Nanopharmaceuticals for improved topical vaginal therapy: Can they deliver?

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Abstract

Nanopharmaceuticals have the potential to revolutionise medical treatment by permitting the design of more potent, less toxic "smart" therapeutics, ultimately leading to personalised medicine. This review summarises the challenges and potential uses of nanodelivery system for the topical drug therapy of vaginal diseases. The vaginal route of drug administration remains a challenge in the development of novel drug therapies, including nanomedicines. We attempted to provide an unbiased overview of currently investigated nanodelivery systems, some of which remain to be extensively studied under laboratory conditions, and some of which are already in clinical trials. Most nanodelivery systems are aimed at improving the treatment of vaginal infections, including HIV prevention. Promising new approaches in nanopharmaceutical design are discussed in this review, as well as the controversies related to mucoadhesiveness of nanopharmaceuticals.

Key words: vagina, mucosa, topical therapy, nanomedicine, drug delivery

Contents

1. Introduction

Nanomedicine has the potential to revolutionise medical treatment by permitting the design of more potent, less toxic "smart" therapeutics. It has been extensively described in numerous reviews, which have discussed the rationales, challenges, efficacy, safety, and regulatory issues related to the development of nanoscale drug delivery systems, i.e., nanopharmaceuticals (Desai, 2012; Duncan and Gaspar, 2011; Riehemann et al., 2009; Svenson and Tomalia, 2005). The aim of this review is to focus on the potential uses of nanopharmaceuticals as delivery systems for topical vaginal therapies. We attempted to provide an unbiased overview of currently investigated nanodelivery systems, some of which still require extensive study under laboratory conditions, and some of which are already in the clinical trial stages. Throughout the review, we have used the terms "nanodelivery systems" and "nanopharmaceuticals" as defined in the recent review by Duncan and Gaspar (2011), i.e., in terms of what is defined as nanomedicine, nanopharmaceuticals and the flexibility/rigidity of the size boundaries. We have included delivery systems with size ranges below 1 μm, although some of these systems may be considered either nano- or micro-delivery systems. In writing this review, we have focused on the potential of each proposed nanodelivery systems with respect to topical vaginal drug delivery, considering the uses of nanodelivery systems in other routes of drug administration to be beyond the scope of this review.

2. The vaginal environment

Although it is referred to as mucosal tissue, the vagina does not have secretory glands; rather, a mixture of fluids originating from a number of different sources composes the moist surface film of this tissue. This mucus coating has several important physiological functions and plays an

important role in drug absorption. The composition, volume, and rheological properties of vaginal fluids are affected by age, the stage in the menstrual cycle, and sexual arousal, thus influencing the release pattern of a drug delivery system administered into the vagina. Furthermore, it is well established that changes in the volume, viscosity, and pH of the vaginal fluid may affect the efficacy of administered drug delivery systems. Importantly, due to the self-cleansing action of the vaginal tract, the residence times of dosage forms and delivery systems will be reduced, unless they are modified for this specific route of drug administration (das Neves et al., 2011b; Robinson and Bologna, 1994; Sassi et al., 2008). More details on the features of the vaginal environment that affect drug efficacy can be found in the reviews by Gupta et al. (2011), Lai et al.(2010), Mallipeddi and Rohan (2010), and Yu et al. (2011). One particularly important characteristic of any novel delivery system destined for topical administration within the vagina is a low propensity to cause genital irritation and systemic toxicity. This consideration is particularly relevant for therapy in pregnant patients. The effects of pH, the dilution of the delivery system in the vaginal fluid, and/or the presence of semen, on each delivery system are discussed in the following sections.

3. Mucoadhesion as a means of prolonging vaginal residence time

It is important to emphasise that the efficacy of drug therapy based on the use of nanoparticles *in vivo* may be restricted by the short residence times of these particles within the vagina. To overcome this limitation, an extended and intimate contact of nanocarriers with the vaginal mucous is required; such contact can be successfully achieved using mucoadhesive polymers. Over the last two decades, mucoadhesive nanopharmaceuticals have been extensively investigated as means of improving drug delivery in different mucosal tissues, including the

vagina. The use of mucoadhesive nanopharmaceuticals could ensure prolonged and intimate contact with the mucus, which is a prerequisite for subsequent events leading to the enhanced delivery of drugs to the underlying tissue, sustained and controlled drug release or the protection of unstable drugs (Andrews et al., 2009; das Neves et al., 2011a).

Mucoadhesive drug delivery systems function through the attraction between the mucus and polymeric drug carriers (Peppas and Huang, 2004). However, mucoadhesive particles can cause considerable disruption of the protective mucus microstructure in the vagina, thus allowing foreign particles, such as pathogens and other potentially toxic nanomaterials, to penetrate the mucus barrier more easily (McGill and Smyth, 2010; Wang et al., 2011). The issue of desired/unwanted mucoadhesiveness has been highlighted in the past several years and is further discussed below. In addition, new methodologies for the study and characterisation of mucoadhesion at the nanoscale are required to fully understand the actual interaction between nanopharmaceuticals and the mucus and to utilize the findings for the optimization of nanomedicine (das Neves et al., 2011b). It is equally important to study the effects of the repeated administration of mucoadhesive nanoparticles into the vagina, as very little is known about topic.

4. Nanoscale drug delivery systems with potential in topical vaginal drug delivery

We have attempted to classify the studied/proposed delivery systems according to date that the delivery system was first proposed or developed, how close each system is to achieving therapeutic success or clinical evaluation. These systems are not characterised in details, rather, we have focused on their applicability to vaginal therapy. We have highlighted, where applicable, the potential uses of particular system for the treatment of specific vaginal diseases to allow the reader to access to this information in a straightforward manner.

4.1. Liposomes

Liposomes are biocompatible colloidal nanoparticles that are characterised by their lipid composition, particle size distribution, number of lamellae and inner/outer aqueous phases, all of which dictate their stability and interaction characteristics. Liposomes have been studied for more than 40 years in many different therapies, due to their ability to carry a wide variety of drugs, their structural versatility, and their physiological compatibility (Torchilin, 2005).

