Expert Opinion

Nanoparticle-based vaginal drug delivery systems for HIV prevention

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Importance of the Field: Several strategies are being investigated for the prevention of heterosexual transmission of HIV. Of these, topical vaginal drug delivery systems, microbicides, are being actively pursued. HIV prevention by means of a topical microbicide has several drug delivery challenges. These challenges include the vaginal mucosal barriers and potential degradation of the drugs in the vaginal lumen due to pH and enzymes present. Also, new drugs being evaluated as microbicides have specific mechanisms of action, which in some cases require drug targeting to a specific site of action. Nanoparticles provide a delivery strategy for targeted or controlled delivery to the vagina which can be applied in the field of HIV prevention.

Areas covered in the review: This review summarizes nanoparticulate systems and their use in mucosal delivery to date. The sexual transmission of HIV along with the various targets to prevent transmission are discussed as well as the potential opportunities, challenges and advantages in using a nanoparticle-based approach for microbicidal drug delivery.

What the reader will gain: This review provides a general understanding of vaginal drug delivery, its challenges, and nanoparticulate delivery systems. Additionally, insight will be gained as to the limited existing application of this technology to the field of HIV prevention.

Take home message: To date, few studies have been published that exploit nanoparticle-based microbicidal delivery to the vagina. The use of nanoparticles for vaginal drug delivery provides an approach to overcome the existing barriers to success.

Keywords: HIV prevention, nanoparticles, topical microbicides, vaginal drug delivery

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1. Introduction

Sexually transmitted infections (STIs) such as human immunodeficiency virus (HIV), herpes simplex virus (HSV), chlamydia and gonorrhea are spread predominantly through unprotected heterosexual vaginal intercourse [1]. Women are more susceptible to STIs for several reasons, which include biological vulnerability, absence of widely accepted female-oriented prevention methods, socio-economic status and an inability to negotiate safe sex practices. The probability of male to female transmission is higher than that for female to male owing to anatomical circumstance and the fact that most current barriers used are male controlled. The need for female controlled methods for STI prevention is evident. Infections caused by bacteria, including chlamydia, gonorrhea, and syphilis, can be cured with proper diagnosis and treatment, whereas no definite curative therapies exist at present for viral infections such as HIV, HSV, and human papilloma virus (HPV), leaving prevention as the only option available thus far [2].

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The most promising mode for prevention now being studied is the use of microbicides. Microbicide products would inactivate pathogens deposited into the vagina or rectum during sexual intercourse. Microbicides help in the prophylactic inhibition or prevention of STI transmission and offer the benefit of a female controlled option for STI prevention. Several vaginal microbicide products are under development. Early active agents studied included drug substances that had nonspecific activity against HIV. Some of these products advanced to the clinic but unfortunately those evaluated so far have not been effective. However, drug substances with specific mechanism of action are now being evaluated as microbicides. These drug candidates may benefit from more advanced drug delivery strategies to achieve effective dosage forms. Within these strategies nanoparticulate delivery may play a significant role.

2. The vagina

An understanding of the anatomy and physiology of the target organ, in this case the vagina, is important for successful formulation/delivery of a microbicide product. The efficacy and toxicity of candidate microbicide drug substances can be impacted by their stability in the vaginal environment and permeability into the vaginal tissue. The vagina is a collapsed fibromuscular tube (7 – 10 cm) that extends from the exterior of the body to the uterus. The tissue morphology of the vagina varies with respect to anatomical region. The vagina and ectocervix are comprised of multilayered stratified squamous epithelium. Regarding the cervix, the transformation zone is the area where the stratified squamous epithelium of the ectocervix changes abruptly to the single columnar layer of the endocervix. This zone is highly immunoreactive [3]. The thickness of vaginal/cervical epithelium can vary with age and menstrual cycle status [4]. The vagina is a dynamic complex system that contains fluids which may provide both barrier and target to delivery, all the essential elements for an effective immune response, and a normal vaginal flora, which provides an innate defense system that should not be altered. The normal bacteria in the vagina maintain a vaginal pH between 3.5 and 5, but it should be noted that this pH can be altered by the presence of semen in the vagina and in some disease states such as bacterial vaginosis and trichomoniasis, which result in elevated vaginal pH levels that are closer to neutral range.

3. Sexual transmission of HIV

The mucosal surface, an interface between host and environment, is the portal of entry for STIs. The female genital tract is the primary route of heterosexual transmission of HIV [5,6]. Conflicting reports exist as to the ability of the virus to infect intact mucosal epithelial cells [7-9]. Immune cells such as T cells, macrophages and dendritic cells located predominantly in the subepithelial layers of the vaginal and cervical mucosa are the targets for HIV infection [10,11]. HIV transmitted during sexual intercourse by semen or other biological fluids penetrates the stratified squamous epithelium of the vagina and the ectocervix or the columnar epithelium of the endocervix to infect the target cells. Langerhans cells present in the mucosal epithelium may also play a role in transporting the virus to the subepithelial layers [12,13]. Several mechanisms have been proposed for the *in vivo* transmission of HIV, including direct infection of the epithelial cells, transcytosis through the epithelial cells, epithelial transmigration, uptake by intraepithelial Langerhans cells and migration through physical breaches in the epithelial cells [9,14].

