Co association of mucus modulating agents and nanoparticles for mucosal drug delivery

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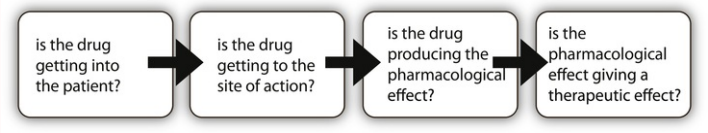
Abstract

Nanoparticulate drug delivery systems (nDDS) offer a variety of options when it comes to routes of administration. One possible path is crossing mucosal barriers, such as in the airways and in the GI tract, for systemic distribution or local treatment. The main challenge with this administration route is that the size and surface properties of the nanoparticles, as opposed to small molecular drugs, very often results in mucosal capture, immobilization and removal, which in turn results in a very low bioavailability. Strategies to overcome this challenge do exist, like surface 'stealth' modification with PEG. Here we review an alternative or supplemental strategy, co-association of mucus modulating agents with the nDDS to improve bioavailability, where the nDDS may be surface modified or unmodified. This contribution presents some examples on how possible co-association systems may be achieved, using currently marketed mucolytic drugs, alternative formulations or novel agents.

Keywords: ~~m~~Mucus; ~~m~~Mucolytic; ~~d~~Drug delivery

~~1.~~1 Introduction

Effective delivery of active pharmaceutical agents (APIs) to their site of action is a prerequisite for functional drug therapy [1], and as such drug delivery considerations form part of any drug development process (Fig~~figure.~~ 1). Oral drug administration involves drug uptake across the gastrointestinal mucosa and as such is a form of mucosal delivery. However, even though the oral administration route is the most widely utilized administration route for APIs [1], the historic experience gained from effective oral delivery of small molecule APIs does not fully address the challenges associated with delivering nanoscale drug formulations over the gastrointestinal, or indeed any other, mucosal surface.



~~Figure 1.~~**Fig. 1** Schematic of the processes involved in drug therapy.

alt-text: Fig. 1

~~1.1.~~1.1 Nanoscale drug delivery systems and mucosal delivery

Nanoparticles have significant potential in drug delivery applications. Nanoscale drug delivery systems may enable formulation of APIs that are otherwise too hydrophobic to achieve uptake or protect APIs that are unstable in the in vivo environment such as biologics, which tend to be rapidly degraded by endogenous enzymes. Nanoscale drug delivery systems also have the potential to improve bio-distribution and pharmacokinetics and may be targeted to the site of action increasing efficacy and reducing off target side effects [2]. Mucosal delivery covers multiple non-invasive delivery routes, in the gastrointestinal, respiratory and genitourinary tracts. These routes may be utilized for local treatment of mucosal diseases but also for systemic drug administration [3].

Currently, the majority of nanotherapeutics approved or in clinical development are for intravenous or other injected delivery routes [4,5] thus ensuring good systemic bioavailability of the API (active pharmaceutical ingredient), something that is particularly important when the cost of goods for the active agent is high. Nevertheless non-invasive administration routes offer clear advantages and are also represented both among approved formulations and in development pipelines [4,6,7].

1.2.1.2 The mucus barrier

Mucosal barriers are characterized by the presence of extracellular mucus secretions and/or a mucin rich cell glycocalyx. Nanoparticles, as a result of their size, experience a much greater reduction in mobility in extracellular mucus when compared to small molecules [8–12] and therefor the barriers to mucosal delivery of nanoparticles differs significantly from that of small molecule APIs. Indeed, if one considers the role of mucus as a protective barrier it is clear that nanoscale drug formulations may have both size and surface features in common with, for example, the viruses and bacteria the mucus has evolved to protect the underlying cells against [8,13]. Given that mucus can provide a significant extracellular barrier to nanoparticles, strategies to overcome this barrier [8,14,15] are a valuable part of any nanoparticle drug development process. One available approach, and the focus of this review, is to address the mucus directly and attempt to modulate its barrier properties, thereby improving the access to the underlying cells for nanoscale drug formulations ~~(Tables 3-5)~~.

Mucus is a complex material, and the mucus barrier is multifactorial, with steric, interactive and dynamic components, all of which may be potential targets for modulating the barrier properties of mucus [8,16]. To understand these barrier functions and strategies for their modulation we must consider the physiochemical nature and physiology of the mucus gel itself. Mucus is a physical hydrogel containing around 95% water, where the gel matrix is composed primarily of polymeric mucin molecules. A mucin subunit consists of a protein backbone and oligosaccharide sidechains, which may form up to 80% of the weight of the molecule. Mucin subunits are combined through disulfide bonds to form multimers and these multimers then interact with each other through non-covalent interactions such as hydrogen bonding, hydrophobic interactions, electrostatic interactions and Van der Waals forces to form a hydrated network [8,15]. Unlike many other biopolymer gels the mucus gel does not contain specific well defined junction zones [17] but nevertheless maintenance of the gel matrix involves multiple intermolecular interactions over and above simple molecular entanglements, forming a heterogeneous matrix structure with a wide pore size distribution [18,19]. Both the sequence of the protein backbone (mucin gene product) and the structure of the oligosaccharide side chains of the mucin vary with physiological location. The steric component of the mucus barrier is related to the pore size of the gel matrix. In its simplest form entities with a diameter larger than the pore size (cut off size) will be unable to penetrate the mucus barrier [19]. Given the heterogeneous nature of the mucus gel matrix there may be steric hindrance to the movement of particles even much smaller than the nominal cut off size and in some cases smaller particles may be more hindered than larger particles as they have access to a greater fraction of the matrix and may become temporarily ‘trapped’ in small pores.