The potential uses of liposomes in topical vaginal therapy were recognised relatively late compared to their applications in parenteral and skin delivery. In late 1990ties, two groups explored the applicability of liposomes in vaginal drug delivery. Jain and coworkers (1997) proposed the use of liposomes for the intravaginal delivery of progesterone, whereas Foldvari and Moreland (1997) developed and clinically evaluated liposomes containing interferon alpha for the treatment of genital papilloma virus infections. Following those pioneering studies, several different research groups focused on liposomes as a means of improving local vaginal drug delivery (Table 1).

Table 1.

4.1.1.. Fungal and bacterial infections

The potential of liposomes in the topical treatment of vaginitis was originally reported by Pavelić et al. (1999). Three commonly applied drugs in the treatment of vaginal infections (clotrimazole, metronidazole, and chloramphenicol) were entrapped in conventional lecithin liposomes and tested for *in vitro* stability in pH values corresponding to those of the pre-and post-menopausal vaginal environments, as well as for *in situ* stability in the presence of cow vaginal mucosa. To

improve liposomal stability and the overall applicability of the formulation, liposomes were further incorporated into bioadhesive Carbopol hydrogels. *In vitro* release studies performed in the vaginal fluid simulant demonstrated the prolonged release of all three drugs from respective liposomal hydrogels (Pavelić et al., 2004a; 2005b). The sustained release of clotrimazole from proliposomes destined for vaginal therapy was also reported by Ning and coworkers (2005a, 2005b). Moreover, the same group confirmed the adequate antifungal activity of the developed system and the absence of visible changes in the morphology of exposed vaginal tissue. Two different approaches have been proposed to further improve the local delivery of liposomal drugs to the infected vaginal site. One of these approaches is to enhance the ability of liposomes to penetrate through the mucus using deformable (elastic) liposomes (Vanić et al., 2013). Elastic vesicles are known to penetrate deeper into the skin than conventional liposomes (Elsayed et al., 2007). We extended their applicability to vaginal delivery and have achieved the promising results in the delivery of metronidazole using deformable liposomes that are able to enhance the permeability of the drug, as shown in the *in vitro* model of the epithelial barrier (Vanić et al., 2013). Another approach that we are currently developing is the use of pectin- and chitosan-containing mucoadhesive liposomes to prolong the residence time in the vaginal mucus.

4.1.2. Viral infections

A considerable number of the nanopharmaceuticals developed for vaginal administration involve antiviral drugs and protective agents (Gupta et al., 2011). The topical delivery of microbicides represents a remarkable strategy for preventing HIV transmission through sexual intercourse, which is the predominant mode of HIV transmission worldwide. Microbicides delivered via nanodelivery systems are expected to inhibit the virus at its point of entry through the vaginal mucosa, and prevent all subsequent steps leading to host infection, as well as to block viral

replication and provide a high genetic barrier to the development of drug resistance in the virus. The system is also expected to exhibit an acidic pH similar to that of the normal vagina, should not affect normal vaginal flora, and will be non-toxic to the protective genital mucosa. The system should also be compatible with latex male condoms (Omar and Bergeron, 2011). In addition, it should be considered that microbicide-containing delivery systems can be diluted by the presence of vaginal fluid (arousal) or contact with semen (unprotected intercourse) (Sassi et al., 2008).

Malavia's group tested the ability of liposomes to inhibit the infection of transformed cells that express virus-specific receptors. The reaction of the vaginal tissue to liposome formulations after intravaginal administration was found to be benign in female mice, indicating their potential use in the prevention of HIV infection in women (Malavia et al., 2011). The MC1220 microbicide incorporated into liposomes in adult female rhesus macaques provided partial protection against the vaginal transmission of virus (Caron et al., 2010). The liposomal MC1220 formulation was reported to cause less vaginal irritation than the control formulation (nonxynol-9) in a rabbit model (Mourtas et al., 2010). The encapsulation of octylglycerol (OG) into liposomes resulted in a greater efficacy against HIV, *Herpes simplex* virus (HSV), and *Neisseria gonorrhoeae* than the conventional gel formulation. The efficacy was maintained for over two months. Moreover, no toxicity was observed for the liposome formulation containing octylglycerol in *ex vivo* testing in a human ectocervical tissue model or in *in vivo* testing in a macaque model (Wang et al., 2012). The potential use of liposomal interferon α into genital wart tissues to provide a localised treatment for human papillomavirus (HPV) infections was shown in one of the first studies reporting vaginal applications of liposomal drugs. The preliminary clinical testing demonstrated the complete resolution of cervical lesions in a female patient at the end of the therapy and a decreased number of lesions in a male patient at the end of the observation period (Foldvari and

Moreland, 1997). Pavelić and colleagues (2005a) evaluated the ability of liposomes to provide a sustained and controlled release of acyclovir. Their findings suggested that the lipid composition (surface charge), and vehicle determined the fate of liposomes in *in vitro* simulations of vaginal conditions.

4.1.3. New challenges and opportunities in the vaginal application of liposomes

The vaginal administration of *nucleic acids* offers significant potential in the prevention of different viral infections that are responsible for such diseases as genital herpes, acquired immune deficiency syndrome, and cervical cancer (Cristofaro and Ramratnam, 2006). The vaginal treatment of cancer or viral infections with gene-silencing approaches is more challenging because it requires the delivery of small interfering RNA (siRNA) molecules through the mucosal barrier, while avoiding rapid nuclease degradation so that the molecules may be taken up by the cervicovaginal epithelium. Considering the protective barrier function of mucus against all foreign particles (Cone, 2009), the potential to improve delivery through the use of mucus-penetrating nanoparticles is of great importance (Ensing et al., 2012a, 2012b). An attractive siRNA delivery, based on PEGylated liposomes, was presented recently. Wu and coworkers (2011) developed a novel biodegradable alginate scaffold system containing muco-inert PEGylated lipoplexes that assure the extended vaginal presence of the lipoplexes *in vivo* and facilitate the delivery of siRNA into the vaginal epithelium. Interestingly, despite evidence that conventional lipoplexes were smaller than the PEGylated lipoplexes, conventional lipoplexes failed to deliver nucleic acids into the vaginal tissue (Wu et al., 2011). These findings are consistent with the research of Lai and colleagues (2010), who demonstrated that large particles (200-500 nm) were transported much more rapidly than smaller particles (100 nm) in the human cervicovaginal mucus.

