Overview of the advantages and disadvantages of different mucosal sites for the delivery of nanoparticles

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CONTENTS

ABSTRACT

Nanoparticles (NPs) can often improve the efficacy of therapeutic actives, and their delivery to mucosal sites allows for unique and localized effects compared to parenteral delivery. Sites of mucosal surfaces includes the eyes, nasal cavity, lungs, and the entire gastrointestinal tract from mouth to anus, and offers extensive areas for the delivery of therapeutics. However, each mucosal site has unique physiological properties that affect aspects such as stability during the transit to the mucosal surface, release of the active molecules, and absorption of NPs into the body. The required NPs properties also differ based on if the goal is for absorption of intact NPs or release of the active molecules at the mucosal site. Therefore, the interaction of the NPs, with the medium that is in contact with the mucosal surface, the mucus layer, and the epithelial cells, must all be considered during the formulation process. This chapter focusses on the advantages and disadvantages of delivering NPs through each major mucosal site and offers indications on NPs properties that may be ideal for each site.

KEYWORDS

Mucosal, delivery, nanoparticles, ocular, nasal, lung, oral, vaginal

1 INTRODUCTION

Nanoparticulate delivery systems have garnered much attention in the past few decades and constitutes a large area of interest in current research. Nanoparticles (NPs) exhibit unique properties through size, surface, solubility and other modifications, which can offer advantages relative to the conventional forms of drug molecules [1]. Through these unique characteristics and properties, NPs have been investigated for a range of therapeutic applications, including drug delivery, diagnostics and immunotherapy for various pathologies [2]. In addition, nanoparticles can be delivered through different routes, which can result in unique responses and localization of the effect [1].

The mucosal surface of the body is considerably large and represents an extensive area for therapeutic delivery. Sites of mucosal surfaces includes the eyes, nasal cavity, lungs, and the entire gastrointestinal tract from mouth to anus. The delivery of NPs to these mucosal sites allows for unique localized effects and other advantages compared to parenteral delivery [3]. This chapter focusses on the advantages and disadvantages of delivering NPs through each major mucosal route.

1.1 Justification for mucosal delivery of nanoparticles

From a logistical perspective, the mucosal sites are generally more convenient for administration, as it is minimally invasive and may enable greater access to therapeutics without requiring qualified personnel for administration [2]. It also translates to reduced risks for the patient, as, there is less opportunity for body fluid contamination and disease transmission without needles. The implications are also associated with economics, as there is reduced cost associated with administration due to the reduction in logistics which would otherwise be required for parenteral administration. Furthermore, the regulations and requirements for the manufacturing of parenteral formulations are more burdensome compared to those of mucosal routes.

An equally important aspect of the mucosal surface is the therapeutic effects associated with delivering to localized sites for localized ailments. The ability to restrict the exposure of the drug to the intended mucosal site for targeting means that it reduces the potential for adverse effects through reduced drug concentration outside of the target area, and equally increases drug efficacy through increased concentration at the target site. Due to the unique physiology at different mucosal sites such as the proximity to important organs, type of epithelial layer or composition of immune cells, the delivery to different mucosal sites can also lead to unique effects compared to systemic administration [2].

Although NPs formulations such as polymeric NPs and liposomes can be tailored to accommodate for the specific requirements of different mucosal routes [1], each of these routes still presents with issues that must be overcome or makes it unfavorable for administration. It is important to acknowledge the balance of the advantages and disadvantages for each mucosal route, when considering NPs delivery.

1.2 General physiology of mucosal sites

Although mucosal sites in the body differ considerably in many factors in accordance to their functions and location (Table 1), there are a number of general features which can be considered as broadly analogous (Figure 1). Mucosal surfaces are generally composed of epithelial cells that act as a barrier between the body and the environment. Before any nanoparticulate material can reach this epithelium, it must generally first pass through a viscoelastic layer of mucus that lines the epithelium and separates it from the environment [4]. The mucus is a hydrogel composed mainly of proteins known as mucins, and functions not only as a barrier to protect the epithelium from pathogens and pernicious material, but also prevents particle uptake by trapping and clearing them before they can interact with the epithelium [4]. Even when the NPs manage to reach the epithelium, the tightly connected cells that function to control the movement of material into and out of body generally limit NPs entry into the blood through. Overall, the mucosal surfaces can act as barriers for NPs drug delivery through physical and chemical mechanisms.

2 ADVANTAGES AND DISADVANTAGES OF SPECIFIC MUCOSAL SITES

2.1 Ocular

The unique anatomical and physiological structure of the eye comprises a myriad of structures that work together to provide the sense of sight, and aims to protect the eye against foreign substances. Despite numerous efforts, efficient ocular drug delivery remains challenging for researchers, and conventional invasive and non-invasive treatments, cannot guarantee high residence time of the drug in the tear film (2-5 min for topical applications of drug in the form of eye drops [5, 6]). The low absorption represents the major issue yet to be overcome, and it is primarily due to clearance mechanisms that include efflux pumps, aqueous turnover, vitreous flow and ocular drug metabolism [7]. Recently, numerous NP-based formulations intended for both ophthalmologic and systemic diseases, have been developed aiming to overcome ocular barriers, target specific ocular tissue and avoiding non-specific drug tissue accumulation. Several nanocarrier systems including polymeric NPs, liposomes, niosomes and dendrimers, have been widely studied as potential ocular drug delivery systems. The development of nanotechnology based formulations also contributes to the creation of novel devices including nanoparticles-loaded contact lenses, and innovation in the field of imaging and screening.

