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Recent advances in electrospun nanofiber vaginal formulations for women's sexual and reproductive health

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Abbreviations¹

**Abbreviations:** CFU, colony-forming units; DSC, differential scanning calorimetry; FTIR, Fourier-transform infrared spectroscopy; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; HSV, herpes simplex virus; MPT, multipurpose prevention technologies; PCL, polycaprolactone; PEO, poly(ethylene oxide); PLA, poly(lactic) acid; PLCL, poly(L-lactide-co-caprolactone); PLGA, poly(lactic-co-glycolic acid); POP, pelvic organ prolapse; PP, polypropylene; PrEP, pre-exposure prophylaxis; PVA, poly(vinyl alcohol); PVP, poly(vinyl pyrrolidone); SEM, scanning electron microscopy; XPS, x-ray photoelectron spectrometry.
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Abstract

Electrospinning is an innovative technique that allows production of nanofibers and microfibers by applying a high voltage to polymer solutions of melts. The properties of these fibers – which include high surface area, high drug loading capacity, and ability to be manufactured from mucoadhesive polymers – may be particularly useful in a myriad of drug delivery and tissue engineering applications. The last decade has witnessed a surge of interest in the application of electrospinning technology for the fabrication of vaginal drug delivery systems for the treatment and prevention of diseases associated with women's sexual and reproductive health, including sexually transmitted infections (e.g. infection with human immunodeficiency virus and herpes simplex virus) vaginitis, preterm birth, contraception, multipurpose prevention technology strategies, cervicovaginal cancer, and general maintenance of vaginal health. Due to their excellent mechanical properties, electrospun scaffolds are also being investigated as next-generation materials in the surgical treatment of pelvic organ prolapse. In this article, we review the latest advances in the field.

Keywords: Drug Delivery, Electrospinning, Mucoadhesion, Nanofibers, Polymers, Vaginal drug delivery.

1. Introduction

According to the earliest human writings, women have long administered products to the human vagina for clinical benefit. The medical papyri of the ancient Egyptians describe various concoctions intended for application to the human vagina: 'Shemshemet' (the Egyptian word for 'cannabis') mixed with honey was prescribed to "cool the uterus and eliminate its heat" (E 821, Ebers Papyrus 821, 1550 BCE.) (Ghalioungui, 1987); women were recommended to mix crocodile dung and acacia gum with sour milk or honey and to form the resulting thick paste into...
a pessary for administration to the vagina for prevention of pregnancy (Christin-Maitre, 2013; Haimov-Kochman et al., 2005); the Eber's papyrus describes use of ground corn and celery ground in cow’s milk for induction of labour (Haimov-Kochman et al., 2005). David Macht's 1918 seminal article – entitled 'On the absorption of drugs and poisons through the vagina' – is widely regarded as one of the earliest to apply modern scientific methods to the administration of drugs to the human vagina. Prior to its publication, it was widely believed that the vagina was incapable of absorbing pharmacological agents systemically, and that the various vaginally-administered products commonly used by women at the time – including medicated douches, tampons, ovules, and 'uterine wafers' – only acted locally (Macht, 1918).

Today, a diverse range of dosage forms is routinely used to administer pharmaceutical drugs to the human vagina for both local and systemic effect, including tablets, capsules, gels, creams, ointments, rings, films and foams (Barbosa et al., 2018; Johal et al., 2016; Notario-Pérez et al., 2020). Each type of vaginal dosage form may offer certain advantages over others, depending upon the nature of the drug to be administered and the intended therapy. For example, vaginal rings can provide sustained or controlled release of highly potent steroid hormones drugs over many weeks or months (Murphy et al., 2018; Nave, 2019); aqueous vaginal gels are useful in administering relatively large doses of hydrophilic drugs (Ciocacu et al., 2020). Other advanced drug delivery systems have also been reported, including mucoadhesive or stimuli-responsive (i.e. pH-sensitive or thermogelling) drug delivery systems (Martín-Illana et al., 2021; Pandey et al., 2020; Vigani et al., 2019). Judicious selection of the most appropriate vaginal dosage form based upon a detailed knowledge of the physicochemical properties of the drug, the target clinical indication, the required drug dose, the intended duration of treatment, and the potential end user is critical for maximising clinical outcomes.

Unsurprisingly, vaginal drug products have primarily been used to treat diseases directly associated with women's sexual and reproductive health. The most common indications for marketed vaginal products include: treatment of vaginal infections (various azole antifungals), treatment of bacterial vaginosis (metronidazole or clindamycin), treatment of vaginal atrophy (various estrogens), cervical ripening to help induce labour (dinoprostone or misoprostol), hormonal contraception (various progestins, or combinations of a progestin and an estrogen) and luteal phase support (progesterone) (Barriga Pooley et al., 2020; Chen et al., 2016; Child et al., 2018; Johal et al., 2016; Lethaby et al., 2016).

Advances in engineering and manufacturing technologies often find new applications within pharmaceutics and medicine. Hot melt extrusion and injection molding technologies – originally established in the 1930s for use in the plastics and food industries – are now common applied to the manufacture of drug-releasing vaginal rings (Major and McConville, 2015; Murphy et al., 2020).
First introduced in the 1970s to overcome swallowing difficulties with conventional oral dosage forms, fast-dissolving orodispersible thin films manufactured by solvent casting or hot melt cast extrusion methods are now applied to the manufacture of drug-releasing vaginal films (Notario-Pérez et al., 2020; Romano et al., 2008). Similarly, recent advances in 3D-printing and additive manufacturing technologies are also being applied to vaginal drug delivery, including vaginal rings (Fu et al., 2018; Janusziewicz et al., 2020; Tiboni et al., 2021; Welsh et al., 2019).

Although the technique of electrospinning (involving the use of electrostatic forces to spin fine fibers) has been known since the late 19th century (Cooley, 1902; Ghosal et al., 2018), its use in medical and pharmaceutical applications – initially for wound dressing and implantable vascular grafts – was only reported much later in the 1970s (Annis et al., 1978; Martin et al., 1978). Recent advances, including the ability to manufacture core-shell nanofibers (Sun et al., 2003) and 3D structures (Kim et al., 2018; Rafiei et al., 2020), have stimulated interest in the fabrication of drug delivery systems, including applications in the field of vaginal drug delivery (Son et al., 2014; Thakkar and Misra, 2017).

Among the different nanosystems, nanofibers have been widely explored not only as drug delivery systems but also as healthcare materials (for example, for use in tissue engineering and wound dressing). Nano and microfibers are defined as nanomaterials with two external dimensions of similar magnitude and a third dimension considerably larger. While the two similar dimensions are frequently nanometric (in some cases micrometric depending on electrospinning conditions), the larger dimension reaches several centimeters (Meireles et al., 2018). Interest in these fibers is due to their unique properties, such as their efficiency in encapsulating other substances including drugs, high surface/volume ratio, low density, tuneable porosity and excellent mechanical properties. These properties are also useful in enhancing the release, biodistribution and retention of drug substances in the vagina (Chindamo et al., 2021). Also, depending on the characteristics of the polymers and the manufacturing techniques used in the fabrication of fibers, drug release can occur via different mechanisms, including biodegradation of the matrix or permeation (offering both fast and sustained drug release rates) (Anup et al., 2021; Pérez-González et al., 2019). Interestingly, encapsulation of nanoparticles in electrospun fibers has been frequently proposed, which provides opportunities for modulating drug release (Fathollahiipour et al., 2015), offering antimicrobial properties (Zhan et al., 2017) and increasing the residence of the systems through mucopenetration (Krogstad et al., 2017).

In this manuscript, we (i) provide a brief overview of vaginal anatomy and physiology, (ii) describe the general techniques and principles underlying the production of electrospun fibers, and (iii) review the recent scientific literature describing the application of electrospun fiber
technology to the manufacture of vaginally administered formulations and devices for the
treatment or prevention of conditions or diseases associated with female sexual and reproductive
health.