A study of the well-known natural antioxidant and anti-inflammatory agent curcumin, encapsulated in liposomes for the topical treatment of vaginal inflammation demostrated that liposome-solubilized curcumin and curcuminoids significantly enhanced the antioxidant and anti-inflammatory activities of curcuminoids *in vitro* (Basnet et al., 2012). An *in vitro* model for the assessment of the biopharmaceutical properties of liposomally-associated curcumin was developed, and passive diffusion was found to be the main mechanisms of curcumin penetration through the vaginal mucosa (Berginc et al., 2012). The results indicated that the transport was found not influenced by transporters or metabolising enzymes. Although the permeability of liposome-associated curcumin was relatively low, its binding to the mucosal tissue was found to be significant, thus indicating its potential to assure relatively high levels of curcumin in the vaginal tissue with limited systemic absorption (Berginc et al, 2012).

4.1.4. Improving the viscosity and stability of liposomal dispersion: Liposomes-in-gel

The major disadvantage of using liposomes for vaginal drug delivery is their liquid nature and their consequently low residence time within the vagina. Different types of vehicles have been proposed to assure prolonged retention time in the vagina. Liposomes are known to be compatible with viscosity increasing agents such as methylcellulose and polymers derived from acrylic acid (Carbopols) (Foldvari, 1996; Škalko et al., 1998). Carbopol resins exhibit strong bioadhesive properties, and are often used to prepare hydrogels that are suitable as vehicles for the incorporation of liposomes, because they provide the required viscosity and mucoadhesiveness (Pavelić et al, 2001, 2004a, 2004b). The use of polycarbophil hydrogels to deliver granulocyte-macrophage colony-stimulating factor (GM-CSF) has exhibited potential for the treatment of HPV, these vesicles enabled the release of GM-CSF over a seven day period in the HPV models (Hubert et al., 2004).

An additional advantage of Carbopol resins is their ability to form a gel when exposed to pH levels above 4.0 (Dittgen et al., 1997). Therefore, Carbopol gels with lower pH values may additionally act on vaginal infections by maintaining the vaginal pH at approximately 4.5 (das Neves and Bahia, 2006). Low viscosity hydrogels prepared from acryclic polymers alter the hydration level of the vaginal tissue and can be applied as moisturizers for the treatment of vaginal dryness (Robinson and Bologna, 1994).

FIGURE 1

Liposomes in Carbopol gels

Pavelić and coworkers (2001) investigated two different Carbopol gels as a vehicle for administrating liposomes vaginally. They performed *in vitro* release studies of liposomally-associated calcein incorporated into a hydrogel, in a buffer with a pH of 4.5, and demonstrated the extended release of the liposomal calcein from both gels, compared to that of free calcein. The sustained release of the liposomally-associated high-molecular-weight markers (FITC-dextrans 4400 and 21200, respectively) indicated that the release was inversely proportional to the molecular weight of the marker (Pavelić et al., 2004b). To follow the release of liposomally associated drug from both liposomes and the vehicle, they applied a modified method proposed by Peschka et al. (1998). This method enables the determination of intact liposomes released from the gel into the buffer (receptor medium), which is not feasible using the Franz diffusion cells method. There are several possible and likely interconnected mechanisms by which liposomes-associated markers or drugs could be released from the liposomes-in-gel system (Figure 1). The simplest pathway involves the leakage of the marker (drug) from liposomes inside the gel (Figure 1, path 1) and the diffusion of the free marker (drug) through the agarose

layer into the receptor compartment (Figure 1, path 2). The third pathway is represented by the release of the intact liposomes from the gel into the receptor compartment (Figure 1, path 3) and, the fourth pathway includes the release of the liposomally-entrapped drug from intact liposomes (which have penetrated the gel) inside the receptor compartment (Figure 1, pathway 4) (Pavelić et al., 2001, 2004b).

The effect of lipid composition and surface charge on the stability of liposomes and the release of acyclovir from liposomes-in-gel in vaginal fluid simulant (VFS) with or without mucin were studied, and positively charged vesicles were found to be the most stable in the VFS containing mucin. This higher stability of positively charged vesicles is most likely a consequence of the interaction of the negatively charged mucin with the positively charged liposomal membrane. The incorporation of liposomes in the bioadhesive hydrogel further improved their stability and applicability for the prolonged and controlled release of acyclovir (Pavelić et al., 2005a). Carbopol gels were also reported to be capable of preserving the original size distributions of liposomes (Pavelić et al., 2004a, 2004b, 2005b).

A rheological characterisation of the influence of liposome addition on the viscosity of Carbopol 974P NF and Carbopol 980 NF gels indicated that negatively charged egg phosphatidylcholine/phosphatidylglycerol (PC/PG) liposomes alone had no significant effect on the physical properties of liposomal gels (Pavelić et al., 2001). The incorporation of positively charged and sterically stabilized liposomes did not affect the rheological properties of the gels, whereas the gel viscosity was significantly increased in the presence of positively charged liposomes (Boulmedarat et al., 2003). Mourtas and colleagues (2008) demonstrated that the liposome composition (membrane rigidity) and lipid concentration, but not liposome size, determined the rheological modulations caused by the addition of liposomes in gels. Whereas the addition of PC liposomes exhibited the minimum effect on the rheological properties, the

addition of hydrogenated-PC (HPC) and HPC/cholesterol liposomes caused the significant changes in the same rheological characteristics, with the changes being proportional to the lipid concentration (Mourtas et al., 2008). A recent review summarised the rheological characteristics and performances of vaginal gels (Yu et al., 2011). The complex and dynamic environment of the vagina requires a complete understanding of the rheological performances of vaginal gels. The establishment of suitable rheological tests to appropriately define such characteristics may facilitate the selection of a gel that avoids leakage after administration. The ideal gel vehicle should provide adequate coating with minimal leakage which is difficult to achieve beacuse it requires the formulation of a gel with a precise viscoelastic balance (Yu et al., 2011).