The ideal NPs delivery system should enhance the permeation and control the release of the drug, enabling high drug loading efficiency to reduce the instilled volume, and hopefully increase the patient compliance through avoidance of more than two administrations per day [8]. Furthermore, the NPs must protect the drug from the metabolic degradation. In particular liposomes have been shown so far to provide protection of entrapped genetic material and enhance its adsorption [9]. These lipid bilayer vehicles can be considered as a possible strategy to formulate several potent actives, although they still have limitations including their limited drug loading efficiency, harsh and aggressive conditions for preparation, and also difficulties related to the sterilization of the formulation. The susceptibility of phospholipids to oxidative degradation in air [10] can be easily overcome by using a similar non-ionic surfactant-based vesicular system called niosomes, which are more chemically stable and can encapsulate both hydrophilic and hydrophobic drugs [11].

Much of the published data regarding ocular drug delivery via NPs systems suggest that the particle composition, size and surface properties, play significant roles in the in-situ retention time and cellular uptake of the active. In order to avoid ocular irritation and blurred vision the NP should have an appropriate particle size and a narrow particle size distribution. The drug time of action is particle size-dependent; smaller particles lead to higher absorption into ocular tissues from the precorneal pocket, larger particles lead to slower drug dissolution [12]. Moreover, surface properties including the particle surface charge, are key factors affecting the particle distribution between the vitreous humor and retinal layers [13]. Positively charged carriers show higher cellular uptake and retention time, and due to the negatively charged surface of the corneal epithelium, it is possible that the initial interaction is electrostatic in nature [14]. The literature also shows that formulations of positively charged liposomes containing a poor water-soluble drug, such as acyclovir, exhibit sustained penetration of the drug across the cornea, increasing the extent of absorption [15], which could potentially be useful for the treatment of herpes keratitis.

Another method for increasing the precorneal residence time of the active, is encapsulating within NPs with mucoadhesive properties. Polyethylen glycol (PEG), chitosan and hyaluronic acid are the most common polymer used to improve the mucoadhesion, because of their ability to contact intimately with corneal and conjunctival surfaces. Chitosan-coated systems compared to non-coated ones, exhibit unique behaviors, which can potentially be utilized to target different regions of the eye. A comparative *in vivo* study for chitosan coated vs non-coated, indomethacin NPs, were conducted in rabbits and showed that the surface coating helped to increase the half-life of indomethacin relative to non-coated formulation [16]. Such NP formulations that can increase the residence time at the ocular surface could be one avenue for ocular NP formulations with improved efficacy.

To summarize, the ocular mucosa presents with a number of disadvantages; the main ones being the high degree of clearance, and limited systemic applications. Despite these limitations, there is potential for the development of suitable NPs capable of encapsulating a wide range of drugs and that can increase the absorption of the active for local pathologies. The retention of NPs at the ocular surface is one of the potential strategies for increasing the absorbance of the active by enabling sustained release.

2.2 Nasal

Intranasal (IN) delivery of therapeutics is widely practiced for treating local nasal conditions such as sinusitis, rhinitis, coryza, nasal bleeding, and nasal polyps, using with anti-inflammatory steroids, antihistaminic, vasoconstrictors, and numerous other drugs. The IN route has also recently, garnered attention as a potential alternative route for systemic drug delivery; most importantly, for drug delivery to the brain and for vaccination [17]. The formulation of active therapeutics into NPs for the nasal route is an avenue for improving the efficacy, as it has been shown to enhance the potential effects of active therapeutic molecules, compared to their conventional formulations [18].

The nose is a portal of entry for the respiratory system, and responsible for filtration and humidification of the inspired air. The nasal cavity extends from the nasal vestibule to the pharynx (around 160 cm^2 surface area) and is halved by the nasal septum. The mucus (around 5 μm thickness) forms a viscous elastic layer, and contains salts and mucin that confers a slightly acidic pH (6.5) and negative charge. In addition, hydrolytic enzymes such as aminopeptidases that degrade proteins, antibodies plus other molecules, are especially abundant. The nasal cavity has 3 regions that differ in their epithelial and functional characters; the vestibule, turbinate and olfactory regions. The vestibule has the epithelial change from skin to stratified squamous epithelium, with abundant hairs representing the first filtering mechanism for inhaled particles (aerodynamic diameter > 10 μm). The turbinate forms the main nasal cavity and highly perfused warming chambers. It is lined by pseudo-stratified columnar mucous-secreting epithelium that aids in trapping inspired particles. It has ciliated and non-ciliated cells, with both immotile and motile microvilli, that play a double-edged role by increasing the surface area of absorption of NPs, as well as limiting the drug absorption through mucociliary clearance. The olfactory region, formed from pseudo-stratified non-ciliated columnar epithelium, is a recognized target for brain drug delivery through olfactory nerves and/or para- or trans-cellular transport. The nasal mucosa is part nasal-associated lymphoid tissue (NALT) that is rich in M cells and dendritic cells (DCs) and has been investigated as a delivery route for NP vaccine formulations [17, 19-21].

