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Mucoadhesion across scales: Towards the design of protein-based adhesives

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ABSTRACT

Mucoadhesion is a special case of bioadhesion in which a material adheres to soft mucosal tissues. This review elucidates our current understanding of mucoadhesion across length, time, and energy scales by focusing on relevant structural features of mucus. We highlight the importance of both covalent and non-covalent interactions that can be tailored to maximize mucoadhesive interactions, particularly concerning proteinaceous mucoadhesives, which have been explored only to a limited extent so far in the literature. In particular, we highlight the importance of thiol groups, hydrophobic moieties, and charged species inherent to proteins as key levers to fine tune mucoadhesive performance. Some aspects of protein surface modification by grafting specific functional groups or coupling with polysaccharides to influence mucoadhesive performance are examined. Insights from this review offer a physicochemical roadmap to inform the development of biocompatible, protein-based mucoadhesive systems that can fulfil dual roles for both adhesion and delivery of actives, enabling the fabrication of advanced biomedical, nutritional and allied soft material technologies.

1. Introduction

Mucoadhesion is a special case of bioadhesion in which materials adhere to mucosal membranes [1], so that it is directly involved in, *e.g.*, coating and protecting damaged tissues [2]. While mucoadhesive materials are not inherently classified as high cellular uptake drug delivery systems [3], their prolonged residence within the mucosal environment may offer advantages such as localized and sustained delivery [4]. This offers further benefits as compared to parenteral or oral administration of non-mucoadhesive materials, including enhanced patient compliance [5] and painless drug delivery directly to the bloodstream [6]. Mucoadhesive properties are also pivotal in the development of mucosal vaccines for cancer immunotherapy [7], and for nasal drug delivery systems [8]. The key mechanisms that explain mucoadhesion are derived from the ones that underpin bio-adhesivity in general [9]. However, the presence of multiple length scales in mucoadhesive interactions [10] requires specific consideration. Here, we review the current literature critically to offer insights into the mechanisms for the mucoadhesive process, relating multiple length, strength, and time scales for the observed phenomena.

Substantial progress in the development of mucoadhesive materials is already documented in several reviews [11], encompassing how mucus interacts with hydrogels [12], nanocarriers [13,3], and the implications for transmucosal drug delivery [14]. Some reviews focused on the comparison between mucoadhesion and mucopenetration technologies [15–17], alongside recent advances in the field for a diverse array of applications employing polymeric mucoadhesives such as PEG, cellulose derivatives, chitosan, xanthan gum, pectin, and alginate [18–22]. Proteins as therapeutic agents have also been incorporated in polysaccharide-based matrixes [23]. However, apart from lectins, known for their cell membrane recognitions and subsequent cellular adhesion [24], limited attention has been paid to protein-based mucoadhesives. A recent review [25] highlights the potential benefits of biodegradable, naturally-occurring proteinaceous materials, such as whey protein [26], in enhancing the bioavailability of encapsulated active ingredients *via* mucoadhesion due to their high functionality, biocompatibility, and cost-effectiveness. In the commercial space, a milk-protein-based mucoadhesive Loramyc® (Europe) is currently used for the oral treatment of mucosal disease *i.e.* candidiasis [27]. While the interaction between various food proteins and salivary mucins has been

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well-documented [28–30] in the context of oral processing and sensorial properties, we know of no review that explores the role of proteins as potential mucoadhesive materials in the light of their interactions with mucin.

Therefore, in this review, we discuss the mechanisms of interaction of mucoadhesive systems with mucus across length, time, and strength scales with a specific focus on protein-based mucoadhesive materials. First, we give a comprehensive introduction to mucus, including the structure of mucin. We also discuss its key macro and micro-scale rheological properties alongside understanding its alteration owing to site and disease conditions. This gives us a stepping stone to understand the mucosal disparities across sites and disease condition to highlight the range of tailored properties a mucoadhesive material may need on site of administration. We then provide underpinning principles of the length, time and strength scales that may affect mucoadhesion highlighting key covalent and non-covalent interactions. We then focus on mucoadhesive interactions of proteins. The impacts of surface modification of proteins, such as by grafting specific functional groups to enhance covalent or non-covalent interactions, are also discussed. We have compared the less-studied protein mucoadhesion with the more established landscape of glycan-based mucoadhesion, where appropriate. Oil-based mucoadhesives, including emulsions, are outside the scope of this review. Informed by the importance of the multiscale understanding and summarizing the various mucosal interactions, we pinpoint the design principles relevant to fabricating the next-generation protein-based mucoadhesives for advanced biomedical technologies.

2. Mucus: what do we know so far?

2.1. Composition, function, and structure

Mucus is a highly hydrated biological barrier [31] in an aqueous bioactive environment [32] that includes inorganic salts, lipids, nucleic acids, mucinous glycoproteins, *etc.* [33]. Mucin glycoproteins belong to the MUC gene family that, in humans, includes 21 known protein-coding MUC genes [34], each of which has a specific tandem repeat size on their amino acid sequence and is expressed within specific tissues. Mucins can be classified into three main groups based on their structure: secreted oligomeric gel-forming mucins (GFM), secreted monomeric non-gel-forming mucins (NGFM) and monomeric membrane- (or cell-)associated mucins [35]. While secreted mucins make up the outer layer of mucus, membrane-associated ones are found closer to the cell walls.

Fig. 1a-b shows a schematic illustration of the general bottle-brush structure of secreted mucins, with a protein backbone that contains *O*-linked oligosaccharide substitutions. The protein backbone is mainly composed of serine, threonine and proline (s/t/p regions) [36]. Secreted mucins bear cysteine-rich domains (CYS), which are responsible for intra-domain disulfide bonds [37]. The glycan substitutions usually first appear with *N*-acetylgalactosamine (GalNAc) groups linked to serine and threonine. The glycan chains are further elongated, varying from 6 to 18 monosaccharides, *via* repeated sequences of *N*-acetylglucosamine (GlcNAc), galactose (Gal) and *N*-acetyllactosamine (LacNAc) groups (Fig. 1b), which may be terminated with carboxyl (sialic acid) or sulfate groups (heparin, for example) [38], making mucins negatively-charged at neutral pH, with an isoelectric point (IEP) between pH 2 and 3 [39]. Other terminal groups include fucose, a sugar molecule that contains a methyl group and one fewer hydroxyl group than galactose and glucose, which enhances hydrophobic interactions with other materials [40,41] and thus may contribute to mucoadhesive performance of hydrophobic materials. Membrane-associated mucins relate to the cell's glycocalyx formation and have other structural features, such as a characteristic cytoplasmic tail, epidermal growth factor (EGF) domains and transmembrane domains [31].

In the airway epithelium, the presence of ciliated cells (Fig. 1a) is associated with another important aspect of the mucus hydrogel structure. This structure is described as a gel-on-brush system, with distinct structural characteristics across different domains, namely the mucosal and periciliary-glycocalyceal layers [42]. The mucus gel layer, rich in GFM, exhibits a more ordered structure [38]. The periciliary layer (PCL), constituting the brush part of the system, is found closer to the membrane epithelial cells, where the cilia are located, containing membrane-bound mucins and tethered mucopolysaccharides. It is important to note that the membrane-associated mucins do not form highly viscous solutions like GFM. However, the tight mesh structure of the PCL layer prevents mucus from penetrating the interciliary space and plays a key role in controlling the water distribution between the two layers due to osmotic forces [42]. This dense PCL structure also has strong barrier properties, as evidenced by the mesh size of this layer: particles with 5 nm in diameter can reach the cell surface, while particles with radii >40