Other gel-like vehicles for the incorporation of liposomes

Several novel vehicles e-g-, thermosensitive gels composed of poloxamers 407 and 188 incorporating cationic liposomes have been proposed for the vaginal administration of the poorly soluble antifungal drug amphotericin B (Kang et al., 2010). Chen and coworkers (2012) suggested the use of a pH- and temperature-sensitive liposome gel based on the cleavable methoxy polyethylene glycol 2000-hydrazone-cholesteryl hemisuccinate (mPEG-Hz-CHEMS) polymer. The dual-sensitive liposome gel was stable at neutral and basic pH values but was degradable under acidic conditions, such as those in the vagina. The gel was designed to form a thermogel at body temperature and to degrade under locally acidic vaginal conditions, releasing the entrapped active compound. Another group proposed the use of a post-expansible hydrogel foam aerosol made of propylene glycol-embodying liposomes (PG) liposomes as a novel vaginal delivery system with a prolonged residence time (Wei-Ze et al., 2012). A different approach based on the development of lyophilised liposomal gel formulations consisting of HIV-1 envelope glycoprotein (CN54gp140) encapsulated in liposomes dispersed within a hydroxyethyl

cellulose (HEC) aqueous gel has been proposed for mucosal immunisation against HIV-1 infection (Gupta et al., 2012).

4.2. Dendrimers

Dendrimers are a new class of precisely constructed hyperbranched structures constructed by the repeated stepwise addition of branched subunits to a reactive core (Gong et al., 2005). They consist of three distinct architectural domains: i) the multivalent surface, ii) the interior shells surrounding the core, and iii) the core to which the dendrons are attached (Svenson and Tomalia, 2005). Their properties depend mainly on the functional groups present at their surfaces. Though the careful choice of functional groups, it is possible to develop a wide variety of tailor-made structures with broad potential to serve as new nanopharmaceuticals. One of the most promising lines of dendrimer research addresses their potential as antimicrobials. Most dendrimer structures are based on poly(amido amine) (PAMAM) or poly(propylene imine) (PPI) dendrimers (Rojo and Delgado, 2007).

4.2.1. Fungal infections

Very limited data are available about the potential use of dendrimers for the treatment of *Candida albicans*. Tulu and coworkers (2009) compared the antifungal activity of water-soluble dendritic macromolecules with those of nystatin, ketoconazole, and clotrimazole under *in vitro* conditions, and found the dendrimers to be equally or comparatively more potent. The inclusion of lipids in the new dendrimeric lipopeptides resulted in stronger antifungal activity, expressed in a multimodal and dose-dependent manner (Janiszewska et al., 2012).

4.2.2. Viral infections

It is well known that many viral-host cell interactions are initiated by viral protein binding to specific carbohydrates on the cell surface. Derivatised dendrimers offer multivalent ligands that are able to inhibit viral binding, thereby inhibiting infection. They are expected to serve as the scaffolds required for the development of effective antivirals (Rosa Borges and Schengrund, 2005).

HIV

Most of the research on dendrimers targeting HIV infections has focused on the development of microbicides for topical use (Rojo and Delgado, 2007). Among several promising potential microbicidal dendrimers, a gel form of the negatively charged dendrimer SPL703, developed by Starpharma Pty Ltd, appears to be the leading candidate. SPL703 was the first dendrimer-based drug submitted to the United States Food and Drug Administration (2003) for any dendrimer-based drug, and its first clinical trial was completed in 2004 (McCarthy et al., 2005). In 2006, the formulation was granted fast track status by the FDA for the prevention of HIV (Rupp et al., 2007). SPL7013 (16,581 Da), the sodium salt of a sulphonated dendrimer, has a polyanionic outer surface that allows for multiple interactions with target sites able to block the replication of HIV-1 and chimeric simian/HIV-1 viruses in both *in vitro* and *in vivo* conditions in macaques (Jiang et al., 2005). SPL7013 is composed of a divalent benzhydryl amide core and four generations of lysine branches that confer hydrophobicity and a high anionic charge (Tyssen et al., 2010). Patton and colleagues (2006) evaluated the potential of the dendrimer–based (SPL7013) microbicide gel formulations in a pigtailed macaque model, and confirmed its safety. SPL7013 binds the gp120 proteins on the surface of HIV, which normally bind to CD4 receptors on human cells (Rupp et al., 2007). These data enabled the further clinical evaluation of the formulation in humans, currently in phase III clinical trials (Duncan and Gaspar, 2011).

VivaGel® is a topical water-based gel containing of 3 % (w/w) SPL7013 in BufferGel®, a Carbopol-based acidic buffering gel that enhances the natural protective action of the vagina. The pH of the formulation is physiologically compatible with that of the normal human vagina. Its characteristics and efficacy and toxicity data were summarised in a review by Rupp et al. (2007). As a combined system with dual action, it is one of the most promising microbicidal formulations currently under clinical investigation. A detailed characterisation revealed that the dendrimer is not entropically trapped in Carbopol-based gels and is therefore available to physically interact with the virus (Mumper at al., 2009). During the first human testing in healthy, HIV-uninfected women, VivaGel® applied once daily for seven days was found to be safe and well tolerated in sexually abstinent women at strengths of up to 3 %, without any evidence of genital irritation (O'Loughlin et al., 2010). The study was followed by clinical testing in 18 to 24-year old women in the United States and Kenya, who received either VivaGel® or a placebo twice daily (vaginally) for 14 days. Adverse genitourinary effects, although mild, were found to be more common among the women receiving VivaGel® (Cohen et al., 2011). A clinical study of *ex vivo* antiviral potency and the local retention of VivaGel® in healthy, sexually abstinent, HIV-uninfected women following a single vaginal administration of the, confirmed that high levels of HIV-1 and HSV-2 inhibitory activities were maintained for up to 3 h post-dose (Price et al., 2011). In addition, 3 % SPL7013 gel was found to be safe, and no toxicity or systemic absorption was detected (Chen et al., 2009).

Chonco et al. (2012) recently reported the use of a new anionic carbosilane dendrimer (2G-S16) that exhibited high levels of biocompatibility, with promising anti-viral activity in an *in vitro* model. No irritation, inflammation, or vaginal lesions were detected in female New Zealand rabbits upon repeated administration of the dendrimer.