One main advantage of delivery through the nasal route is the large, highly vascularized surface area, which is known to be relatively-permeable and leading to fast circulatory drug levels. The conditions of the IN route is also relatively less harsh compared other sites such as the GI tract, and allows the bypass of first-pass hepatic metabolism. It is also a site in contact with the lymphatic system, opening the opportunity for the delivery of vaccine formulations [20, 21]. Another avenue for the utilization of the unique nasal site is the delivery of active therapeutics to the brain, though the olfactory epithelium, avoiding the brain barrier. For the patient, the nasal route is easily accessible, allows for self-administration and is well-tolerate.

There are however, numerous limitations and challenges for NP delivery at the nasal site. The limited drug absorption and rapid mucociliary clearance, means that designing nano-based formulations which provide drug stability and desired release properties suitable for local nasal delivery and systemic delivery, is still challenging [19]. To address these hurdles, various mechanisms have been employed to enhance the nasal drug solubility, retention and uptake. The use of the solubility and/or permeation enhancement agents have shown promising results. Solubility enhancers modify the formulation characteristics after delivery, to increase the availability of the drug [19]. Permeation enhancers alter the permeability of the nasal mucosa, temporarily reducing the mucociliary and enzymatic clearance, and prolonging the drug retention. Examples include bile salts, peptidase inhibitors and cyclodextrins among others, which have been widely investigated [22]. Mucoadhesive materials have also been investigated to enhance the mucosal retention and reduce its clearance. Examples include naturally occurring polysaccharides such as chitosan, which exhibits biocompatible, mucoadhesive properties, and is commonly used for as part of NP carrier

formulations. Chitosan has also been used in a variety of dosage forms from a solution to dry powder [22-24]. In addition, PEGylated NP carriers have exhibited promising absorption profiles compared to non-PEGylated counterparts.

2.3 Lung

Administration through the pulmonary route has been successful for delivering therapeutics intended to treat local respiratory problems, such as asthma, chronic obstructive pulmonary disease (COPD), lung malignancies and lung infections, as well as systemic diseases through delivery of therapeutic molecules, such as proteins/peptides, genetic material, hormones or vaccines [25, 26]. NPs can be a successful platform for enhancing the efficiency of the pulmonary drug delivery, not only for the local conditions but also for systemic administration [27]. The pulmonary route has very complex structure that is divided into two parts; conducting and respiratory areas. Each area exhibits different physiological and functional properties that presents unique challenges for NP delivery.

The conducting area of the lungs extends from the nose, trachea, main bronchi, and branching until the respiratory bronchioles, resulting in a surface area of 2-3 m^2. One major immediate limitation for NP delivery into the lungs is the significant influence that the aerodynamic diameter has on the deposition within the different regions of the lungs. Particles in the size range of 1-5 μm are generally considered as the appropriate range for lung deposition [28, 29], which means that NPs alone are not suitable for direct inhalation. There are however, solutions such as formulation of NP in microcarriers or inhalation via nebulizers, which can temporarily increase the aerodynamic diameter for appropriate lung deposition [30, 31]. The lining of the conducting airways is also a barrier for NP delivery as it is composed of pseudo stratified columnar epithelium, which secrete mucous, express motile cilia, and is lined with a surfactant layer. Epithelial tight junctions limit the translocation of molecules and NPs across the epithelium, and the strong mucociliary clearance mechanisms that filter the inspired air from any particles or bacteria, present major challenges for NPs delivery [32]. The humid environment represents another challenge for the hygroscopic NPs, which undergo increases in their particle size, and subsequently is favored for mucociliary clearance. The state of the conducting area of the lungs can also be affected by different diseases like asthma, cystic fibrosis and COPD [33], which may consequently increase the resistance for the air flow and limit the delivery of aerosolized NPs formulations.

The respiratory area of the lungs extends from respiratory bronchioles to the terminal bronchioles and alveolar sacs, with a wide surface area approximately 120-140 m^2. The lining epithelium is very thin compared to the conducting epithelium (0.2-2 μm, 60 μm thickness respectively) and is an attractive target for NP delivery. It includes alveolar cells type I (main cells, flat) and type II (irregular shape, secreting lung surfactant) with tight and gap junctions, with a thin layer of lung surfactant [34]. The alveolar epithelium has a plethora of wandering cells, for example, DCs, macrophages, mast cells and lymphocytes. This however represents a double-edged sword, as these cells contribute to the clearance of NPs, but at the same time, could be used as the initiator of immune responses in case of vaccination therapy [35, 36]. NPs of various forms have been proposed and investigated, for potential induction of immune responses in the lungs, as the pulmonary route is the entry site for pathogens [31, 37, 38]. An ideal response would induce the production of secretory IgA and plasma IgG antibodies specific for the pathogens [36]. Furthermore, NP formulations of antimicrobials have also shown great potential for use in established infections [39-41]. In terms of NPs uptake by the epithelium, the alveolar epithelium exhibits high permeability and dense vasculatures for its gas exchange functions, and subsequently makes it an attractive site for NPs delivery. NPs are known to be translocated past the epithelium through transcytosis or paracytosis, and is influenced by particle size. Like the conducting area, the respiratory area of the lungs is also affected by different diseases, such as emphysema, pneumonia, lung cancer and tuberculosis [27]. Therefore, it is important to consider the interaction of NPs with the mucosal site in different states.