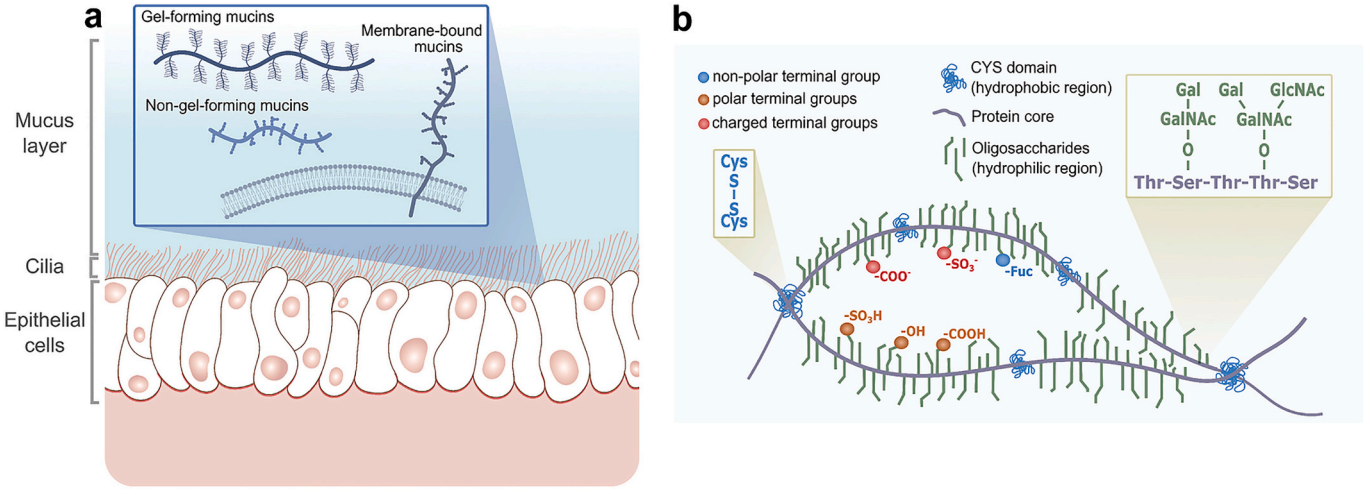

Fig. 1. Diagram of mucus structure (a) showing the membrane cells, cilia, and mucus gel with a schematic representation of membrane- (or cell-) bound mucins, secreted gel-forming mucins and secreted non-gel-forming mucins. Structural illustration of the gel-forming mucin structure (b) is represented with a purple and blue protein core (mostly hydrophobic regions) and green hair-like glycan chains (mostly hydrophilic regions). The glycan chains may be terminated in either polar or negatively charged groups represented in orange and red, respectively, depending on the pH and ionic strength of the surrounding environment. Non-polar terminal groups such as fucose can also be present at the termini of the glycan chains. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

nm are completely excluded from the PCL [43]. Lastly, the structure of this layer also allows the cilia to move and aids in defense mechanisms for the removal of foreign pathogens from the airways, for example.

Beyond mucus clearance, membrane-associated mucins have a crucial role in inhibiting viral infections due to a repulsive steric hindrance mechanism [44], while secreted mucins do not exhibit this protective effect. When considering mucosal adhesion, the structure of the secreted mucins is therefore of utmost importance, as further discussed later in this review, whilst the entire structure of the gel layer may impact the mucosal penetration phenomena.

2.2. Physicochemical properties of mucus

Mucus is a gelled network that coats epithelial cells, serving as a barrier in biological environments [45]. For humans, the range of thickness for the mucus layer starts at a few μm (in the ocular region) [46] and goes up to 1000 μm in the stomach [47]. In the oral cavity, it is around 100 μm [48], while the precise thickness of the mucus layer remains uncertain for some regions such as the small intestine [49]. Uncertainties in literature values probably reflect the difficulty of performing measurements *in vivo*, especially in the gastrointestinal tract, which has two layers, a loosely adherent one and a firmly attached layer [50]. The thicknesses and cell types of each mucosal layer may impact the systemic delivery of drugs [6].

Before we can discuss specificities of mucoadhesion, we need to understand mucus, and, as the following sections will show, there is no universal property for mucus, and the properties vary a lot depending on conditions at different organs. Herein, we follow Lai and co-authors [51] and describe some physical properties of mucus in terms of macro- and micro-rheology, for different body sites and health conditions. Macroscale rheology relates to the elastic or storage modulus (G') and to the viscous or loss modulus (G"), covering the non-Newtonian bulk behavior of mucus, while the microscale rheology pertains to the microstructural organization, covering pore size distribution, specific chain entanglements and inherent microheterogeneities.

2.2.1. Macrorheology of mucus

The viscoelastic properties of mucus are crucial for its tissue protective function and are attributed to the presence of gel-forming mucins (GFM), whose properties are influenced by other proteins such as calcium-binding proteins and keratin [52]. The non-Newtonian behavior of mucus encompasses a shear-rate dependent behavior and a time dependent behavior. For mucus, the shear-rate dependence is characteristic of a pseudoplastic or shear-thinning material, displaying resistance to deformation at low shear rates and fluid-like behavior at higher shear rates [38]. Once sheared, the viscoelastic properties of mucus recover in a rapid and reversible way [51]. This recovery on the other hand is related to the time-dependent behavior as mucus is a thixotropic material with rapid recovery of gel properties after its disruption, as shown for pig stomach mucus [53]. These properties allow mucus to effectively coat and safeguard tissues, providing a barrier against external agents, and avoiding the gravity-induced flow towards the alveoli, for instance [51]. A more formal explanation of this mucus restructuring behavior includes its consideration as a metastable arrested phase separation gel [53], in which dense regions become trapped in less-dense regions within the gel network.

At the macroscale, under quiescent conditions, mucus generally exhibits a higher G' than G". The phase angle ($\delta = tan^{-1}(G''/G')$), taking values between 0 and 90°, signifies the viscoelastic behavior of a gel, under linear rheology. A lower value of δ trending towards 0°, corresponds to mucosal gels with increased elastic character. Diseased cystic fibrosis (*CF*) mucus, for instance, shows $\delta = 16.2 \pm 0.6^{\circ}$ at 1 rad/s [54]. Higher phase angle values approaching 90° indicate a lower elastic character, and at 90°, the material behaves as a purely viscous material with both G' and G" increasing with frequency, often following an approximate power-law relationships. Amongst different types of animal mucus, Lai and coauthors (2009) reported a high variation in the phase angle values, ranging from 6 to 60 (from 0.01 to 100 rad/s) [51], demonstrating how significant the variations in the rheological properties are throughout the literature.

Table 1 provides values for G' in mucus from the respiratory tract at different angular frequencies showing how disease conditions may affect mucosal rheology. It has been demonstrated that evaluating G' and G" values at different shear frequencies requires multiple measurements to improve the reliability of rheological data for human *CF* sputum [55]. Irrespective of the frequencies used, it is clear that diseases conditions such as purulent rhinitis and bronchitis may directly impact the G' of mucus by two-three folds. Complex viscosity values, which can be obtained from G' and G" values, are also reported throughout the literature for mucus in various disease conditions [51,56–58].

Fig. 2 illustrates schematically how apparent viscosity values vary for typical diseased and healthy human mucus samples. The bulk viscosity of mucus secretions may vary by several orders of magnitude and may show varying degrees of shear thinning behavior, depending on body site and state of health. Under chronic sinusitis conditions, nasal human mucus is reported to have a viscosity value varying from 1.6 Pa s to 0.38 Pa s between 1 and 10 Hz [56]. Some pulmonary diseases such as cystic fibrosis (*CF*) are associated with an increase in the elastic character of mucus as well as increased viscosities, leading to a viscosity of 70 Pa s at 0.2 Hz for human *CF* sputum [54], whilst other conditions like bronchorrhea may lead to a decreased mucus viscosity [60]. Comparing viscosity values published at different strain rates or frequencies can be challenging; therefore, the literature commonly presents rheological measurements for mucus collected from different organisms, with comparisons between different groups and control groups. It is important to highlight that such comparisons are only meaningful if the measurements are done at the same frequency or shear rate. For example, Bucher and coauthors demonstrated a significant increase in nasal mucosal viscosity for patients with postnasal drip diagnosis, when compared to a control group of healthy patients [61].

It is important to consider all varying factors when comparing literature values, including sample properties as well as experimental procedure and method of analysis. For example, the non-linear rheological property of yield stress has been recently reviewed for mucus, and the obtained values for yield stress depend not only on the animal species, type of mucus, the region from which the mucus is taken, and state of health, but also on the rheological model used to fit the data [63]. Other sources of variability in the rheological parameters of mucus found in the literature arise from the use of different measurement techniques and from challenges in effectively controlling mucin concentration [43]. It is also important to consider the specific experimental regime being evaluated since mucus exhibits both transient and permanent interactions [64].

Some experimental parameters, such as shearing conditions (steady shear or dynamic oscillatory shear) and testing procedures (*e.g.*, creep recovery assays to fully identify viscoelasticity) can also lead to differences in results. For example, dynamic oscillatory shear (Fig. 2) is typically performed in the small strain limit and so does not strongly perturb the mucus structure, whilst steady shear might involve large deformations and structural reorganization of the material. As one might

Table 1
G' values obtained for nasal secretions and chronic bronchitis sputum for groups of patients ([55,59]).