2. The vaginal route of administration

2.1. Vaginal anatomy and physiology

The vagina – along with the uterus, the fallopian tubes and the ovaries – is a primary organ of the
female reproductive system (Fig. 1A). It is a S-shaped collapsed fibromuscular organ (the anterior
and posterior walls contact each other) measuring 40–100 mm in length and 25–45 mm in width
and extending internally from the cervix to the introitus (Barnhart et al., 2006). However, baseline
dimensions are known to vary considerably with parity (i.e. number of vaginal births), age and
height, such that single product size is not considered optimal for all women. Despite these
differences in vaginal dimensions, all vaginal drug products are offered in one size only; only
vaginal pessaries for treating pelvic organ prolapse are offered in different sizes (Oliver et al.,
2011; Qureshi et al., 2008).

The vagina comprises two compartments deposed at an angle of ~130°, a lower convex
compartment and an upper compartment that is practically horizontal when the woman is standing
(Funt et al., 1978). The lumen of the vagina has multiple folds called rugae, which gives the
vagina a high surface area and extensibility. The vaginal tissue is composed of four layers: the
outer stratified squamous epithelium of variable thickness (Fig. 1B, 1C); a muscularis layer
containing smooth muscular fibers running in circular and longitudinal direction that give the
vagina great elasticity, and an adventitia layer, composed of collagen and elastin and containing
many lymphatic and vascular channels. Although often referred to as a mucosal tissue, the vagina
does not contain any secretory glands; instead, the vagina is coated in secretions comprising
transudate originating from the endometrium, cervix and vestibular glands. The extent of
innervation varies along the vagina length; while peripheral nerves innervate the lower quarter,
the rest of the cavity presents autonomic innervation. For this reason, the deepest sections of the
vagina (including the posterior fornix and the external cervical os) is less sensitive, and
formulations placed in this area rarely cause discomfort.

The vagina is highly vascularized, making it useful for the systemic administration of drugs. It
receives blood from the descending branches of the uterine, vaginal and internal pudendal arteries.
Drainage from the lower area occurs through the pudendal vein, while the upper area drains
through the vaginal, uterine and bladder veins into the vena cava, thereby avoiding hepatic first-
pass effects (Alexander et al., 2004; de Araújo Pereira and Bruschi, 2012; Machado et al., 2015).
Also there is considerable evidence for a vagina-to-uterus transport mechanism (the ‘uterine first
pass effect'), attributed to the direct transfer of vaginally administered drugs from the vaginal veins to the uterine artery (Bulletti et al., 1997; Ziegler et al., 1997).

**Figure 1.** A – Representation of the female reproductive system, including fallopian tubes, uterus, cervix, and vagina. B and C – Histological sections of the vaginal tissue.
The human vagina is replete with microorganisms (Carson et al., 2021). The healthy vaginal microbiota is dominated by Lactobacillus spp., most notably L. crispatus, L. jensenii, L. gasseri and L. iners. Conversion of the glycogen found in the vaginal cells into lactic acid by endogenous Lactobacillus spp. helps maintain the weakly acidic vaginal pH (typically 3.8–4.2) associated with a healthy vaginal ecosystem (de Araújo Pereira and Bruschi, 2012). Other facultative anaerobic bacteria also found to a lesser extent, including Gardnerella vaginalis, Atopibium vaginae and Prevotella spp. Any disruption to the microbial balance within the vagina – and particularly loss of the lactobacilli and overgrowth of the anaerobes – can result in vaginitis, such as bacterial vaginosis, yeast infection, and trichomoniasis (Chindamo et al., 2021; Zupančič et al., 2019). During menstruation or intercourse, the pH of the vaginal fluid increases due to the presence of increased vaginal transudates, cervical mucus and slightly alkaline seminal fluid.

Two different glands can be found in the vagina: Bartholin’s glands are two 5mm diameter oval glands found in the posterior region of the vaginal opening. These glands secrete mucus into the vaginal vestibule (Lee et al., 2015). Skene's glands are tubuloaveolar structures that resemble pre-pubescent male prostate glands and which produce a fluid that contains prostate specific antigen and prostate acid phosphatase. This fluid – commonly referred to as female ejaculate – is often expelled during female orgasm (Dwyer, 2012; Rodriguez et al., 2021). Vaginal fluid is a complex mixture of vaginal transudate, secretions from the Bartholin and Skene glands, exfoliated epithelial cells, residual urine, and fluids from the upper reproductive tract, such as cervical mucus or tubal fluids. It contains enzymes and enzyme inhibitors, proteins, carbohydrates, amino acids, alcohols, hydroxyl-ketones, aromatic compounds and inorganic salts (Owen and Katz, 1999; Raffi et al., 1977; Wagner and Ottesen, 1980). The rate of production of the fluid varies during the stages of the menstrual cycle, and sexual arousal induces greater production of vaginal fluid, for which the composition can also vary. Vaginal fluid is produced at a rate 6g/day in women in reproductive age, and this rate is significantly reduced (up to 50%) in postmenopausal women (Hussain and Ahsan, 2005; Owen and Katz, 1999). Pathological conditions can also alter the characteristics of vaginal fluid; for example, bacterial vaginosis, vulvovaginal candidiasis, and trichomoniasis are associated with reduced Lactobacillus spp populations and increased vaginal fluid pH. Variations in the volume and composition of vaginal fluid are important factors for consideration when developing vaginal drug delivery systems. The epithelium expresses various enzymes that may also compromise the stability of the administered drug (de Araújo Pereira and Bruschi, 2012; Hussain and Ahsan, 2005; Owen and Katz, 1999). Of course, the characteristics of the drug molecule intended for vaginal administration will also be critical for efficacy; properties such as molecular weight, lipophilicity and ionic charge of the drug must be taken into account.
As with all mucosal membranes, drug absorption across the vaginal mucosa can occur via different transport pathways, including transcellular, paracellular, pinocytosis and receptor-mediated transport. For most drug molecules administered vaginally having relatively low molecular weight and moderate hydrophobicity (McBride et al., 2019), the transcellular pathway predominates. Changes in the thickness of the epithelial layer and the characteristics of the vaginal fluid (pH, volume, viscosity or thickness of the layer) can modify the absorption rate. For example, an increase in the volume of vaginal fluid may increase both the rate of dissolution of the dosage form and the quantity of drug dissolved, while an increase in viscosity may slow diffusion of the drug to the vaginal mucosa. In addition, small lipophilic molecules can permeate the vaginal mucosa more easily than large lipophilic or hydrophilic molecules. Furthermore, the presence of enzymes and other compounds can compromise drug stability.

It is desirable that the dosage form remains in the vagina long enough for the entire dose to be released, and – for some indications, such as HIV prevention – also allows uniform distribution of the drug throughout the vagina (Chindamo et al., 2021; Hussain and Ahsan, 2005).

2.2. Mucoadhesion and mucopenetration

Mucoadhesion – a specific type of bioadhesion – involves interactions between a mucoadhesive polymer (Table 1) and the mucus associated with a mucosal tissue. If the interactions are particularly strong, mucoadhesive formulations can be retained on the mucosa long after administration, often permitting longer duration of drug release and increased drug bioavailability.

**Table 1.** Common mucoadhesive polymers for drug delivery systems, information retracted from (Asane et al., 2008; Notario-Pérez et al., 2020; Pérez-González et al., 2019).