The interactive component of the mucus barrier is related to the high potential of the mucin molecules for forming varied intermolecular non-covalent interactions such as hydrogen bonding, hydrophobic interactions, electrostatic interactions and Van der Waals forces [8]. As such entities meeting the mucus (such as bacteria, viruses or indeed nanoparticles) that have surface structures able to take part in these types of interactions may become functionally entrapped. This entrapment effect is also somewhat size dependent since each single interaction is weak in absolute energy terms and it is the multiplicity of interactions that occur simultaneously for nanoscale particles that lead to functional entrapment. The dynamic component of the mucus barrier is primarily related to the continual secretion and removal of mucus, which means that any entity must diffuse ‘upstream’ through mucus to access the underlying cells [8]. Additionally, the mucus exists in locations with mechanical deformation both from the mucosal and luminal side and gel matrix has an inherent flow character where matrix forming interactions are broken and remade to adapt to such deformations and maintain a continuous layer [20]. This flow character, particularly when exposed to shear forces, may aid nanoparticle mixing (and therefore penetration) into the mucus matrix, however, unless the mixing occurs throughout the full depth of the mucus layer it is unlikely to functionally enable mucosal nanoparticle uptake.

Mucus is a complex material and in addition to the mucin molecules, the secreted mucus gel may contain a wide variety of additional components such as proteins (including enzymes), lipids, surfactants, nucleic acids and oligo and polysaccharides of both physiological and pathological origin [8,10,11,21]. These non-mucin components may interact directly with the mucin matrix or may act as filler material within the pores of the matrix structure, and have the potential to alter the barrier properties of the mucus gel. Strategies to use mucus modulating agents to improve mucosal nanoparticle drug uptake must then address one (or more) of these barrier components. Such strategies are summarized in ~~table~~Table 1 and further discussed in ~~s~~Section 4 of this review.

~~Table 1.~~Table 1 The barriers of mucus, their possible modifications and interactions.

alt-text: Table 1

~~b~~Barrier component	Governed by	Modification strategies	Modification may also alter…
Steric	Pore size in the matrix Viscosity of the aqueous phase	Increase hydration in the mucus inducing swelling and diluting the aqueous phase Depolymerize macromolecular matrix components Disrupt mucin interactions	The interactive barrier

Interactive	Interaction potential of mucins	Competitive inhibition/masking of mucin interactions	The steric and the deformation induced dynamic barriers
Dynamic	Mucus secretion Deformation induced changes in mucin matrix	Pharmacological alteration of mucus secretion (outside the scope of this article)	

We can consider that all three barrier properties are to some degree interlinked, with the same intermolecular interaction sites having the potential to be involved in entrapping particles as part of the interactive barrier and in contributing to the matrix structure, with the dynamic nature of the gel leading to potential exchange of roles over time. As a result of this interventions that alter one feature of the mucus barrier may also contribute to an alteration in another feature of the barrier, even if this is not the primary goal. The mechanisms behind the interconnectedness of these barrier elements are summarized in tableTable 2.

Table 2.Table 2 The interplay between the different mucus barriers.

alt-text: Table 2

Influence of	mMechanism
Steric barrier on interactive barrier	Pore size and intermolecular distance in matrix influences number of potential simultaneous interactions with a given nanoparticle
Interactive barrier on steric barrier	Immobilizing interactions of non-mucin components with the mucin matrix can reduce effective pore size Strong mucin – non mucin interactions can induce mucin clustering leaving larger pores
Interactive barrier on dynamic barrier	Interactions with non-mucin components may alter the mobility of mucins within the matrix and alter the response to applied deformation
Dynamic barrier on steric barrier	Deformation induced making and breaking of matrix forming interactions may alter pore size and/or pore size distribution
Dynamic barrier on interactive barrier	Mucus secretion provides a continual supply of new mucins with high interaction potential

Table 3.Table 3 Summary of essential elements linked to modifying barrier properties by increased mucus hydration.

alt-text: Table 3

Strategy	Evidence	Pro	Con
Osmotically active agents (hypertonic)	Increased cellular fluid secretion Increased pore size Reduced steric barrier	Generally safe Reduced steric barrier Decreased sol viscosity	Initial mucus de-swelling Mucus swelling increasing distance for NP diffusion Network interactive barrier unchanged
Hypotonic aqueous formulations	Epithelium fluid uptake «Tidal flow» Mucus gel swelling Increased pore size	Generally safe Flow directed towards the epithelium Reduced steric barrier Decreased sol viscosity	Mucus swelling increasing distance for NP diffusion Network interactive barrier largely unchanged

Table 4.Table 4 Summary of essential elements linked to modifying barrier properties by use of mucolytic agents.