4. Targets for HIV prevention

Several microbicidal products have been studied for their activity against HIV-1 *in vitro* by targeting the virus at different stages of its infection cycle [15-19]. The appropriate vaginal drug delivery system for these agents depends on the mechanism of action of the microbicide drug chosen. The first step in the life cycle of HIV is fusion of the viral envelope with the target cell and subsequent release of its genome into the cell. Microbicidal agents that disrupt the viral membrane before attachment of the virus to the host cell can be delivered to the vaginal lumen without deeper penetration into the vaginal mucosa. Once the viral membrane is destroyed, the viral genome of such a virus loses its infectivity. Sequential interactions of the HIV envelope glycoproteins gp120 and gp41 with CD4 receptors followed by interactions with CCR5 or CXCR4 co-receptors initiates HIV–target cell fusion [20]. Delivery systems for microbicidal agents such as CCR5 inhibitors that act by blocking these receptors or co-receptors in the target cells must be able to penetrate the epithelial barrier to reach the target cells. Several studies have been reported suggesting an alternative endocytotic mechanism of HIV entry. Fusion of HIV with endosomes and micropinosomes has been observed by electron microscopy [21,22]. Augmentation of HIV infection by blocking endosomal acidification [23] and its reduction by the inhibition of clathrin-mediated enodcytosis [24] support the endocytotic mechanism of HIV entry. Recently, Miyauchi *et al.* [25] reported the occurrence of complete HIV-1 fusion with the cells in endosomes of the epithelial and lymphoid cells by means of dynamin-dependent mechanisms. According to these studies, HIV entry does not occur at the cell membrane, but inside the endosomes. This might be the reason for the failure of the drug delivery systems that target the intermediate conformations of viral envelope glycoproteins to offer complete protection against HIV infection. Drug delivery systems such as nanoparticles that permeate the cell membranes and block the fusion events inside the cell may be more effective and hence, may be more desirable.

Once fusion of the HIV membrane with the host cell occurs, the viral RNA genome is released into the cell where it undergoes reverse transcription followed by integration of

the pro-viral DNA into the host chromosome. Subsequent to translation, immature viral particles egress the cell by assembly of the viral proteins at the cell membrane. Structural rearrangement subsequent to virion budding generates a mature virus that can infect other cells. Microbicides that act as reverse transcriptase inhibitors, integrase inhibitors, or protease inhibitors all interfere at several stages of the viral life cycle, as indicated by their mechanism of action. Drug delivery systems that can deliver microbicides to the subepithelial layers as well as penetrate the target cells to release their drug intracellularly are preferable as they improve the efficacy of the microbicides by ensuring sufficiently high drug concentrations at the target site of action. Nanoparticles provide one option for such a drug delivery strategy. Owing to their small size, nanoparticles can enter the target cells via energy dependent or independent mechanisms, such as endocytosis, receptor-mediated transport or facilitated transport. The nanoparticles that enter the epithelial layers can then be transcytosed to reach the subepithelial layers and release their drug intracellularly.

5. Nanoparticulate drug delivery systems

Nanoparticles vary in size from 10 to 1000 nm and may or may not contain drug substance. Drug loading is generally achieved by encapsulation, entrapment or dissolution/dispersion. The nomenclature for these systems includes nanocapsules, nanospheres, or nanoparticles. Different materials have been used in the preparation of nanoparticles, and material choice in some cases is driven by application. Synthetic or semi-synthetic polymers such as chitosan, poly(alkyl)cyanoacrylates, poly(lactic acid) (PLA), poly(lactic-*co*-glycolic acid) (PLGA), poly(ethylene glycol-*co*-(lactic-glycolic acid), poly (caprolactone) and polymethyl methacrylate are the most commonly used materials for the manufacture of nanoparticles intended for drug delivery. This is owing to the use of these materials resulting in greater manufacturing reproducibility. Also, synthetic or semi-synthetic materials lead to better control and stability of nanoparticle properties such as chain length, side chains and molecular mass [26]. The performance of the polymer can be adapted to the intended application by controlling the molecular mass or co-polymer composition, which influences properties such as degradation rate, thermal sensitivity and pH sensitivity, to list a few.

The physicochemical characteristics of nanoparticles such as size, shape, texture and composition can be influenced by the manufacturing method [27]. Manufacturing methods for nanoparticles can be broadly categorized into two groups – *in situ* polymerization [28] and the use of preformed polymers [29,30]. Toxicity resulting from the presence of residual monomers in the final product, instability of drugs in the presence of monomers, oligomers and catalysts, inability to produce biodegradable nanoparticles, and lack of complete control over final nanoparticle characteristics make the former method less popular than the later [26]. Use of preformed polymers enables a better control over the physicochemical properties of the nanoparticles produced [26]. This, along with the ability to use biodegradable polymers in the manufacturing process, makes this the preferred method of nanoparticle manufacture.

