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Review

Electrospun fibers for vaginal anti-HIV drug delivery

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ABSTRACT

Diversity of microbicide delivery systems is essential for future success in the prevention and treatment of HIV in order to account for the varied populations of women all over the world that may benefit from use of these products. Recently, a novel dosage form for intravaginal drug delivery has been developed using drug-eluting fibers fabricated by electrospinning. There is a strong rationale to support the idea that drug-eluting fibers can be designed to realize multiple design constraints in a single product for topical HIV prevention: fibers are able to deliver a wide range of agents, incorporate multiple agents via composites, and facilitate controlled release over relevant time frames for pericoital and sustained (coitally-independent) use. It is also technologically feasible to scale-up production of fiber-based microbicides. Electrospun fibers may allow for prioritization of physical attributes that affect user perceptions without compromising biological efficacy. Challenges with using fibers as a microbicide include issues related to vehicle deployment, spreading and retention in the vaginal vault. In addition, studies will need to address the interaction of the fibers with the mucosal environment, including unknown safety and toxicity. Sustained release fiber microbicides capable of delivering multiple antiretroviral drugs while simultaneously exhibiting tunable degradation or dissolution of the fibers is also a challenge. However, electrospun fibers are a promising new platform for vaginal delivery of anti-HIV agents and future research will inform their place in the field. This article is based on a presentation at the "Product Development Workshop 2013: HIV and Multipurpose Prevention Technologies", held in Arlington, Virginia on February 20–21, 2013. It forms part of a special supplement to *Antiviral Research*.

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1. Introduction

Topical microbicides are a critical component of the prevention portfolio to combat sexual transmission of HIV. One of the priorities in the field is to confront the low levels of user adherence that have been documented in clinical trials of microbicides. The early termination of VOICE due to low user adherence compromised evaluation of efficacy (Marrazzo et al., 2013). Vaginal drug delivery systems (DDS) play a principle role in bridging biological efficacy and behavioral adherence, which collectively determine the overall impact of a topical microbicide product. It is unlikely that a single DDS technology will solve the prevention needs for all women,

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particularly for adolescent women in low-resource settings where the crises of women's reproductive health is complicated by issues of poverty, malnutrition, poor education, and gender inequality. For this reason, the portfolio of available microbicide DDS needs to be numerous and diverse.

The advancement and development of any single microbicide DDS must prioritize the capacity of the DDS to design for physical attributes that impact user perceptions without compromising the design for biological efficacy. The ultimate decision to use a microbicide product is influenced by a complex combination of product attributes (sensory perceptions, dosing frequency, coital association), user demographics (age, race, culture), and perceived risks for HIV as well as perceived benefits from product use. These user perceptions are the first barrier to designing effective microbicide products, but are often the last consideration in product development. In addition to being acceptable to users, the product must deliver bioactive compounds, possibly in combination, over a relevant period of time (both coitally dependent and independent), be affordable, and have the ability to be manufactured on a production scale. Future development of products will likely incorporate design iterations informed by user perception as well as biological efficacy.

A number of microbicide products are in various stages of the development pipeline, but the lead technologies include gels, films, tablets and intravaginal rings (IVRs) (Abdool Karim et al., 2010; Akil et al., 2011; Garg et al., 2010; Malcolm et al., 2010). There are a number of advantages and disadvantages associated with the leading DDS. Vaginal gels are relevant for pericoital or daily use but have limited ability to deliver physicochemically diverse agents, are not amenable to sustained protection and are messy and may leak out of the vaginal cavity after application. Tablets are easily formulated and manufactured but may leave a grainy residue in the vaginal cavity after dissolution (Garg et al., 2003). IVRs are currently the only sustained release dosage method and have enhanced product stability as a solid dosage form, but are relatively complicated and expensive to fabricate (Malcolm et al., 2010). Vaginal films are also relevant for pericoital use, have demonstrated capability for delivering physicochemically diverse agents, and exhibit enhanced product stability compared to semi-solid dosage forms such as gels. However, vaginal microbicide films have reported loadings of <~1%, and their low overall mass may preclude delivery of sufficient doses of certain APIs (Akil et al., 2011; Mahalingam et al., 2011; Sassi et al., 2011). In addition, the bulk physical properties of films must be controlled to avoid sharp edges and corners that could induce abrasion upon application and use. It is also not clear if current films will be amenable for sustained drug delivery and coitally independent applications.