Herpes simplex viruses (HSV)

The first report of the anti-HSV activity of dendrimers was published in 2000 by Bourne and colleagues, who tested dendrimers against HSV-1 and HSV-2 (Bourne et al., 2000). SPL7013 was confirmed as a potent anti-herpes microbicide in both mouse and guinea pig models of herpes simplex infection (Bernstein et al., 2003). Even stronger evidence came from the dendrimer SPL7013, which exhibited strong anti-HSV activity through the inhibition of virus internalisation for both HSV-1 and HSV-2, without any toxicity to the vaginal epithelium. Moreover, its activity was not affected by acidic pH or by the presence of human serum proteins. Interestingly, in addition to the inhibition of virus attachment and entry, it also inhibited the later stages of HSV replication, indicating its broad therapeutic potential (Gong et al., 2005; Tyssen et al., 2010). VivaGel® is also currently being investigated as a preventive treatment against HSV-2 indication (its second Investigational New Drug Application by Starpharma and the United States National Institute of Health).

Peptide-derivatised antiviral dendrimers consisting of a peptidyl branching core and covalently attached surface peptide units appear to be gaining more attention in recent years. Dendrimers derived from the M6 prototype (a tetrabranched lysine-based dendrimer), namely, SB105 and SB105_A10, were found to inhibit the *in vitro* replication of HSV-1 and HSV-2 by inhibiting virion attachments to the cell-surface heparin sulphate proteoglycans. The antiviral activity was found to be dose-dependent (Luganini et al., 2011).

4.2.3. Gram-positive and Gram-negative bacteria

In comparison to the extensive studies of the antiviral potency of dendrimers, relatively little is known about their potential as antimicrobial agents against Gram-positive and Gram-negative

bacteria (Tulu et al., 2009), and most of the work has focused on ocular *Pseudomonas aeruginosa* and *Staphylococcus aureus* infections. PEG-modified poly(amidoamine) dendrimers displayed promising antibacterial potential (Callabretta et al., 2007). Dendrimers were also studied as solubilising agents (carriers) for the antibacterial drugs nadifloxacin and prulifloxacin (Cheng et al., 2007). The topical cervical application of G_4-PAMAAM-OH (generation-4 neutral dendrimer) dendrimers exhibited the potential to treat *Escherichia coli*-induced ascending uterine infection in a guinea pig model of chorioamnionitis (Wang et al., 2010). However, their potential as a treatment against bacterial vaginosis remains to be exploited.

4.2.4. *In situ* dendrimer-based gels

In situ-forming hydrogels, such as thermosensitive hydrogels, which form gels at body temperature, have earned increased attention recently (Navath et al., 2011). Dendrimer-based intravaginal have shown promise as topical microbicides, even in the treatment of challenging infections such as HPV. An *in situ*-forming biodegradable hydrogel obtained by the cross-linking of a thiopyridil functionalised PAMAM dendrimer with eight-arm polyethylene glycol (PEG) was developed for the sustained intravaginal delivery of amoxicillin designed to treat ascending genital infections during pregnancy. The PEGylation of dendrimers reduced their cytotoxicity while maintaining their antibacterial properties. The sustained release of the antibiotic and its antibacterial activity provided the dual-acting mechanism (Navath et al., 2011).

4.3. Nanoparticles for improved topical vaginal therapy

Nanoparticles are defined as solid colloidal particles with a size range under 500 nm, in which therapeutic agents can be entrapped, encapsulated or chemically linked to their surface. They are characterised by their high stability, ability to incorporate a broad spectrum of drugs, and ability to modulate their pharmacokinetics which enable prolonged, controlled, or targeted drug delivery (Mohanraj and Chen, 2006). As a result, the efficacy of the drug can be significantly improved, particularly for drugs with a narrow therapeutic window or low bioavailability. The properties that govern the changes in pharmacokinetic parameters and drug bioavailability are determined by the physicochemical features of the nanoparticles, particularly the surface-exposed molecules, as well as their charge and size. Therefore, the major goals in designing nanoparticles as a delivery system are to control the particle size, surface properties and release of pharmacologically active agents to achieve site-specific drug activity at the therapeutically optimal rate and dose regimen (Li and Huang, 2008).

Different types of nanoparticles were investigated as vehicles to improve the vaginal delivery of drugs. These nanoparticles may be broadly classified as polymeric (synthetic and natural), inorganic, or solid lipid nanoparticles. A new class of mucus-penetrating nanoparticles was recently introduced, and is described in a separate chapter.

4.3.1. Polymeric nanoparticles

Polymeric nanoparticles are formulated using either natural or synthetic polymers with a high level of biocompatibility to reduce cytotoxicity and maximise tissue compatibility. The only synthetic polymers that have yet been approved by the United States FDA for human use are:

poly-D,L-lactic acid (PLA), polyglycolic acid (PGA), poly(lactic-co-glycolic acid) (PGLA), poly-e-caprolactone (PCL), and poly(methylmethacrylate) (PMM) (Lembo and Cavalli, 2010).

Most of the nanoparticles that have been investigated for vaginal application were developed for microbicide delivery (Cutler and Justman, 2008; das Neves et al., 2010, 2012; Mallipeddi and Rohan; 2010). A combination of antiretroviral agents in a single formulation has been suggested as a promising strategy to increase antiviral activity and overcome the limitations of conventional treatments (Ferguson and Rohan, 2011). This concept was illustrated by Date et al. (2012), who developed raltegravir- and efavirenz-loaded PLGA nanoparticles incorporated in a thermosensitive vaginal gel for pre-exposure prophylaxis of HIV. The sustained intracellular release of raltegravir and efavirenz from this gel was confirmed. PLGA nanoparticles were also investigated to enhance the tissue uptake, permeation, and targeting of the anti-HIV agent PSC-RANTES (Ham et al., 2009). The evidence that HIV can be present in human semen during the intercourse has led to another antivirus protection approach, namely the development of semen-triggered topical delivery systems. One such system has been prepared by loading tenofovir and its prodrug into nanoparticles composed of the widely accepted PLGA and methacrylic acid copolymer (Eudragit® S-100), which is known for its solubility in alkaline environment. The developed pH-sensitive nanoparticles (250 nm) were non-cytotoxic in cell culture and exhibited significant pH-responsive release of anti-HIV microbicides in the presence of human seminal fluid simulant. However, the particle stability of this preparation, as well as its *in vivo* safety and efficacy, must be evaluated further (Zhang et al., 2011). Mucoadhesive chitosan-based nanoparticles carrying tenofovir, exhibited a controlled release of this drug (Meng et al., 2011). Polymeric poly(ε-caprolactone) (PCL) nanoparticles carrying dapivirine exhibited improved

intracellular delivery, antiviral activity, and related cytotoxicity of the incorporated drug (das Neves, 2012).

