers' shell thicknesses to their outer radii (δ/R_O) was significantly different between fibers electrospun with variable Q_S/Q_C ($P < 0.05$). Furthermore, δ/R_O increased with higher shell-to-core flow rates ($P < 0.05$) (Fig. 1H). We also used XPS to interrogate the surface atomic compositions of representative EC/PVP coaxial fibers prepared with different core drug loading and shell to core flow rate ratios (Table 1). The surface atomic compositions of all EC/PVP coaxial fibers closely matched that of pure EC, supporting a coaxial fiber structure. However, for thinner shells ($Q_S/Q_C = 0.5$), the top 10 nm of coaxial fibers showed small amounts of nitrogen and fluorine that are characteristic of PVP (N only) and maraviroc (both N and F). We also observed that the fluorine signal increased 2.5-fold in response to a 2.8-fold increase in maraviroc loading in the thin-shelled fibers. Neither maraviroc nor PVP were detected on the surface of thick-shelled coaxial fibers, which had an average shell thickness 1.6-fold greater than their thin-shelled analogs (Fig. 1H). Thus, thicker EC shells were necessary to completely prevent maraviroc or PVP from reaching the fiber surface during the electrospinning process.

3.2 Sink release of maraviroc from electrospun fibers

We found that all three compositions of electrospun materials (EC/PVP, EC/EC, and EC fabrics) could sustain maraviroc release, but core-shell fabrics demonstrated more control over release rates and greater versatility than did uniaxial EC fabrics (Fig. 2). In general, increasing shell thickness (Q_S/Q_C) slowed drug release, and increasing core drug loading increased the amount of maraviroc release. For example, EC/PVP fibers with the thinnest shells provided 4 h to 24 h of maraviroc release whereas those with the thickest shells provided at least 120 h of maraviroc release (Fig. 2A). We found that we could control the

magnitude and duration of maraviroc release from EC/PVP fabrics by simply setting the Q_S/Q_C ratio during electrospinning, and then independently adjusting the core drug loading (Fig. S1). In contrast to EC/PVP fabrics, all EC/EC fabrics sustained maraviroc release over at least 120 h (Fig. 2B). As with the EC/PVP fabrics, thinner shells accelerated maraviroc release from EC/EC formulations and higher core drug loading increased the total amount of drug that was released (Fig. S1). By comparison, uniaxial fabrics were less versatile and programmable. For example, although uniaxial EC fabrics could sustain maraviroc release over 120 h, modest increases in drug loading from 23 wt% to 33 wt% resulted in accelerated drug release from these formulations (Fig. 2C). In contrast, core-shell fabrics responded more measuredly to increases in drug loading. We observed strong relationships between fiber composition, fabric wetting, and drug release (Fig. S3–S4), suggesting that the presence of a high integrity EC shell was critical to our formulations' success.

3.3 The importance of drug-polymer compatibility

To investigate the generalizability of the core-shell release kinetics, we exchanged maraviroc for metronidazole in both the core-shell and uniaxial fabric formulations. Metronidazole is a gynecologically relevant small molecule antibiotic, and is less lipophilic than maraviroc in its neutral (as formulated) state [25]. In addition, metronidazole has been shown to form stable crystalline solids due to hydrogen bonding and strong pi-pi interactions [26]. As such, we expected metronidazole to be less stable than maraviroc in fibers, particularly those made exclusively of the hydrophobic polymer EC. We electrospun 6 EC/PVP core-shell, 6 EC/EC core-shell, and 2 uniaxial fabric formulations of metronidazole (Table S3) and examined their drug release characteristics (Fig. S5). SEM imaging revealed that metronidazole was surface crystallizing out of the core-shell fibers, particularly from the EC/EC fibers, and forming large drug crystals on the fabrics' surface (Fig. S6). As we expected, these surface drug crystals accelerated the release of metronidazole compared to maraviroc. Although metronidazole release was tunable, most core-shell formulations suffered from an initial burst release and sustained drug release out to just 48 hours. Interestingly, while uniaxial EC fibers containing 33 wt% metronidazole burst released all their drug content within 1 h, uniaxial EC fabrics containing 50 wt% metronidazole released metronidazole continuously for 96 h with only 10% burst release (Fig. S5A). This suggests that, as drug loading increased beyond a critical threshold, metronidazole may have phase separated from EC to form a solid drug core within the uniaxial fabric. These results highlight that drug-polymer compatibility is important for sustained release from electrospun core-shell fabrics.