<table>
<thead>
<tr>
<th>Synthetic polymers</th>
<th>Semisynthetic polymers</th>
<th>Natural polymers</th>
</tr>
</thead>
<tbody>
<tr>
<td>poly(acrylic acid)</td>
<td>methylcellulose</td>
<td>chitosan</td>
</tr>
<tr>
<td>poly(methyl vinyl ether)</td>
<td>hydroxypropyl methylcellulose</td>
<td>alginate</td>
</tr>
<tr>
<td>poly(vinyl pyrrolidone) (PVP)</td>
<td>methyl ethyl cellulose</td>
<td>pectin</td>
</tr>
<tr>
<td>high molecular weight</td>
<td>hydroxyethyl cellulose</td>
<td>natural gums (i.e. tragacanth or xanthan gum)</td>
</tr>
<tr>
<td>poly(ethylene oxide) (PVA)</td>
<td>hydroxypropyl cellulose</td>
<td>zein</td>
</tr>
<tr>
<td>poly(hydroxyethyl methacrylate)</td>
<td>biopolymeric thiomers</td>
<td>gelatin</td>
</tr>
<tr>
<td>synthetic polymer thiomers</td>
<td></td>
<td>carrageenan</td>
</tr>
<tr>
<td>poly(ethylene oxide) (PEO)</td>
<td></td>
<td>xanthan gum</td>
</tr>
</tbody>
</table>

Different polymers exhibit different mucoadhesive characteristics and the degree of mucoadhesion depends upon factors such as molecular weight, viscosity of the polymeric formulation, the ionic charge on the polymer, the porosity of the formulation, and the degree of molecular branching of the polymer. It is generally accepted that the mucoadhesion process
comprises three stages: (a) water from the aqueous medium diffuses between the polymeric chains; (b) physical crosslinking occurs between the polymer chains and the mucus via intermolecular forces; (c) consolidation of adhesion occurs with the formation of covalent bonds between the mucus and the polymer (dos Santos et al., 2020; Edsman and Hägerström, 2005; Khutoryanskiy, 2011; Mackie et al., 2017).

Mucopenetration has also been proposed for optimal delivery of drugs to the vaginal tissue (Ensign et al., 2013; Lechanteur et al., 2017; Wu et al., 2011; Zierden et al., 2021). Unlike mucoadhesion which seeks to immobilize the formulation on the surface of the mucosa, mucus-penetrating strategies are intended to facilitate diffusion of the drug-loaded particles through the cervicovaginal mucus. Mucopenetration is based on a superficial functionalization that allows the particles to diffuse unobstructed through the mucus layer. A commonly employed strategy is to coat the drug carrier – often nanoparticles or lipid vesicles – with PEG. The use of particles with high charge density, which mimic viral infection mechanisms, has also been proposed. Also, assemblies of zeta potential shifting polymers allow the preparation of negatively-charged particles which slide through the mucus due to the presence of sialic and sulphate moieties; later, during diffusion, these particles revert to positively charged, thus remaining strongly anchored to the mucus by electrostatic interactions, which favours the subsequent penetration though the epithelial layer (Schattling et al., 2017). A common strategy to cause this phenomenon involves the use of negatively charged particles having positively charged moieties on the surface of the particles; these moieties are often attached via phosphate bonds. In contact with the alkaline phosphatase present in the mucus, the bond is broken and the particle reverts to electronegativity or electroneutrality (Le-Vinh et al., 2019; Wolf et al., 2020). Mycolysis is an alternative form of mucopenetration in which the formulation diffuses through the mucus to access the epithelium by either breaking the disulfide bonds present in the mucin network or via thiol-disulfide exchange (Schattling et al., 2017) (Fig. 2).

Figure 2. Graphical representation of mucoadhesion, mucopenetration and mycolysis.
Mucoadhesive and mucopenetrating drug delivery systems have generated great interest within the scientific community, particularly for vaginal application (de Araújo Pereira and Bruschi, 2012; Valenta, 2005). Recently, vaginal dosage forms offering a combination of mucoadhesive and mucopenetrative strategies have also been reported (Krogstad et al., 2017).

3. Electrospinning

3.1. Techniques and parameters

The most commonly employed technique to prepare fibers is electrospinning (Fig. 3A). When the polymer is dissolved in a volatile organic solvent, the technique is referred to as 'solvent electrospinning'. Here, a syringe is loaded with the polymer solution and forced through a fixed diameter needle at a constant rate using a syringe pump. A collector is placed at a specified distance from the end of the needle. With this system, a voltage supplier is required; the positive electrode is connected to the needle while the ground electrode is connected to the collector. Upon reaching a critical voltage, the surface tension of the polymer solution is exceeded and the drop at the end of the needle deformes into a conical shape known as the 'Taylor cone' (Fig. 3B). The stretching of the jet from the end of the needle to the collector allows the fibers to be formed and dried before being collected in the collector (Fig. 3C) (Dziemidowicz et al., 2021).

![Electrospinning Setup](image)

**Figure 3.** A – Conventional electrospinning setting; B – Taylor cone and stretching of the polymer solution or melt jet; C – the collected electrospun fibers.

Although fibers are usually prepared from polymer solutions, they can also be obtained from molten polymers in what is known as 'melt electrospinning'. Here, the formation of the jet leads to the solidification of the polymer fiber. It is essential that the polymer used to prepare the fibers is stable above its melting temperature, and the working temperature must be carefully selected and monitored during the process. Also, higher voltages are required to obtain the fibers by melt electrospinning compared to solvent electrospinning. The main advantage of this technique over solvent electrospinning is the absence of toxic solvents (Góra et al., 2011). Both melt and solvent
electrospinning allow the production of monolithic fibers. If a single polymer is used, it is known as 'single-fluid electrospinning'. However, 'blend electrospinning' is also possible using polymer blends, resulting in monolithic fibers containing two or more polymers (Fig. 4A). Moreover, the dispersion of nanoparticles in the polymer solution or melt makes it possible to obtain hybrid systems in which the nanoparticles are dispersed inside the electrospun fibers (Fig. 4B) (Dziemidowicz et al., 2021; Hu et al., 2020; Selvaraj et al., 2018).

Coaxial electrospinning – involving simultaneous use of two different polymer solutions – was initially introduced in 2002 and allows the preparation of a greater variety of nanofiber structures. Two syringes are needed to drive the solutions through a needle having two concentric holes. By applying the required voltage, the solution ejected through the inner hole forms the core of the nanofiber, while the solution ejected through the outer nozzle forms the sheath (Fig. 4C). A particular advantage with this technique is that the internal solution (forming the core) does not have to be electrospinnable, thereby extending the range of substances (including drugs) that can be incorporated into the fibers. However, this technique is also more challenging, since interactions between the solutions can affect production of the fibers (Han and Steckl, 2019; Yoon et al., 2018). Another interesting approach is 'emulsion electrospinning', based on emulsifying an aqueous solution or dispersion as the internal phase of an organic solution. Again, the outer organic phase must be electrospinnable, while the internal phase does not. The result is that the internal phase is encapsulated (continuously or discontinuously) within an electrospun polymeric fiber (Fig. 4D) (Parham et al., 2020).

**Figure 4.** A – Different electrospun fibers structures, depending on the technique used for their manufacture: monolithic fiber obtained through single polymer or polymer blend electrospinning; B – nanoparticles loaded electrospun nanofiber; C – core-shell electrospun nanofiber obtained through coaxial electrospinning; D – emulsion electrospun nanofiber.
Multiple parameters can affect the formation, structure, porosity and arrangement of fibers. The selected voltage is critical for fiber formation; if the voltage is lower than that necessary to overcome the surface tension, the jet will not form and fibers will not be obtained. As the voltage increases, the fiber diameter usually decreases due to more efficient solvent evaporation. However, excessive voltage can lead to spherical deformation of the fibers, commonly referred to 'beaded fibers'. The most common voltages used are in the range of 15–25 kV (Pérez-González et al., 2019).

Other factors to be considered include the characteristics and behaviour of the polymer solution. With solvent electrospinning, the concentration and molecular weight of the polymer determine the viscosity of the solution, which affects the morphology and distribution of the fibers. The range of concentrations at which the polymer becomes entangled in the solution must be taken into account; if the concentration is lower than the minimum entanglement concentration, particles rather than fibers will be formed, and the process is referred to as 'electrospraying'. At other concentrations, beaded fibers may be produced. Finally, if the polymer concentration is sufficiently high to exceed the maximum entanglement concentration, well-formed fibers will be obtained. At excessively high polymer concentrations, needle blockage can occur and therefore no fibers will be produced. Most studies indicate that the optimum viscosity for electrospinning is 100–2000 cP (Pérez-González et al., 2019; Rodríguez-Tobías et al., 2019).