To summarize, the main advantages of the pulmonary delivery are the large surface area for adsorption, good vascularization, and relatively high permeability of the epithelium compared to other mucosal administration sites. There are also limited proteolytic enzymes that could degrade NPs and the

encapsulated active. The pulmonary route can be used to treat both local and systemic diseases and subsequently absorbed actives do not encounter first pass metabolism. For the patient, the delivery method is non-invasive, uses smaller doses for local lung conditions which can result in less potential side effects [42]. In terms of the challenges for NPs delivery at the lungs, numerous physical and biological barriers can make sufficient NPs delivery difficult. The delivery of NPs, even to the epithelium is a challenge on its own, as airway narrowing and branching play a role in particle impaction away from the alveoli and respiratory barrier. The high humidity within the lungs also affects hygroscopic particles, favoring their clearance. The intrinsic clearance mechanisms of the lungs can contribute to the loss of NPs in the forms of mucociliary clearance in the conducting airways, and NPs phagocytosis by alveolar macrophages in the respiratory airways. Lastly, pulmonary diseases can affect the state of the airways and subsequently delivery of the NPs into the lungs and also the interaction of NPs with the mucus and surrounding cells. The NPs formulation should have sufficient biocompatibility and biodegradability, as to minimize any potential toxicity and inflammatory response that may elicit adverse effects, and careful exclusion of any toxicity or inflammatory should be ensured [42, 43].

2.4 Oral

The potential sites for oral delivery starts directly in the mouth cavity, and extends all the way to the rectum, forming the largest continuous mucosal surface in the body and functioning as the interface between the body and the environment [44]. Despite the large surface area, the unique environmental conditions in these areas is a challenge for NPs delivery, due to the variety of conditions.

However, there are a number of unique effects that can be induced from the oral route using NPs [45]. The localized delivery, sustained release and potential for targeting are some of the NPs properties that could be utilized to improve efficacy. There are also many specialized sites of immune cells found throughout the oral route, which can interact with NPs differently compared to the non-particulate form of the active molecule. The immune system of the oral route makes up a large part of the mucosa-associated lymphoid tissue (MALT), and has the capacity to dictate how the immune system responds to encountered antigen [2]. All of these unique effects, including drug delivery and immunology, can however vary depending on the region of the oral route; oral cavity, GI tract and rectum.

2.4.1 Oral cavity

The oral cavity is the first region the NPs encounter through oral administration. The area is composed of stratified squamous epithelial lining that covers the highly vascular tissue and features low proteolytic enzyme activity. There are numerous delivery forms, such as sublingual, buccal, disintegrating, effervescent, and chewable systems. The oral cavity is subsequently considered as an appropriate area for the treatment of local pathologies, and also a potential portal for systemic delivery, due to the rich blood supply and relatively high permeability [46].

The delivery of NPs to the oral cavity presents with a number of advantages over the regions further down the GI tract. Firstly, the method of administration is relatively convenient for the patient. There is no need to swallow tablets or capsules, which could be advantageous for the elderly or the very young. The conditions in the oral cavity are also less degradative compared to the stomach and intestine, allowing for the delivery of sensitive molecules, and also NPs, that could otherwise potentially be degraded [46]. In addition, the pharmacokinetics of the formulations is likely to be unaffected in the presence of food, compared to the GI tract. One practical circumstance is for the formulation of sustained release NPs, which can allow prolonged effects for drugs with short half-lives [46]. The absorption at the buccal site also avoids first-pass metabolism, which allows for a favorable pharmacokinetic profile for affected drugs. An example of such drug is the sustained release of imidazopyridines, which has a rapid onset of action but can be limited by short half-life [47]. Another possible benefit is potential NPs internalization by epithelial cells, allowing for the delivery of active molecules to the local cells [48]. These NPs properties allow for the treatment of conditions, which are local to the oral cavity, enabling active drugs to exert effects without causing unwanted side effects at

unaffected regions. Such local pathologies include inflammatory and ulcerative diseases [49], oral cancer [48], dental caries, and oral infections [50].

One of the major disadvantages associated with delivery in the oral cavity is the continuous secretion and movement of saliva, which results in high clearance, and compromises the retention of the NPs within the oral cavity [51]. To address this limitation, there have been numerous research into mucoadhesive formulations, which can enhance the residence time of NPs and active molecules. For example, incorporating polymers such as chitosan [52], mucoadhesive films [53, 54], and buccal tablets containing NPs [49], have shown promising results for concepts which could eventually lead to products on the market.