Several techniques are used to characterize the physicochemical properties of nanoparticles, including size, shape, surface morphology, surface chemistry, drug loading and release characteristics. The shape and size are very important properties of the nanoparticles, which have to be optimized within a narrow size distribution. Electron microscopy techniques, including scanning and transmission electron microscopy, Fourier transform infrared microscopy, scanning probe microscopy, optical tweezers, dynamic light scattering and size exclusion chromatography, are used to characterize the shape and size distribution of the nanoparticles. These techniques are also used to measure the surface topography of the nanoparticles and their interaction with their targets. Zeta-potential measurements are commonly used to measure the net surface charge of the nanoparticles. Drug loading, encapsulation efficiency and release characteristics of the nanoparticles are characterized *in vitro* using standard dissolution techniques.

Nanoparticles constitute a versatile drug delivery system, owing to their ability to overcome physiological barriers and guide the drug to specific cells or intracellular compartments either by passive or by ligand-mediated targeting mechanisms. Entry of nanoparticles into the cells can occur by means of energy dependent or independent mechanisms, such as endocytosis, pinocytosis, fluid phase diffusion, receptor-mediated transport, or facilitated transport. Endocytosis and receptor-mediated transport are the primary mechanisms of intracellular uptake of nanoparticles [31]. The matrix of the nanoparticles is designed to escape the endolysosomal compartments to release their drug in the cell cytoplasm. Receptor-mediated transport involves binding of the nanoparticles by specific cell surface receptors that take up the nanoparticles on the exterior and release them intracellularly [32,33].

Nanoparticles offer several advantages, such as the protection of drugs against degradation [34], targeting of drugs to specific sites of action [35], and delivery of biological molecules such as proteins [18,36], peptides [37], and oligonucleotides [38]. Nanoparticles are now being investigated for many therapeutic applications to overcome typical drug delivery challenges such as conformational stability, physicochemical stability, enhanced cellular uptake of poorly permeable drugs [36,39], reduced cellular and tissue clearance of drugs [40], sustained drug delivery [41,42], and reduction of immunogenic response. The therapeutic efficacy and safety of drugs can be improved significantly by targeted delivery of the nanoparticles. Targeting moieties used to achieve site-specific nanoparticle delivery can be broadly categorized as: i) permeability enhancing targets (transactivating transcriptional factor [TAT] peptide, integrin); ii) stimuli-sensitive targets (temperature, pH, magnetic field, ultrasound); and iii) cell- or tissue-specific targets (folate receptors, transferrin receptors, epidermal growth factor receptor). A target site may have several specific

attributes that can be targeted by either single or multicomponent targeting moieties. The use of multiple targeting moieties in a nanoparticulate system may improve their efficiency in terms of site specificity [43]. However, incorporation of multiple targeting moieties into a single nanoparticle increases the complexity of the formulation processing required, which may result in lower yield, higher production cost and scale-up difficulty.

Nanoparticles can provide both hydrophobic and hydrophilic environments, depending on their polymeric composition. This flexibility in design can be used to enhance drug solubility and overcome associated problems such as precipitation in the biological environment and toxicity. This improved drug stability and enhanced bioavailability in the biological environment achieved by nanoparticulate delivery can provide protection from rapid clearance and destabilization, thereby making administration of a lower drug dose possible [40].

The small size of nanoparticles allows them to penetrate through cells and deliver drugs intracellularly without risking extracellular degradation. Also, their small size increases their ability to be transported through mucosal barriers such as mucus and the epithelial and subepithelial cells by passive or active transport mechanisms [31]. It should be noted that the smaller the size of the nanoparticle, the harder it is to control the drug release kinetics from the nanoparticle. Drug diffuses out of smaller nanoparticles faster, resulting in burst effects and failure to obtain sustained or controlled drug delivery over prolonged periods of time [44,45]. The drug release kinetics can be improved by increasing the size of the nanoparticles, which results in higher drug loading and prolonged drug release with lower burst effects [41,42]. However, increasing nanoparticle size may lead to hindered transport through mucosal barriers. Optimization of particle size with desired release characteristics is a challenge with nanoparticulate delivery systems.

There are several more challenges with respect to nanoparticle formulations. Owing to the small size of nanoparticles, their higher surface area as compared with micro and macro systems provides a high surface energy, which can result in stability issues such as aggregation [26]. Aggregation during storage or *in vivo* on administration are both challenges with regard to the formulation of nanoparticles. In addition, owing to their high surface-to-volume ratios, nanoparticles have an extremely limited drug reservoir volume, which, when combined with the conventionally low entrapment efficiencies obtained for nanoparticles, reduces the amount of drug that can actually be loaded into the nanoparticles [45]. A significant amount of drug may also be lost during loading because of the nature of the processes involved in nanoparticle production. Use of hydrotropic polymers and crosslinking of the polymeric nanoparticles provide potential approaches for improving the amount of drug loading and nanoparticle stability [46]. However, so far few studies have been conducted to address this issue.