Current challenges and opportunities

Nanoparticles can form stable complexes that can stabilise siRNA (Mahajan et al., 2012). Biodegradable polymeric nanoparticles (Woodrow et al., 2009) have been proposed as an alternative to lipoplexes (Wu et al., 2011) for the delivery of siRNA. PLGA nanoparticles could be densely loaded with siRNA and, led to efficient and sustained gene silencing when applied topically to vaginal mucosa. More important, they were found to be less irritating (Woodrow et al., 2009) than siRNA lipoplexes (Wu et al., 2011). siRNA-loaded PLGA nanoparticles were reportedly able to prevent lethal intravaginal HSV-2 infection in mice, increasing their survival for up to 28 days (Steinbach et al., 2012).

4.3.2. Inorganic nanoparticles

The inorganic nanoparticles are much smaller than polymeric nanoparticles, with sizes commonly under 20 nm. They are prepared via the surface modification of inorganic oxides, and these modifications are important for providing diversity in the size, shape, solubility, long-term stability, and attachment of selective functional groups (Mahajan et al., 2012).

The concept of using multivalent gold nanoparticles to inhibit HIV fusion was first introduced several years ago. Bowman and colleagues (2008) employed 2-nm mercaptobenzoic acid-modified gold particles as a nanoscale platform for the construction of multivalent therapeutic.

After the conjugation of these nanoparticles to SDC-1721 (a fragment of the potent HIV inhibitor TAK-779), HIV fusion was significantly inhibited, whereas free SCC-1721 exhibited no inhibitory effect.

Another strategy based on the use of silver nanoparticles as virucidal candidates was recently proposed by Lara and collaborators (2010a). Polyvinylpyrrolidone (PVP)-coated silver nanoparticles exerted anti-HIV activity through linkage to gp120 in a manner that prevents CD4-dependent virion binding, fusion, and infectivity. When incorporated into a non-spermicidal gel, PVP-coated silver nanoparticles prevented the transmission of cell-associated and extracellular HIV-1. In addition, they were non-toxic to cervical tissue explants, even after 48 h of continuous contact. Interestingly, an exposure time of only 1 minute was sufficient to prevent HIV-1 transmission, and after 20 min of pretreatment with PVP-coated silver nanoparticles and subsequent washing, the cervical culture remained protected for 48 h, demonstrating the long-lasting effect of this treatment (Lara et al., 2010b).

4.3.3. Solid lipid nanoparticles

Solid lipid nanoparticles (SLNs) were proposed as an alternative to polymeric nanoparticles. They are spherical particles in the nanometer range, built of solid lipids and emulsifiers. SLNs also have the potential to be considered as carriers for improved vaginal drug delivery (Alukda et al., 2011) due to numerous potential advantages, including the potential for sustained/controlled drug release and targeting, an increase in drug stability, the ability to incorporate a wide variety of drugs and their biocompatibility. SLNs can be manufactured at the commercial scale more easily than liposomes (Mehnert and Mäder, 2001).

Polylysine-heparin-functionalised SLNs have been designed and evaluated for the vaginal delivery of the hydrophobic microbicide agent tenofovir. The nanoparticles appeared to be non-cytotoxic to vaginal cells for 48 h and were able to enhance the cellular uptake of hydrophobic microbicide (Alukda et al., 2011).

4.4. Other nanopharmaceuticals

Several nanopharmaceuticals are emerging in the literature as potential drug delivery systems. Most of these agents, such as cyclodextrin complexes, micelles, or niosomes have been extensively studied for drug administration via different routes and are relatively understudied in the context of vaginal drug delivery.

4.4.1. Cyclodextrines in topical vaginal therapy

Cyclodextrin complexation has been proposed as a means to increase the bioavailability of the drug through the inclusion of poorly soluble drugs in cyclodextrin complexes (Bilensoy et al., 2006). Examples from several studies suggest that cyclodextrines could improve antimicrobial vaginal therapy. The incorporation of clotrimazole into a β-cyclodextrin complex and further inclusion into a mucoadhesive, thermosensitive vaginal gel composed of Pluronic F127, Carbopol 934, and hydroethylcellulose, resulted in the controlled release of the incorporated drug, providing antimycotic activity over a longer period of time (Bilensoy et al., 2006). Francois and coworkers (2003) evaluated an itraconazole formulation based on hydroxypropyl-β-cyclodextrin

that considerably enhanced drug solubility and generated a mucoadhesive system in the presence of other ingredients. The cyclodextrin-based vaginal cream formulation of itraconazole was found to be safe, well tolerated, and retained in the vaginal cavity over long period of time. The increased solubility of amphotericin B was confirmed after its inclusion in hydroxypropyl-γ-cyclodextrin complexes, whereas the thermosensitive, pH-dependent gel formulation ensured the constant release of the drug in the acidic environment (Kim et al., 2010).

The cyclodextrins were also studied as nanocarriers for anti-HIV agents. UC781, a highly potent microbicide with poor water solubility, was included in different cyclodextrins, improving its virus inhibitory potency (Yang et al., 2008).

4.4.2. Polymeric micelles

Polymeric micelles are nanodelivery systems that are formed through the self-assembly of amphiphilic block copolymers in an aqueous environment. Both the inherent and modifiable properties of polymeric micelles make them particularly well suited for drug delivery purposes, namely, drug solubilisation, controlled drug release and targeting (Aliabadi and Lavasanifar, 2006). Among the several routes of administration tested, polymeric micelles have been investigated for enhanced vaginal microbicide therapy. Nanoviricides®, a trade mark owned by NanoViricides Inc., is a polymeric single chemical chain with covalently attached ligands that specify the virus target. These polymeric micelles are designed to target a specific virus type, attach to the virus particle, and engulf or coat the virus particle, thereby neutralising the virus's infectivity and further, destabilize and possibly dismantle the virus particle. This approach is certainly promising with respect to the challenge of developing of an efficient nano-microbicide.