3.4 Impact of changing release media on drug release from electrospun fabrics

Media dependent release is often described in microbicide formulation research [27]. Such studies inform on the appropriateness of a given media for identifying differences between formulations and deepen our understanding of how formulations respond to environmental variability. Therefore, we repeated our release studies using citrate buffer with 0.1% vol/vol Tween 20, vaginal fluid simulant (VFS), and cDMEM cell culture media, which have measurable differences in surface tension and pH (Table S4).

We identified buffer surface tension and pH as two important media-specific factors affecting maraviroc release from core-shell fibers. We found that reductions in surface

tension of the release media triggered a faster maraviroc release from both core-shell and uniaxial fabrics (Fig. 3). Comparisons of water uptake into fabrics and core polymer mass loss from fibers in the various release media suggested that reduced media surface tensions enhanced spontaneous wetting of the electrospun fabrics, particularly those with thin outer shells of EC, thereby accelerating drug release (Fig. S8–S9). Of particular note, we observed that EC/EC core-shell fibers with the thickest shells maintained sustained release of maraviroc even in the lowest surface tension buffer (Fig. 3E), demonstrating the potential for increased robustness of core-shell fabrics over uniaxial fabrics for small molecule drug release. Media pH also played a big role in determining maraviroc release rates from core-shell fiber formulations. For example, cDMEM's mildly basic pH slowed drug release from EC/PVP and EC/EC fibers (Fig. 4) as compared to the release from similar compositions into mildly acidic VFS (Fig. 3A–B), which had a similar surface tension (Fig. 4). We were able to accelerate drug release into the media by vortexing fiber samples in cDMEM for 10s at the start of the release study. This observation is likely explained by a pH-dependent deionization of maraviroc above its pKa of 7.8, at which point maraviroc becomes hydrophobic and poorly soluble.

3.5 Formulation toxicity and anti-HIV activity of formulated maraviroc

The eluates from maraviroc-loaded EC/PVP and EC/EC fabrics collected after release into cDMEM, both with and without vortexing (see Section 3.4), were tested for toxicity against the TZM-bL reporter cell line and showed no cytotoxic effects (Fig. 5). Antiviral testing of fiber eluates with the same cell line confirmed that maraviroc released from fibers into cDMEM for 120 h retained its potency against HIV-BaL *in vitro*. The IC₅₀ values of maraviroc both from release eluates and as a neat drug were 5–11 nM, which is consistent with values seen previously in equivalent assays with HIV-BaL and TZM-bL cells (Fig. 5) [1,2,28]. Thus, formulation into core-shell fibers and release for 120 h into cDMEM at 37°C had no deleterious effect on the anti-HIV potency of maraviroc.

3.6 Layered composite fabrics for single-dose rapid and sustained maraviroc release

Fabrics that could provide both rapid and sustained release of maraviroc were created by layering rapidly dissolving uniaxial PVP fabrics containing 28 wt% maraviroc [2] with EC/PVP core-shell fabrics containing 20 wt% maraviroc. The layered composites were joined by solvent welding or mechanical pressure (Fig. 6A,B). Both techniques created composites that retained their soft, flexible properties without delaminating. Release studies of these composite materials into citrate media showed that the 28 wt% maraviroc PVP fibers rapidly hydrated and released maraviroc in less than 20 minutes without inducing wetting of the sustained release EC/PVP fabrics (Fig. 6C). Thus, the dissolution of PVP immediately adjacent to an EC surface did not significantly alter the relationship between EC/PVP fibers and the release media. This ensured sustained release from EC/PVP fabrics over the next 120 h. As a result, release from composite materials was nearly equivalent to the superposition of the individual fabric's release profiles weighted by their mass fractions in the final composites.