Another potential problem associated with nanoparticle delivery systems is their potential for immunologic response [45]. Opsonins such as complement proteins C3, C4, C5 and immunoglobulins bind to the surface of the nanoparticles, rendering them visible to the immune system [47]. This results in activation of the immune system, leading to phagocytosis of the nanoparticles by macrophages of the mononuclear phagocytic system (MPS) and failure of the nanoparticles, and hence the drugs, to reach their target site of action. Nanoparticles that cannot be degraded by the phagocytes are sequestered in the MPS organs, most commonly the liver and spleen [47,48]. If such nanoparticles are made of non-biodegradable polymers, their accumulation in those organs leads to toxicity and other negative side effects [45,48,49].

Despite the challenges, the application of nanoparticles has been very diverse and includes local as well as systemic targeted delivery. Nanoparticles have been studied extensively in the areas of pulmonary delivery [50,51], delivery to the central nervous system [52,53], and the cardiovascular system [54]. Other areas for which the application of nanoparticles has been studied extensively include delivery of anticancer drugs [55,56], hormones, and vaccines [57,58].

6. Nanoparticles for mucosal delivery

Mucosal drug delivery involves delivery to the nasal, respiratory, buccal, ocular, rectal and vaginal surfaces. The mucosa consists of the epithelium itself and a supporting loose connective tissue, called the lamina propria, which lies immediately beneath the epithelium. Deeper connective tissue, called submucosa, provides support for the mucosa. The epithelial layer can be either a single layer, as in the intestine and the bronchi, or stratified and multilayered, as in the mouth, cornea and vagina. Single-layered epithelia consist of specialized cells that secrete mucus onto the surface of the epithelium. The multilayered stratified epithelium contains, or is adjacent to, tissue that contains mucus-secreting cells. Mucosal routes are used primarily for local drug delivery, but systemic delivery of drugs through mucosal surfaces is also used. One advantage of systemic delivery by the mucosal surface is that it offers a means to avoid first-pass metabolism effects associated with the oral delivery route.

The literature provides several studies dealing with the application of nanoparticles in mucosal drug delivery. Several review articles deal with the application of nanoparticles in mucosal delivery, which can be referred to for more information [37,57,59,60].

6.1 Barriers to mucosal nanoparticle delivery: the epithelium

The common physical barriers specific to all the mucosal surfaces that limit systemic or subepithelial delivery of drugs/nanoparticles are the epithelium and the mucus. Epithelial cells restrict the paracellular permeability of solutes, drugs and

particulate systems between adjacent cells by the formation of protein complexes on their apical side, known as tight junctions [60,61]. These tight junctions are dynamic in that they allow for alteration in permeability to facilitate the passage of solutes, ions, water and even cells of the immune system, such as neutrophils. However, the paracellular route represents only a small fraction of the overall surface area of the epithelium. Nevertheless, materials that alter the permeability of the tight junctions have been investigated to improve the delivery of nanoparticles systemically or to basal layers of the mucosa. Chitosan and its derivatives have been used extensively for the manufacture of nanoparticles for mucosal delivery owing to their ability to increase the permeability of the tight junctions. Chitosan nanoparticles have been used for the oral and nasal delivery of peptides such as insulin and tetanus vaccine [36,51,58,62,63]. Cyclodextrins are another class of compounds that have been shown to enhance the permeability through the cells. Recently, nanoparticles made of chitosan and cyclodextrin were investigated for the nasal delivery of insulin [50,64]. *In vivo* studies in rats showed a significant reduction in the blood glucose levels on nasal administration of these hybrid nanoparticles.

The apical and basolateral membranes that consist of lipid bilayers form most of the mucosal epithelial barrier. Small molecules can be transported transcellularly through membranes by partitioning into and out of the lipid bilayers. Transport of nanoparticles and hydrophilic macromaterials occurs by their interaction with specific protein and/lipid elements on the apical surface, known as receptors, and subsequent formation of vesicular structures. These interactions are highly regulated, minimizing nonspecific uptake by the cells [60,65].