	Nasal human mucus – Healthy	Nasal human mucus - Purulent Rhinitis	Bronchitis sputum
G' (Pa) at 1 rad/s	1399 ± 1.8 [59]	689 ± 310 [59]	520 ± 422 [59]
G' (Pa) at 100 rad/s	3123 ± 2.6 [59]	1774 ± 2.6 [59]	980 ± 722 [59]

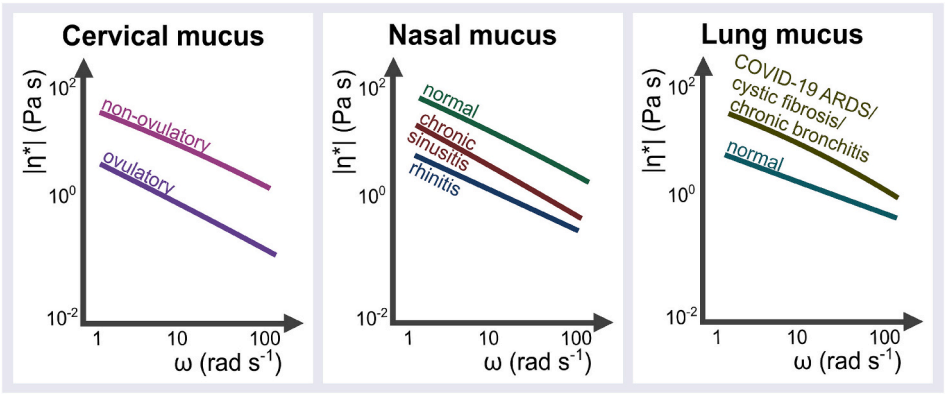

Fig. 2. Schematic representation of typical complex viscosity as a function of shear frequency plotted on logarithmic axes for different mucus samples. For the original values, the reader is referred to [51]. COVID-19 acute respiratory distress syndrome (ARDS) causes thick sputum samples, like *CF*, as reported previously [62].

expect, for complex fluids such as mucus, the shear-thinning profile and the viscosity obtained as a function of shear rate in steady shearing conditions do not match the complex viscosities measured in an oscillatory frequency sweep (reported in Fig. 2), *i.e.*, the Cox-Merz rule is not followed. In general, the complex viscosity $|\eta^*(\omega)|$ obtained *via* dynamic oscillatory measurements is higher than the apparent viscosity $\eta(\dot{\gamma})$ obtained using steady shearing conditions. This was reported for both *ex-vivo* porcine gastric material [65] and for rehydrated porcine gastric mucin solutions [66]. For mucin extracted from porcine small intestine, an increase in mucin concentration was responsible for a weak network identified only at low shear stresses (up to 3 Pa) [52]. This trend has also been reported for healthy non-ovulatory cervical mucus [51], for example, where the reported values for the $|\eta^*(\omega)|$ are approximately one order of magnitude higher when compared with $\eta(\dot{\gamma})$. For mucus, the Cox-Merz rule fails due to transient physical interactions which are dominant over the covalent ones, and the resulting microstructure depends on the deformation applied.

2.2.2. Mucus-mimetics

The use of materials capable of mimicking the mucus properties offers advantages such as increased sample availability, reduction in the use of animals, and avoidance of heterogeneity or contamination issues that can lead to experimental inaccuracies. The design of mucus and mucin mimetics focuses on reproducing specific properties of the mucus hydrogel, including adhesivity, coating capacity, and rheology. Although not within the scope of this review, mucus mimetics are important to study mucoadhesion, develop mucoadhesive materials, and perform *in vitro* characterization before *in vivo* pre-clinical trials. Readers may refer to previous reviews [67–69] emphasizing such mimetic work. To give just one example here, Wagner and coauthors [10] compared various physicochemical parameters of native mucus with gels made from purified or commercial mucins and synthetic polymers. They demonstrated that no single model may represent all the mechanical, biological, and chemical properties of mucus simultaneously, but mucin gels can exhibit qualitatively similar properties to native mucus when specific characteristics are considered.

In terms of rheology, a significant challenge when using rehydrated mucins from freeze-dried mucus to study mucoadhesion is that these rehydrated materials may not have the same composition, structure, and rheological behavior as native mucus. The native rheological properties of mucus may be somewhat recaptured by cross-linking rehydrated mucins. For example, crosslinking porcine gastric mucin using glutaraldehyde produces gel-like samples with bulk elastic and viscous moduli ranging between 10 and 100 Pa and 1–10 Pa, respectively [70]. These values are comparable to the desired values for native porcine gastric mucus. Another study used the principles of click chemical reactions with tetrazine or norbornene functionalization to crosslink mucins, resulting in implantable hydrogels that exhibit a macrogel-like rheological behavior [71]. These hydrogels not only have a predominant elastic character but also display immune-modulating properties that depend on the crosslinking architecture of mucin [72]. Mucin crosslinking can also be achieved by employing a polyethylene glycol-based 4-arm PEG-thiol (PEG-4SH) agent using MUC5B and MUC5AC from bovine submaxillary and porcine gastric mucins, resulting in bulk elastic moduli on the order of 200–400 Pa, higher than the expected values for human airway mucus (10–50 Pa), also composed of MUC5B and MUC5AC; however, dilution can reduce the G' values [73]. Therefore, working with a close replica of mucus in terms of rheological properties is crucial to develop the just right mucoadhesive formulation.

2.2.3. Microrheology and mucus penetration

Mucoadhesion may involve mucosal penetration when multiple properties are combined in one material. Understanding such mucosal penetration phenomena requires a thorough understanding of mucus microstructure. Mucosal penetration is usually associated with smaller length scales, as the rate and extent of mucosal penetration depends on mucus mesh size. Microrheological experiments prove valuable in this regard, offering the capability to quantify local viscoelastic properties. To conduct such experiments, colloidal beads are suspended in the sample and their motion is measured, in response to an applied force, which can stem from intrinsic thermal fluctuations within the sample from or external forces. The former approach, termed passive microrheology, relies on the dependence of probe diffusivity on thermal fluctuations, enabling determination of the fluid's viscosity [74]. Conversely, the latter approach involves applying external forces, such as magnetic fields, inducing probe movement, with the material's response revealing insights into its complex viscosity.

Although some studies indirectly deduce microstructural properties of mucus from bulk rheological experiments [75], in reality, microscale phenomena such as particle permeation, bacterial colonization, and the subsequent response of immune cells cannot be accurately predicted due to the presence of microheterogeneities in the mucus [54,76–78]. Therefore, investigations carried out using microscopic techniques with fluorescent probes, enabling particle tracking microrheology measurements [79], are more reliable as far as mucosal penetration is concerned. The mucosal barrier selectivity arises from its structure and biochemistry [80]. From a structural point of view, no matter the surface chemistry, if the particle is larger than the mesh-pore size of the mucosal network and does not have mucolytic action, it will either not permeate or will soon be retained. Hence, "microrheology" experiments using such large particles tend to deform the mucosal network and so will report rheology close to the macroscopic rheology with a strong elastic character. On the other hand, particles smaller than the mesh-pore size

can permeate mucus and their motion typically does not deform the mucosal network, instead being subject only to viscous drag from the fluid medium. Microrheology carried out with such small particles will report a viscous response typically quite different from the macrorheology. As a result, microrheology measurements are strongly correlated with mucus penetration. Clearly, the mesh pore size directly affects the diffusional transport and sustained drug delivery capability of particles that do not exhibit specific mucosal interaction or bio-adhesion. The mesh pore size of mucus can vary from 20 to 2000 nm, depending on the body location and health status [80]. In the specific case of human cervicovaginal mucus, the reported range is up to 200 nm [81,82].

Some key factors influencing mucosal penetration include particle surface chemistry and size. Regarding size, it is known that smaller molecules tend to diffuse through mucus [83]. In terms of surface chemistry, particles coated with poly(ethylene) glycol (PEG) with low molecular weight and dense PEGylation present diminished mucoadhesive interactions. This allows particles with sizes up to 500 nm to penetrate thick mucosal layers, such as the human cervicovaginal mucus (CVM) and chronic rhinosinusitis mucus (CRSM) [84,85]. The explanation relies upon the avoidance of polymer and mucin chain interpenetration, due to the low molecular weight, together with the dense coating that can shield a polymer hydrophobic core such as poly(sebacic acid) (PSA), preventing adhesive interactions [83,86]. To achieve penetration through the channels within the mucus mesh, it is fundamental to avoid the mechanisms that typically promote strong mucoadhesion. In this sense, the design of the mucus penetrating system can be tailored based on the chemical nature of the drug or active ingredient being carried. For instance, hydrophobic moieties found in bovine serum albumin or lysozyme lead to entrapment in the mucus mesh due to specific interactions with mucin. In addition to PEGylation, another approach to enhance particle transport through human mucus involves coating particles with Pluronics molecules, which are triblock copolymers of polyethylene and polypropylene oxide [87,88]. Another strategy is the utilization of mucolytic agents such as papain/poly (acrylic acid) nanoparticles, that are capable of disrupting the mucosal barrier [89]. The mucolytic strategy can be particularly beneficial in the case of chronic diseases that increase the elastic character of mucus, such as in case of cystic fibrosis [51].