However, a comprehensive explanation for the observed efficacy has yet to emerge, and its effectiveness is tentatively attributed to the complex mechanism of action of the nanomaterials used as drugs (du Toit et al., 2010).

4.4.3. Niosomes

Niosomes are non-phospholipid vesicles, composed of nonionic surfactants such as mono and dialkyl polyglyceryl ethers or polyoxyethylene glycols. Their morphology is similar to that of lipsoomes, but their structures are distinct. They have also been investigated as carriers for sustained and targeted drug delivery in vaginal therapy (Sankhyan and Pawar, 2012). The incorporation of clotrimazole in a niosomal gel provided the extended release of the drug and adequate antifungal activity (Ning et al., 2005b). In addition, niosomes have been proven as promising nanopharmaceuticals for the topical vaginal application of the anti-HIV drug tenofovir disoproxil fumarate. The prolonged release of the drug from niosomes embedded in a gel was achieved, and no signs of irritation of the rat vaginal mucosa were detected (Patel and Patel, 2011). Furthermore, other microbicidal agents, such as -2 RANTES, have been loaded into a special type of niosomes (Novasomes 7474) and studied for their microbicide efficiency (Kish–Catalone et al., 2006; Singh et al., 2011). These Novasomes were able to release –2 RANTES *in vitro* in a dose-dependent manner over a period of 30-120 min, while retaining the HIV-1 suppressive activity of the drug. Furthermore, a cynomolgus macaque model was used to evaluate the efficacy of -2 RANTES vaginal microbicide formulations in blocking vaginal challenge with the R5-tropic SHIV $_{162P3}$. The majority of the challenged animals pretreated with Novasomes alone or containing an antiviral agent were protected from viral infection. Surprisingly, Novasomes alone exhibited a potent prophylactic effect against SHIV $_{162P3}$, suggesting that the

physical nature of Novasomes, i.e., the presence of surfactants produced a physical barrier between the cervicovaginal epithelium and the incoming virus (Kish-Catalone et al., 2007).

4.4.4. Nanoemulsions

The long-term stability, ease of preparation via spontaneous emulsification, and high solubilisation of drug molecules make nanoemulsions an attractive carrier for the improved vaginal delivery of lipophilic drugs (Tadros et al., 2004). Although often described as microemulsions in the literature, it appears more appropriate to refer to these systems as nanoemulsions because their droplet size is typically less than 200 nm.

Several studies have evaluated the characteristics of nanoemulsions destined for the vaginal delivery of antimicrobial agents. The undesirable liquid nature of the formulation has been overcome by the addition of different polymers and gelling agents. The resulting gel-microemulsions (GM) have been studied in the context of contraceptive delivery (D'Cruz and Uckun, 2001; D'Cruz et al., 2005) and were shown to be more effective than commercially available nonoxynol-9 gel. Additionally, the repeated intravaginal application of spermicidal GMs has been found to be safe and not causing any local, systemic or reproductive toxicity (D'Cruz and Uckun, 2001). The antifungal activity and retention on vaginal mucus of a GM preparation were found to be superior to a commercial product containing clotrimazole (Bachhav and Patravale, 2009).

4.5. Mucus-penetrating nanoparticles: Rationale, general considerations, and potential uses

Although mucoadhesive nanodelivery systems have shown the potential to improve vaginal therapy, recent investigations by Lai and colleagues (2007) demonstrated that these mucoadhesive properties are questionable with respect to the ability to deliver drugs across the mucus to the deeper epithelial layers, which are often the targeted site. Because the mucus is secreted and shed continuously, mucoadhesive nanoparticles often remained captured and wrapped in the mucus without the attainment of default target (Cone, 2009). Viruses can infect the vaginal mucosa due to their ability to efficiently overcome a mucus barrier. Thus, the strategy for engineering viral-like nanoparticles (< 200 nm) has been suggested as an alternative method for overcoming mucosal obstacles (Cone, 2009; Lai et al., 2007).

The surface properties of particles play an important role in their retention and delivery capacities once in contact with the mucus. The surface charge and chemistry determine the attraction or repulsion of the mucin fibres, whereas the size of the particle controls its ability to fit within the mucin mesh pores. Therefore, it is clear that the diffusion rate of the nanoparticles is a consequence of bonding to mucin fibres and particle size. Stronger bonds between mucin and the particle' surface are associated with lower diffusion rates (das Neves et al., 2011a). This model was confirmed by research from the Hanes team, who have shown that large nanoparticles (200-500 nm), if densely coated with polyethylene glycol (PEG) with neutral hydrophilic surfaces, diffused much more rapidly through undiluted human cervicovaginal mucus, than did corresponding particles of smaller size (100 nm). Such behaviour is a consequence of the heterogeneous structure of the mucus (pore sizes). As in size-exclusion chromatography, smaller particles diffused through smaller pores or were retained in pockets of mucus, resulting in an

overall decrease in the transportation rate. In contrast, larger particles were unable to diffuse into small pores and therefore diffused more rapidly through larger channels (Lai et al., 2007). Similar results were obtained for biodegradable PEGylated PLGA nanoparticles with a net-neutral surface; these particles were found within epithelial cells, the underlying submucosal stroma, and fibroblast cells of the vaginal tissue (Cu et al., 2011).

Non-mucoadhesive PEGylated polystyrene nanoparticles of various sizes were investigated to gain insight into the spacing between mucin fibres in the healthy human cervicovaginal mucus. It was found that the average pore size is approximately 340 nm, much larger than a virus. However, the addition of non-ionic surfactant significantly reduced the average pore size of the mucus to approximately 130 nm, indicating that hydrophobic interactions between the lipid-coated protein regions on mucins can cause mucin fibres to self-condense and/or group into thick cables. Viruses and other particles that are smaller than the native pores, such as HSV (180 nm), are not trapped by the steric obstruction of gel mucus but rather by adhesive interactions with the mucus and move down through the mucus at a considerably slower rate than 200-nm non-mucoadhesive PEGylated nanoparticles (Lai et al., 2009b; 2010). Thus, topical applications of surface-active agents, such as vaginal microbicides, might be used to change the mucus pore size to achieve therapeutic or protective effects (Lai et al., 2010). Similar to HSV, cell-free HIV may be captured in the mucus, but only under acidic conditions (pH 4) due to the mucoadhesive interactions of the virus after the neutralisation (e.g., in the presence of ejaculate), HIV is capable of diffusing through the cervicovaginal mucus (Lai et al., 2009a).