alt-text: Table 4

Strategy	Evidence	Pro	Con
N-acetyl cysteine	Breaks S—S bonds in multimeric mucin Liquefies mucus gels Increased mucus mobility	Apparent increased NP mobility (moving with mucus rather than through	Increases sol phase viscosity Increased viscous drag Increased interaction potential Corona build-up and increased NP size Reduced first line of defense due to mucus structure breakdown
DNase	Depolymerizes DNA within mucus Reduces mechanical properties of mucus gels	Mucin network not affected Increased pore size	Increases sol phase viscosity Increased viscous drag

		Reduced steric barrier	Increased interaction potential Corona build-up and increased NP size

Table 5. Summary of essential elements linked to modifying barrier properties by inhibiting mucus interactions.

alt-text: Table 5

Strategy	Evidence	Pro	Con
Modified NP surface (PEGylation)	Increased NP mobility Reduced NP electrostatic and hydrophobic interaction potential	Increased NP mobility	NP surface modification needed Increased complexity
Mucus pretreatment; Triblock polymer	Amphiphilic polymer masking hydrophobic interaction sites Increased polystyrene NP mobility	No NP surface modification Works with hydrophobic NP's	Potential solubility issues related to amphiphilic polymer micelle formation Treatment 'window' (dynamic barrier) Effect on surface charged NP's unclear
Mucus pretreatment; Oligo-guluronate	Charged oligomer Increased NP particle mobility and uptake in a number of mucus systems	No NP surface modification Works with a number of charged NP's Easily soluble Non-toxic	Treatment 'window' (dynamic barrier) Potential reduced first line of defencedefense due to opening mucus structure in treatment 'window'

2.2 Why use mucus modulating agents to improve mucosal nanoscale drug delivery?

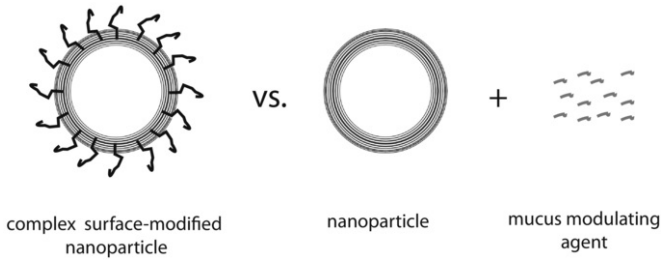
Given the important physiological functions of mucus and the existence of 'stealth coatings' [19] to prevent nanoparticles becoming entrapped in mucus (both reviewed elsewhere in this issue) one must address the question of what motivation exists for using mucus modulating agents to improve mucosal nanoscale drug delivery. Whilst there may be many specific motivations, two broadly applicable elements to consider here are

1. Existing pathologically induced alterations in mucus and
2. the industrial scale manufacture of nanomedicines.

Mucosal delivery routes may be used for formulations where the goal is systemic drug distribution but they may also be used for local administration of drugs to treat mucosal diseases. In the latter case the disease itself may give rise to changes in mucus volume and composition, potentially resulting in both altered barrier properties and an impairment in physiological function [22,23]. Under these circumstances mucus modulating agents can act not only to improve nanoparticle drug mobility in mucus and thereby functional drug therapy, but also to improve aspects of physiological function such as mucocilliary clearance in lung diseases as in the case of guluronate oligomers, which in addition having the potential to modify mucus barrier function (sSection 4.3), are also under development as an API to enhance mucociliary clearance in cystic fibrosis [24,25].

Functional drug therapy in the clinic is also wholly dependent on effective industrial production of the drug product even before market authorisation is achieved, with the European Medicines Agency EMA stating that "In certain cases, it is considered necessary to provide production scale validation data in the marketing authorisation dossier at the time of regulatory submission, for example when the product is a biological/biotech product or where the applicant is proposing a non-standard method of manufacture" [26]. In the case of nanoparticle drug delivery systems, particularly those designed to deliver biologics, the challenges in upscaling to industrial production are significant [5], both in terms of the technological challenges in producing the nanoparticles to the required specifications [27] and in keeping the unit cost low enough that it can be accepted by payors in the health systems [28]. The more complex the nanoparticle DDS is, in terms of number of components or required surface modifications, the greater these challenges become [27,28] and it has been noted that scaling up from preclinical small batch production is not always possible even for clinical trials [5] Whilst some researchers have focused on the scalability of nanoparticle production [29] it has generally not been a focus when investigating novel functional drug delivery systems. As such, commercial considerations may drive an interest in strategies that can enable relatively simpler, and therefore more easily manufactured, nanoparticles to function effectively in a therapeutic context. One such strategy is to utilize a mucus modulating agent to reduce the effective mucus barrier met by a nanoparticle for mucosal delivery, and thus improve drug uptake, which is a fundamental prerequisite for therapeutic efficacy. In this manner one highly complex manufacturing challenge (the complex surface modified nanoparticle) may be replaced by two comparatively simpler manufacturing processes (Figfigure. 2). Of course, any benefit from manufacturing simplicity must be considered as part of a total drug development strategy. The use of two agents in combination has potential implications for pre-clinical and clinical development, including toxicology, safety, efficacy etc. in terms of the potential for

increases in both costs and timelines, and challenges with dosage optimization, and should not be regarded as a panacea.