2.2.4. *Relating macro and microscopic properties*

The literature contains several reports that discuss the relationship between macro and micro-scale rheology in the investigation of mucus matrix architecture. For instance, in the specific case of *CF* sputum from patients, the use of mucolytic agents resulted in an increase in pore size of the mucus gel, improving the transport properties of mucus, while reducing its viscoelastic properties [90,76]. Lai and coauthors demonstrated that nonoxynol-9 (N9), a commercial spermicide and microbicide, can alter the microscale rheological parameters of human cervicovaginal mucus (CVM) by reducing hydrophobic interactions between mucin fibers without significantly affecting its macroscopic viscoelasticity [91]. When comparing the ease of operation between macro and micro-scale rheology instruments, macrorheology experiments offer certain advantages, such as simplicity and the avoidance of bespoke instrumentation. For example, the severity of *CF* can be classified by analyzing the storage modulus (G') of sputum, with 88 % predictive efficacy [92]. New methods are also being explored to improve sputum handling protocols before rheological measurements, such as a non-destructive vortex homogenization, which can significantly reduce the variability of rheological parameters [93].

Fig. 3a compares schematically elastic and loss modulus measured on macro and micro scales [94]. Macrorheological properties were investigated in terms of bulk G' and G" using a conventional rheometer, and the microscale ones were measured using optical tweezers. As previously mentioned, at the macroscale, mucus has G' > G", whereas it is opposite at smaller length scales, with G" > G' (considering frequencies

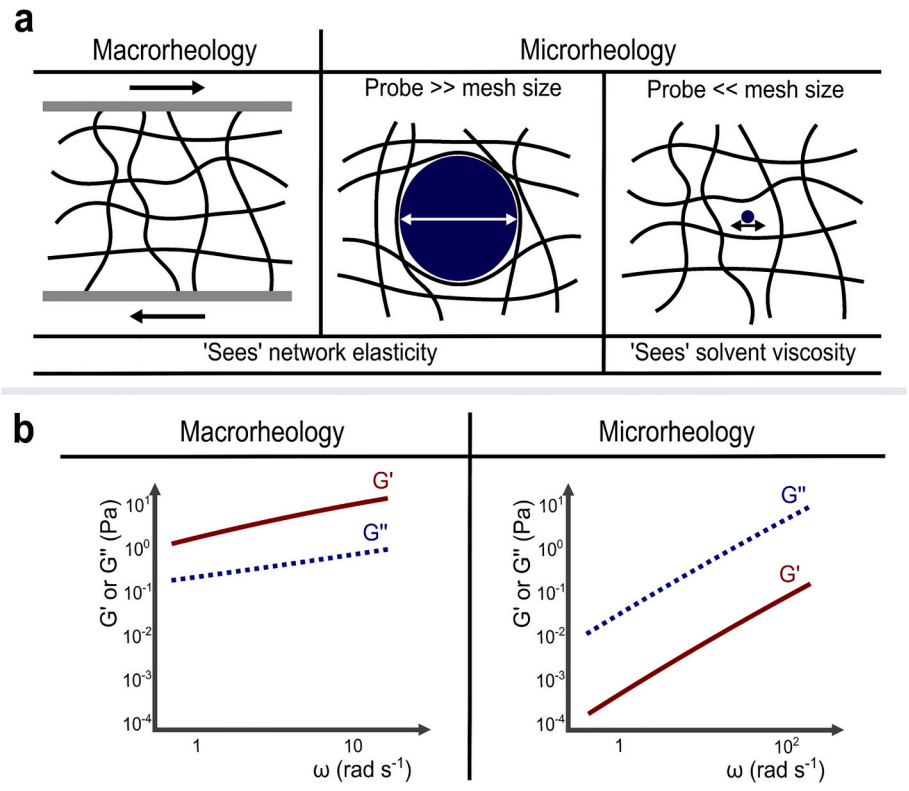

Fig. 3. Schematic illustration (a) Length scale of the measurement determining the rheological outcome of the gel network in mucus. b) Comparison between macro and micro-scale rheological properties of mucus from bronchial epithelial culture samples derived from healthy patients. The schematic representation was drawn based on group samples [94].

from 0.6 to 300 rad/s) (Fig. 3b). These macrorheological parameters were obtained using a gap size of 128 μm at the edge of a 1° cone geometry, and the microrheological ones using mucus-inert beads composed of different materials (silica, melamine resin and carboxylated melamine resin), with different sizes (diameters from 1 to 5 μm). This allows us to deduce information about which length scales are being characterized, and how different length scales impact the results. At smaller length scales mucus is less elastic, by at least two orders of magnitude. Such discrepancies between macro and micro scales are concentration-dependent and particularly observed at lower mucin or mucus-simulants concentrations, where the network mesh size is large, becoming negligible once a certain volume fraction is reached (in this case, close to 1 wt%), given that at this point, the viscosity of the continuum is measured by the 1–5 μm beads. Deviations from the expected viscoelastic response for the continuum are usually associated with smaller particle sizes, when the pore size matrix architecture or structural inhomogeneities influence the observed results [95]. Regarding the relation between particle surface charge, size and the viscoelastic response, it was found that higher G' and G" values were associated with 5 μm melamine beads when compared to 3 μm carboxylate-melamine beads [94]. Other reports have also indicated that elasticity dominates the macroscale, while viscous dissipative behavior is more prominent at the microscale, as observed in mucus from a marine worm [96], healthy respiratory human mucus [97], and mucin mimetic crosslinked materials [98].

Regarding structural visualization employing imaging techniques, Meziu et al. [99] employed environmental scanning electron microscopy (ESEM) and confocal laser scanning microscopy (CLSM) to contrast the hydrated and freeze-dried structures of mucus. Hydrated mucus exhibited a granular structure, whereas freeze-dried samples revealed a porous architecture with enlarged pore size, as observed through both CLSM and ESEM imaging techniques. Although this study did not delve into the microrheology of the mucus sample considering the two handling protocols, it underscores the importance of considering all variables when studying microrheology, including the impact of freeze-drying on a native mucus sample.

Considering the relationship between mucus mechanical properties and the length scale considered during the measurement, it is also important to emphasize the need to consider the appropriate length scales when studying mucosal systems [100] and attempting to design mucoadhesive materials. For instance, while at the macroscale the self-healing nature of mucus can be observed, at the microscale, the mechanical properties of the gel together with specific interactions will dictate the diffusion of bioengineered peptides or active ingredients.

2.2.5. Impact of concentration, pH, and salts on rheological properties

Exchange of sodium (Na^+) and calcium (Ca^{2+}) ions during mucus secretion plays a key role in controlling mucus rheological properties. Replacing divalent Ca^{2+} ions with monovalent Na^+ ions triggers a transition from a highly compacted state to a hydrated mucus [101]. The compact state reflects the ability of polyvalent cations (Ca^{2+} in this case) to cause a 'correlation attraction' between polyanions (segments of mucin in this case). When the Ca^{2+} are replaced by Na^+, the number of counterions doubles once electroneutrality is achieved, increasing the osmotic pressure. As a consequence, water molecules move towards the Na^+ ion-rich material, causing the gel to hydrate and expand significantly, from 1000 to 3000-fold [102]. This feature relies on bicarbonate secretion, which acts as a calcium-chelating agent and is also influenced by the pH difference between the gastric lumen (acidic) and epithelial surfaces (neutral) [103,104]. The addition of calcium to a mucin-containing biological fluid such as saliva for instance results in the formation of aggregates with a 6-fold higher molecular weight than the original oral mucin fraction [105]. The formation of high molecular weight aggregates induced by Ca^{2+} has also been observed for MUC2, the major colonic mucin [103]. For porcine intestine mucus, the addition of Ca^{2+} resulted in the formation of a network with increased elastic character, both at the macro and microscopic scales [52].