Other important electrospinning parameters include the flow rate and the distance from the needle to the collector. For example, if the flow rate is too low, the continuous jet required for the formation of fibers will not form. High flow rates are also problematic, causing droplets to be formed rather than a jet due to the insufficient electric field. The distance to the collector can affect the morphology and diameter of the fibers. Generally, the preferred distance is in the 10–20 cm range (Bhattarai et al., 2018). Environmental conditions (temperature and humidity) can also modify the formation of fibers. Low relative humidities lead to rapid evaporation of the solvent and resulting in fibers with high diameters, while high ambient humidities slow down the evaporation of the solvent and produce small-diameter fibers (Pelipenko et al., 2013). Lara-Espinoza et al reported that an increase in temperature reduces the viscosity and surface tension of solutions, producing thinner fibers (Lara-Espinoza et al., 2018). However, excessive temperatures can cause premature jet termination and thereby prevent the formation of continuous fibers.

The major limitation with current electrospinning methods includes long manufacturing times, use of organic solvents and challenges with scalability. Research efforts focused on scaling the production of fibers for industrial manufacture have increased substantially, and include techniques such as multi-nozzle or needleless electrospinning. It is now possible to produce high
batch volume of fibers, although high surface area and production speed are necessary. By
optimising and controlling processing parameters (including temperature and humidity), fibers
can be produced having excellent reproducibility and accuracy. Also, organic solvents released
during evaporation can be collected using a solvent recovery system and recycled. Several
companies have already marketed industrial-scale electrospinning equipment and nanofiber-
based products for different applications, and, with increasing interest in the technology, the
market is expected to grow significantly in the coming years (Omer et al., 2021; Xue et al., 2019).

Aside from electrospinning, alternative techniques to obtain fibers are beginning to emerge, such
as centrifugal spinning and pressurised gyration, although literature in this field is still rather
limited due to the novelty of these techniques. Fibers can be obtained with higher performance
and lower energy consumption, which may be interesting for the industrial production of these
systems. Furthermore, these techniques could avoid needle clogging, and allow production of
aligned or random fibers with desirable morphologies (Basnett et al., 2021; Hong et al., 2019).
With centrifugal spinning, a tank loaded with a polymeric solution is attached to a motor. The
rotation of this symmetrical axis creates a balance between the centrifugal and capillary
forces. With pressurised gyration, a pressure differential is generated between the interior and exterior of
the reservoir, which induces ejection and stretching of the polymer dissolution jet through
perforations in the vessel. The drying of the fibers occurs spontaneously due to the raised surface
of the jet. Concentrically, there is a stationary collector that allows the formed fibers to be obtained
(Alenezi et al., 2019). This technique also allows the manufacture of core-sheath fibers, offering
advantages similar to those described previously for coaxial electrospinning. A recently reported
core-sheath fiber manufacturing system based on the use of a novel spinneret allows the
concentric ejection of two polymer solutions at the same time (Mahalingam et al., 2020, 2019).
Pressurised gyration is being evaluated for development of systems with potential vaginal
application, including mucoadhesive fibers loaded with progesterone (Brako et al., 2018).

4. Electrospun nanofibers for vaginal administration

4.1. Advantages of electrospinning for vaginal drug delivery

Electrospinning is a versatile technique, since it allows production of fibers with different
structures (e.g., aligned, patterned, non-woven) and porosities. Electrospun fibers are often
produced using mucoadhesive polymers, which are known to be useful for improving the
retention of vaginal dosage forms (Pérez-González et al., 2019). Given the importance of
mucoadhesion in improving the efficacy of vaginal drug delivery systems, the high surface area
of electrospun fibers probably represents the greatest advantage of these systems compared to
conventional solid and semi-solid vaginal dosage forms. The exposure of functional
mucoadhesive groups ensures good interaction between the mucus and the formulation leading to
an increase in the mucoadhesion capacity compared to other systems with a lower surface/volume ratio. Also, the interconnected non-woven architecture can further enhance this characteristic, supporting the application of fibers for other mucosal routes, including nasal and ocular (Sofi et al., 2020).

Various methods have been reported for drug loading of electrospun fibers, either before or after fabrication of the fibers. The simplest method involves dissolving the drug together with the polymer in the solution to be electrospun. The rapid drying that occurs during electrospinning (typically ~ 0.1s) means that solid amorphous drug dispersions are usually obtained. The amorphous form of the drug lends itself to relatively fast dissolution rates (compared to the crystalline form of the drug) and increased apparent solubility and permeability of the drug; this is particularly useful for drugs with bioavailability due to poor solubility (Dziemidowicz et al., 2021). For certain formulations, particularly those containing highly soluble excipients or actives that tend to dissolve rapidly, a burst drug release is observed. Various strategies can be employed to mitigate the burst and to sustain drug release, including use of hydrophobic polymers, surface immobilization of the drug, or encapsulation by coaxial or emulsion electrospinning (Mohammadian and Eatemadi, 2017). Core-sheath fibers are frequently employed for this purpose, since the use of a functional sheath polymers can lead to sustained release of the drug during the residence time of the formulation (Sofi et al., 2020). Indeed, some polymers can also introduce a stimuli response for drug release into the system (Sayin et al., 2019).

The mechanical properties of fibers are easily tuned by controlling the manufacturing parameters. Although essential for any dosage form, optimisation of mechanical performance is particularly important for the vaginal dosage forms, as the formulations are required to be strong and smooth (Sofi et al., 2020). Today, semisolid gel and creams are the most widely used dosage forms for vaginal drug administration. However, some studies have reported that women prefer solid formulations with a smooth texture, a small size for transport and administration, and a practical dosing frequency (weekly or even more spaced) (Laborde et al., 2018)

4.2. Sexually transmitted infections

Although transmission of the human immunodeficiency virus (HIV) occurs via a number of modes, sexual transmission accounts for the majority of new infection, particularly in Sub-Saharan Africa (Walker et al., 2003). The envelope protein on the virus surface binds to certain host cells containing the CD4 receptor, forcing the cells to produce new viral particles and leading to their early destruction. With time, the immune system weakens, and without the intervention of modern antiretroviral therapy, leads to acquired immunodeficiency syndrome (AIDS). At this stage, the patient is susceptible to developing severe illnesses such as tuberculosis, bacterial infections or Kaposi’s sarcoma. Highly active antiretroviral treatments (HAART) effectively
prevent disease progression (and transmission). An estimated 38 million people were infected with HIV in 2019 (United Nations Joint Programme on HIV/AIDS (UNAIDS), 2021). For a variety of biological and sociocultural factors, women – and particularly young women – have a significantly higher risk of acquiring HIV infection via sexual transmission compared to men, due to the relatively large mucosal surface area of exposure, intravaginal practices, high incidence of bacterial vaginosis, the underdeveloped cervix and low vaginal mucus production in young women, gender inequalities within society, and various social and economic pressures associated with poverty (Chersich and Rees, 2008; Kim and Watts, 2005; Myer et al., 2005). The greater vulnerability of the female gender to HIV makes it necessary to develop new prevention systems (United Nations Joint Programme on HIV/AIDS (UNAIDS), 2021; World Health Organization (WHO), 2020a).

An estimated 491 million people aged 15–49 worldwide have genital herpes, a sexually transmitted infection caused by herpes simplex virus type 2 (HSV-2). Risk of HSV-2 transmission is significantly increased when erosions and ulcers are present on the external genitalia and during periods of viral shedding, although it is also possible to infect when asymptomatic. Furthermore, a synergy between HSV-2 and HIV-1 has been observed; HSV-2 infection, regardless of the presence of symptoms, enhances CD4+ T-cell proliferation in the vaginal tissue, and these cells are the target for HIV-1. Thus, therapeutic strategies to prevent sexual transmission of HSV-2 can impact prevalence of both genital herpes and HIV-1 infection (Schiffer and Gottlieb, 2019; World Health Organization (WHO), 2020b).

Vaginal formulations have gained great interest in the field of preventing the transmission of STIs, especially HIV, culminating in the recent approval of a vaginal ring device offering sustained release over 28 days of the antiretroviral drug dapivirine (Baeten et al., 2016; European Medicines Agency, 2020; Nel et al., 2016). However, certain vaginal formulations also suffer drawbacks, including low retention times due to leakage or messiness (for gels and creams), discomfort after administration, or repetitive dosing regimens. Fibers have emerged as a new option for vaginal drug administration, with particular focus on HIV microbicides targeted at HIV acquisition (Nunes et al., 2021).