6.2 Barriers to mucosal nanoparticle delivery: mucus

Mucus is a dynamic semipermeable covering on the mucosal surface that mediates interactions between the epithelial cells and their environment by maintaining osmotic balance, enabling exchange of nutrients, water, gases, hormones and odors, providing lubrication, while at the same time protecting the epithelium and underlying cells from pathogens such as bacteria, viruses and any waste or foreign particles [4,66]. Mucus is secreted and cleared away continuously at different rates depending on the surface that it covers, activity at the surface, and age [66,67]. The thickness of the mucus layer is dependent on the balance between the rate of secretion and the rate of degradation and shedding. The rate of mucus shedding is stimulated by the presence of toxic and irritating substances, enabling their efficient and rapid removal. Mucus consists primarily of glycoproteins (mucins), lipids, inorganic salts and water. Mucins are the structure-forming components of mucus, imparting characteristic gel-like and viscoelastic properties, which are responsible for its adhesive and cohesive properties. The mucin content is ~ 1 – 3% by weight [59]. Mucins and other mucus constituents entangle by reversible linkages such as disulfide bonds to form a mesh whose pore size is ~ 100 nm [67]. Water accounts for ~ 98% of mucus by weight [67]. Mucus present in the cervicovaginal tract is similar in its components and rheological characteristics to that found in the airways of the lungs, gastrointestinal tract, nose, eyes, and epididymis [68]. MUC5B is the principal glycoprotein identified in these secretions [69]. Nanoparticles must pass through the cervicovaginal mucus layers, which are up to a few hundred micrometers thick, to reach the underlying epithelia and avoid mucus clearance mechanisms [60].

The viscoelasticity of mucus is highly dependent on hydration, which is regulated by ionic balance by the underlying mucosal epithelium. The viscoelastic properties of the mucus are associated with its ability to perform its physiological functions, such as regulating the sperm motility in the vagina, ciliary movement in the respiratory tract, or prevention of pathogen transfer to the underlying mucosa. At the macroscopic level, mucus is a viscoelastic gel that seems to prevent the passage of proteins or pathogens or even small molecules [67]. It has non-Newtonian flow that is responsible for its ability to lubricate the surfaces it covers without interfering with normal physiological functions. However, certain proteins, pathogens and even large particles depending on their surface characteristics were found to overcome the mucosal barrier to reach the underlying epithelium [68,70], implying that the properties of the mucus at the microscopic scale are different. Understanding their role is key to the development of drug delivery systems such as nanoparticles which can diffuse efficiently through the mucus. The microrheological studies of mucus indicate that at the microscale and nanoscale, mucus is heterogenous with domains of low viscosity fluid interspersed between high viscosity mucins [67]. Other factors that influence the viscoelasticity of the mucus include lipids secreted into the mucus, calcium ions, pH, trefoil factor and non-mucin glycoproteins [66,68]. Mucus viscoelasticity, required for normal physiological functions such as ciliary clearance in the respiratory epithelia and sperm motility through the cervicovaginal tract, plays an important role in the transport of substances to the mucosa. In the cervicovaginal tract, the viscosity of the mucus varies with the menstrual cycle. The human sperm can swim only through the ovulatory mucus, which is much thinner than non-ovulatory mucus, which is virtually impermeable for the sperm [4,60].

The cohesive and adhesive properties of the mucus can act as a target or as a barrier to drug delivery systems. The adhesive properties of the mucus have been exploited for the development of mucoadhesive delivery systems, which adhere to the mucus or mucosal surface by means of ionic, covalent, hydrogen, van der Waals or hydrophobic interactions [71]. Delivery systems such as nanoparticles can protect drugs from the surrounding environment such as pH and enzymes at the absorption site to allow absorption of the drug entrapped in the nanoparticle matrix. Imparting mucoadhesive properties to nanoparticles can reduce their clearance from these sites and ensure their prolonged retention at the mucosal surface, resulting in improved absorption of poorly absorbable drugs

such as proteins and peptides. Several strategies have been used to generate mucoadhesive nanoparticles. They include using mucoadhesive polymers as the matrix forming material, or surface modification of the nanoparticles with mucoadhesive polymers.

Several polymers such as poloxamers, pectins, chitosans, polyacrylates and their derivatives have been used to impart mucoadhesive properties to the nanoparticles by surface coating. Emulsion solvent diffusion, emulsion polymerization, and phase separation are some of the commonly used methods for the preparation of mucoadhesive nanoparticles. Chitosan and its derivatives are the most widely used polymers to prepare mucoadhesive nanoparticles because of their dual advantage of improving nanoparticle mucoadhesiveness while enhancing mucosal penetration by opening tight junctions between the mucosal epithelial cells. Liu *et al.* prepared mucoadhesive nanoparticles using chitosan for intranasal SiRNA delivery [72]. Chitosan in combination with alginate as polyplexes is another approach that has been pursued for developing mucoadhesive nanoparticles for oral insulin delivery [73]. Lauryl succinyl chitosan obtained by the amide linkage of lauryl group-substituted succinic anhydride with chitosan was used to deliver insulin by means of the gastointestinal tract in rats [74]. The lauryl group was used to enhance the mucoadhesive properties and enhance paracellular permeability of the drug via hydrophobic interactions and the succinyl group was used to improve mucoadhesive properties by balancing the hydrophilic properties of the polymer. Chitosan and carbopol were linked by irreversible covalent linkages to polymethyl methacrylate nanoparticles to impart mucoadhesive properties through emulsion polymerization by Cui *et al.* [75]. The improvement in the mucoadhesive properties of the nanoparticles due to chitosan is because of its polyelectrolytic cationic charges, which interact with the negatively charged mucosal surface. Carbopol, on the other hand, imparts negative charge to the nanoparticles. These negatively charged carbopol nanoparticles could interact with the mucus glycoproteins through physical entanglement strengthened by hydrogen bonding to form a strong gel network with the mucus. Mucoadhesive PLGA nanoparticles coated with chitosan were developed by Kawashima *et al.* using emulsion solvent diffusion methods in water and in oil for the oral delivery of elcatonin [76]. PLGA nanoparticles coated with carbopol to improve their mucoadhesive properties have been prepared by a solvent displacement method for intramural delivery of rapamycin, an immunosuppressive and antiproliferative agent [35].