The pH value and the presence of salts can also influence the mucosal structure by affecting mucin conformations [33]. The mucosal pH varies depending on the body surface, ranging from as low as pH 1 in the gastric lumen when food is being digested, to neutral pH in the respiratory tract and alkaline pH in the pancreas. In studies using a model of purified solutions of porcine gastric mucins, authors have observed a pH-dependent transition from a viscoelastic solution at pH 6 to a gel at pH 4 [106]. These results were recently reproduced for artificial mucus, indicating a negative correlation between mucus cross-link density and pH [107,108]. This pH-induced structural change explains, for example, how bacteria swim through the mucus layers until they reach the epithelial surface [109]. A recent and in-depth investigation using *ex vivo* pig gastric mucus showed that the viscoelastic properties of native mucus are maintained when considering pH values higher than 3 and lower than 8.5, highlighting the differences in using native mucus instead of purified mucin solutions [110]. In the specific case of diseased mucus in cystic fibrosis (*CF*), recent studies have shown that mucus concentration directly impacts the mucus clearance mechanism in the lung much more than pH does, affecting both micro and macro scales [111].

While for purified pig gastric mucins the viscoelastic response is highly pH-dependent [106], these results do not necessarily hold true for native human mucus. For example, Wang et al. [112] demonstrated that human cervicovaginal mucus (CVM) remains stable over a wide range of physiological pH values. This stability was observed in terms of microstructure and bulk rheology. Although the size distribution for the pore sizes covered a wide range (~50 nm to >1 μm), the authors were able to identify that minor structural changes happen when comparing CVM micro/macrostructures at pH 1–2 to pH 8–9. In summary, mucus may vary significantly across body sites and thus one mucoadhesive might not fit all purposes. Nevertheless, deriving general physics behind adhesion to mucus is key for fabricating mucoadhesives. Having understood the mucus, we next focus on principles governing mucoadhesion across length and timescales.

3. Principles of mucoadhesion across time and length scales

The mucoadhesive process encompasses multiple interactions occurring simultaneously, and gaining a comprehensive understanding of the forces involved can provide valuable insights into the design of the next generation of mucoadhesive materials. To begin, we provide a brief overview of macromolecular interactions and then offer a unique perspective on mucoadhesive interactions based on length and strength scales, as shown in Fig. 4. It is important to emphasize that even weak interaction at the individual bond level can also lead to strong adhesion when multiple interactions occur together (*i.e.*, polyvalent interactions). This becomes especially relevant once multiple binding sites at the mucin structure are considered. We discuss the interaction of mucoadhesive with mucin at smaller length scales followed by interaction of mucoadhesives with mucus at large length scales.

3.1. Mucin-mucoadhesive interactions at smaller length scales

Classical chemistry explains the adhesive process through various intermolecular interactions, categorized by distinct types of bonds or interaction forces, either solvent-dependent interactions or steric forces [113]. These include covalent, ionic, hydrophobic, hydrogen, and van der Waals interactions. Fig. 4a illustrates how each interaction at smaller length scales plays a role in the mucoadhesive process. As 95 % of the mucosal gel is water, the aqueous nature of mucin and the high dielectric constant of water prevent the classic ionic bonds as found in the rigid lattice of crystalline salts from happening. Nevertheless, electrostatic interactions (Fig. 4a) occur between the primary amino groups of positively-charged mucoadhesives such as chitosan [114], and negatively charged sialic and sulfonic acid residues, which are the terminal

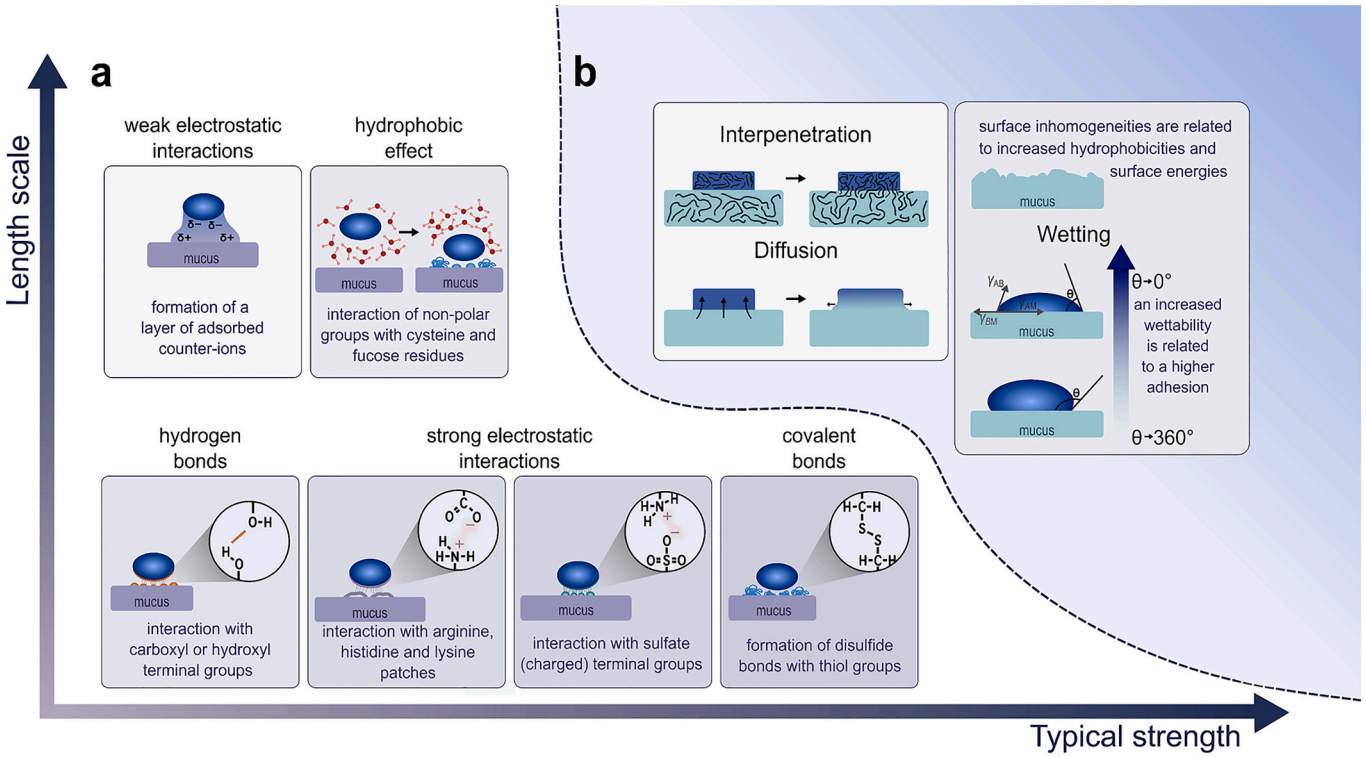

Fig. 4. Interactions involved in the mucoadhesion process elucidated at (a) smaller and (b) larger length scales. At smaller length scales (a), weak electrostatic interactions happen when adsorbed counter-ions interact with oppositely charged mucin. The hydrophobic effect is represented in terms of non-polar groups within the mucoadhesive material preferring to interact with cysteine and fucose groups in mucin (in blue), rather than interacting with polar water molecules (oxygen in red, hydrogen in pink). Hydrogen bonds (in orange) may form with carboxyl or hydroxyl terminal groups in mucin. Strong electrostatic interactions occur directly between negatively charged mucoadhesive materials with positively-charged arginine, histidine, and lysine patches at the mucin protein core (in purple), or between positively charged materials with sulfate terminal groups at the glycosidic section of mucin molecules (in blue). Covalent (disulfide) bonds (in blue) arise from the interaction of free thiol groups present in mucin and in the mucoadhesive material. At larger length (and strength) scales (b), the interactions with mucus are dominated by polymer interpenetration, water diffusion/uptake, and wetting. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

groups on the oligosaccharide side chains. This type of interaction is influenced by pH, with respect to the pk_a values of sialic acid (2–2.6) and sulfuric acid (−2). Notably, the negative charge character of mucin may be less predominant in the gastric environment, where the pH is around 1.5–2.0, a condition in which other interactions with mucin are revealed. Once the negative charge of mucin is less-predominant, strong electrostatic interactions occur between negatively charged mucoadhesives such as alginate [115] and positively charged arginine, histidine and lysine amino acids at the mucin core, which are exposed in non or lightly-glycosylated regions of the protein backbone [116].

Mucoadhesive interaction with dominance of hydrogen bonding (Fig. 4a) can occur either with the protein core or the glycosylated regions of mucin. This type of bonding is favored in mucoadhesive materials containing hydrogen-bonding chemical groups, such as the carboxyl and hydroxyl groups found in anionic polysaccharides like polyacrylic acid, pectin, and alginate [9,117]. While van der Waals interactions are considered secondary interactions in the mucoadhesive process [118], hydrophobic interactions can play a significant role in mucoadhesion (Fig. 4b). Transitions in the character of a mucin film from hydrophilic to hydrophobic have been observed [119], as a conformational change happens from random coil to anisotropic structure when the pH is shifted from 7 to 2, with the exposure of hydrophobic residues at the lowest pH value [120]. The entrapment of synthetic polystyrene on mucus, for example, has been proposed as a hydrophobic-driven phenomenon [45].