Unfortunately, the delivery of NPs through the epithelium presents another significant barrier, as the multiple layers of epithelial cells promotes low translocation through the epithelial layer [46]. This means that the oral cavity route is limited to lipophilic drugs, and consequently indicating that NPs formulated to release active molecules in the form of proteins, nucleotides and polysaccharides would face a difficult task of sufficient delivery into the systemic circulation. Permeation enhancers which can overcome this limitation have been suggested as a possible means of enhancing NPs or active molecule absorption, and offers a possible avenue for addressing absorption of non-lipophilic active molecules [46]. The GI tract membrane is also thought to be robust enough to handle the temporary effects of permeation enhancers,

The lack of NPs formulations, for the oral cavity on the market, may be an indication of the difficulties of overcoming these limitations and suggests that there is a need for further novel approaches which could enhance retention and permeability of the NPs or active molecules within the oral cavity.

2.4.2 GI tract

As with the oral cavity, the administration through the oral route is arguably the most convenient for the adult patient due to possibility of self-administration and lack of pain, compared to parenteral routes. Not only do oral formulations promote compliance, but they also enable greater access, as they negate the requirement for qualified personnel for administration [44]. This also translates to reduced safety risks, as there is less opportunity for body fluid contamination and disease transmission without needles. From a regulatory and manufacturing perspective, oral formulations may also be favorable due to the production and preparation without aseptic processes [55].

Physiologically, the intestinal tract is generally an attractive mucosal area for delivery due to the high absorptive processes for smaller molecules and abundant vasculature that exists under the large surface area of the intestinal tract. Despite most of the absorbed material entering into the portal blood due to the relatively higher rate of flow compared to the lymph [56], the lymph is thought to be favorable for colloids or large molecules, as the capillaries of the lymphatic endothelium have greater permeability compared to the blood capillaries. The lymphatic pathway also avoids hepatic first pass metabolism, which can be a source of degradation for some molecules. From a drug delivery perspective, the GI tract is acknowledged as a difficult area for delivery that presents with challenging conditions, but NPs formulations can be useful for overcoming some of these limitations and achieving effective drug delivery [55].

One way that NPs can improve delivery of the active molecule through the GI tract, is through increasing solubility of the active drug. Many new drugs are hydrophobic and difficult to deliver, which can hinder delivery, absorption and subsequent bioavailability. By formulating the active molecule into a NPs form, saturation solubility and dissolution rate can be increased, enabling sustained release and potentially greater bioavailability [57]. Active molecules can also be encapsulated inside carrier NPs, not only improving the solubility, but also allowing for controlled release. It is also possible to formulate the NPs to initiate release upon changing conditions, such as when the formulation gets past the harsh acidic conditions in the stomach into the small intestine.

One of the biggest advantages of NPs formulations is the ability to prevent or minimize degradation of the encapsulated actives by the degradative GI environment. The pH of the GI fluid varies along the GI tract, starting with highly acidic conditions in the stomach, to a neutral or slightly alkaline pH in the intestine and colon. The GI fluid also contains phospholipids, surfactants, enzymes and buffering agents, which serve to facilitate the degradation of ingested material. There are numerous approaches to formulating NPs that can maintain sufficient stability within these conditions, including NPs surface coating approaches such as with PEG [58] and chitosan [59], using particle ingredients resistant to disruption or degradation, and increasing the membrane stability through covalent links [59].

Another way in which NPs can improve bioavailability is by targeting specified sites of the GI tract. Attachment of specific ligands on the surfaces of NPs can direct the NPs to certain cells and can improve the proximity of the NPs to the desired site and potentially increase the chances for absorption or interaction [2]. This targeting also applies to specific regions of the GI tract for targeting specific conditions such as for gastric ulcers in the stomach and ulcerative colitis in the small intestine. This can be achieved by pH, adhesion, or time dependent systems [44], which releases the active molecules in the affected area and reduces side effects elsewhere.

In addition to delivery of conventional therapeutic molecules, NPs vaccines through the oral route offers unique benefits in terms of the types of immune responses generated, as they not only induce mucosal immunity locally in the GI tract, but can stimulate other parts of the MALT through activated cells in the gut-associated lymphoid tissue (GALT) [60][61]. The main form of lymphoid tissue in the GI tract are the Peyer's patches (PP), which are unique due to the presence of phagocytic M cells that demonstrate the unique ability to transcytose nanoparticulate matter, from the intestine to the underlying immune system through adsorptive endocytosis, fluid phase endocytosis and phagocytosis [62]. NPs made from various materials, such as inorganic materials like gold and silica, and organic particles such as liposomes and polymeric NPs, have been investigated for oral use, and have exhibited immunostimulatory effects, that could be useful for immunotherapy and vaccine applications.

Despite the many benefits of administration through the GI route, it is remains one of the most complicated delivery routes. The absorption of active therapeutic molecules such as proteins have been challenging, with one of the main hurdles being the potential instability in the conditions of the GI tract [63], which can degrade the active drug or the particle before sufficient absorption can occur. The gastric pH can range from 1.5 to 2.9, and the presence of degradative enzymes presents a challenge for delivery of active molecules and NPs. The NPs must exhibit the sufficient capacity to protect the encapsulated material in these conditions, as encapsulated materials can degrade through acid catalysis, and proteins can potentially lose activity through changes in the intra-molecular bonds that disrupt secondary and tertiary structures [64]. This is the reason that oral doses, especially for proteins, are required to be significantly higher compared to doses given by the subcutaneous route for comparable effect [65], as 94-98% of ingested proteins are digested by the GI proteases [64].