Many different classes of antiretroviral drugs have been considered for vaginal administration for prevention of sexual transmission of HIV, including nucleoside reverse transcriptase inhibitors (i.e. tenofovir and its prodrug forms tenofovir disoproxil fumarate and tenofovir alafenamide), non-nucleoside reverse transcriptase inhibitors (i.e. dapivirine, efavirenz), integrase inhibitors (i.e. raltegravir) and entry inhibitors (i.e. maraviroc) (Date et al., 2012; Gallay et al., 2017; Ilomuanya et al., 2020). Many of these drugs have been formulated as electrospun fibers. Maraviroc-loaded nanofibers have been reported for pericoital administration (Ball and
Woodrow, 2014). The fibers contained a relatively high 28 wt% loading of maraviroc in either PVP or PEO, demonstrating the ability to load relatively high quantities of drugs in nanofibers compared to some other solid dosage forms. Addition of Tween 20 as a wetting agent improved the release of maraviroc from PVP fibers; the fibers were able to release the drug over a few minutes. The PVP-based fibers were optimized by coating them with ethylcellulose using coaxial electrospinning, extending their in vitro release for up to 5 days. Finally, the combination of a layer of monolithic PVP fibers with a layer of coaxially electrospun fibers provided rapid initial release followed by a period of sustained release, which may be useful in quickly obtaining therapeutic concentrations in the vagina and then maintaining concentrations over a prolonged period (Ball et al., 2016).

Electrospun fibers containing tenofovir disoproxil fumarate and emtricitabine have also been reported for vaginal PrEP (Nunes et al., 2021). Liposomes containing both drug substances were first prepared using the thin film hydration method. The authors then prepared two different types of nanofibers – the first containing both drugs dispersed in PCL nanofibers, and the second containing the liposomes in PVA nanofibers. The fibers were tested for morphology, mechanical and physicochemical performance (e.g., scanning electron microscopy (SEM), differential scanning calorimetry (DSC), X-ray photoelectron spectroscopy (XPS) and texture analysis), and in vitro release. They also evaluated fluorescent quenching of mucin in vitro as a proxy for mucoadhesion. Pharmacokinetic evaluation in mice showed fast release of the drugs (15–30 min), supporting use of the PCL nanofibers for on-demand vaginal administration. Interestingly, nanofibers loaded with liposomes provided higher vaginal concentrations of both drugs in a single vaginal administration compared to 5 days of oral treatment with doses 25-fold higher. Vaginal concentrations were up to 30-fold higher than those observed in oral treatment. The fibers loaded with dispersed drugs also led to higher vaginal concentrations compared to the oral treatment.

Electrospun nanofiber formulations have also been reported offering release of drug-loaded nanoparticles. Krogstad et al described nanofiber formulations comprising the mucoadhesive polymers PVA and PVP and mucopenetrating nanoparticles prepared from PEGylated poly(lactic-co-glycolic acid) (PLGA) (Krogstad et al., 2017). The resulting fibers were able to adhere to mucus and, since both polymers were hydrophobic, their dissolution in aqueous medium provided rapid release of the nanoparticles, which were then able to penetrate the mucus and deliver etravirine to the vaginal tissue.

Acyclovir, a potent nucleoside analogue, is widely used to treat HSV-2, and vaginal administration may be beneficial in the treatment of recurrent genital herpes (Corey et al., 1982). Various acyclovir-based vaginal formulations have been described for the prevention of sexual transmission of the virus (Asvadi et al., 2013; Sánchez-Sánchez et al., 2015; Ursell et al., 2014).
Electrospun nanofibers of PLGA and poly(L-lactide-co-caprolactone) (PLCL) offering sustained release of acyclovir for up to one month have been reported (Aniagyei et al., 2017). The fiber formulation showed high biocompatibility and provided both physical and chemical barriers to the HSV-2 during the release period.

Griffithsin – a lectin derived from algae with known potent activity against transmission of HIV, HPV, HSV-2 and other viruses (Emau et al., 2007; Lusvarghi and Bewley, 2016; Mori et al., 2005) – has been formulated in various nanofiber-based formulations. A PLGA nanofiber-based drug delivery system for the release of griffithsin for the prevention of HIV has been reported (Grooms et al., 2016). Monolithic PLGA nanofibers were prepared under different conditions, and subsequently surface-functionalized with griffithsin at different concentrations (5–0.00005 nmol/mg of nanofiber). In vitro studies demonstrate that griffithsin could effectively inhibit the virus with little toxicity in the cell lines studied. Griffithsin-loaded electrospun fibers have also been developed based upon poly(n-butyl acrylate-co-acrylic acid) and methoxy-poly(ethyleneglycol)-b-poly(lactide-co-glycolide) (Tyo et al., 2019). These polymers were selected for their mucoadhesive and pH properties. The fibers obtained were able to release griffithsin in response to pH, with minimal release under acidic conditions acidic and triggered release at neutral pH. In this way, the activity of the formulation would remain latent until contact with alkaline (and potentially infected) semen. The fibers were also found to provide immediate protection from the moment release began. Furthermore, after the pH-stimulus, the release was sustained for a minimum of 72 h.

Recently, Tyo et al published two articles describing griffithsin-releasing electrospun fibers for dual protection against HIV-1 and HSV-2. Such combination strategies are referred to as ‘multipurpose prevention technology’ products. The first article, reporting the fabrication of griffithsin-loaded nanofibers for immediate release, describes the use of the hydrophilic, mucoadhesive and biocompatible polymers PEO, PVA and PVP, and evaluation in vitro for HIV-1 and HSV-2 and also in vivo for HSV-2 (Tyo et al., 2020a). The fibers provided complete and dual-purpose protection against in vitro infections with both viruses, and griffithsin was capable of reducing HSV-2 transmission in mice. When transmission did occur, symptoms were milder.

The second article describes the use of three-layered nanofiber systems based on a nanoparticle-electrospun fiber composite for the controlled release of griffithsin against HIV-1 and HSV-2 (Tyo et al., 2020b). The two outermost layers were formed using polycaprolactone (PCL) fibers, while the intermediate layer was formed using a composite of PEO fibers and methoxy poly(ethylene glycol)-b-poly(lactide-coglycolide) nanoparticles. Griffisithin was loaded into the nanoparticles (Fig. 5), and could be released for up to 90 days. Furthermore, the system was tested in vitro against HIV-1 infection and in vivo against lethal HSV-2 infection in a murine model.
Figure 5. Scheme depicting the vaginal delivery system developed by Tyo et al. (A) The multi-layered dosage form consisted of outer hydrophobic layers and an inner hydrophilic layer containing griffithsin-loaded nanoparticles. (B) Different thickness of the outer layers were prepared and investigated. Reprinted from (Tyo et al., 2020a), with permission.

4.3. Vaginitis

The term ‘vaginitis’ encompasses multiple pathologies characterized by abnormal and malodorous vaginal discharge and irritation, leading to itching or burning. There are multiple possible causes, the most common being vulvovaginal candidiasis (20–25% of the cases), bacterial vaginosis (40–50%), or trichomoniasis (15–20%). Other non-infectious causes, such as allergic or atrophic vaginitis, represent less than 10% of cases. Bacterial vaginosis and trichomoniasis are associated with increased risk of HIV transmission (Paladine and Desai, 2018).