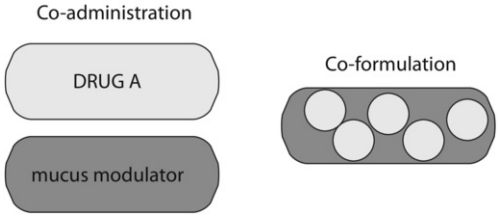


~~Figure 2.~~Fig. 2 Nanoparticle plus mucus modulating agent as an alternative to complex surface modified nanoparticles.

alt-text: Fig. 2

~~3.~~3 Co-association of nanoparticles and mucus modifying agents

In this review we consider the term co-association to cover both co-administration and co-formulation of nanoparticles and mucus modifying agents. In the case of co-administration, the mucus modulating agent and the nanoparticle are contained within two separate dosage entities, which are administered at the mucosal surface either simultaneously or in immediate succession. In the case of co-formulation, the nanoparticle and the mucus modifying agent are contained within the same dosage entity, for example within the same capsule, which may be coated to release the contents in a particular intestinal segment, or spray dried together to form microparticles for inhalation [1] (Fig~~figure~~. 3). A third situation that may fall within the scope is the non-covalent association of mucus modifying agents on a nanoparticle surface, however given that the nanoparticle itself may be formed through non-covalent interactions, such as in the case of polyplexes, in this situation the surface modification could also be considered to be part of the nanoparticle itself rather than a co-associated entity. It should also be noted that nanoparticles themselves may have the potential to induce alterations in the functional properties of mucus, including the barrier properties, although these changes are hypothesized to be the result of nanoparticle entrapment/interaction within the mucus matrix and as such may not have a great influence on the uptake of the nanoparticle itself [30–32].



~~Figure 3.~~Fig. 3 Schematic of co-administered and co-formulated nanoparticle and mucus modulating agent. With co-administration the nanoparticle and the mucus modulating agent are contained within two separate dosages whereas co-formulation combines them in a single dosage.

alt-text: Fig. 3

For a co-association of a nanoparticle and a mucus-modifying agent to provide an effective pharmaceutical treatment both need to arrive at the mucosal surface in a functional state without negative alterations as a results of physiological processes. Additionally, the mucus modifying agent must bring about the modifications of the mucus barrier within a timescale where the nanoparticle is present and in a functional state. As such pharmaceutical formulation technology will have a central role to play in the development of clinical products, as it has for mucosal delivery of small molecule APIs today [33,34]. In the case of co-administration of a nanoparticle and a mucus modifying agent there may be the possibility to utilize mucomodulating agents that are already in clinical use, for example DNase (~~s~~Section 4.2), *n*-acetyl cysteine (~~s~~Section 4.2) or mannitol (~~s~~Section 4.1) by co-prescribing these with the nanoparticle drug, however this does not remove the need for a clinical development pathway.

~~4.~~4 How may the mucus barrier be modified?

Theoretically it is possible to target all three components of the mucus barrier; the steric, the interactive and the dynamic. However, in this review the focus is on co-association of mucus modulating agents and nanoparticles, which excludes systemic administration of drugs that reduce mucus secretion (the major component of the dynamic barrier). As such we will focus on agents which alter mucus barrier properties after direct application to the mucus

gel, and thus consider primarily modification of the steric and interactive components of the barrier in terms of induced alterations in matrix architecture and pore size and strategies to reduce mucus – nanoparticle interactions (Tables 3-5).

~~4.1.~~4.1 Strategy 1. Increased mucus hydration

Increasing the hydration level of mucus can reduce barrier properties by

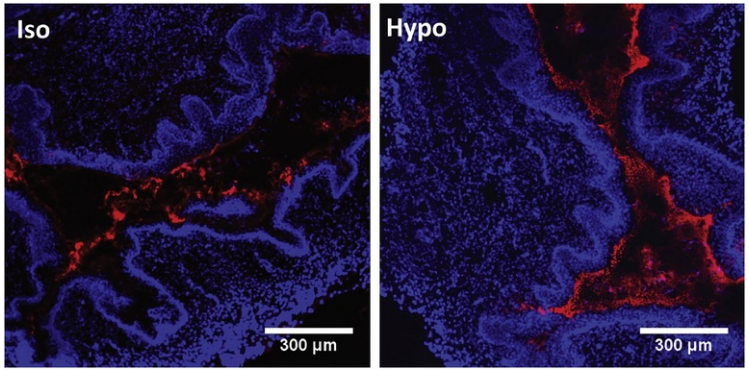
a) gel swelling, which increases the average pore size of the gel matrix [35–37]

b) the dilution factor, which decreases the viscosity of the sol phase within the pores of the mucus gel [37]