Polymeric NPs can be susceptible to surface and bulk erosion, resulting in loss of encapsulated material and loss of the initial particle characteristics [64]. Alternatively, lipid NPs can be broken down by disruption of the membrane or surface by enzymes and surfactants contained in the GI fluid. Even without full degradation of the NPs, particle properties such as size and surface characteristics may change as a result of the different pH conditions, and presence of components in the GI fluid which may adsorb to the particle surface to change the surface characteristics or promote aggregation. This means that testing of potential GI tract formulations in bio-relevant fluids is required, in order to evaluate the state of particle characteristics through the various conditions of the GI tract. An alternative solution is the formulation of NPs in vehicles such as tablets, which can release the NPs once it reaches the targeted site of the GI tract [66].

Assuming that the NPs and active drug survives the degradative conditions, another major limitation of the GI route is the barrier presented by the mucus and epithelial layers. The mucosal surface of the GI tract is covered by a 50-500 μm viscoelastic layer of mucus [67].The outer loosely adherent mucus layer

has a high turnover due to peristalsis, and the firmly adherent mucus layer is unyielding and, cannot be removed mechanically without compromise of the epithelium. Interaction of NPs with the mucus layer is influenced by certain particle characteristics, as hydrophobic particles with sizes smaller than 500 nm, were found to have faster diffusion and increased penetration through the mucus layer respectively [68]. There are however, conflicting opinions on how surface charge might affect uptake. There have been suggestions that positively charged particles have a greater chance for uptake as the overall negative charge of the mucus may potentially result in a greater likelihood for interaction and retention [4]. However results using different surface coating polymers have shown negative and uncharged particles to have greater affinity for the underlying PP [68]. Recent literature suggests that particles which penetrate the outer loose mucus layer and adhere to the deeper, firmer layer are optimal for delivery to the underlying epithelium [4].

Despite the large surface area of the intestinal mucosa, there is very little particulate uptake through conventional intestinal epithelia due to the low rate of endocytosis occurring at the enterocytes [55]. There have been uptake of inert particles via transcellular and para-cellular pathways but this generally limits the uptake of NPs to sites such as the PPs, which only makes up 1% of the total intestinal surface and takes up less than 0.01 % of the administered dose [69]. Furthermore, NP aggregation upon exposure to GI fluid could have a large influence on the degree of uptake, as particle size has been correlated to transcytotic uptake by PP M cells [68]. The failure of particles to maintain their size and surface properties could ultimately result in poor in vivo responses. Even after absorption, the active molecule travels directly to the liver where hepatic first-pass metabolism occurs, potentially reducing the active molecule concentration further. In addition to the various macroscopic barriers for absorption, the state of the GI tract is also susceptible to influence from ingested food [45]. The fed or fasted state can influence the motility of the GI tract and subsequently affect the retention of nanoparticles at sites.

2.5 Vaginal

The vaginal route has been widely investigated as an alternative way of drug administration, mainly for the advantages it presents in terms of avoiding the GI environment and the hepatic first pass effect. Recently, researchers have been focusing on the advantages of using NPs to improve vaginal delivery of drugs or the use of this route for immunization purposes [71].

The encapsulation of drugs in NPs such as liposomes, polymeric particles, inorganic NPs, niosomes and dendrimers offers many advantages compared to the traditional vaginal formulation [72]. The increase in solubility and bioavailability of the drug, together with the possibility of developing formulations that exhibit controlled [73] and prolonged [74] release of the drug, will lead to the decrease in the administered dose and of systemic side effects.

Firstly, although the use of NPs *in vivo* may be limited by their short residence times within the vagina, mucoadhesive polymers have been employed to overcome the poor retention issue that NPs may present, given the tight attraction between the mucus and the polymeric carrier [75]. Chitosan and alginate NPs showed prolonged contact with the mucus, thus being the first step for the delivery of drugs to the underlying tissues. [76]. However, it is imperative to mention that mucoadhesive particles can damage the vaginal mucosa facilitating the penetration of pathogens and toxic materials into the mucus, leading to infections of the area [77, 78]. Lai et al [79], also demonstrated that the mucoadhesive properties of NPs can interfere with their capability of delivering drugs across the mucus to reach the epithelium; NPs often remain captured into the shed of the mucus without showing the desired effect [80].

NPs have exhibited promising activity for the delivery of macromolecules, such as proteins and nucleic acids, which are degraded if administered alone in other routes. As commonly acknowledged, NPs have a protective effect against enzymatic attacks, given that they are too large to gain access to the drug entrapped within the nanocarrier [3]. Recent studies revealed that niosomes containing insulin have enhanced effects, compared to vaginal administration of the free insulin. Moreover, it has been demonstrated that vaginal administration of insulin-loaded niosomes have a similar bioavailability when compared with its subcutaneous administration [81].

Furthermore, the vaginal administration of molecules such as RNA entrapped in NPs, offers the advantage of avoiding nuclease enzymes that are present in the mucus, thus allowing RNA to reach the underlying epithelium without being degraded [82].