4. Discussion

The core-shell fiber formulations investigated in this work allowed us to examine the unique design features related to coaxial electrospinning of fiber-based vaginal microbicides. Using hydrophobic EC as a shell and either EC or water-soluble PVP as a core, we formulated a diverse set of model core-shell fabrics containing the HIV entry inhibitor maraviroc. Our previous work on solid dispersions of maraviroc in electrospun PVP fabrics showed rapid drug dissolution in less than 20 minutes [2]. In this study, we found that coating similar PVP-maraviroc fibers with an EC shell of variable thickness could facilitate sustained release of maraviroc from 24 hours to over 5 days. Therefore, core-shell fibers could extend the release duration of maraviroc by 70 to more than 350 times compared to the previously reported PVP uniaxial fibers. Our previous attempt to sustain maraviroc release from uniaxial fibers by blending the hydrophobic polymers poly(lactic-co-glycolic acid) and polycaprolactone was less successful at modulating both the duration and linearity of the release than the coaxial electrospinning methods used in this study. For example, blended polymer fibers showed a shorter timeframe of continuous drug release of less than 2 days, and maraviroc release was less linearly responsive to adjustments in blend composition than were other water-soluble antivirals [4]. Similarly, here we have demonstrated that drug release does not respond linearly to drug loading in uniaxial EC fabrics. In contrast with these uniaxial fabrics, EC/EC and EC/PVP core-shell fabrics enhance the versatility and programmability of maraviroc release. For example, EC/EC fabrics release maraviroc quite slowly and continuously, and both the reduction of shell/core flow rate ratio during electrospinning and the incorporation of PVP into the fiber core can gradually and predictably reduce the timespan for maraviroc release to just a few hours. Because of their controllable nano-architecture, core-shell fabrics promise superior sustained release characteristics compared to uniaxial electrospun fabrics, particularly for hydrophilic drug delivery.

An additional advantage of core-shell fabrics is improved robustness to variations in release conditions. Maraviroc-based microbicides have previously been evaluated in the context of different release media in order to draw comparisons between physicochemically distinct formulations. For example, Forbes et al. compared the release of maraviroc from hydroxyethylcellulose and silicone-based microbicide gels in both simulated vaginal fluid and mixtures of water and IPA to demonstrate polymer swelling dependent drug release *in vitro* [27]. In this work, we examined release in up to 4 different media with varying surface tension and pH. A number of reports have demonstrated that the surface characteristics of electrospun fibers impact fabric wettability [29,30] and thus the robustness of drug release to changes in release media composition. Coaxial electrospinning permitted high core drug loading without significant enrichment of maraviroc on the fiber surface (Table 1), which we previously observed in uniaxial fibers made from PVP [2]. This mitigated the risk of sudden transitions to burst release in low surface tension or drug-ionizing media. We observed that core-shell fibers maintained the ability to sustain maraviroc release better than equivalently loaded uniaxial EC fabrics in release media with low surface tension. In summary, we found that coaxial electrospinning could be used to enhance the versatility, tunability, and robustness of drug release from maraviroc-loaded fabrics.

We also identified potential limitations for the use of our core-shell fibers for vaginal microbicides. First, significant considerations to the core and shell polymers, solvent compatibility, and electrospinning parameters were required to obtain discrete and defect-free core-shell fiber structure. For example, in order to solubilize metronidazole, trifluoroethanol rather than ethanol was used as the core solvent. Although this change produced regular coaxial fibers, we observed that metronidazole phase separated out of EC shells, suggesting that there was still suboptimal compatibility between the polymers used and the drug compound. Metronidazole has been shown to readily form crystals [26] and is also less lipophilic than maraviroc in its neutral state. This may make metronidazole less compatible than maraviroc with EC, affecting both drug stability and subsequently drug release. For drugs like metronidazole that are stabilized by hydrogen bond donors [31], a polyvinyl alcohol-core may be superior to a PVP-core. Secondly, while EC shells provide controlled release characteristics, the polymer is not biodegradable and may preclude long-term vaginal use. Alternative polymers, including rapidly degrading but hydrophobic polyurethanes [32], tyrosine-derived polycarbonates [33], or poly(ortho esters) [34] might greatly improve shell integrity and biocompatibility while maintaining or improving release characteristics from electrospun fibers, especially in low surface tension media or other conditions conducive to accelerated drug release. Finally, coaxial electrospinning for fiber-based microbicides may add complexity and cost to the formulation [35]. However, recent advances in slit-surface electrospinning improve the outlook for large-scale manufacture of core-shell fabrics [36]. Despite these limitations, coaxial electrospinning warrants further development for fiber-based microbicides.