Recently, a novel dosage form for intravaginal drug delivery has been developed using drug-eluting fibers fabricated by electrospinning, a technique that applies electrostatic forces to form polymeric fibers. Electrospinning is an elegant and facile method for formulating a solid-dosage form microbicide product. The process of electrospinning fibers is well-established (Schiffman and Schauer, 2008), efficient and relatively inexpensive and, since most synthetic and many biological polymers can be electrospun, there is a wide array of possible formulations envisioned for diverse antiretroviral (ARV) drugs. A number of physicochemically diverse drugs have already been encapsulated into electrospun fibers (Taepaiboon et al., 2006), typically with high loading and encapsulation efficiency (Agarwal et al., 2008). Fiber-based "fabrics" are typically soft and non-abrasive, highly flexible, lack sharp corners and can realize a number of geometries (sheets, tubes, coatings). In addition, there is no leakage or mess expected with delivery of fibers into the vaginal cavity. Therefore, there is a strong rationale to support that drug-eluting fibers can be designed to realize multiple design constraints in a single product for topical HIV prevention.

2. Electrospun fibers for drug delivery

Two recent publications investigated electrospun fibers as a platform for vaginal delivery as a topical microbicide for HIV prevention (Ball et al., 2012; Huang et al., 2012). Huang et al. encapsulated the reverse transcriptase inhibitors etravirine or tenofovir disproxil fumarate (Viread) into fibers based on cellulose acetate phthalate (CAP) (Huang et al., 2012). CAP has documented anti-HIV activity, which is thought to be mediated by interactions of the polymer with HIV glycoproteins. In addition, CAP undergoes a solution-to-gel phase transition in response to pH due to the phthalate function group of CAP (pKa of ~5.5). In the low pH environment of the vagina (pH of ~4–5) CAP is a semi-solid, but dissolves upon a semen-induced increase in pH. Thus, CAP fibers were designed to dissolve and release antiretrovirals within seconds to minutes after exposure to semen (Fig. 1). CAP fibers were found to be safe *in vitro* at concentrations of up to 1.8 mg/mL, with minimal toxicity in both TZM-bl cells and vaginal epithelial cells. Exposure to only 0.05 mg/mL of CAP fibers resulted in 50% HIV neutralization and complete neutralization was achieved upon incorporation of 17.8% (wt. drug/wt. polymer) TDF, for a final concentration of TDF of ~0.1 μg/mL.

Ball et al. fabricated nanofiber meshes from a polymer blend of poly-L-lactic acid (PLLA) and polyethylene oxide (PEO) with an assortment of antiretroviral and contraceptive agents for the dual prevention of HIV transmission and unintended pregnancy (Fig. 2) (Ball et al., 2012). Fibers were loaded with maraviroc (MVC), an inhibitor of CCR5-mediated HIV fusion, or 3′-azido-3′deoxythymidine (AZT). Both ARV drug-fibers were found to be non-toxic to TZM-bl cells and macaque ectocervical explants. ARV drug-loaded fibers also had comparable HIV inhibition to the free drugs and maintained IC50 levels at concentrations of 0.90 nM for MVC and 120 nM for AZT. Both MVC and AZT were found to exhibit burst-release from the fibers, but sustained release on the timescale of weeks was achieved by incorporation of

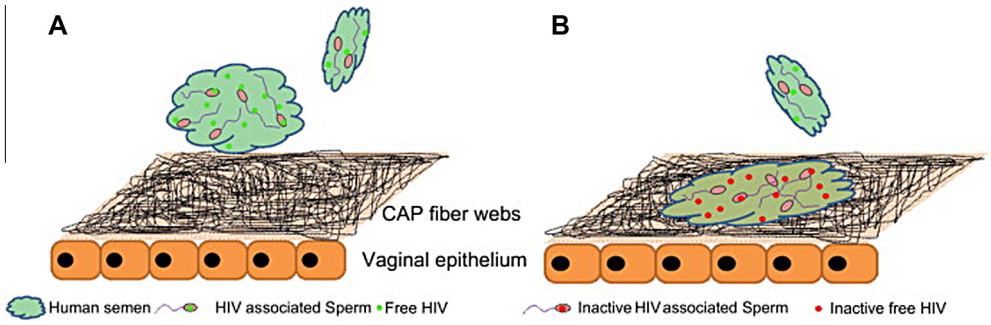

Fig. 1. Schematic by Huang et al. depicting a layer of vaginal epithelial cells covered by a web of electrospun fibers containing antiretroviral drug before (left) and after (right) contacting human semen contaminated with HIV. Used with permission from Elsevier (Huang et al., 2012).