Strong covalent bonds can form between thiolated mucoadhesives and cysteine-rich domains of mucus glycoproteins, containing thiol groups [121] (Fig. 4a). Thiolation of mucoadhesives has been achieved by attaching cysteine, homocysteine, 2-iminothiolane, thioglycolic acid, and glutathione groups to a diverse array of polymers, including polycarbophil [121–123], alginate [124], poly(acrylic acid) [125,126], poly (methacrylic acid)-starch [127], carboxymethylcellulose [123], chitosan [128–130], or through the functionalization of materials like bovine serum albumin [131,132]. The improvement in mucoadhesive properties can be attributed to the formation of disulfide bonds between the thiolated materials and mucin, which are structural connections commonly found in biological substances. These disulfide bonds can be formed *via* thiol exchange or oxidation reactions and in terms of strength, the improvement in the mucoadhesive properties vary from 2- to 140-fold [133]. Thus a combination of covalent and non-covalent interactions may be exploited to enhance mucoadhesive interactions with mucins in smaller length scales.

3.2. Mucus-mucoadhesive interactions at larger length scales

At larger length scales, the interaction can be probed between the mucoadhesive materials and the mucus (mucin-rich hydrogel) rather than the mucins. Wetting is related to the ability of a mucoadhesive material to spread on a substrate. If we consider the mucus as a substrate, the wettability of a liquid mucoadhesive will be influenced by the surface and interfacial energies of the mucus (Fig. 4b) as can be represented by Dupré's equation:

$$W_{AM} = \gamma_A + \gamma_M - \gamma_{AM} \quad (1)$$

where, thermodynamic work of adhesion W_{AM} between two surfaces (A and M) is related to the surface tension of adhesive A (γ_A) and mucus

M (γ_M), and to the interfacial tension between them (γ_{AM}). These surface and interfacial energies are, in their turn, related to the contact angle θ by the Young's Eq. [134],

$$cos\theta = \frac{\gamma_M - \gamma_{AM}}{\gamma_A} \quad (2)$$

The presence of a textured and chemically heterogeneous surface affects the shape of liquid droplets after they adhere to a surface, and these wettability inhomogeneities are usually found in biosystems. Another important aspect to be considered is the presence of water in both hydrogels (mucus and mucoadhesive material), which leads to an impractical determination of the contact angle in the steady state [135]. Even so, it is still generally true that for lower contact angles, there is a higher affinity from the mucoadhesive for the mucosal surface [136].

Suitable wetting is a requirement for liquid and semisolid mucoadhesives, as it indicates the material's spreadability. For instance, when hydroxyethyl cellulose or vinylpyrrolidone-vinyl acetate copolymer (film-forming agents) were added to derivatives of acrylic acid, enhanced wetting properties were observed, as determined by measuring the advancing contact angle using the sessile drop method [137]. Wetting characteristics are also improved when considering non-flat surfaces, as a characteristic surface morphology (Fig. 4b) contributes to the mechanical interlocking of the mucoadhesive, which plays a role in the adhesion phenomena [138]. A high surface roughness leads to an increased contact area, thereby facilitating the adhesive process. Even when evaluating solid (instead of liquid) dosage forms of mucoadhesives, the measurement of the contact angle between biological fluids and the solid can aid in comprehending specific surface properties (such as surface energy) of the materials being used [137].

Separately, the diffusion of mucoadhesives within the mucin network leads to chain interpenetration and entanglement effects (Fig. 4b). In terms of mucoadhesive macromolecules, some important parameters which affect chain diffusibility include the molecular weight, chain length and flexibility, and presence of functional groups [138], along with their hydrodynamic size, mobility [139], conformation, and miscibility [140]. Another important parameter is how available the chains of the mucoadhesive are to form these entanglements. For mucoadhesive particles coated by high-density grafting of low molecular weight PEG chains, a decreased mucoadhesion was found due to the avoidance of chain entanglements and interpenetration, while lower PEG grafting densities were associated with enhanced mucoadhesive properties [141].

In the specific case of a mucoadhesive material undergoing rapid gelation once exposed to aqueous environments, water uptake from the mucus to the gelling mucoadhesive occurs, resulting in the formation of an adhesive joint. This water uptake is directly linked to a rapid swelling behavior, which enhances the diffusion process between the polymer chains and mucin network, thereby increasing the mucoadhesivity [142]. The critical degree of hydration required to observe mucoadhesion has also been a subject of discussion [143,144], as data suggests that for some excipients, the main mechanism of active release is patch swelling (when with time, water uptake increases and the active is slowly released) [145]. A recent modeling approach for mucoadhesion suggested that fully swollen hydrogels are not as mucoadhesive as dehydrated ones, since the adhesive interaction forms due to a competitive interaction for the solvent that ultimately leads to water uptake from the mucosal layer. This hypothesis was experimentally validated using polyacrylamide gels, with an adhesive stress of 1 kPa [146]. To sum it up, larger length scale interactions between the mucoadhesive materials and mucus tend to be dominated by wetting, diffusion and interpenetration effects.

3.3. Time scale in mucoadhesion

The concept of a two-stage mucoadhesive process was derived from mechanisms observed for adhesion to biological surfaces [134]. Besides the time scale that will be described for the contact and consolidation stages, there is another time-dependence effect when measuring adhesive forces. The experimental characterization of semisolid adhesive materials involves performing peel and tensile tests, during which the interfacial toughness or tensile strength is measured [147]. For soft bioadhesives, some energy dissipation to the bulk occurs as the surfaces are being separated, due to their viscoelastic character [148]. This viscoelastic dissipative property is particularly important in the context of soft bioadhesives, as for these materials the amount of dissipation is closely related to the rate at which the materials are being pulled apart [149]. Using sufficiently low peel rates (with respect to the adhesive relaxation or equilibration times) would allow for these measurements to be performed in an equilibrium state or steady condition. On the other hand, experiments that use higher peel rates will be impacted by viscoelastic energy dissipation. In this sense, for these adhesive measurements, the ideal dimensionless Deborah number (*De*), which represents the ratio between the system relaxation time and the measurement time [150], would be lower than 1. In this context, the proper design and testing of adhesive materials is closely related with their rheological properties, as well as with the specific bonds and interactions at the surface, which will be covered in section 4.

Understanding the concept of timescales in mucoadhesion is important for developing effective mucoadhesive materials, although it can be challenging to differentiate experimentally and determine the precise duration of each step. This is particularly relevant in certain body regions, like the gastrointestinal tract, where natural peristalsis can disrupt weaker adhesive forces before the consolidation step [151]. Additionally, mucus turnover (synthesis, secretion, and degradation) is a critical process to consider in some regions such as the intestinal tract. Spontaneous mucus secretion in humans occurs at a rate of approximately 240 μm/h [49] at the gastrointestinal tract, and mucus turnover can be a limiting factor for the residence time of mucoadhesive materials.

3.3.1. The contact stage

The contact stage occurs when a mucoadhesive approaches the mucus layer, establishing an initial contact. In the case of liquid mucoadhesives, this stage is accompanied by wetting. Previous studies applied the Derjaguin, Landau, Verwey, Overbeek (DLVO) theory to describe bacterial adsorption to surfaces, and some authors considered extending the theory to explain this initial stage of the mucoadhesive process. This involves considering varying magnitudes of attractive and repulsive potentials as mucoadhesives approach mucus [1]. However, it is important to acknowledge that the presence of surface inhomogeneities in both the mucosal surfaces and the mucoadhesive can significantly impact the application of this theory when interpreting *in vivo* results.

3.3.2. The consolidation stage

The consolidation stage comes after the initial adhesive contact, and in this stage strong adhesion is established. Just as in the initial stage, the specific characteristics for the consolidation stage will vary depending on the size, surface properties and dosage form of the mucoadhesive, as well as on the nature of the interactions being formed. For instance, when using thermoresponsive systems with a transition temperature (liquid to solid viscoelastic) near 20 °C, the liquid wettability ensures uniform distribution once exposed to the tissue. This wetting step is followed by a sol-gel transition, given that the human body temperature is around 37 °C, allowing prolonged adhesion (more than 24 h) [152].