Our results add to the literature on controlled release of hydrophilic small molecule drugs from coaxial fibers. Most reports of tunable and sustained release from coaxial fibers describe the delivery of macromolecules (e.g., proteins and nucleic acids) [8–11] or hydrophobic small molecules [10,12–14], where sustained release is aided by their large size, poor solubility or favorable partitioning into insoluble polymers. For hydrophilic small molecules that are often incompatible with hydrophobic polymers used for sustained release, low drug loading into the core of a coaxial fiber can facilitate slow drug release [15–17,21]. Here, we show high drug loading (up to 39 wt% in core-shell fibers) of a hydrophilic small molecule drug and sustained release over at least 5 days *in vitro* by ensuring compatibility between maraviroc, EC, and PVP. As discussed previously, incompatibility of metronidazole with EC presented a challenge for sustained drug release. Interestingly, He et al. also recently reported on sustained release of metronidazole from gelatin/polycaprolactone core-shell fibers containing 5 wt% to 33 wt% drug [19]. He et al. also observed metronidazole crystals forming at higher drug loadings, which generated 30% to 60% burst release from gelatin/polycaprolactone fibers and reduced the duration of a sustained release phase from 6 days to 2.5 days. Taken together, these results underscore the importance of formulation stability for controlled release.

In a separate study investigating sustained release of a water-soluble drug from core-shell fibers, Sohrabi et al. demonstrated sustained release of ampicillin over 30 days from poly(methyl methacrylate)/nylon-6 core-shell fibers loaded with 20 wt% antibiotic [18]. These results are comparable to those we obtained with EC/EC fibers, although we monitored drug release over a shorter time period. Sohrabi et al. found that PMMA/nylon-6

core-shell fibers released 30% of encapsulated ampicillin within the first 6 h in phosphate buffered saline, and postulated that this initial burst release phase was due to the presence of drug at the fiber surface. In contrast, we found that high flow rate ratios of shell to core electrospinning solutions prevented maraviroc from reaching the fiber surface, helping to explain the lack of burst release from our core-shell fabrics in citrate buffer. Nguyen et al. formulated poly(lactic acid)/poly(ethylene glycol) core-shell fibers loaded at up to 7 wt% with salicylic acid [20]. As in our study, their fiber characterization and release data showed that a minimum shell to core flow rate ratio was necessary to achieve complete encapsulation of the core materials, preventing rapid dissolution of fiber core contents and sustaining 3 wt% salicylic acid release over 5 days without an initial burst release. Our work represents a significant contribution to the existing literature on coaxial electrospinning for hydrophilic drug delivery. By successfully encapsulating maraviroc within stable EC/EC and EC/PVP core-shell fabrics, we were able to modify relative shell thickness and core drug loading to achieve broad tunability and minimal burst release from fibers loaded with 4 wt% to 39 wt% maraviroc.

Finally, we have presented composite materials for maraviroc release that represent a first step toward developing a new class of fiber-based anti-HIV microbicides capable of instant and sustained drug release from a single dose. Such a product may prove capable of providing both instant and multi-day protection against sexually transmitted infections, including HIV. A few existing microbicide delivery systems, including silicone elastomer vaginal gels [27] and tablets based on osmotic pumps [37] have also recently begun to address this gap. Such products could be particularly useful for delivering maraviroc, which is required at high doses for efficacy but absorbed rapidly in the vagina [38,39]. We combined both rapid- and sustained release fibers to linearly superpose their release characteristics using either solvent welding or mechanical pressure to fuse stacked fabrics. Adjusting the ratio of specific PVP and EC/PVP fabric formulations in stacked composites enabled simple tuning of the amounts and timing of burst and sustained release kinetics. Our approach to fabricate composites is different from that of Yu et al., who previously utilized EC core, PVP shell fibers to accomplish two-phase release [13], and is more similar to that of Okuda et al. or Falde et al., who created stacked composites by sequential electrospinning to achieve two-stage delivery of drugs [40] or stacked composites with hydrophobic “shield” layers [30]. In contrast to these strategies, our post-processing of pre-fabricated electrospun fabrics into layered composites may be advantageous for scale up, rapid prototyping, and independent evaluation of component materials. Future studies will focus on applying this class of materials to relevant microbicide models for HIV prevention.