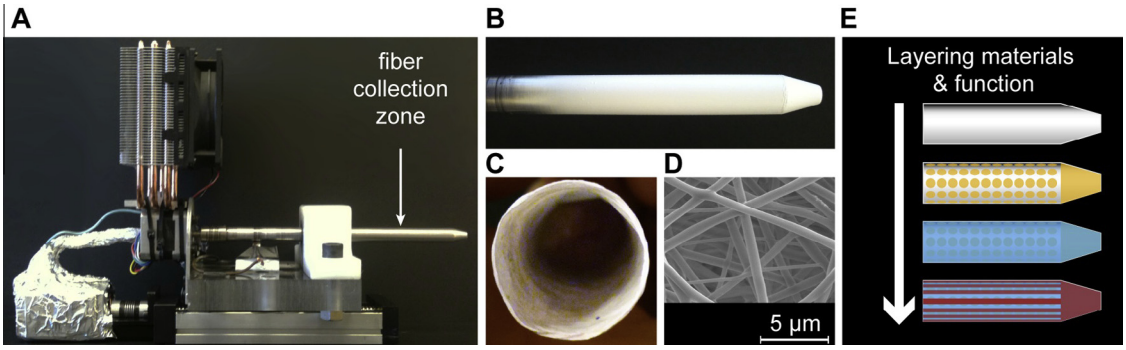

Fig. 2. Potential manufacturing geometry as presented by Ball et al. including: (A) A two-axis mandrel electrospinning rig for fiber collection, (B) a mandrel in the shape of a tampon applicator that may be suitable for vaginal delivery, (C) a fiber mesh removed from the mandrel with a hollow interior, (D) a scanning electron micrograph of fibers with a porous microstructure, (E) a schematic of a potential way to produce a layered mesh for a multifunctional material (Ball et al., 2012). Used with permission from PLOS ONE.

poly-(D,L)-lactic acid into the fibers. Glycerol monolaurate (GML) was also incorporated into the fibers and was found to inhibit sperm motility and viability at concentrations of 0.05–0.5% wt./vol. With the inclusion of GML, the fibers formed both a chemical and physical barrier to sperm. Ball et al. also electrospun fiber meshes in the geometry of a tampon applicator and observed release of a dye to assess coverage of the vaginal lumen and cervix in a mouse model.

These reports establish the potential for using electrospun fibers as a platform for vaginal drug delivery of antiretrovirals and contraceptives. In addition, the drug-eluting fiber platform may represent a discreet, female-controlled and reversible method of HIV prevention. The ability to combine antiretrovirals with other STI prevention or contraception offers an advantage that may increase user acceptability. For example, high-risk groups of women who have low perceived risk of HIV acquisition may be more likely to employ a dual protection product that incorporates HIV prevention with a contraceptive.

Electrospun fibers offer a number of significant advantages that enable their potential for use as a vaginal microbicide platform. They have been used to deliver a wide range of agents (Zeng et al., 2003), and can be fabricated into composites for incorporation of multiple agents. Composites are created by spinning multiple solutions at one time (Theron et al., 2005), layering different fiber meshes (Vaz et al., 2005), coaxial spinning (Jiang et al., 2005) or emulsion spinning (Xu et al., 2006b). It is also possible to formulate fibers with controlled release over time frames relevant for both pericoital and sustained (coitally-independent) use. Electrospun fibers have also been successfully formulated for rapid (Cui et al., 2006) and sustained (Chew et al., 2005) release of drugs. The ability to scale-up manufacturing of microbicide products for use in human clinical trials as well as widespread use is essential. The infrastructure for mass-production of electrospun fibers has already been established, as electrospinning is used widely in industrial applications (Frenot and Ioannis, 2003). Finally, the ability to design a fiber-based product to account for user preference could enhance performance regarding user adherence. Electrospun fibers offer a variety of possible geometries and mechanical properties that could enable user-centered design. In the following sections, we will discuss the delivery of diverse APIs using electrospun fibers, the ability to achieve quick and sustained release profiles, and current challenges with drug delivery from electrospun fibers.