Nanotechnology-based vaginal drug delivery systems containing benzydamine for the treatment of non-specific vaginitis have been reported in an effort to overcome some drawbacks with this drug, including poor local pharmacokinetics and toxicity issues in vaginal administration (Tuğcu-Demiröz et al., 2021). A nanoparticle-fiber composite system comprised benzydamine-loaded chitosan nanoparticles dispersed in a PVA solution prior to electrospinning has been evaluated for mechanical and physicochemical properties, in vitro drug release and ex vivo permeability. The presence of the nanoparticles within the nanofibers significantly increased the fiber diameter compared to those loaded with free drug. The mucoadhesiveness and mechanical properties of the fibers were improved due to presence of the nanoparticles. These fibers also resulted in sustained in vitro release for up to 8h. Finally, in ex vivo penetration experiments, benzydamine penetrated more slowly when it was loaded into nanoparticles.
Vulvovaginal candidiasis is generally treated with azole-type antifungal agents that inhibit the synthesis of ergosterol and other sterols, which otherwise disrupt the cell membrane of the pathogen and modify its permeability, leading to cell death by elimination of fundamental intracellular structures. Clotrimazole – the drug of choice for treating vaginal candidiasis – is usually administered orally although topical, transdermal and vaginal administration are also possible. However, complicated fungal vaginitis is usually treated with fluconazole, administered orally once a week for 6 months. This regimen has multiple drawbacks, including systemic toxicity (including hepatotoxicity), discomfort for the patient, and low bioavailability. For this reason, vaginal administration of fluconazole may be useful. Traditional formulations for vaginal administration of azoles include creams, gels, tablets and capsules. However, it is necessary that the formulations allow the correct dispersion of this lipophilic drug in the vagina, avoiding its clearance. Recent efforts have sought to optimise distribution of the drug in the vaginal cavity, and to space the frequency of administration (Nematpour et al., 2020; Sharma et al., 2016). Nematpour et al. developed a hybrid electrospun system comprising alginate/PVA fibers mixed with dextran fibers (Nematpour et al., 2020). Co-electrospinning was used, which uses two different solutions at the same time (Fig. 6), and the clotrimazole was added to the dextran solution before electrospinning. For comparison, the authors also prepared films using through a solvent-casting method. The systems were evaluated for physicochemical properties, ex vivo mucoadhesion, antifungal effect and in vitro drug release. The fibers showed two-fold higher mucoadhesion compared with the films, probably due to the higher surface area. However, from the perspective of mechanical properties, both drug delivery systems were suitable for vaginal administration. Moreover, the fibers showed a more sustained release, over at least 140 min. Both systems were non-toxic and inhibited in vitro the growth of Candida albicans and Candida dubliniensis.
Figure 6. Hybrid electrospinning setting proposed by Nematpour et al. Reprinted from (Nematpour et al., 2020), with permission.

Sharma et al. prepared PVA-based nanofibers for the vaginal delivery of fluconazole for the treatment of complicated fungal vaginitis (Sharma et al., 2016). Electrospinning was performed using a PVA solution of the drug, and the fibers were studied for physicochemical properties, swelling behaviour, in vitro release, mucoadhesion and antimicrobial activity. Homogeneous fibers with reduced diameters (150–180 nm) showed sustained release of fluconazole for 6 h. Although the presence of the drug increased the diameter of the fibers, (thus reducing the specific surface area), good mucoadhesive capacity was found for the loaded systems. The fibers also showed enhanced capacity compared to the free drug to inhibit the growth of Candida albicans and Lactobacillus acidophilus. The authors propose two explanations for this observation: PVA may be acting as a penetration enhancer, facilitating the permeability of the drug through the cell membranes of the pathogen; alternatively, the rapid drying of the solution during electrospinning causes the drug to solidify in an amorphous state within the fibers, thus improving its effectiveness.

Metronidazole is the drug of choice for treatment of bacterial vaginosis and trichomoniasis. It is a nitroimidazole-derived antibiotic with broad spectrum activity against most gram-negative and gram-positive anaerobic bacteria as well as various protozoans, including Trichomonas vaginalis. As part of efforts to enhance retention and bioavailability of this drug when administered vaginally, Tuğcu-Demiröz et al., prepared metronidazole-loaded electrospun fibers using PVP as the fiber-forming polymer (Tuğcu-Demiröz et al., 2020). The concentration of polymer greatly affected morphological, mechanical and mucoadhesive properties of the nanofibers. Ex vivo permeability studies demonstrated that PVP nanofibers enhanced metronidazole permeability.
compared to gels and solutions. Drug release occurred within 20 min, likely due to the 
hydrophilicity of PVP.

4.4. Preterm birth

According to the World Health Organization, preterm birth, defined as a delivery that occurs
before 37 weeks of gestation, currently affects about 1 in 10 births (World Health Organization
(WHO), 2018). Preterm births can be further classified as: late (34–36 weeks), moderate (32–33
weeks), very (28–31 weeks) or extremely (<28 weeks). Rates of survival and neurological
prognosis are enhanced as the weeks of gestation increase (da Fonseca et al., 2020). Vaginal
administration of progesterone in the second trimester of gestation for women with a cervical
length less than 2.5 mm has been shown to reduce singleton preterm deliveries (<34 weeks of
gestation) by 34% (Romero et al., 2016). Also, in women with a short cervix and a twin
pregnancy, the risk of preterm delivery of less than 33 weeks is reduced by 31%. Thus, reducing
preterm births significantly reduces neonatal mortality (Romero et al., 2017).

Cam et al prepared and tested poly(lactic) acid (PLA) fibrous patches from both progesterone-
loaded electrospun nanofibers and nanofibers obtained through pressurized gyration (Cam et al.,
2020). Both systems offered similar in vitro release and good cytocompatibility with Vero cells.
The patches were also able to reduce uterine contractions as effectively as the standard oral
treatment. While the patches obtained by electrospinning showed better dispersion of the drug,
the patches obtained by pressurized gyration showed a higher manufacturing yield. Nevertheless,
both systems could be an option to prevent preterm birth thanks to their high bioavailability and
low frequency of dosing.

4.5. Cervicovaginal cancer

Cervicovaginal cancer affects more than 500,000 new women and causes ~300,000 deaths each
eyear (World Health Organization (WHO), 2020c). Although most cervicovaginal cancers can be
treated with surgery and chemotherapy, adverse effects associated with systemic administration
of antineoplastic drugs are common. Localized administration of these drugs vaginally may
overcome this drawback. Cisplatin is currently considered the gold-standard for treating this type
of cancer. However, its intravenous administration is associated with long-term toxicity
(Aggarwal et al., 2017).

Vaginal delivery of cisplatin using electrospun nanofibers may prove useful for treatment of
cervicovaginal cancer. 10wt% cisplatin-loaded PEO/PLA electrospun nanofibers showed good
mucoadhesion in a mouse following vaginal administration (Zong et al., 2015). The cisplatin was
adequately distributed to the vagina, uterus, rectum and tumour with very low concentrations in
peripheral tissues, indicating good balance between safety and efficacy compared to parenteral
administration. PCL/chitosan based nanofibers have also been reported for vaginal administration
of cisplatin, demonstrating sustained release for up to 30 days via erosion and diffusion mechanisms (Aggarwal et al., 2017). The fibers showed high mucoadhesion strength, with values of around 500N/m². In mice, the fibers were shown to substantially reducing tumour size 14 and 21 days after administration, and histopathology studies confirmed that the fibers showed greater efficacy against tumor cells compared to free cisplatin.

4.6. Drug co-delivery in multipurpose prevention technologies

Multipurpose prevention technology (MPT) are multi-drug products – often for vaginal application – that are intended to simultaneously prevent unintended pregnancy and protect against sexually-acquired HIV and other sexually transmitted infections (Fernández-Romero et al., 2015; Malcolm et al., 2014; Romano et al., 2013). Various drug-releasing vaginal ring devices have been widely reported (Boyd et al., 2016; Dallal Bashi et al., 2019; Murphy et al., 2016; Smith et al., 2017; Thurman et al., 2013), as well as other vaginal formulations, including films (Li et al., 2019; Politch et al., 2021) and tablets (McConville et al., 2016). In general, co-delivery of drugs – such as with conventional combination drug and fixed dose combination products – is challenging, since each drug often has different dosing requirements and there is the potential of drug-drug interactions when the drugs are contained in the same compartment (Fernandes et al., 2020). Drug delivery products that contain a single drug capable of preventing multiple pathologies, such as tenofovir (HIV and HSV-2) or griffithsin (section 4.1), may also be considered as MPTs. However, in this section, only formulations intended for the co-delivery of drugs with different therapeutic targets will be discussed.

A MPT system must fulfill four basic requirements (Ball et al., 2012): it should (i) release multiple drugs with different physicochemical and pharmacokinetic properties from a single formulation; (ii) allow good drug dispersion in the vaginal mucosa; (iii) be easily applied, portable and discreet for women; (iv) be inexpensive; and (iv) be readily reversible in terms of contraceptive provision.