5. Conclusion

This study investigates potential microbicide development using coaxial electrospinning. Our *in vitro* release data support the hypothesis that modulations of the core-shell structure in coaxial fibers tune the rate of maraviroc release. Specifically, we observe that increasing shell thickness sustained the release of maraviroc. Furthermore, we demonstrate the importance of drug-polymer compatibility for sustained release from coaxial fibers by replacing maraviroc with metronidazole. Moreover, we show that drug release rates from coaxial fibers are subject to the effects of surface tension and pH of the release media. *In*

vitro cytotoxicity and anti-HIV data suggest that eluates from antiretroviral-loaded coaxial fibers are non-toxic and that released maraviroc maintains its potency. Finally, we assemble core-shell fabrics into composites with uniaxial fiber-based microbicides. These composites exhibit both a rapid release phase and a sustained release phase. Future work will focus on formulation of other anti-HIV agents, improved biocompatibility, user perceptions of fiber-based microbicides, and *in vivo* drug release characteristics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

EC	Ethyl cellulose
PVP	polyvinylpyrrolidone
Q_s	shell flow rate
Q_c	core flow rate

References

1. Ball C, Krogstad E, Chaowanachan T, Woodrow KA. Drug-Eluting Fibers for HIV-1 Inhibition and Contraception. *PLoS ONE*. 2012; 7:e49792. [PubMed: 23209601]
2. Ball C, Woodrow KA. Electrospun solid dispersions of maraviroc for rapid intravaginal pre-exposure prophylaxis of HIV. *Antimicrobial Agents and Chemotherapy*. 2014
3. Blakney AK, Krogstad EA, Jiang YH, Woodrow KA. Delivery of multipurpose prevention drug combinations from electrospun nanofibers using composite microarchitectures. *Int J Nanomedicine*. 2014; 9:2967–2978. [PubMed: 24971008]
4. Carson D, Jiang Y, Woodrow KA. Tunable Release of Multiclass Anti-HIV Drugs that are Water-Soluble and Loaded at High Drug Content in Polyester Blended Electrospun Fibers. *Pharm. Res*. 2015
5. Krogstad EA, Woodrow KA. Manufacturing scale-up of electrospun poly(vinyl alcohol) fibers containing tenofovir for vaginal drug delivery. *International Journal of Pharmaceutics*. 2014; 475:282–291. [PubMed: 25169075]
6. Huang C, Soenen SJ, van Gulck E, Vanham G, Rejman J, Van Calenbergh S, et al. Electrospun cellulose acetate phthalate fibers for semen induced anti-HIV vaginal drug delivery. *Biomaterials*. 2012; 33:962–969. [PubMed: 22018388]
7. Lederman M, Offord R. Microbicides and other topical strategies to prevent vaginal transmission of HIV. *Nature Reviews Immunology*. 2006
8. Jiang H, Hu Y, Zhao P, Li Y, Zhu K. Modulation of protein release from biodegradable core-shell structured fibers prepared by coaxial electrospinning. *J. Biomed. Mater. Res*. 2006; 79B:50–57.