2.1. Breadth of API delivery using electrospun fibers

Small molecules, peptides, nucleic acids, antibodies and nanocarriers all have potential for use in vaginal delivery systems for topical HIV prevention. The physico-chemical diversity of these agents demand that next generation microbicide dosage forms enable delivery of these agents alone and in combination. The wide variety of polymers that can be electrospun allows for the specific formulation of a single agent, and multiple agents can then be combined by layering fibers, using a multi-jet configuration to produce interwoven fibers, or by combining multiple drugs in a single fiber. In addition, the vaginal environment consists of a low pH, degradative enzymes, microflora and other potential components that may inactive many microbicide agents before they can be effective (Richardson and Illum, 1992). Encapsulating drugs in electrospun fibers offers a way to protect the active compounds until they are released. The electrospinning process is carried out at room temperature and, because many polymers can be electrospun using only water as the solvent, the fabrication process is gentle.

Many studies have shown that electrospun fibers are capable of encapsulating drugs with a wide range of solubility (Prabaharan et al., 2012). Electrospun fibers can also be used as a way to increase the bioavailability of therapeutic agents through solid dispersion. When incorporated into a DDS, the drug may exist as a crystalline, amorphous or molecularly dispersed solid. The ability of the drug to be released may depend on the solid state, with an inverse relationship between the degree of crystallinity, solubility and thus bioavailability. Verreck et al. incorporated itraconazole, a highly water insoluble drug, into electrospun fibers to produce dispersions of amorphous drug, which was confirmed by no evident melting endotherm for itraconazole as analyzed by differential scanning calorimetry (Verreck et al., 2003). Similarly, Yu et al. observed well-dispersed amorphous ketoprofen, a water-insolube non-steroidal anti-inflammatory drug (NSAID), and hypothesized that the distribution was due to hydrogen bonding between the drug and polymer nanofibers (Yu et al., 2010). Thus, due to their solid dosage form and wide range of applicable materials, electrospun fibers offer the potential for excellent bioavailability of drugs.

Biological agents such as peptides, antibodies, enzymes and nucleic acids have also been proposed for vaginal delivery, but current delivery vehicles limit usage. The challenge resides in delivering biologic agents to the vaginal mucosa without altering their activity. Loss of activity arises from instability in liquid based platforms or high temperature or abrasive solvents used in processing. A wide range of biologics have been encapsulated into electrospun fibers, including proteins (Wang and Hsieh, 2008), viruses (Liao et al., 2009), DNA (Saraf et al., 2010), siRNA (Cao et al., 2010), bacteria (Lopez-Rubio et al., 2009) and cells (Townsend-Nicholson and Jayasinghe, 2006). Maretschek et al. were able to tune the release of a model hydrophilic protein, cytochrome C, from a fiber blend of PLLA and PEG (Maretschek et al.,

2008). Human β-nerve growth hormone and basic fibroblast growth factor have been encapsulated within (Chew et al., 2005) or deposited on the surface of nanofibers (Patel et al., 2007), with intended use in tissue engineering. Liao et al. and Saraf et al. encapsulated adenovirus (Liao et al., 2009) and plasmid DNA (Saraf et al., 2010), respectively, using coaxial spinning for gene delivery purposes. Cao et al. achieved controlled release of siRNA from PCL nanofibers for 28 days with a silencing efficiency comparable to conventional siRNA transfection (Cao et al., 2010). Though many of the applications of electrospinning have been focused on cancer therapy or tissue engineering, the foundation of incorporation of biologics into electrospun fibers has been clearly established. Therefore, fibers are poised to have real application in antibodies against HIV, antigens or genes for vaginal mucosal vaccines and bacteria for the treatment of bacterial vaginosis.