Co-delivery of drugs from electrospun nanofibers can be achieved by a variety of methods, by either encapsulating multiple drugs within the same monolithic fiber or loading the drugs into separate fibers (Fig. 7). If loaded in the same fiber, both drugs can be dispersed in a monolithic fiber (Fig. 7A). However; coaxial electrospinning is also frequently used, allowing one drug to be formulated in the core and the other in the sheath (Fig. 7B). With the drugs encapsulated in different fibers, there are two possibilities for their combination – sequential arrangement of layers containing the different drugs (Fig. 7C) or via co-electrospinning in which the fibers loaded with the different drugs are mixed homogeneous and randomly (Fig. 7D) (Blakney et al., 2014; Wang and Windbergs, 2019).
Figure 7. Possibilities for loading multiple drugs into a single product based on electrospun fibers (green represents drug A, red represents drug B). (A) monolithic fibers with two dispersed drugs, (B) coaxial fibers with one drug in the core and one in the sheath, (C) fibers loaded with two different drugs, arranged in layers (multilayer electrospinning), (D) fibers loaded with two drugs, randomly mixed (co-electrospinning).

One of the major clinical indications being considered for MPT strategies is the prevention of unintended pregnancy. Rates of unintended pregnancy remain alarmingly high, particularly in developing countries due to the difficulties in accessing modern and highly effective contraceptive methods. For many women, negotiating safe sexual practices (such as condom use) is not possible. The development of women-controlled products that can be self-administered and do not require partner consent of their partner are urgently needed (Fernandes et al., 2020). Although there have been no reports of electrospun nanofiber formulations intended solely for contraception, several studies have described MPT products that include a contraceptive component.

Ball et al. have described nanofibers for the co-delivery of anti-HIV drugs maraviroc and zidovudine, the anti-HSV drug acyclovir, and glycerol monolaurate (a novel spermicidal substance) (Ball et al., 2012). They prepared fibers for encapsulation of both hydrophilic and hydrophobic drugs using different proportions of PLA and PEO. These fibers were able to release a variety of molecules, and were effective against HIV-1 in vitro. Furthermore, the drug-loaded meshes physically obstructed sperm penetration. The combination of the physical barrier to semen and the ability to immobilise sperm represents a promising contraceptive strategy, and the addition of other drugs for the prevention of STIs make these electrospun fiber-based systems a promising candidate for a vaginal MPT product.
Blakney et al explored the potential for co-delivery of the antiretroviral drug tenofovir and the contraceptive progestin levonorgestrel using PVA electrospun monolithic fibers containing both drugs and separate fibers containing each of the drugs which were subsequently combined interwoven or layer-stacked (Blakney et al., 2014). Levonorgestrel release was hardly affected by the microstructure of the system, while tenofovir release was slower when mixed with levonorgestrel in the same fiber. The anti-HIV activity of tenofovir was maintained after electrospinning. This study also demonstrated that the effect of microstructure on drug release depends on the drug properties.

4.7. Lactobacillus delivery

Different species of the genus Lactobacillus are known to prevent the proliferation of pathogenic microorganisms in the vagina. Although not yet completely understood, the inhibition may occur through various mechanisms, such as competition for nutrients and adherence, the production of secondary metabolites like hydrogen peroxide or lactic acid, and stimulation of the host immune system. Candidiasis and bacterial vaginosis occur due to an imbalance between the normal microbiota of the vagina and endogenous or exogenous bacteria or yeast. The characteristic increase in vaginal pH associated with bacterial vaginosis is due to the loss of Lactobacilli population and the concomitant reduction in lactic acid production. Therefore, vaginal administration of Lactobacillus species may be an effective treatment or prevention strategy against candidiasis and bacterial vaginosis, and may prove particularly useful in light of the recent increase in microbial resistance with conventional drug therapies (Nagy et al., 2014; Ribeiro et al., 2020). Also, certain species of Lactobacillus can prevent transmission of the HIV-1 and HSV-2. For example, L. crispatus has been shown to trap HSV-2, providing a physical barrier against infection (Mousavi et al., 2018). Inflammation in the vagina results in the activation of CD4 + T lymphocytes and is therefore associated with increased risk of HIV transmission (Masson et al., 2015) A high proportion of certain microorganisms, such as Gardnerella vaginalis, can induce vaginal inflammation, while Lactobacillus species, such as L. crispatus, can lower inflammation (Gosmann et al., 2017). Monoglycerides are also capable of inhibiting enveloped viruses such as HIV-1 and HSV-2, although the mechanism is not yet known. Therefore, secretion of the reuterocyclin analogue glycerol monolaurate by Lactobacillus spp may reduce the risk of being infected with these STIs (Welch et al., 2020). The composition of the vaginal microbiota may also impact pregnancy health and the frequency of preterm births. Thus, the vaginal administration of Lactobacillus spp may be useful in reducing preterm births (Chu et al., 2018).

Development of practical and effective vaginal formulations suitable for administration of Lactobacilli will likely require incorporation of a relatively large quantity of bacteria into the formulation with prolonged stability to ensure its efficacy after storage. For these reasons, solid formulations are the preferred option. Compared to competing technologies such as
microencapsulation or lyophilisation, electrospinning may prove particularly interesting, since it allows the encapsulation and drying of bacteria in a single step process. However, there are few reports in the literature dealing with the encapsulation of Lactobacilli in electrospun nanofibers (Zupančič et al., 2019).

Nagy et al investigated the encapsulation of *L. acidophilus* into electrospun PVA/PVP nanofibers (Nagy et al., 2014). Some loss of biological activity was observed after one week at ambient temperature, while better stability was reported at 7 °C and -20 °C, with a large amount of bacteria preserved in the fibers after 90 days. Greater stability of the bacteria in the electrospun nanofibers could even be achieved using stabilizers or oxygen-free packaging. Škrlec et al developed and characterized electrospun nanofibers for the encapsulation of *L. plantarum* (Škrlec et al., 2019).

Using PEO as the fiber-forming polymer, they were able to achieve a relatively high loading efficiency of ~10⁹ colony-forming units (CFU) per mg of nanofibers. Preparation of the fibers with the lyoprotectant trehalose significantly improved the stability of the bacteria out to 24 weeks at ambient temperature. Further, 90% of the Lactobacillus were released in PBS in less than 30 min. Subsequently, Zupančič et al encapsulated nine species of *Lactobacillus* and one species of *Lactococcus* in electropsun PEO nanofibers (Fig. 8), using a single polymeric solution for each bacterium (10¹⁰ CFU/mL). Incorporation of all the microorganisms into the nanofibers was successful, although bacterial survival after electrospinning varied considerably. Although these results are promising, the study highlights the need for further investigation to fully understand bacterial survival during the electrospinning process.

![Figure 8. SEM images of different *Lactobacillus* species (high and low magnification in columns 1 and 3, respectively) and electrospun nanofibers loaded with those species (high and low magnification in columns 2 and 4, respectively). Reprinted from (Zupančič et al, 2019), with permission.](image-url)
Silva et al. produced PVA-based electrospun nanofibers loaded with *L. gasseri* and *L. rhamnosus* (Silva et al., 2021). *L. rhamnosus* showed higher survival after electrospinning compared with *L. gasseri* (93% and 84%, respectively), and both bacteria showed low stability at 25 °C (less than 7 days) and 4 °C (28 days for *L. rhamnosus* and 56 days for *L. gasseri*), making necessary the use of -20 °C to preserve their survival. Two strategies were investigated to improve the stability of nanofibers loaded with this species – the addition of protective substances (skim-milk lactose, skim-milk lactose-glycerol and glycerol) and oxygen-excluding packaging. The authors concluded that storing the nanofibers in oxygen-excluding packaging was the best option, extending the bioactivity of *L. rhamnosus* during up to 360 days at 4 °C.