9. Jia X, Zhao C, Li P, Zhang H, Huang Y, Li H, et al. Sustained Release of VEGF by Coaxial Electrospun Dextran/PLGA Fibrous Membranes in Vascular Tissue Engineering. *J Biomater Sci Polym Ed.* 2011; 22:1811–1827. [PubMed: 20961491]
10. Su Y, Su Q, Liu W, Lim M, Venugopal JR, Mo X, et al. Controlled release of bone morphogenetic protein 2 and dexamethasone loaded in core-shell PLLACL-collagen fibers for use in bone tissue engineering. *Acta Biomater.* 2012; 8:763–771. [PubMed: 22100346]
11. Jiang H, Wang L, Zhu K. Coaxial Electrospinning for Encapsulation and Controlled Release of Fragile Water-soluble Bioactive Agents. *Journal of Controlled Release.* 2014:1–32.
12. Jiang Y-N, Mo H-Y, Yu D-G. Electrospun drug-loaded core-sheath PVP/zein nanofibers for biphasic drug release. *International Journal of Pharmaceutics.* 2012; 438:232–239. [PubMed: 22981688]
13. Yu DG, Wang X, Li XY, Chian W, Li Y, Liao YZ. Electrospun biphasic drug release polyvinylpyrrolidone/ethyl cellulose core/sheath nanofibers. *Acta Biomater.* 2013; 9:5665–5672. [PubMed: 23099302]
14. Li Z, Kang H, Che N, Liu Z, Li P, Li W, et al. Controlled release of liposome-encapsulated Naproxen from core-sheath electrospun nanofibers. *Carbohydrate Polymers.* 2014; 111:18–24. [PubMed: 25037324]
15. Viry L, Moulton SE, Romeo T, Suhr C, Mawad D, Cook M, et al. Emulsion-coaxial electrospinning: designing novel architectures for sustained release of highly soluble low molecular weight drugs. *Journal of Materials Chemistry.* 2012; 22:11347.
16. Wang C, Yan K-W, Lin Y-D, Hsieh PCH. Biodegradable Core/Shell Fibers by Coaxial Electrospinning: Processing, Fiber Characterization, and Its Application in Sustained Drug Release. *Macromolecules.* 2010; 43:6389–6397.
17. Kiatyongchai T, Wongsasulak S, Yoovidhya T. Coaxial electrospinning and release characteristics of cellulose acetate-gelatin blend encapsulating a model drug. *J. Appl. Polym. Sci.* 2013; 131 n/a–n/a.
18. Sohrabi A, Shaibani PM, Etayash H, Kaur K, Thundat T. Sustained drug release and antibacterial activity of ampicillin incorporated poly(methyl methacrylate). *Polymer.* 2013; 54:2699–2705.
19. He M, Xue J, Geng H, Gu H, Chen D, Shi R. Fibrous guided tissue regeneration membrane loaded with anti-inflammatory agent prepared by coaxial electrospinning for the purpose of controlled release. *Applied Surface* 2015
20. Nguyen TTT, Ghosh C, Hwang S-G, Chanunpanich N, Park JS. International Journal of Pharmaceutics. *International Journal of Pharmaceutics.* 2012; 439:296–306. [PubMed: 22989981]
21. Tiwari SK, Tzezana R, Zussman E, Venkatraman SS. Optimizing partition-controlled drug release from electrospun core-shell fibers. *International Journal of Pharmaceutics.* 2010; 392:209–217. [PubMed: 20227472]
22. Chou S-F, Carson D, Woodrow KA. Current strategies for sustaining drug release from electrospun nanofibers. *J Control Release.* 2015
23. Owen DH, Katz DF. A vaginal fluid simulant. *Contraception.* 1999; 59:91–95. [PubMed: 10361623]
24. Ritger PL, Peppas NA. A simple equation for description of solute release II. Fickian and anomalous release from swellable devices. *Journal of Controlled Release.* 1987
25. Sobel JD, Ferris D, Schwebke J, Nyirjesy P, Wiesenfeld HC, Peipert J, et al. Suppressive antibacterial therapy with 0.75% metronidazole vaginal gel to prevent recurrent bacterial vaginosis. *Am. J. Obstet. Gynecol.* 2006; 194:1283–1289. [PubMed: 16647911]
26. Ramukutty S, Ramachandran E. Crystal growth by solvent evaporation and characterization of metronidazole. *Journal of Crystal Growth.* 2012; 351:47–50.
27. Forbes CJ, Lowry D, Geer L, Veazey RS, Shattock RJ, Klasse PJ, et al. Non-aqueous silicone elastomer gels as a vaginal microbicide delivery system for the HIV-1 entry inhibitor maraviroc. *Journal of Controlled Release.* 2011; 156:161–169. [PubMed: 21864598]
28. Chaowanachan T, Krogstad E, Ball C, Woodrow KA. Drug Synergy of Tenofovir and Nanoparticle-Based Antiretrovirals for HIV Prophylaxis. *PLoS ONE.* 2013; 8:e61416. [PubMed: 23630586]