In addition to delivery of biologics, electrospun fibers may also offer potential to deliver drug-loaded nanoparticles. There is currently no well-established vehicle for vaginal delivery of nanoparticles, and the combination of nanofibers and nanoparticles could allow for enhanced retention of these carriers. As a solid dosage form, the fibers may overcome the issues associated with delivering nanoparticles in a vaginal gel, such as aqueous stability and long-term storage of the particles. Coumarin-6, a model compound, was released from PLGA nanoparticles encapsulated in PVA/PEO blend nanofibers (Beck-Broichsitter et al., 2010). Release was modulated over 2–8 h depending on the PVA to PEO ratio. For example, nanoparticles showed faster release in vitro of coumarin-6 compared to nanoparticle composites with PVA fibers. FGF-2 was released from heparin-containing polyelectroyle complex nanoparticles encapsulated in chitosan fiber networks over a period 30 days, with maintained bioactivity of the encapsulated drug (Volpato et al., 2012). Furthermore, Volpato et al. showed the preservation of nanoparticle structure when encapsulated in electrospun fibers using fluorescent dyes and confocal microscopy. The duo of nanofibers and nanoparticles may broaden the delivery capabilities of fibers as a vaginal microbicide.

2.2. Rapid and sustained release from drug-eluting fibers

Drug release from polymeric fibers depends on the erosion and degradation of the polymer used and the diffusive properties of the incorporated drugs. Release of drugs from gels is governed by both erosion and diffusion and depends on hydration, swelling and dissolution of the polymer (Sinha Roy and Rohera, 2002). The vaginal film matrix also swells, but release largely depends on diffusion of the drug from the polymer matrix at low loading (Donbrow and Friedman, 1975). In contrast, intravaginal rings undergo very minimal swelling and erosion, and relies on diffusion of the drug from the polymer matrix (Woolfson et al., 2006). Electrospun fiber meshes similarly rely mostly on diffusion for drug delivery, although erosion of individual fibers has been observed (Luong-Van et al., 2006).

By utilizing a variety of polymers and blends of polymers, researchers have achieved both rapid (<15 min) and sustained (>7 days) release of various drugs from electrospun fibers (Table 1). This is a huge advantage in vaginal delivery of antiretroviral drugs, as there are a number of scenarios in which vaginal microbicides might require different timescales for delivery. Quickly dissolving fibers could be useful for a vaginal microbicide intended for pericoital use, in which rapid delivery of antiretroviral drugs would be imperative to act against immediate viral exposure. Long-term release is coitally independent and would be preferable in a situation where a user could dose monthly and have sustained levels of drug in tissue for HIV prevention.

Several groups have demonstrated rapid drug release from electrospun fibers by using quickly dissolving polymers such as PVA, PEO, alginate and chitosan. The use of quickly dissolving polymer fibers allows for burst release of drug. Li et al. used PVA nanofibers to burst-release caffeine and riboflavin. They observed dissolution of fibers within 5 s and 100% release of caffeine and 40% release of riboflavin within 60 s (Li et al., 2013b). Macri et al. used tyrosine-derived polycarbonate terpolymer fiber mats to deliver a hydrophilic peptide with complete release within 9 h (Macri et al., 2012).

Table 1
Reported release kinetics of small molecule drugs from polymeric fibers.

Time-frame of drug release	Released compound	Fiber and mesh properties	% Released at 24 h	Time to release:		Refs.
				50%	100%	
Rapid release (<60 min)	Azidothymidine (AZT), acyclovir (ACV), maraviroc (MVC)	**Polymer**: PLLA/PEO **Solvent**: Chloroform/Trifluoroethanol **Fiber diameter**: 200–700 nm **Drug loading**: 1 wt.%	100%	<1 h	<1 h	(Ball et al., 2012)
	Cefoxitin	**Polymer**: PLGA (75:25) **Solvent**: Dimethlyformamide **Fiber diameter**: 200–400 nm **Drug loading**: 1–5 wt.%	100%	<1 h	<1 h	(Feng-Lei et al., 2009; Kim et al., 2004)
Intermediate release (1–3 d)	Bis-chloroethylnitrosourea (BCNU, Carmustine®)	**Polymer**: PEG-PLLA **Solvent**: Chloroform **Fiber diameter**: 700–1400 nm **Drug loading**: 5–30 wt.%	60–80%	5–15 h	70 h	(Forward and Rutledge, 2012b; Xu et al., 2006a)
	Ciprofloxacin	**Polymer**: PVA/PVAc **Solvent**: Water/Acetic acid **Fiber diameter**: 400–500 nm **Drug loading**: 10 wt.%	40–50%	<50 h	>100 h	(Jannesari et al., 2011; Persano et al., 2013)
Sustained release (>7 d)	Paclitaxel	**Polymer**: PLGA (50:50) **Solvent**: Dichloromethane **Fiber diameter**: 700–2500 nm **Drug loading**: 10 wt.%	13–22%	>20 d	>60 d	(Ball et al., 2012; Xie and Wang, 2006)
	SN-38	**Polymer**: PCL/PGC-C18 (90:10) **Solvent**: Chloroform/methanol **Fiber diameter**: ~7000 nm **Drug loading**: 0.1–1 wt.%	~30%	~40 d	>70 d	(Yohe et al., 2012)