This may be a particularly relevant strategy in lung diseases where the mucus is known to be dehydrated compared to the normal state [23,38,39], but may also confer benefits in other situations. Blackmon and co-workers have recently published an elegant method demonstrating real time increases in the pore size, and corresponding reduction in the steric barrier properties, of mucus in mucus secreting cell cultures upon exposure to osmotically active agents (hypertonic saline) that drive cellular fluid secretion and hence increase mucus hydration. This study utilizes mucus secreting Calu-3 [40] or cultured human bronchial epithelial cells [41] as the model mucosa and diffusion sensitive optical coherence tomography to image the hydration of the mucus, which is determined by the diffusivity of gold nanorods. It may be argued that there are kinetic challenges connected to the use of hypertonic solutions of osmotically active agents to induce increased cellular fluid secretion without affecting the mucus volume in the first instance, which could initially lead to an increased steric barrier by mucus de-swelling. Indeed some results from Blackmon et al. do indicate that mucus hydration initially decreases before subsequently increasing as cellular fluid secretion is initiated [40]. Here the relative homogeneity/isotropy of mucus is likely to be important. If one considers the that the mucus layer is not isotropic but rather exhibits a natural variation both in depth and composition then this initial shrinking could lead to an increased discontinuity of the mucus layer, which in turn could lead to improved access the osmotically active agent (and any concomitant NPs) to the epithelium, something that may lie behind the phenomenon of mucus lifting from the epithelium as seen in the Blackmon study [40].

Ibrahim and co-workers have also investigated the effects on hydration on the barrier properties of mucus, utilizing mannitol to enhance transport of nanoparticle gene carriers through sputum [37]. Mannitol is an osmotically active inert sugar alcohol that can be administered to the lungs as a dry powder inhalation. Mannitol as an API is characterized as a mucolytic (R05CB) by the World Health Organisation [42] although it has no lytic ability and its presumed mode of action is, as for hypertonic saline, as an osmotic agent that draws water from the mucosal surface to increase mucus hydration. Also in this case kinetic challenges exist, as presumably the mannitol is first solubilized in water drawn out of the surface mucus layer before it is able to diffuse through mucus to the epithelium and draw water from the cellular compartment into the mucus. It should be noted that Ibrahim and co-workers report that mannitol improves nanoparticle mobility in model sputum in the absence of cells (or other water containing compartment) [37]. It is possible these results reflect water being drawn from the model sputum, thus dehydrating it, and reducing the effective NP concentration in an aqueous non-sputum phase, and this reduction in NP concentration has been erroneously attributed to NP diffusion into the sputum phase.

Another strategy, which is likely to increase mucus hydration as a secondary effect although this is not the primary goal, has been employed by the group of Justin Hanes. Hypotonic aqueous formulations have been used as a vehicle for nanoparticle administration at the absorptive mucosal surfaces of the vagina and colorectum (Fig~~ure~~. 4). These formulations induce fluid uptake by the epithelium which creates a tidal flow that drives the nanoparticle formulation through the mucus [43,44]. These studies have utilized mucus penetrating (‘stealth’) nanoparticles which have inherently good mucomobility, but it can be assumed that the presence of hypotonic aqueous formulations will also promote mucus gel swelling, an increase in mucus pore size and a reduction of viscosity in the sol phase [35,45]. The functional result of these hypotonic aqueous formulations (as opposed to their isotonic counterparts) is nanoparticle distribution through the depth of the mucus layer and to the cell surface, a prerequisite for cellular uptake.



~~Figure 4.~~Fig. 4 Vaginal distribution of 100 nm fluorescent MPP administered in isotonic (iso) or hypotonic (hypo) solution. MPP distribution in transverse mouse vaginal cryosections. Tissues were collected and frozen immediately after particle administration. Images are representative of $n = 3$ mice. Cell nuclei stained blue with DAPI.

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alt-text: Fig. 4

~~4.2.~~4.2 Strategy 2. The use of mucolytic agents

Mucolytic agents can be defined as agents that depolymerise (lyse) mucins or one of the other polymeric components of mucus or sputum, with DNA and actin being the most common non-mucin targets. Mucolytics may be used as a therapy in their own right or to enhance the delivery of other therapeutic agents across secreted mucus barriers. Whilst mucolytics have been investigated for their ability to improve therapeutic delivery at a variety of mucosal surfaces [46,47], they have most widely been used in the context of pulmonary delivery in cases of lung disease (particularly cystic fibrosis) where the mucus is abnormally thick and viscous with a high load of non-mucin components [21,48–50].