29. Yohe ST, Colson YL, Grinstaff MW. Superhydrophobic materials for tunable drug release: using displacement of air to control delivery rates. *J. Am. Chem. Soc.* 2012; 134:2016–2019. [PubMed: 22279966]
30. Falde EJ, Freedman JD, Herrera VLM, Yohe ST, Colson YL, Grinstaff MW. Layered superhydrophobic meshes for controlled drug release. *J Control Release.* 2015; 214:23–29. [PubMed: 26160309]
31. Mallapragada SK, Peppas NA, Colombo P. Crystal dissolution-controlled release systems. II. Metronidazole release from semicrystalline poly(vinyl alcohol) systems. *J. Biomed. Mater. Res.* 1997; 36:125–130. [PubMed: 9212397]
32. Rockwood DN, Woodhouse KA, Fromstein JD, Chase DB, Rabolt JF. Characterization of biodegradable polyurethane microfibers for tissue engineering. *J Biomater Sci Polym Ed.* 2007; 18:743–758. [PubMed: 17623555]
33. Macri LK, Sheihet L, Singer AJ, Kohn J, Clark RAF. Ultrafast and fast bioerodible electrospun fiber mats for topical delivery of a hydrophilic peptide. *Journal of Controlled Release.* 2012
34. Heller J, Barr J, Ng SY, Abdellauoi KS, Gurny R. Poly(ortho esters): synthesis, characterization, properties and uses. *Advanced Drug Delivery Reviews.* 2002; 54:1015–1039. [PubMed: 12384319]
35. Moghe A. Co- axial Electrospinning for Nanofiber Structures: Preparation and Applications. *Polymer Reviews.* 2008
36. Yan X, Marini J, Mulligan R, Deleault A, Sharma U, Brenner MP, et al. Slit-surface electrospinning: a novel process developed for high-throughput fabrication of core-sheath fibers. *PLoS ONE.* 2015; 10:e0125407. [PubMed: 25938411]
37. Rastogi R, Teller RS, Mesquita PMM, Herold BC, Kiser PF. Osmotic pump tablets for delivery of antiretrovirals to the vaginal mucosa. *Antiviral Research.* 2013; 100:255–258. [PubMed: 23973812]
38. Veazey RS, Ketas TJ, Dufour J, Moroney Rasmussen T, Green LC, Klasse PJ, et al. Protection of Rhesus Macaques from Vaginal Infection by Vaginally Delivered Maraviroc, an Inhibitor of HIV-1 Entry via the CCR5 Co-Receptor. *J Infect Dis.* 2010; 202:739–744. [PubMed: 20629537]
39. Malcolm RK, Forbes CJ, Geer L, Veazey RS, Goldman L, Johan Klasse P, et al. Pharmacokinetics and efficacy of a vaginally administered maraviroc gel in rhesus macaques. *Journal of Antimicrobial Chemotherapy.* 2013; 68:678–683. [PubMed: 23111849]
40. Okuda T, Tominaga K, Kidoaki S. Time-programmed dual release formulation by multilayered drug-loaded nanofiber meshes. *Journal of Controlled Release.* 2010; 143:258–264. [PubMed: 20074599]

Highlights

- Electrospun core-shell fabrics provide broadly tunable release of maraviroc.
- Good drug-polymer compatibility and shell integrity improve fiber robustness.
- Formulated maraviroc maintains full biological potency following release.
- Multi-fabric composites can generate biphasic rapid and sustained maraviroc release.

Fig. 1.