Extended release kinetics of drugs from polymer fibers are also highly desirable for vaginal microbicides. A once-monthly dosage form, like vaginal rings, has potential to overcome challenges related to low user adherence. Long-term drug release from fibers has been demonstrated by a number of groups (Cui et al., 2006; Yohe et al., 2012). Sustained release is achieved using a variety of polymers and techniques that have been optimized for slowing release, including coating the fibers to prevent burst release (Chunder et al., 2007), coaxial or emulsion spinning to create a shell around a core fiber of drug (Li et al., 2013a), or crosslinking the polymer (El-Refaie et al., 2007). Jannesari et al. observed that by decreasing drug loading of ciprofloxacin HCl in PVA/PVAc nanofibers and increasing thickness, release could be extended to 80 days (Jannesari et al., 2011). Xie et al. delivered paclitaxel, a chemotherapy agent for the treatment of C6 glioma, for up to 60 days from PLGA micro- and nanofibers (Xie and Wang, 2006). Because many sustained delivery fiber systems require the use of slowly degrading materials, the product might require removal, much like vaginal rings. By manipulating either polymer chemistry or physical attributes of the fibers, sustained delivery of therapeutics is achievable.

2.3. Challenges in electrospun fibers for vaginal delivery

While electrospun fibers are a promising new platform for vaginal delivery of microbicides, there are a number of challenges that must be confronted. Co-delivery of multiple drugs from a single electrospun fiber mat with precisely controlled release profiles may be difficult. In addition, delivering multiple, physicochemically diverse agents may require a novel approach to design of the electrospun fiber vehicle, such as manipulation of microarchitecture or use of polymer blends. Unlike drug-loaded nanoparticles, which are able to penetrate tissue and deliver their payload intracellularly, fibers are limited by the pharmacokinetics at the site of administration. The maximum achievable drug loading in fibers will likely be drug-dependent, as high loading into polymer solutions may negatively alter the ability to electrospin the solution. However, our lab has successfully electrospun fibers containing 60% tenofovir by mass with high encapsulation efficiency on a production-scale electrospinning instrument (unpublished results). Future research into the area of the physics of electrospinning may offer more control over fiber sizes, drug distribution and release profiles (Sill and von Recum, 2008). A clear mode of administration, i.e. the way the fibers will be inserted into the vaginal cavity, has not been established. It is also unclear whether any residual polymer film will persist in the vaginal cavity, which may impact user acceptability.

In vivo testing of biocompatibility of fibers for vaginal drug delivery has not been conducted, and safety and toxicity studies will need to be performed before efficacy can be fully evaluated. Ball et al. did not conduct *in vivo* safety, toxicity or release kinetics but did observe release of dye and coverage of the cervicovaginal tract from ICG-loaded fibers in mice (Ball et al., 2012). However, there are a number of examples of fiber materials used *in vivo* that would support their safety and ability for sustained drug delivery. Silva et al. are currently conducting a Phase III clinical trial to evaluate efficacy of an electrospun fiber-based patch that releases nitric oxide for the treatment of diabetic ulcers (Silva et al., 2007). Ranganath et al. used an electrospun fiber patch to deliver paclitaxel in the treatment of malignant glioma in a mouse model and found increased tumor inhibition when compared to acute administration of Taxol (Ranganath and Wang, 2008). Furthermore, Hong et al. observed therapeutic effects based on mechanical properties as well as sustained antibiotic release from an electrospun fiber patch used after laparotomy (Hong et al., 2008). Though vaginal testing has yet to be completed, promising results from alternate routes of administration of drug-eluting electrospun fibers indicate the feasibility of their use *in vivo*.