Despite the extensive research on mucoadhesive nanoparticles for improving targeting of drugs to or through mucosal surfaces, their application is limited by several drawbacks. The residence time of mucoadhesive nanoparticles at the site of action is determined by the turnover rate of the mucus, which is again affected by several factors, such as the mucosal site, physiological conditions and the presence of irritants [77,78]. The second major limitation is that the mucoadhesive nanoparticles interact with and adhere to mucus and hence may be unable to reach the underlying epithelium or subepithelial tissues, making them inefficient as intracellular delivery systems [59]. To overcome this limitation, nanoparticles that adhere to the underlying epithelial surface have been reported [79,80]. However, they interact predominantly with mucus and thus are not completely capable of efficiently delivering the drugs to the mucosal epithelium. One of the approaches to overcome this problem is to use mucolytic agents as adjuvants to disrupt the mucus. Several mucolytic agents, such as *N*-acetyl-L-cysteine [81], recombinant human DNase (rhDNase) [82,83], thymosin β4 [84], and so on, have been studied for their ability to disrupt the mucus network and improve the transport of nanoparticles. However, the diffusion rates of the nanoparticles through the mucus treated with such mucolytic agents did not show significant improvement. In fact, studies conducted by Sanders *et al.* [83] with human rhDNase suggest a potential reduction in the diffusion of the nanoparticles owing to the action of the mucolytic agent. The use of mucolytic agents may also result in increased susceptibility of the underlying mucus membranes to infections by pathogens, as these agents essentially act by reducing the barrier properties of the mucus. These and other side effects of mucolysis decrease enthusiasm for the use of mucolytic agents as a strategy to improve the transport of nanoparticles through the mucus. To overcome these challenges, nanoparticles that can penetrate the mucus efficiently without interacting with it are now being engineered.

One of the guides to overcoming the mucus barrier comes from nature, that is, the manner in which viruses can overcome this barrier to reach the underlying epithelial surfaces. Small viruses (< 100 nm) such as polio, adenovirus, rotaviruses and hepatitis B virus have been shown to diffuse rapidly in cervicovaginal and other mucus without hindrance. However, larger viruses such as HSV (~ 180 nm) were slowed 100 – 1000-fold by the mucus compared with water [85]. Based on these observations, the pore size of the mucus mesh was assumed to be ~ 100 nm and it was assumed that nanoparticles have to be fabricated at dimensions < 100 nm to efficiently overcome mucosal entrapment. Several studies have compared the diffusion of nanoparticles through the mucus and reported faster diffusion with smaller particles [82,85,86]. However, Lai *et al.* have reported, to the contrary, that larger nanoparticles diffused faster than smaller ones at lower concentrations in cervicovaginal mucus [68].

Surface properties such as net charge and hydrophilicity of the nanoparticle play a crucial role in their ability to be transported through the mucus. Negatively charged polystyrene nanoparticles of ~ 60 nm were found to be strongly entrapped by cervicovaginal mucus [85]. The flexible chains of the mucus also have high densities of hydrophobic domains, which results in the formation of polyvalent low-affinity adhesive interactions and thus entrap foreign materials [59]. The carboxyl and sulfate groups on the mucin proteoglycans impart a net negative charge to the mucus. The negative charges are expected to repel the polystyrene nanoparticles

and hence not stick to them, but the hydrophobic interactions lead to their entanglement with the mucin. These interactions pose a challenge, particularly in the design of polymeric nanoparticles as most of the polymers used for this purpose are either hydrophobic (PLGA, polyanhydrides) or have a net charge (polylysine, chitosan). Viruses capable of rapid transport in mucus possess hydrophilic surfaces that are densely coated equally with positive and negative charges, creating a net-neutral shell that minimizes hydrophobic and electrostatic adhesive interactions [59,66,87]. Mimicking viral surface properties may provide a way to enhance the diffusion of nanoparticles through mucus. Successful engineering of neutral surfaces with such high densities of cationic and anionic charges as in the case of viral proteins has not been reported so far. Lai *et al.* engineered nanoparticles with neutral surface charge whose hydrophobic groups were masked from mucus interaction [68]. In these studies, the surface of polystyrene nanoparticles was modified by covalently linking to a low molecular mass PEG. The hydrophilic and neutral PEG chains improved the transport of the nanoparticles through human mucus. The rate of transport was found to be dependent on nanoparticle size, density of surface coverage and PEG molecular mass. Higher surface density and lower molecular mass of PEG resulted in better transport of the nanoparticles through the mucus. The transport rates were indeed improved several orders of magnitude by surface modification.