Coaxial electrospinning produces core-shell EC/PVP fibers containing maraviroc. (A) A diagram of the setup used for coaxial electrospinning. (B)–(C) SEM micrographs of EC-shell and EC- or PVP-core fibers with the highest core maraviroc loading. (D) SEM of core-shell fabric cross-sections reveals a highly porous network of regularly sized core-shell electrospun fibers. (E)–(G) SEM micrographs of EC/PVP-25 fibers in cross section with cores removed for shell to core flow rate ratio $Q_S/Q_C = 1.0$ (E), 2.0 (F), and 4.0 (G). (H) Higher Q_S/Q_C leads to thicker fiber shells (δ/R_O), $P < 0.05$, $n > 10$.

Fig. 2. Sustained release profiles of maraviroc from core-shell and uniaxial fibers into pH 4 citrate buffer media. (A) Release from core-shell EC/PVP fibers with 100% wt/wt core loading and variable Q_s/Q_c . (B) Release from core-shell EC/EC fibers with 10% wt/wt core loading and variable Q_s/Q_c . (C) Release from uniaxial EC fibers with 10% to 100% wt/wt drug loading. $n = 3$.

Fig. 3.

Effect of release media on drug release from maraviroc-loaded electrospun microbicides.

Row 1: release of maraviroc from (A) EC/PVP-100, (B) EC/EC-100, and (C) EC-10 to EC-100 fibers into VFS. Row 2: release of maraviroc from (D) EC/PVP-100, (E) EC/EC-100, and (F) EC-10 to EC-100 fibers into citrate buffer + 0.1% Tween 20. In general, core-shell fabrics maintain superior tunability and sustained drug release in low surface tension media. Note that the y-axis scale is different in uniaxial release panels. $n = 3$.

Fig. 4.

Maraviroc release from core-shell fibers into cDMEM was significantly slower than from the same fibers into other media (Fig. 2,3). Fibers had to be vortexed at the start of the release study to trigger air displacement and encourage drug release. No maraviroc release into cDMEM was detected for non-vortexed EC/EC-100 2.0 fibers. Arrows represent 10 s vortex treatments with the vortexer set to maximum. Data represent mean and SD (error bars) of 2 independent experiments.

Fig. 5.

A) Fiber eluates into cDMEM were nontoxic to TZM-bL cells. Thus, antiviral activity was due to specific activity of maraviroc against HIV-BaL. The mean concentrations of maraviroc in undiluted eluates were: $8.7 \mu\text{M}$ (EC/EC-100 2, vortex), $0 \mu\text{M}$ (EC/EC-100 2, no vortex), $11.1 \mu\text{M}$ (EC/PVP-100 4, vortex), $6.7 \mu\text{M}$ (EC/PVP-100 4, no vortex). B) The antiviral activity of maraviroc following release from shell-core EC/PVP or EC/EC fibers into cDMEM with vortexing (Fig. 4) was equivalent to that of neat maraviroc. Data

represent mean and SD from pooled technical duplicate wells of 2 independent release studies.

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Fig. 6.

Composite fabrics demonstrate superposition of component release profiles. (A) Solvent welding construction method. (B) Fold and press construction method. (C) Maraviroc release behavior under sink conditions in 37°C citrate media. The dashed lines represent the mean expected amount of maraviroc release from the rapidly dissolving PVP fabrics with 28 wt% maraviroc based on their contribution to the overall mass of the layered composites.

Table 1

Chemical surface analysis of core-shell fibers by XPS.

Formulation	% C	% N	% O	% F
Pure EC	64.0 (0.1)	---	36.0 (0.1)	---
Pure PVP	75.4 (0.3)	11.6 (0.4)	13.0 (0.1)	---
Pure MVC	76.1 (0.4)	11.8 (0.2)	5.9 (0.4)	6.1 (0.2)
EC/PVP-25 0.5	67.7 (0.2)	1.7 (0.4)	30.4 (0.3)	0.2 (0.1)
EC/PVP-25 4.0	66.5 (1.9)	---	33.5 (1.9)	---
EC/PVP-100 0.5	68.8 (0.5)	0.8 (0.6)	29.9 (1.1)	0.5 (<0.1)
EC/PVP-100 4.0	68.2 (0.2)	---	31.8 (0.2)	---

Values are listed as the mean (SD) of n = 2 samples.