Several studies have been carried out so far to develop nanopharmaceuticals for the vaginal delivery of antimicrobial, antiviral and antifungal drugs, as useful strategies to prevent infections, or transmission of dangerous pathogens. Ensign et al, demonstrated that acyclovir encapsulated in mucus-penetrating NPs, when administered prior to the virus infection, would protect the Herpes Simplex Virus infection of 53% of the treated mice [83]. Malavia et al, developed liposome formulations that were capable of inhibiting HIV infections, having potential use in the prevention of HIV infection in women [84]. Moreover, it was demonstrated that the encapsulation of octylglycerol in liposomes enhanced its activity against HIV HSV and *Neisseria Gonorreae* with a prolonged released of the drug compared to the traditional gel formulations [85]

However, despite several advantages associated with the delivery of NPs through the vaginal route, it needs to be mentioned that NPs show stability-related problems due to their short shelf life [71]. This can be overcome by incorporating NPs in adequate micro]carrier systems in order to be delivered, even though it has been found to be challenging when in need of achieving specific release profiles. Moreover, the mucus layer that cover the vaginal epithelium represent a barrier to overcome to achieve a uniform distribution of the drug and its prolonged retention in the vaginal tract [86].

3 CONSIDERATIONS FOR MUCOSAL NP DELIVERY

In order for NPs to gain mainstream adoption as mucosal therapeutic delivery vehicles, there are a number of hurdles to overcome. Each mucosal site has unique physiological properties that NPs formulations must cater towards (Table 1), but the ideal properties are mutual; NPs are required to exhibit sufficient stability during the transit to the mucosal surface, must be retained long enough for release, and must deliver or release the active molecule at the desired site and the appropriate rate. The required NPs properties also differ based on if the goal is for absorption of intact NPs or release of the active molecules at the epithelium. Therefore, the interaction of the particle with the medium that is in contact with the mucosal surface, the mucus layer, and the epithelial cells, must all be considered during the formulation process.

The GI tract is arguably the most studied for systemic delivery of the NPs or active therapeutic, due to convenience and high possibility of absorption, but other routes such as the lungs and nose are also commonly investigated for local pathologies. One of the main advantages for each mucosal site is the localization of treatment, improving the drug concentration at the area, and subsequently reducing the potential for side effects associated with systemic distribution. Another advantage is the lack of needles required for administration, which offers ease of logistics, as well as relatively pain-free administration. There are of course limitations associated with mucosal sites, including the low bioavailability. The distinctive features of the mucosal sites, and the advantages and disadvantages, have been summarized in Tables 1 and 2 respectively, and gives an indication of how varied the conditions are.

The immune response is another unique feature at some mucosal sites. There are local differences in the composition of immune cells and tissues, and the resulting immune response can differ, based on the site. This is a point of consideration for the induction of the desired immune response by immunotherapy/vaccine NP formulations at the desired locations. The presence of immune cells also means that the awareness of the immunological consequences, such as inflammation, are also required for formulations even which are not primarily designed to induce immune response.

Future mucosal NPs formulations would therefore ideally address points of interest such as the stability of the particles before they reach the mucus layer, whether retention or penetration at the mucus layer is desired, interaction of the particles with the epithelial or immune cells for uptake, and subsequent release of the active molecules.

4 CONCLUSIONS

The delivery of NPs to mucosal sites offer unique advantages and challenges. Each mucosal site differs in physiology and subsequently requires adaptation of the formulation to optimize the NP interaction with the barriers associated with absorption or delivery.

5 REFERENCES

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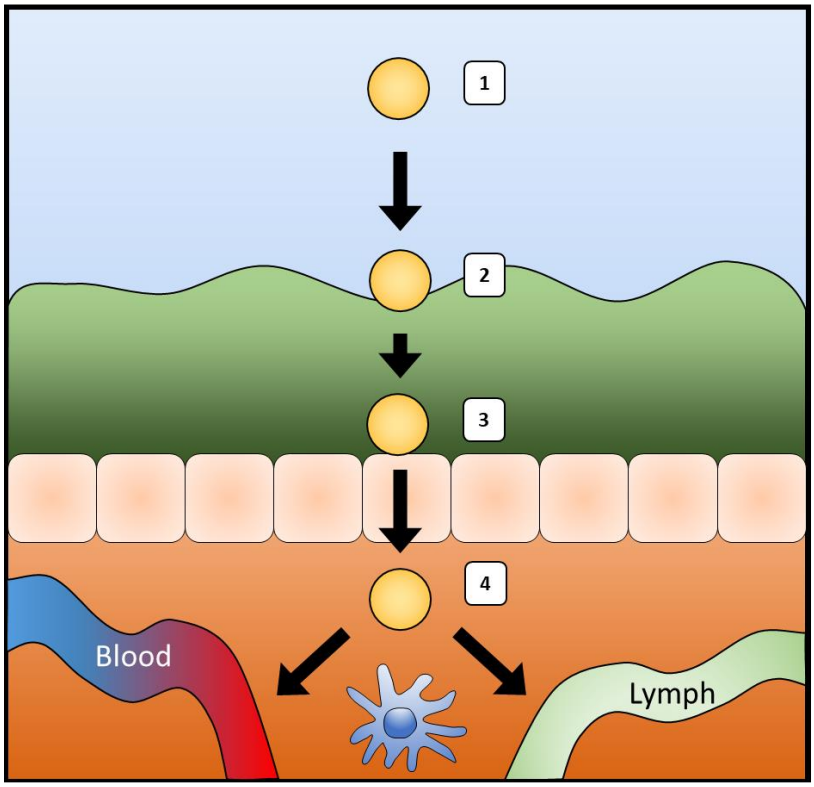
Abbreviations