6.3 Further barriers specific to vaginal drug delivery

Apart from the general characteristics of the mucosal barriers mentioned above, there are more barriers that are specifically present in the female reproductive system that will have to be considered when developing vaginal nanoparticle drug delivery systems. The vaginal pH in a normal menstruating woman varies from 3.5 to 5.0. However, this may change under a variety of circumstances, such as intercourse, menstrual cycle and pathogenic infections of the vagina. Bacterial vaginosis, a most common vaginal infection, may increase the vaginal pH to > 4.5. Gardnerella vaginalis, a common infection of the vagina, may increase the vaginal pH to 6.0. The pH is elevated even further beyond 6.0 with Trichomonas vaginalis infection [88]. The presence of blood and other components during menstruation and the presence of semen during intercourse also elevate vaginal pH. Apart from these pH variations, the intracellular pH in the epithelial cells and subepithelial layers is different and relatively higher compared with that in the vaginal lumen. Such variations in the pH may affect stability of and drug release from the nanoparticles intended for vaginal drug delivery.

Mucosal surfaces of the female reproductive system have adequate permeability properties for the delivery of nanoparticles. The epithelium lining the oviduct is a simple epithelium with great potential for transport of nanoparticles through it. However, the administration of nanoparticles to this site is very difficult considering the anatomical location. Apart from this, the surface area is limited and the presence of ciliated processes in the oviduct limits the residence time of the administered nanoparticles. The uterus provides a significant mucosal surface area for transport. However, nanoparticulate delivery to the uterus is complicated by the fact that the epithelium is multilayered and undergoes cyclic changes in its properties depending on estrogen/progesterone levels [60]. The vaginal and cervical epithelia can be readily targeted by nanoparticle delivery systems because of their relatively convenient anatomical location and the large surface area that they offer for nanoparticulate delivery. Mucus sloughing can potentially limit the residence time in the cervix and the vagina.

Another consideration for vaginal nanoparticle delivery systems is the presence of innate bacteria in the vaginal vault. Lactobacilli comprise the main bacterial constituent of the vaginal microflora in premenopausal women. The pH of the vagina is maintained by these bacteria, which convert glycogen into lactic acid. Given that the innate microflora is required for a healthy vaginal environment and provides a natural defense system against pathogens, it is important that any nanoparticle delivery system designed should not adversely impact the innate microflora of the vagina.

The identity and quantity of enzymes present in the vagina are not completely known. Several enzymes such as lysozyme, aminopeptidases and antitrypsin are known to be present in vaginal secretions [89,90]. Hydrogen peroxide produced by lactobacilli and protease enzymes present in the vaginal secretions are two major causes of drug degradation, especially of proteins and peptides in the vagina. These constituents are not constant and their activity varies with the menstrual cycle. Enzymatic levels in human cervical mucus are higher during the ovulation period [89]. These enzymes and the associated variations may present a challenge to nanoparticle delivery, especially those made with biodegradable polymers that respond to physiological conditions such as pH and enzymatic activity.

7. Nanoparticles in vaginal drug delivery

Despite the barriers presented there is evidence of success with nanoparticle delivery in the vagina. Intravaginal immunization against HSV-2 infection using biodegradable calcium phosphate nanoparticles as an adjuvant to induce mucosal immunity was investigated in mice [91]. Vaccination of mice by both vaginal and nasal routes with the combination of HSV-2 antigen and calcium phosphate nanoparticles resulted in high IgG and IgA antibody levels at mucosal surfaces and effective neutralizing antibody titers. This led to increased protection from infection when challenged with the HSV-2 virus. Intravaginal immunization resulted in higher mucosal levels of IgG and IgA as compared with intranasal immunization, providing optimal protection against HSV-2 infection.

A biodegradable targeted nanoparticle drug delivery system for PSC-RANTES, a CCR5 chemokine receptor

inhibitor, has been developed in the authors' lab [18]. In these studies, nanoparticles were used not only to protect the active agent from the vaginal environment such as pH and enzymes, but also to facilitate penetration of the drug into the vaginal and ectocervical tissue, allowing drug to reach HIV target cells. PSC-RANTES, the active agent, was encapsulated into PLGA nanoparticles by means of a double-emulsion solvent-evaporation method. *Ex vivo* targeting studies were performed in a Franz cell system with human ectocervical tissue. It was shown that encapsulated PSC-RANTES had a 4.8 times greater uptake into the tissue over non-encapsulated PSC-RANTES during a 4-h exposure time. Furthermore, it was shown that the PSC-RANTES-loaded PLGA nanoparticles targeted the basal layer of the cervical epithelium.