3. Scale-up of electrospun fibers

To date, only academic research laboratories have pursued the investigation of electrospun fibers as a platform for vaginal microbicides. One of the main challenges in translation of this research to a clinical setting, in addition to thoughtful design of the ideal product, is developing a clear strategy for product development and implementation. Clinical translation will require establishing the design, usage, storage parameters and production cost. Currently, parallel investigation into product design, including rationale, form, and deployment of a fiber-based vaginal microbicide as well as rigorous testing in established pre-clinical models for microbicides are acting to inform prototype development (Garg et al., 2010). Manufacturing requirements are a critical component to product realization, so we will discuss in the following sections the scalability and material requirements for a potential fiber-based microbicide product.

3.1. Materials and capital investments

The required materials for a fiber-based delivery system will be highly dependent on the intended use. A quick-dissolving fiber for pericoital use may have different requirements than a sustained-release fiber that is used monthly for HIV prevention. Composites containing multiple drugs, either combinations of antiretrovirals or antiretrovirals and contraception/STI prevention, may necessitate the use of different materials. While there is no consensus on the final form of a fiber delivery system, the first fiber-based microbicide product to be realized may share similarities with vaginal films as this has been a relatively acceptable microbicide delivery mode. The International Partnership for Microbicides found that 85% of women in Burkina Faso, Tanzania and Zambia said they would use a vaginal film as an HIV prevention method (Nel et al., 2011). In addition, the ease of producing a simple geometry that resembles a film will establish the proof-of-principle for manufacturing scale-up of a fiber-based microbicide product. Prioritizing materials that have already been FDA-approved for use in vaginal delivery, such as polyvinyl alcohol (PVA), hydroxyethyl cellulose (HEC), hydroxypropryl methylcellulose (HPMC) and polyurethane (PU) may enable more rapid clinical translation of this new technology. However, a fiber-based microbicide should focus on addressing gaps not currently addressed by the lead dosage forms.

3.2. Manufacturing capability

A number of large-scale electrospinning instruments exist due to applications of electrospinning in industries such as air and water filtration, energy and construction. Because of the large demand for fiber-based technologies, which are expected to reach $2.2 billion by 2020 (Luo et al., 2012), the infrastructure for mass production is already established. However, there may be limitations to current large-scale instrumentation for production of electrospun fiber based dosage forms. Current production-scale instruments are not specifically made for pharmaceutical applications and will require adaption to FDA standards. Likewise, if coaxial fibers prove advantageous for sustained antiretroviral drug delivery, production-scale instruments capable of making this architecture are still in early development.

Production costs can be estimated by assuming that initial fiber-based products will be similar to a vaginal film or gel, as far as dosage requirements and size. Currently, vaginal films are dosed at around 300 mg and vaginal gels are dosed from 2–4 g (gel

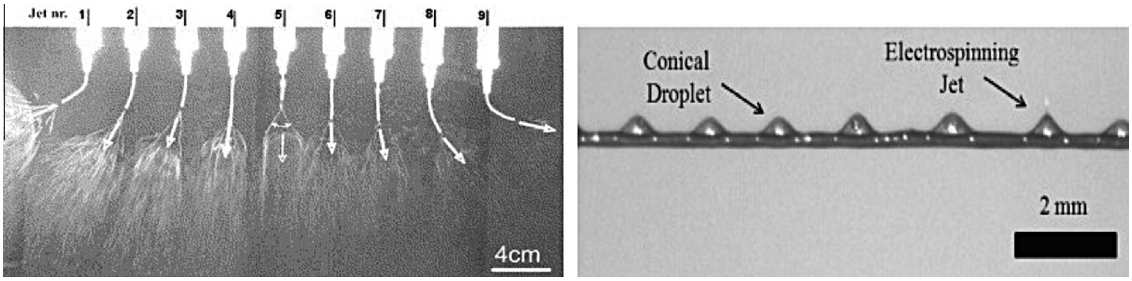

Fig. 3. Comparison of multi-nozzle electrospinning, where multiple Taylor cones form from separate nozzles, (left) and multi-jet nozzleless electrospinning, where multiple Taylor cones form on the same electrode (wire or cylinder) (right). Reprinted with permission from Elsevier (Forward and Rutledge, 2012a; Theron et al., 2005).

Table 2
Comparison of commercial industrial scale instruments for manufacturing of electrospun polymer fibers. (Feng-Lei et al., 2009; Forward and Rutledge, 2012b; Persano et al., 2013).