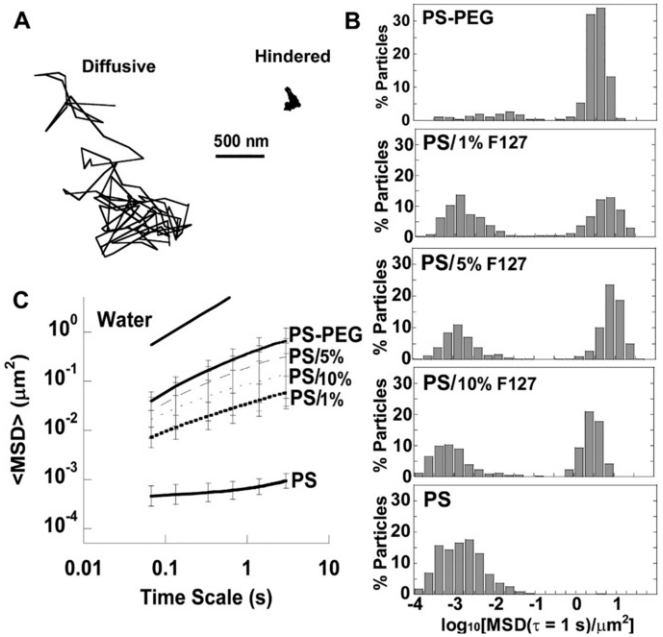
Sanders and coworkers used a diffusion chamber set-up to demonstrate that cystic fibrosis sputum was a significant barrier to the transport of nanospheres, with larger nanoparticles (560 nm) being functionally immobilized [50]. They found that DNAse moderately facilitated the transport of nanoparticles through CF sputum and hypothesized that whilst DNAse reduced the macroscopic viscoelasticity of sputum through depolymerization of DNA it is possible that the resultant DNA fragments accumulated in the sol phase in the mucus gel pores increasing the viscous drag on diffusing particles and hence counteracting improvements in particle mobility brought about by the reduction in polymeric DNA. It is also possible that polymer fragments may adhere to the nanoparticle surface as part of the corona, increasing the effective nanoparticle radius. Similar considerations are brought to light by Dawson and co-workers who, using multiple particle tracking showed that particle transport in cystic fibrosis sputum was highly heterogeneous and that whilst DNAse treatment reduced the bulk viscoelasticity of the sputum the effect on particle transport was primarily to reduce the variability rather than lead to direct improvements in average mobility [48]. Broughton-Head and co-workers, like Sanders used a diffusion chamber set-up to investigate mobility of nanoparticles in cystic fibrosis sputum and in model matrices composed of the major polymeric components of CF sputum [21]. They found that whilst both DNAse and the mucin reducing agent *n*-acetylcysteine could improve nanoparticle mobility it was dependent on the composition of the mucous matrix, with actin being found to limit the effect of these mucolytics on nanoparticle mobility. Interestingly, these studies all utilized nanoparticles with charged surfaces which, unlike 'stealth' coated nanoparticles [19], can interact with the mucin matrix so the interactive barrier of mucus also comes into play here. Suk and co-workers have investigated the barrier posed by cystic fibrosis sputum to highly compacted DNA nanoparticles that had a PEGylated ('stealth') surface to reduce interactions with mucus. They found that despite PEGylation the particles were still somewhat hindered by the CF sputum, perhaps because the PEG coating was not sufficiently dense. In this case *n*-acetylcysteine or *n*-acetylcysteine + DNase where found to increase particle mobility whereas DNAse alone did not [51], which is in broad agreement with the results of Dawson and co-workers [48]. Unlike the situation for hypertonic (osmotically active) agents, where an additional water-containing compartment (the cell) is required for functionality, mucolytic function is achieved directly within the mucus phase. The depolymerisation of macromolecules within mucus will lead to a reduction in matrix cross-link density irrespective of external hydration factors. However, external hydration factors may still influence the degree to which mucus barrier properties are altered by mucolytic induced reduction in matrix cross-link density. Firstly, as hypothesized by Sanders et al. depolymerisation of macromolecules may result in the accumulation of degraded material that increases the viscosity of the sol phase. In this case increased mucus hydration will effectively reduce the concentration of this material in the sol phase thus reducing the viscosity and the barrier properties. Secondly increased mucus hydration can be equated with increased mucus swelling, which will maximize the effect of a reduction in cross-link density on mucus pore size. Particle tracking studies, which utilize a single mucus phase, will not be

affected by changes in mucus hydration unless an additional water phase/compartment is actively included in the experimental setup. In the case of diffusion chamber studies, whilst an additional water phase is present, steric restrictions of the mucus phase such as sandwiching between filters [50] will limit the potential for mucus swelling.

Both *n*-acetyl cysteine and DNase are in clinical use as mucomodulating treatments in lung disease.

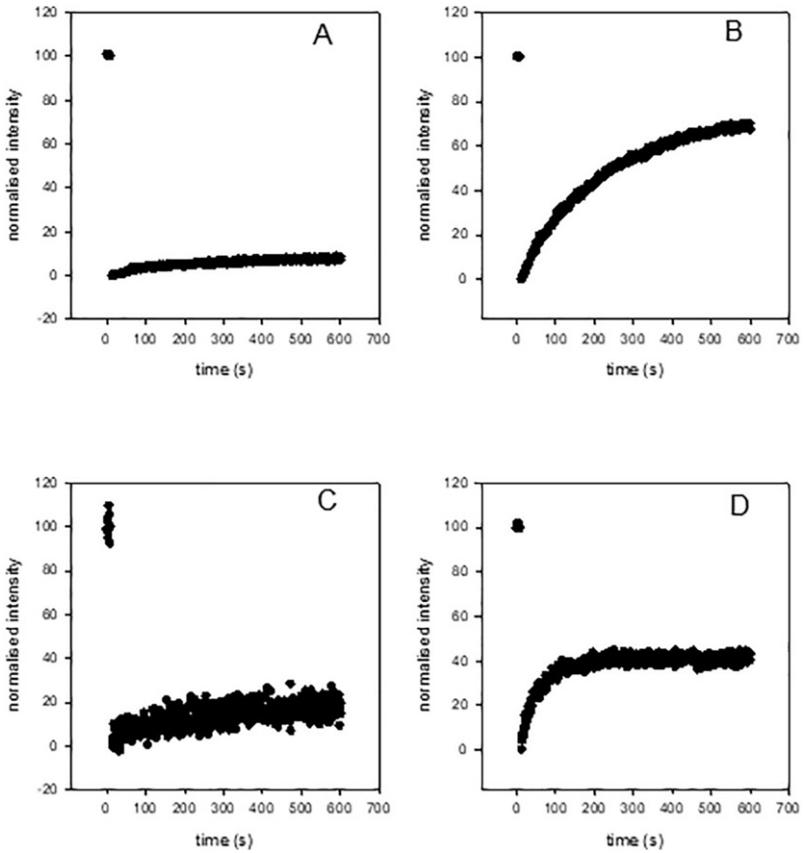
~~4.3.~~4.3 Strategy 3. Inhibition of mucin interactions