Chronic obstructive pulmonary disease	COPD
Gastrointestinal	GI
Gut-associated lymphoid tissue	GALT
Intranasal	IN
Mucosa-associated lymphoid tissue	MALT
Nanoparticles	NPs
Nasal-associated lymphoid tissue	NALT
Peyer's patch	PP

Figures:

Figure 1. Considerations for the delivery of NPs at mucosal sites. The properties of the medium in contact with the mucosal surface, the mucus, epithelium and lamina propria/submucosa, all contribute to the absorption of NPs and/or the incorporated active molecules.



1. Medium
- Degradation in pH and by enzymes
- Corona formation
- Clearance
- Release of actives

2. Mucus
- Clearance
- Retention/penetration
- Release of actives

3. Epithelium
- Translocation across epithelium
- Size and surface characteristics of particles
- Targeting ligands
- Release of actives

4. Lamina propria/submucosa
- Absorption of NPs or actives into lymph or blood
- Uptake and presentation by immune cells

Tables:

Table 1. Summary of the physiology of mucosal sites

Site	Medium in contact with mucosal surface	Mucus	Epithelium	Unique features
Ocular	Air	• Secreted mucins, electrolytes, and water produced by the conjunctival goblet cells • Ocular mucosa is slightly basic with pH ~7.8	• Single layer of basal cells and 4-5 cell layers of nonkeratinized, stratified squamous epithelial cells	• Very low residence time of drug (2-5 mins)
Nasal	Air	• High viscosity and elasticity, rich in mucin containing negatively charged acids, , salts, water, hydrolytic enzymes and antibodies • pH of ~6.5	• Vestibule lining: Stratified squamous epithelium with hairs • Turbinate lining: Pseudo-stratified columnar ciliated epithelium with mucous secreting cells • Olfactory epithelium: Pseudo-stratified non-ciliated columnar epithelium.	• Mucociliary clearance • Thick mucus layer • Bypasses first pass metabolism • Olfactory epithelium provides potential route for brain drug delivery
Lung	Air	• Mucus lined with lung surfactant that undergoes thinning toward the respiratory airways (60 µm to 2 µm) • Limited enzymatic activity and rich in immunoglobulins • Same pH as extravasated blood	• Pseudo-stratified columnar epithelium ciliated and mucous-secreting in the conducting airways. • Alveolar epithelium is composed of almost flat single cells.	• Mucociliary and alveolar macrophage clearance • Bypasses first pass metabolism • Large surface area and densely vascularized
Oral cavity	Air but covered with saliva	• The saliva has pH of 6-7 and contains electrolytes, proteins, enzymes, mucin and immunoglobulins	• Stratified squamous epithelium	• High clearance by saliva • Bypasses first pass metabolism
GI tract	Gastric fluid: • 1-3 pH • Lipases and proteases Intestinal fluid: • 5.7-7.4 pH • Proteolytic enzymes more abundant • Bile salts	• Adherent and non-adherent layers of mucus with thickness of 50-500 µm • pH of 5.2-6.2	• Simple columnar epithelium • Intestinal cells express microvilli	• Highly degradative conditions • Transit time of 0.5-4 hours in stomach, 1-2 hours in small intestine and 12-24 hours in colon • M cells can translocate NPs across intestinal wall at the PP
Vaginal	Air	• Menstrual cycle, menopause and pregnancy are responsible for the diverse composition of the mucus • pH 3.5-4.5	• Nonkeratinized, stratified squamous epithelium. • Highly folded epithelium.	• Large surface area and rich blood supply

Table 2. Summary of the advantages and disadvantages of NP delivery at different mucosal sites

Site	Advantages	Disadvantages
Ocular	• Treatment of local ocular pathologies without unwanted absorbance elsewhere	• Poor bioavailability due to clearance mechanisms • Low patient compliance • Low scope for systemic applications
Nasal	• Ease of administration • Potential for brain drug delivery • Highly vascularized	• Mucociliary clearance • Difficult penetration of mucous layer • Enzymatic degradation
Lung	• Rapid absorption • Highly vascularized • Large surface area • Limited enzymatic degradation	• Narrowing and branching of airways may favor particle impaction away from target site • Mucus and surfactants may cause NP aggregation • Mucociliary and alveolar macrophage clearance
Oral cavity	• Ease of administration • Avoids first pass metabolism	• High clearance due to secretion and flow of saliva • Limited absorption through epithelium
GI tract	• Ease of administration • High surface area • Unique immune make up in the GALT	• Hostile environment can degrade NPs and active molecules • Limited absorption of NPs through the epithelium
Vaginal	• Unique immune make up in the MALT • High residence time of drugs in the site of administration • Potential for prevention of local infections.	• Gender-specific • Hostile environment can degrade NPs and active molecules • Limited absorption of NPs through the epithelium • Mucoadhesive polymer can damage the mucus • Diverse composition of mucus according to age and menstrual cycle.