As a step towards *in vivo* human administration, the team at John Hopkins University explored the diffusive activity of nanoparticles composed of FDA-approved and biodegradable polymers, namely, PEG and PLGA. They confirmed that PEG-PLGA nanoparticles with a neutral surface

charge and a size of approximately 100 nm rapidly diffused through fresh, undiluted cervicovaginal mucus and could be considered as carriers for improved drug and gene delivery to mucosal surfaces (Yu et al., 2012). To evaluate nanopharmaceuticals that could both move through mucus and provide extended release of the entrapped drug, Ensing and colleagues (2012b) coated biodegradable PLGA and polystyrene nanoparticles with low-molecular-weight PEG. These mucus-penetrating nanoparticles exhibited a uniform distribution over the vaginal epithelium and moved more rapidly through mouse cervicovaginal mucus when delivered in hypotonic solution, allowing them to penetrate deep into the vaginal folds within minutes and to remain there for 24 h. In contrast, uncoated (conventional) particles were captured in the thick mucus layer and were unable to reach the tissue below. Although it is clear that further investigations should be conducted, these findings suggest that mucus-penetrating PLGA nanoparticles, particularly PEG-PLGA, are safe and effective drug delivery systems for the prevention and treatment of sexually transmitted diseases (Ensing et al., 2012b). In addition, mucus-penetrating nanoparticles allowed for enhanced gene delivery to mucosal tissues without diminishing the protective function of the mucus and can therefore be used as probes to investigate the micro- and nano-scale properties of complex fluids that cannot always be adequately resolved with conventional macroscopic techniques. Understanding and overcoming the mucus barrier, while maintaining the native barrier function, will significantly improve prophylaxis and therapy for a wide range of epithelial diseases and conditions (Ensing et al, 2012a).

These findings indicate that the issue of mucoadhesion or mucoresistance must be addressed. To summarise the discrepancies and possible mechanisms of drug delivery into the vaginal

epithelial, we propose a set of characteristics of nanodelivery systems that must be clarified to achieve optimal drug therapy (Figure 2).

Figure 2

4.6. Considerations in pregnancy

Any drug therapy administered during pregnancy represents a challenge. Very little is known about the transfer of drugs/carriers from the placenta to the conceptus and specific organs in foetus. The field of perinatal nanomedicine is still very new. Currently, topical intravaginal antimicrobial agents are prescribed to treat ascending genital infections in pregnant women. Pioneering work was recently published by Menjoge and co-authors (2010, 2011) on the transfer of dendrimers across human foetal membranes and placenta. This group reported that drugs conjugated to dendrimers exhibited restricted entry across the human foetal membranes *in vitro* and suggested that dendrimers administered intravaginally could achieve the selective delivery of therapeutics to the mother without affecting the foetus (Menjoge et al., 2011).

Local intravaginal drug therapy remains the preferred treatment of genital infections in pregnant patient, as the use of topical microbicides to treat fungal and bacterial infections is permitted in pregnant patients. Antibiotics are administered intravaginally, to assure a high local drug concentration in the vagina, which cannot be achieved by oral administration (Navath et al., 2011). However, the efficacy and prevention of recurrence associated with these therapies remain to be improved.

4.7. Toxicity issues

Nanotoxiclogy is a relatively new discipline that focuses on the reaction between the highly increased reactive surfaces of nanomaterials, nanoparticles, and so-called "wet biochemistry". Even particles of the same material can exhibit different properties and toxicity due to changes in surface coating, charge, and other properties. To investigate the potentially relevant toxicity issues, nanoparticles can be classified with the respect to their "softness" and/or "hardness". For nanopharmaceuticals, "soft" nanoparticles, such as dendrimer-, latex-, polymer, or protein-based nanoparticles, are particularly interesting (Elsaesser and Howard, 2012). It is important to consider that most nanoparticles tend to agglomerate, and their potential toxicity should be monitored based on both the total surface area of the number of individual nanoparticles, and their agglomerates. It is generally agreed that the toxicity should be monitored not on the "naked" nanoparticle itself, but rather on its surface, the so-called corona, which involves biomolecular interactions with nanoparticle surface (Elsaesser and Howard, 2012). To date, most of the toxicological evaluations of nanoparticles have focused on particles administered via parenteral, pulmonary or topical (skin) routes.

5. Conclusions

Topical vaginal drug therapy remains a challenging treatment modality. Although some of the problems related to the limitations of conventional vaginal dosage forms can be resolved and/or limited by nanopharmaceuticals, issues of mucoadhesion, targeting, and real potential of novel drug delivery system and their optimal features require further attention.

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List of Figures and Figure legends

Figure 1. Incorporation of liposomes containing a hydrophilic marker (drug) into Carbopol gel (A) and possible mechanisms of marker (drug) release (B).

1-leakage of the marker from liposomes inside the gel

2-diffusion of the marker through the agarose layer to the receptor compartment

3-diffusion of intact liposomes from the gel to the receptor compartment

4-release of the marker from liposomes inside the receptor compartment

Figure 2. Influence of the physico-chemical features of nanopharmaceuticals on the vaginal delivery of drugs

List of Tables with Table legends

Table 1. Overview of liposomal formulations investigated for topical vaginal delivery

Chol cholesterol; DMPG dimyristoylphosphatidylglycerol; DOPE dioleoylphosphatidylethanolamine; DOTAP dioleoyltrimethylammoniumpropane; EPC egg phosphatidylcholine; HPC hydrogenated egg phosphatidylcholine; mPEG-2000-Hz-CHEMS methoxy polyethylene glycol 2000-hydrazone-cholesteryl hemisuccinate; PEG_{2000}DSPE poly(ethylene glycol)-2000-distearoylphosphatidylethanolamine; PG phosphatidylglycerol; SA stearylamine; SDCh sodium deoxycholate; SPC soya phosphatidylcholine; T80 Tween 80; S80 Span 80.

Table 2. Dendrimers in vaginal therapy

*review article

HIV human immunodeficiency virus; HSV *Herpes simplex* virus; PAMAM-poly(amidoamine) dendrimer derivative