Polymer interpenetration plays a crucial role when using mucoadhesive polymers with long, flexible, and penetrating chains. On the other hand, in the case of mucoadhesives with low water content, such as poly (acrylic acid), water movement from the mucus gel to the mucoadhesive occurs due to osmotic pressure effects [1]. Other anionic materials such as alginate, carboxymethyl cellulose, polycarbophil and hyaluronic acid

have demonstrated improved mucoadhesivity after chemical modification with sulfhydryl groups. For these materials, the consolidation stage comprises activation by moisture, which plasticizes the system, leading to increased attractive forces due to van der Waals interactions and hydrogens bonds [153]. Fig. 4b illustrates the occurrence of the described processes, with wetting being relevant both in the early stages and during the consolidation stage, where other intermolecular interactions, such as hydrophobic interactions between fucose residues or cysteine knots from the mucin structure and the mucoadhesive material, can also contribute to the overall adhesion process.

Mucoadhesive formulations appear as a promising approach to enhance the duration for which formulations remain in contact with mucosal tissues [154]. This is particularly important once we consider the previously discussed mechanisms for mucosal clearance. Other alternative systems that do not interact strongly with mucus in early stages, such as mucus-penetrating systems, may facilitate the transport and delivery of active ingredients, as they may avoid some rapid clearance mechanisms [46,155]. However, to date, it remains unclear how mucoadhesive and mucus-penetrating materials compare concerning the residence time on mucosal surfaces and the effective release of active ingredients within the human mucosal system.

By introducing solid microparticles prepared from tamarind seed polysaccharide into a mucin solution, the approximate durations of the contact and consolidation stages were experimentally elucidated using cryogenic field emission scanning electron microscopy (cryo-FESEM) [156]. With this system, the contact stage happened in the initial 10 min following particle deposition onto the mucin layer. Subsequently, the consolidation stage started and persisted for 60 min post-particle-deposition, resulting in a smooth gel layer [156]. While the precise determination of the contact and consolidation stage durations remains experimentally limited, conceptualizing a two-stage mucoadhesion process helps in discerning the timings of interactions, which also depend on the form (dry or wet) of the mucoadhesive. Notably, for carbohydrate-based polymers, certain properties such as higher molecular weights have demonstrated an augmented mucoadhesive effect attributable to the formation of mesh entanglements, particularly evident in cellulose-based mucoadhesives [157]. Conversely, polymers with lower molecular weights, despite being associated with reduced mesh entanglement with mucin, exhibit fewer steric restraints during the adhesive process – which allow a higher thermodynamic work of adhesion than polymers with higher molecular weights [157]. The impact of these findings on the mucoadhesion of protein-based mucoadhesives remains unclear and warrants exploration in future studies.

3.4. The role of polymer elasticity

Another important aspect to be considered is the elasticity of the mucoadhesive material. However, even for synthetic materials, there is no general rule on the ideal elasticity for a material to adhere to mucus, as mucoadhesion is a multiscale phenomenon and multiple interactions are involved. Some insights on the impact of polymer elasticity appeared in the 1990s, when it was found that there was a correlation between the contact time of carbomer gels with the eye mucosal layer and the elastic properties of the gels [158]. In general, viscoelastic materials that are able to entangle with the mucus gel should favor mucoadhesion [9]. Nonetheless, excessively high cross-linking density can reduce polymer flexibility and, consequently, its interaction with mucins [159]. How this would influence the adhesion of protein and protein conjugates in the form of nanoparticles is a subject for future investigation. When considering a mucoadhesive that is stiffer than the mucus gel, theoretical considerations suggest that the force of mucosal adhesion is proportional to the square of the difference in the elastic modulus between the mucus and the adhesive material [146]. For the specific case of aqueous poly(methyl vinyl ether-*co*-maleic acid) (PMVE/MA), statistical modeling demonstrated that polymer viscoelasticity is a dominant factor in determining mucoadhesion [160]. For the vaginal retention of semi-solid materials, recent measurements of compliance over time and determination of the residual viscosity of various formulations were able to predict the vaginal retention of the formulations, which further demonstrates the relationship between the viscoelastic properties of materials and their mucoadhesivity [161]. A clear picture of how the deformation of mucus-interacting systems will impact on mucoadhesive interactions still needs to be thoroughly investigated in the future, to entirely describe the role of elasticity.

4. Design principles affecting mucoadhesion of proteins on mucosal tissues

There is an increasing interest in using proteins for mucoadhesion due to their biodegradability, biocompatibility, inherent presence of charged amino acids, and their ease of structuring by chemical and physical processing. Herein, we examine specifically how proteins adhere to mucus. We will start with studies that investigated the fundamental charge interactions between proteins and mucin. Subsequently, we build on these findings considering scenarios where charge interactions are further combined with other contributing factors. At times, we refer to glycan-based mucoadhesives to shed light on certain research gaps in protein-based mucoadhesive materials. It is essential to note, however, that no study comprehensively investigate every conceivable way in which proteins interact with mucin, as certain interactions are assumed over others, due to the complexity of these soft systems. Table 2 and Table 3 provide an overview of studies where protein on its own or protein combined with glycans have been used as mucoadhesive materials.

4.1. Surface charge

One of the mechanisms underlying mucoadhesion is associated with the charge characteristics of the mucoadhesive material. This is pertinent due to the prevailing negative charge of mucin glycoproteins across

Table 2
Protein-based systems interacting with mucus/mucin.

Protein type	Mucus/ mucin type	Condition/ modifications used	Nature of interaction (adhesive with mucin/mucus)	References
Gelatin	Native rat nasal mucosa	Solid gelatin microspheres were used	Electrostatic (positively charged adhesive)	[162]
	Native porcine intestinal mucosa	Thiolated gelatin tablets	Covalent (disulfide bond)	[163,164]
β-lg	Mucin from porcine stomach	Cationic β-lg was synthesized	Electrostatic (positively charged adhesive)	[165,166]
WPI	Native rat intestinal and stomach mucosa	Native or denatured WPI	Electrostatic (positively charged adhesive) at pH < IEP Covalent (disulfide bond) for the denatured form	[167,168]
Keratin	Mucin from porcine stomach	Kerateine and/ or keratose nanoparticles	Electrostatic (positively charged adhesive), hydrogen bonding, hydrophobic interactions Covalent (disulfide bond)	[169]
BSA	Mucin from porcine stomach	BSA modified with N-Acetylcysteine	Covalent (disulfide bond)	[132]

Table 3
Protein or peptide-polysaccharide-based systems interacting with mucus/mucin.

Protein/ peptide type	Polysaccharide type	Mucin type	Conditions/modification used	Nature of interaction (adhesive with mucin/mucus)	References
Homocysteine	Poly(acrylic acid)	Native porcine intestinal mucosa	Protein/polymer conjugate in the form of tablets	Covalent (disulfide bond)	[126]
Gelatin	λ-carrageenan	Mucin from porcine stomach	Protein/polymer mixture in the form of films and microspheres	Not specified	[170]
WPI	Alginate	Native rabbit intestinal mucosa	Protein/polymer microparticles	Electrostatic (negatively charged adhesive)	[171]
Gelatin, Keratin	Chitosan	Native sheep buccal mucosa	Proteins/polymer composite films	Electrostatic (positively charged adhesive) Covalent (disulfide bond)	[172]
Lysozyme	Starch	Native rat intestinal and stomach mucosa	Lysozyme nanoparticles incorporated into oxidized starch microgels	Electrostatic (negatively charged adhesive)	[173]
BSA	Chitosan	Native cow buccal mucosa	Thiolated BSA combined with chitosan into buccal patches	Electrostatic (positively charged adhesive) Covalent (disulfide bond)	[131]
Mucin	Mucin glycans*	Mucin from bovine submaxillary	Mucosome nanoparticles	Not specified	[174]

* Inherent glycosylation of mucin.

most biologically relevant pH values except the gastric conditions, as previously discussed in this review. While the most common protein-based mucoadhesives studies have focused on dietary proteins, intriguingly, the sensation of astringency in the mouth can also be correlated with mucosal adhesion [118]. A proposed explanation for this sensation is a specific mechanism closely tied to the interaction between negatively charged salivary mucins and positively charged proteins, which results in augmented friction between the surfaces within the oral cavity.

The isoelectric point (IEP) of the protein, the pH at which the mean surface charge density is zero, stands out as a crucial factor in mucoadhesion of proteins, as recently highlighted by comparing the mucoadhesive properties of a synthetic polyampholyte with a natural one – bovine serum albumin [175]. The adhesion to mucin is largely driven by proteins with high IEPs such as lysozyme, lactoperoxidase, and lactoferrin, as illustrated in Fig. 5a *via* electrostatic interactions [176], when compared to proteins with a lower IEP value at large ranges of physiologically-relevant pHs. Moreover, the presence of salt, which leads to screening effects of electrostatic charges in colloidal systems [177], may allow for the observation of additional effects, such as hydrophobic interactions or hydrogen bonding in mucoadhesion by proteins.