Interactions between the nanoparticle surface and the mucus gel matrix, the interactive component of the mucus barrier, have been shown to cause a significant reduction in mucomobility of particles small enough to be unaffected by the steric barrier. Strategies to avoid entrapment by the interactive mucus barrier have predominantly been focused on modifying the nanoparticle surface in a manner that reduces the potential for interaction with mucins, most commonly by PEGylation [19]. This strategy is covered elsewhere in this issue of ADDR. However, there have also been examples of the opposite approach, that is attempts to modify the interaction potential of the mucin gel matrix and thus reduce immobilizing interactions with nanoparticles [24,25,52]. Ensign and co-workers have investigated the use of Pluronic F127 (a PEG – PPO – PEG triblock polymer) to pre-treat cervicovaginal mucus rather than to surface modify nanoparticles [52]. They demonstrated that this mucus treatment rendered normally mucus immobilized polystyrene 200 nm nanoparticles mucomobile without altering the mucus pore size or the barrier to herpes simplex virus (Fig~~ure~~. 5). Fig~~ure~~. 5 illustrates examples of nanoparticle trajectories for typically diffusive and hindered (entrapped) nanoparticles, the mean square displacement (a measure of the average distance moved by the particles) over time for particles in treated and untreated cervicovaginal mucus, and the distribution of particle mobilities in the ensemble. Despite significant improvements in average particle mobility a fraction of the particles remain functionally immobile. The authors propose that the Pluronic F127 treatment of mucus blocks hydrophobic binding sites on the mucins, reducing the ability of the mucin matrix to interact with hydrophobic particles, whilst leaving electrostatic interactions, which immobilize the herpes simplex virus unaffected. They also attribute the lack of changes in matrix architecture to the hydrophobic PPO portion of the Pluronic F127, which they hypothesize inhibits insertion into hydrophilic mucin bundles thus leaving the matrix structure intact [52]. Given the physiological protective functions of mucus there is an inherent risk associated with altering mucus barrier properties and potentially leaving the mucosa exposed to pathogenic or damaging agents (these risks are reviewed in another article in this issue). It is therefore a significant finding that it is possible to selectively enhance nanoparticle mucopenetration without a corresponding increase in pathogen mobility based on a detailed understanding of the immobilization mechanism of both NPs and pathogens and the fundamental properties and behavior of the mucus gel. A different mucomodulating action has been demonstrated for short oligomers of guluronic acid [24,25]. These negatively charged, hydrophilic molecules have been shown to alter both the interactive and the steric barrier of both normal physiological mucus from the porcine gastrointestinal tract [24] and cystic fibrosis sputum [25], as well as to interact directly with both pig gastric mucins [24,25] and the purified MUC5AC/MUC5B component of sputum [25] and to increase nanoparticle uptake in an mucus secreting HT29-MTX model [24]. Fig~~ure~~. 6 shows the mobility (by fluorescence recovery after photobleaching) of negatively charged nanoparticles in pig gastric mucus and purified pig gastric mucin in the presence and absence of guluronate oligomers. The particles are functionally immobile before treatment, as seen by the minimal recovery of fluorescence, but after treatment fluorescence recovery, indicative of particle mobility is seen. As with the results with Pluronic F127, an immobile fraction remains after treatment, with more particles gaining mobility in the native mucus as opposed to the purified mucin. Unlike Pluronic F127, the guluronate oligomers appear to exert an influence on the mucin - mucin interactions maintaining the architecture of the mucus gel matrix. This manifests as in an increase in pore-size within the gel matrix, however, the mucus maintains its bulk hydrogel integrity. These changes also alter the rheology of the mucus gel, particularly pathological mucus with a high load of non-mucin components for example sputum from cystic fibrosis patients, and these changes in rheology also increase the mucociliary clearability of the mucus [25]. Increased mucociliary clearability may have negative consequences for drug delivery as a result of reduced contact time between the drug and the mucosal surface. However, it should be remembered that a drug, nanoparticle or otherwise, can only be taken up once it has crossed the mucus layer so ultimately it is the relative timescales of NP diffusion in mucus and mucus clearance that are important in terms of effective drug delivery. This issue has been raised in regard to clinical trials of a liposomal gene therapy in cystic fibrosis [53] where it was noted that concurrent use of mucoactive therapies included hypertonic saline could potentially alter residence time and thus effective gene transfer, although interestingly the potential for hypertonic saline to alter mucus barrier properties was not discussed. The trial results did not appear to be influenced by concurrent use of hypertonic saline [53], which may suggest that if either the barrier modification or increased clearance where clinically relevant then they cancelled each other out. It is also worth noting that pathological mucus, such as cystic fibrosis sputum, is known to pose a greater barrier to NP mobility than normal physiological mucus, something that has been attributed to the high load of non-mucin components [21,48,50]. Given this, it may be possible to titrate the dosage of mucus modifying agents to adjust mucus barrier properties towards the normal state, which may reduce the potential risks associated with a reduced barrier function. Additionally, for agents which modify the mucus barrier by interacting with matrix components and thus shielding potential interaction sites for NPs, the continual secretion of fresh unmodified mucus and the erosion/clearance of the modified mucus will provide a natural limitation to their window of activity.