### 4.8. Electrospun meshes for vaginal grafting

Pelvic organ prolapse (POP) is a pelvic floor disorder that occurs in many women, especially over the age of 40. It occurs due to the weakening of the structures that support the overlying pelvic organs, and leads to the descent of these organs from their natural position. Pregnancy, menopause, advanced age or obesity are known risk factors for POP. Surgical mesh implants can strengthen damaged soft tissues and support the organs that descent during POP. Nowadays, these meshes are administered transvaginally (Farmer et al., 2020). Although use of these implants is clinically effective, up to 4% of patients require further surgery due to complications with the currently marketed mesh, such as chronic inflammation and chronic pain due to the scarring of the vagina. These issue likely arise due to the mechanical features of the knitted meshes that create stress upon mechanical loading, affecting cell behaviour and leading to fibrous tissue formation. Given the expected increases in cases of POP due to increasing life expectancy, new strategies are needed to avoid post-surgery complications. Electrospun meshes may be useful since their structures mimic the natural extracellular matrix, which favours cell attachment and reduces the inflammatory response compared to other materials (Vashaghian et al., 2017).

Wu et al. prepared meshes through the co-electrospinning of PLCL, an elastic, flexible, biocompatible and biodegradable polymer and fibrinogen (Wu et al., 2016). The meshes were evaluated and compared with another knitted polypropylene (PP) meshes in a canine abdominal defective model. Although both PP and PLCL/fibrinogen meshes improved the symptoms of pelvic floor disorders, the latter resulted in faster vascularization, better organization of collagen fibers and better muscle regeneration. However, this was a short-term study; longer-term efficacy studies are required to fully assess any complications.

Vashaghian et al. evaluated different electrospun polymeric meshes – including nylon, PLGA/PCL blend and PCL/gelatin blend (Fig. 9A-D) – as alternatives to PP meshes (Vashaghian et al., 2017). The meshes were characterized to ensure they met the mechanical and physicochemical requirements, before evaluating the cellular responses of POP and non-POP
fibroblasts to the meshes and the extracellular matrix deposition. All electrospun materials exhibited good hydrophilicity and high porosity, resulting in low weight and highly permeable materials. The mechanical properties appeared to be suitable for purpose; the meshes had similar stiffness to the healthy anterior vaginal wall tissue, while commercially available meshes are stiffer. Furthermore, POP and non-POP cells remained functional, adhered to the meshes, proliferated and produced extracellular matrices.

Figure 9. SEM images of electrospun systems for pelvic organ prolapse. Electrospun meshes proposed by Vashaghian et al: nylon (A), PCL/gelatin (B), PLGA/PCL (C) and knitted PP mesh (Scale bar is 2 mm (A and B), 10 mm(C), and 1 mm (D)). Electrospun yarns, as proposed by Aghaei-Ghareh-Bolagh et al, obtained through the electrospinning of a blend composed by tropoelastin:PCL at different ratios; 3:1 (E), 1:1 (F), 1:3 (G), 0:1 (H). Figures 1A-D reprinted from (Vashagian et al, 2016), with permission. Figures 1E-H reprinted from (Aghaei-Ghareh-Bolagh et al, 2020), with permission.

A follow-on study reported the effect of surface texture and mechanical straining on matrix producing fibroblasts (Vashaghian et al., 2019). Meshes were prepared via electrospinning of nylon and PLGA/PCL, and compared with a non-porous PGA/PCL film. All the systems were clamped and seeded with POP fibroblasts. The systems were strained for 24 or 72 h (10% strain, 2 Hz) to mimic a gentle breathing. The synthesis of the new extracellular matrix was more efficient in the electrospun scaffolds, while non-porous films induced an inflammatory response. These results suggest that dynamic conditions enhance the adhesion and the expression of mechano-responsive genes of fibroblasts in electrospun meshes. The combination of the results of both studies shows the potential of these meshes for the surgical treatment of POP.

Paul et al described electrospun PCL scaffolds using melt electrospinning, based on the premise that this new approach might lead to more adaptable meshes through computer aiding design-derived direct writing (Paul et al., 2019). After determining the optimum conditions for obtaining electrospun meshes with the smallest pore and nanofiber diameters, a hydrogel composite was prepared containing Aloe Vera/sodium alginate (1:1 ratio) and endometrial mesenchymal stem cells. That gel was bioprinted onto the PCL electrospun scaffold. This multicomponent scaffold,
was evaluated by SEM, phase contrast microscopy atomic force microscopy, and in vitro degradation and biocompatibility assessments and in vivo performance in mice. The meshes were found to provide an ideal substrate for endometrial mesenchymal stem cells, resulting in increased retention in vivo, promoting good tissue regeneration, and avoiding foreign body reaction or fibrotic encapsulation for at least one week. These multicomponent scaffolds could prove a useful tool for the autologous vaginal grafting using a patient’s own stem cells and could help overcome the current challenges faced in the surgical treatment of POP.

Hympánová et al evaluated in sheep three different meshes for treatment of POP – an ultra-lightweight PP non-degradable textile mesh (Restorelle®), an electrospun biodegradable ureidopyrimidinone-polycar-bonate mesh, and a electrospun non-degradable polyurethane mesh – and compared them with simulated tissue repair (Hympánová et al., 2020). They evaluated a variety of indicators, such as mesh-related complications, vaginal contractility, compliance and host response to the meshes. They found no mesh-related complications and the vaginal contractility was not affected during the study. All meshes were well integrated into the vaginal tissue, and the composition of the connective tissue was similar. The inflammatory response was milder with the electrospun implants compared to the Restorelle® mesh. However, due to the degradation rate of the electrospun biodegradable ureidopyrimidinone-polycarbonate mesh, the authors concluded that the Restorelle® and electrospun non-degradable polyurethane meshes were most suitable for further investigation.

Aghei-Ghareh-Bolagh et al reported manufacture of multimeter electrospun fibrous yarns based on tropoelastin/PCL mixtures (Fig. 9E–H) (Aghaei-Ghareh-Bolagh et al., 2020). Tropoelastin is the monomer subunit of elastin, a component of the extracellular matrix, which is known to support cellular growth. Yarns containing 1:1 ratio of tropoelastin and PCL were degradable and supported cellular growth. Following weaving of the yarns into a mesh, assessments in a sheep model showed excellent integration into the vaginal tissue and low pro-inflammatory response 30 days after surgery.

5. Conclusions and future perspectives

A wide variety of product types are already widely used for administration of drugs to the human vagina, and recent advances in electrospinning may lead to new product types, particularly for therapies aimed at treating or preventing diseases and conditions directly associated with female sexual and reproductive health. Fibers obtained by electrospinning have many useful properties – including high surface area, high drug-loading capacity and the variety of materials that can be used in their manufacture, which may be leveraged to help overcome some of the obstacles associated with other conventional vaginal dosage forms.
However, there also remain various challenges with electrospinning techniques for production of fibers, not least those associated with scaling the technology and manufacturing processes from the research laboratory to a large-scale industrial facility in order to obtain practical production rates. Furthermore, current solvent electrospinning methods rely heavily on use of organic solvents, which are often problematic from safety and regulatory perspectives. For these reasons, further innovations in electrospinning techniques – perhaps based around needless electrospinning, melt-electrospinning methods or pressurised gyration – are necessary for the practical and safe scaled production of fibers.

The application of nanofiber technology to vaginal drug delivery is relatively new. However, advances reported in last decade show promise, particularly for prevention of sexually transmitted diseases, reduction of preterm birth, and the treatment of vaginitis or cervicovaginal cancer. It is now also possible to encapsulate lactobacilli via electrospinning, with potential applications in maintaining a healthy vaginal microbiome and treatment of vaginitis and other STIs. As nanofibers can be a good platform for the administration of multiple drugs or even probiotics in a single dosage form, multiple pathologies might be treated or prevented at the same time. Therefore, next generation electrospun materials should focus on loading multiple drugs within a single product, and with particular application in the field of multipurpose prevention technologies. Combining multiple actives – including of drugs and bacteria, for example – in a single dosage form could provide greater adherence, clinical outcomes and comfort for patients. Another emerging application of electrospun nanofibers is the development of vaginal meshes for the surgical treatment of pelvic organ prolapse. In this field, the manufacturing conditions and the materials selected for electrospinning can produce highly resistant and biocompatible materials. Although no electrospun nanofiber-based vaginal formulation has yet reached clinical trials, we anticipate developments in the direction over the coming years.

**Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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