In recent studies by Woodrow *et al.*, effective and sustained gene silencing was achieved successfully by intravaginal administration of SiRNA-loaded biodegradable PLGA nanoparticles [92]. SiRNA-loaded PLGA nanoparticles were produced using a double-emulsion solvent-evaporation technique. The nanoparticles produced were uniform in size (< 200 nm), morphology and surface charge independent of the initial loading, siRNA target sequence, or polyamine used in the formulation. Spermidine was used as a counterion for encapsulating various siRNA sequences. *In vitro* cell culture studies were conducted to test the gene silencing efficiency, bioactivity and cytotoxicity using SiERK2, targeted against the gene encoding mitogen-activated protein kinase (MAPK1). These studies indicated a dose-dependent and cell-dependent gene silencing effect of the SiRNA nanoparticles. *In vivo* studies on the efficiency of the SiRNA nanoparticles in causing gene silencing were conducted by vaginal instillation of nanoparticles with SiRNA targeted against enhanced green fluorescent protein (EGFP) in transgenic mice. A single topical application of these siRNA nanoparticles resulted in effective and sustained gene silencing throughout the female reproductive tract for at least 14 days. The polymeric SiRNA nanoparticles were less irritating and inflammatory compared with SiRNA lipoplexes and produced significant levels of gene knockdown.

8. Conclusion

Nanoparticles provide a delivery strategy for targeted and controlled delivery to the vagina, which is further applicable in the field of the development of microbicide products for the prevention of HIV and other sexually transmitted infections. Various types of nanoparticles have been studied, including a range of materials and manufacturing methods. Although mucosal delivery is complex, there is evidence of success in the use of nanoparticles for targeted delivery to mucosal surfaces. Importantly, to the topic of vaginal microbicides, although limited literature references exist, nanoparticulate delivery systems have been used successfully in the area of vaginal drug delivery. There is a paucity of published research in the area of the use of nanoparticulate vaginal delivery systems for STI prevention. For this reason, further research is needed in the field.

9. Expert opinion

Intravaginal delivery of small molecules, peptides, proteins and modified bacteria has been investigated for microbicide product development. In this context, nanoparticles can serve not only to protect the active agent, but also to facilitate penetration into the vaginal and ectocervical mucosa, allowing drug to reach HIV and/or target cells of interest.

Many of the drug candidates being evaluated for use in microbicide products have significant drug delivery challenges, including stability and solubility issues, local toxicity, potential for resistance development, and requirement for targeting to specific areas within the mucosa. Nanoparticulate delivery systems may offer a delivery system designed to overcome some of these challenges. By providing a protected encapsulated environment for the drug candidate, nanoparticles can be used to overcome stability, solubility and toxicity issues. The ability to achieve drug targeting using nanoparticles can allow for drug localization to specific target areas within the tissue, limiting systemic uptake of the drug substance. By limiting systemic exposure, resistance development may be avoided. Targeting should also result in a more effective pharmacological product given that the drug will reach its target of action more efficiently. Also, it has been suggested that combinations of anti-HIV drug candidates with different mechanisms of action may result in a more effective product. Nanoparticles can provide a delivery system for such combinations especially in those combinations where compatibility issues may exist between active agents being co-delivered and in cases where different delivery profiles may be necessary for effectiveness.

One of the major challenges in the formulation of vaginal microbicides is the complex vaginal milieu. The cervicovaginal mucus, designed for lubrication of the passages and removal of foreign agents, not only keeps viruses and other infectious agents at bay, but also poses problems for drug delivery. Nanoparticles provide a mechanism to facilitate delivery of active drug candidates through the mucus barrier or can provide targeted delivery to the mucus that bathes the epithelial surface.

The initial drug products evaluated as microbicides were primarily nonspecific in nature with respect to their activity against HIV. The clinical results obtained with these agents have been disappointing [93-97]. However, newer candidates being studied have specific targets of action and will hopefully present more promising evidence of efficacy. With these more specific drug candidates comes a greater challenge for drug delivery. Specifically, in many cases there is a need to target the agent to a specific site of action to obtain maximal efficacy. It has been shown that nanoparticles can successfully enter the

vaginal tissues and deliver drug to this site. Given that this area has been shown to be a potential area for the presence of HIV as well as target cells [10,11,14] for some microbicide drug candidates, the ability to localize drug to this area will result in increased efficacy of the microbicide product.

It has also been suggested that coitally independent dosage forms may be more effective owing to enhanced user compliance than coitally dependent dosage forms. Nanoparticles offer a delivery system through which controlled and extended release of the drug substance at the target site can be achieved. This will provide the advantage of design of dosage forms that can be used before sexual intercourse and provide protection for extended periods of time. There are several drug candidates that are now being evaluated for development as microbicide products. Some of these drug candidates differ with respect to their chemical and physical properties, such as solubility, size and charge, to list a few. For this reason not every microbicide candidate being evaluated will be amenable for nanoparticulate drug delivery. However, for some candidates this drug delivery strategy is applicable.

Although the available literature references are limited for the application of nanoparticles in the area of microbicide product development, this technology is being applied in several cases and data generated will provide the evidence needed to support the use of such delivery systems in the field.

Declaration of interest

The authors state no conflict of interest and have received no payment in the preparation of this manuscript.

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