~~Figure 5.~~Fig. 5 Transport of 200 nm particles in CVM pretreated with high concentrations of F127 (indicated by -/% F127). (A) Representative trajectories of diffusive (left) and hindered (right) PS in CVM pretreated with 1% F127. (B) Distributions of the logarithms of individual particle mean square displacement (MSD) at a time scale of 1 s. (C) Ensemble-averaged geometric MSD ((MSD)) as a function of time scale for PS–PEG and PS, including the theoretical MSD in water (W). Data are means ± SEM (≥ 3 independent experiments, with $n \geq 100$ particles

alt-text: Fig. 5



~~Figure 6.~~ Fig. 6 FRAP curves for 200 nm carboxylate modified fluospheres in native pig gastric mucus (A, B) and 20 mg/mL purified pig gastric mucin (C, D), with (B, D) and without (A, C) 4.8 mg/mL guluronate oligomers added.

alt-text: Fig. 6

~~5.~~5 Gastrointestinal luminal contents and the mucus barrier

The gastrointestinal lumen contains a wide array of components that could potentially alter the barrier properties of mucus, and the composition of the luminal contents varies over time depending on nutrient intake and the digestive process. The differences between fed and fasted state can significantly alter the adsorption of small molecular therapeutics and the challenges are likely to be even greater for nanoparticle therapeutics. Nevertheless, oral drug administration is a highly accepted route for the patient so despite the challenges there is a drive to investigate this route. Whilst a detailed analysis of the effects of luminal contents on the mucus barrier is beyond the scope of this review, we wish to highlight certain elements that are relevant to the strategies for modifying the mucus barrier properties that have been discussed above.

Firstly, whilst guluronate oligomers have been shown to increase the permeability of mucus (including gastrointestinal mucus) to nanoparticles and to improve the expansion of intestinal mucus in cystic fibrosis mice [54], Mackie and co-workers have shown that high molecular weight alginate (a copolymer of guluronate and mannuronate units) rather decreases the permeability of intestinal mucus [55]. This apparent size effect may be related to the ability of high molecular weight alginate to induce additional cross links in mucous systems [56], though Mackie and co-workers found no evidence for direct interactions in their study [55]. Datta and *co*-workers have recently published a study investigating the ability of polymers in the gut to compress the colonic mucus hydrogel [57]. Their data suggest that luminal polymers can compress the mucus layer through a combination of entropic and enthalpic effects in a size dependent manner, with higher molecular weight polymers inducing greater compression [57], which, as the reverse effect of mucus gel hydration discussed above, could be predicted to increase the barrier properties of the mucus. It is possible that similar effects play a role in the alginate induced increase in mucus barrier properties reported by Mackie.

Secondly, Yildiz and co-workers have reported that physiological lipids can increase the barrier properties of mucus [58] and Macierzanka and co-workers have reported that amphiphilic bile salts improve particle, but not bacteria, penetration of intestinal mucus [59]. This is clearly of relevance for oral dosing of nanoparticle therapeutics but is also interesting given that the amphiphilic Pluronic F127 has also be shown to improve particle but not pathogen (in this case virus) mobility in mucus [52]. It is likely that the complex and varied nature of the intestinal luminal contents will influence the reproducibility of intestinal NP uptake.

6.6 Conclusions

It should be evident from the present review that mucus and mucus modifications are very complex areas where manipulation of one barrier more often than not will lead to a change in another barrier function because their highly interlinked nature. On top of that, not all barrier modulation strategies are universally applicable as different mucosal surfaces will provide different challenges (e.g. airway mucus vs. intestinal mucus). At the same time we also hope it has become clear that the overall mucus barrier to nanoparticles can, at least in theory, be modified by co-administration or co-formulation with modulating agents. If such systems will reach a practical/clinical level depends largely on the complexity of the formulation, and hence the probability of a feasible industrial production. Mucus modulation strategies are likely to be most applicable to application where the mucus is already abnormal, where stealth modifications of nanoparticles are difficult from a production point of view or in combination with stealth modifications for optimal uptake of for example extremely costly therapies.

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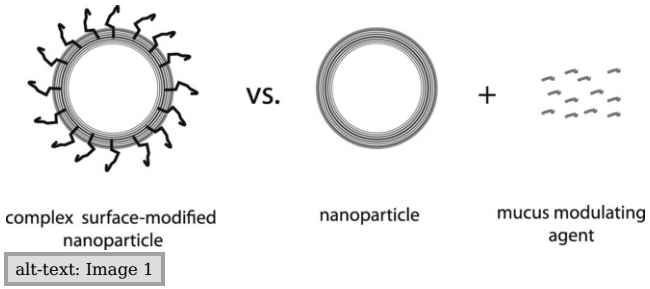
Graphical abstract



alt-text: Image 1

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