Instrument	Process type	Electrode type ([a]number and configuration)	Production Rate (g h^{-1})	Advantages	Disadvantages
Nanospinner 416 (Inovenso Ltd.)	Spinneret-based	Nozzle (multinozzle array)	200 g h^{-1}	Potential for core–shell fibers; higher theoretical production rate	Nozzle clogging, inter-jet interactions, non-uniform fiber deposition, low flow-rate, complex design
Nanospider (Elmarco)	Free-surface	Wire (8 stationary wires)	200 g h^{-1}	No clogging, simple design, self-optimized Taylor cone formation	No fiber alignment; no co-axial fiber architecture; complex cleaning, droplet defects

density ~1.0 g/mL) (Garg et al., 2010). Production yields for large scale instruments could theoretically reach up to 6.5 kg/h, which would correlate to production of 10–20 million doses annually (Luo et al., 2012). Therefore, although production will depend on the specific formulation, scale-up for a fiber-based microbicide is theoretically achievable at a production level that is necessary for human clinical trials (Malcolm et al., 2010). Additional constraints on manufacturing include initial capital investments, availability of raw materials, cost of APIs, utilities and infrastructure, and product reproducibility.

3.3. Scale-up equipment

Currently, commercial instruments available for industrial-scale production of electrospun fibers use either a format involving multi-nozzle or multi-jet nozzleless electrospinning (Fig. 3, Table 2). Multi-nozzle electrospinning refers to the use of multiple nozzles or extrusion holes in a simultaneous fashion for increased productivity. This would be a direct extension of laboratory scale electrospinning rigs, which use only a single nozzle for production of fibers. Several multi-nozzle instruments are commercially available and may incorporate anywhere from 2 to 100,000 production nozzles in a variety of configurations (Varesano et al., 2010). Thus far, multi-nozzle electrospinning has reported the highest achieved production rate, 6.5 kg/h, compared to other reported commercial electrospinning processes (Luo et al., 2012). Commercial units are available from such companies as Inovenso Ltd. and Finetex Technology Inc.

Multi-jet nozzleless electrospinning does not require the use of nozzles or extrusion holes, and produces fibers from a free-surface electrode. Because there are no nozzles, issues with clogging and low flow rates are avoided using this method. Production of fibers occurs with nozzleless electrospinning by deformation of the liquid surface into numerous Taylor cones (Fig. 3). The number of jets and distance between jets is optimized based on the properties of the polymer solution. A number of free-surface electrode configurations exist including cylinders, wires, spheres, magnetic liquids or gas bubbles (Forward and Rutledge, 2012b). Elmarco Inc. (Czech Republic) is the lead instrument manufacturer of industrial-scale instruments for free-surface electrospinning. Currently, reported achievable productivity for both the multi-nozzle and nozzleless electrospinning units are ~200 g/h, which is 30 times lower that the theoretical reported maximum productivity (Persano et al., 2013).

Although the commercially available electrospinning instruments have the desired capacity, none are made specifically for pharmaceutical applications. Therein lie the challenges, and a potentially higher cost associated with scale-up of drug-eluting fibers to meet requirements for good manufacturing practices (GMP). Given the limited information available on the production cost of electrospun fibers, some reports show that the costs of fibers produced by an industrial-scale instrument is \$1–\$5 kg^{-1} (Luo et al., 2012). These reported costs are likely to increase substantially based on the complexity of the GMP pharmaceutical manufacturing process required to produce an ARV-based microbicide, and we estimate that the cost for a 300 mg dose may be on the order of \$0.50–\$3.00 depending on the annual production volume. These estimates are based on incomplete information and rough assumptions for meeting stringent requirements for compliance with ISO and GMP. Based on these estimates, electrospun fibers would be a competitive with other ARV dosage vehicles, such as vaginal films and rings and present another option for a globally accessible dosage form for ARVs.

4. Conclusion

Electrospun fibers are a promising new platform in the context of vaginal delivery of microbicides. Fibers are a solid dosage form that offers a silky texture as well as enhanced product stability. Fibers are capable of delivering a number of therapeutics that may be useful for microbicides, including biologics and nanoparticles, and can exhibit burst, sustained and asynchronous release profiles. Because electrospinning is a well-established technology, there are already commercial-scale instruments available, which would ease

the translation to clinical applications and possibly widespread use. At this point, it is unclear whether fibers will be used for a quick pericoital dosage or a sustained monthly dosage, but the potential for both applications offers an exciting outlook to create innovative new designs as challenges in the field arise.

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