A significant proportion of protein-based mucoadhesive work has focused on whey protein isolate (WPI) (Table 2), which is a complex mixture of proteins that includes β-lactoglobulin (β-lg), α-lactalbumin (α-lac), immunoglobulin G (IgG), lactoferrin (LF), serum albumin, proteose peptone, and caseinomacropeptide proteins [178]; its IEP is around 4.5 [179]. Interactions between whey proteins and mucin glycoproteins have shown strong charge-dependency in interactions. Indeed at pH 6.8, both mucin glycoproteins and whey proteins carry a negative charge, and therefore their natural association is unlikely under their native state. However, when the pH is set to 3.4, salivary mucins remain negatively charged, while whey proteins become positively charged because they are below their IEP. This shift in charge characteristics promotes their interaction. As the pH is further decreased to 2.6, the net negative charge of salivary glycoproteins diminishes, leading to a decrease in the association between the positively charged whey proteins and glycoproteins [180]. Additionally, at this lower pH, mucin proteins may form self-assembled aggregates due to low electrostatic repulsive forces [181], making it harder to distinguish self-assembled mucin aggregates from other proteins-mucin aggregates. In another study, the formation of mucin-BSA complexes was attributed to electrostatic forces occurring at both pH 7.4 and pH 3. At pH 3, where mucin and BSA have opposite charges, electrostatic interactions drove mucoadhesion. At pH 7.4, where both mucin and BSA are negatively charged, it was proposed that due to the strong repulsion between the glycosidic chains of mucin and the surface of BSA, albumin interacted with the positively charged cysteine domains of mucin. This interaction leads to mucin-mucin binding and the assembly of multiple mucin units. Consequently, changes in the secondary structure of mucin were observed, particularly at the highest pH studied, along with an increase in both viscous and elastic responses of the materials [182].

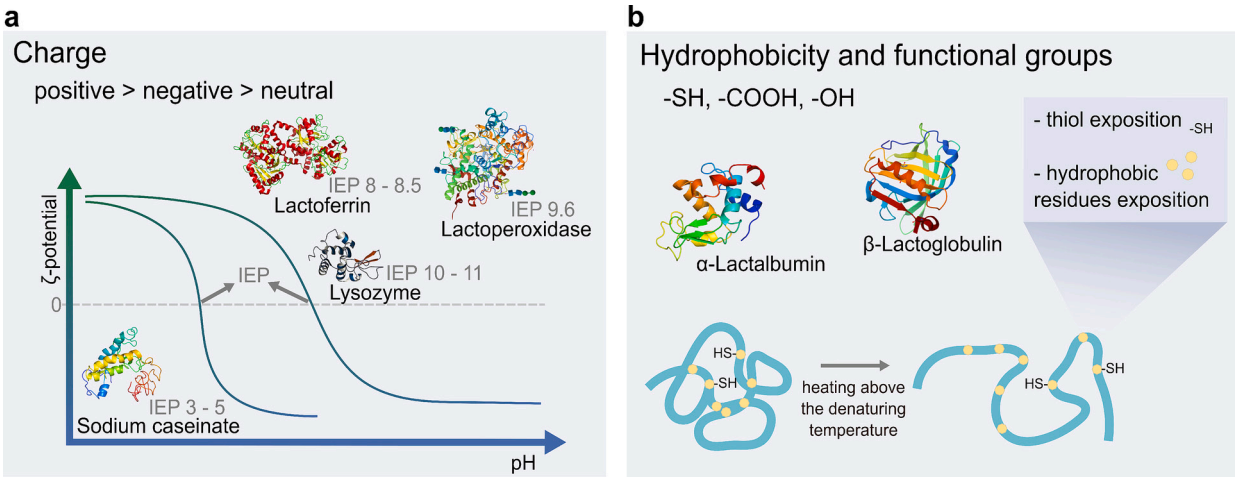

Fig. 5. Surface charge (a) and hydrophobicity and the presence of functional groups (b) are some of the key factors affecting the mucoadhesion of proteins.

Recent efforts in the mucoadhesive field have also focused on modifying β-lg's surface charge aiming to improve its mucoadhesive properties. The effect of modifying this water-soluble protein was compared using two cationizer agents, ethylenediamine (EDA) or polyethyleneimine (PEI). When compared to native β-lg, cationic β-lactoglobulin (C-β-lg) modified with PEI displayed significantly improved mucoadhesive properties, with an increase of more than 400 % as assessed by a quartz crystal microbalance with dissipation monitoring (QCM-D) study [166] (Table 2). Similarly, EDA-modified C-β-lg showed improved mucoadhesion by more than a factor of 2 [165]. These differences were attributed to variations in charge, with C-β-lg cationized with PEI exhibiting a higher ζ-potential of 54.2 mV, while EDA- C-β-lg presented a ζ-potential value of 32.4 mV, both measured at pH 7.0 [166]. Notably, C-β-lg could be assembled into assembled nanoparticles when exposed to organic solvents, offering the potential for encapsulating, and gradually releasing active ingredients. When exposed to ethanol or acetone, C-β-lg self-assembled into nanoparticles with 75–172 nm in size [165]. The size and surface properties impact directly on how particles interact with mucin, during the contact and consolidation stages of the adhesive process. Indeed, focusing on larger length scales (Fig. 4b), analysis of the wettability properties of these particles revealed that those with a higher degree of hydrophilicity exhibited improved mucoadhesion, while the more hydrophobic ones demonstrated enhanced cellular uptake, as measured using Caco-2 cell cultures [183]. Cellular uptake of particles is a complex process which involves size-exclusion in an internalization mechanism known as endocytosis, leading to the intracellular delivery of actives [184].

In a comparative study of mucosal adhesion of two milk proteins, casein and β-lg, it was observed that casein exhibited stronger adhesion to porcine mucus in an *in vitro* test using fluorescence microscopy; while β-lg was completely washed out from buccal mucosa after 20 washes with artificial saliva, casein was still adhered even after 50 washes [185]. The investigation into the underlying reasons for this difference revealed that β-lg possessed a higher thiol content than casein and greater hydrophobic characteristics, which may have been expected to drive greater mucoadhesion, as further discussed in the next section. While the thiol group in β-lg originates from the Cys-121 domain, which contains a free thiol group, the enhanced hydrophobic character is associated with the Cys-121 neighboring groups and resultant from the protein three-dimensional folded structure [186] (Fig. 5b). However, other factors contributed to the difference. ζ-potential measurements indicated negative charges for both proteins, with casein presenting a weaker surface charge compared to β-lg (-10.6 ± 2.1 and -22 ± 0.9 mV, respectively). This partially explains the heightened mucoadhesivity observed for casein, as β-lg's more negative charge led to increased repulsive interactions with the (also) negatively charged mucin.

Gelatin, produced by the partial hydrolysis of native collagen derived from animal tissues, is a protein commonly employed in the formulation of novel mucoadhesive platforms for drug delivery application. Gelatin can be utilized either on its own [162] or combined with other materials, such as poly(acrylic acid) [187]. Morimoto and co-authors [162] conducted studies involving the preparation of microspheres using acidic gelatin (with an IEP of 5.0) and basic gelatin (IEP of 9.0) to investigate their efficacy as mucoadhesive materials *in vivo* (Table 2). Despite both positively and negatively charged materials exhibiting adhesion to the nasal mucosa, the positively charged material showed an approximately one quarter increase in adhesion ability to nasal mucosa when compared to the negatively charged formulation. This heightened adhesiveness significantly contributed to the nasal absorption of salmon calcitonin, a peptide capable of regulating calcium homeostasis [162].

Lysozyme (LZ) is a positively charged protein found in mucosal secretions and on the cell walls of animals and plants, often isolated from chicken egg white. It serves as a natural antimicrobial agent within mucous gels, and the specificity of the interaction between lysozyme and mucin has been the subject of investigation. Due to its high isoelectric point (between 10 and 11) (Fig. 5a), LZ interacts with mucin through electrostatic interactions across a range of physiological pH values. However, at more acidic pH values (pH 3), the binding of LZ with pig gastric mucin weakens as the mucin molecules tend to self-assemble under these conditions. The introduction of salt to the system screens the charged groups on both the PGM and LZ surfaces, leading to a significant reduction in the attractive forces that drive their interaction. Furthermore, LZ has four intramolecular disulfide bonds, and it has been demonstrated that the eight thiol groups only play a role in the formation of aggregates when LZ is in a denatured state. At native conditions, no disulfide bond formation is detected between LZ and PGM [188].

Similar to chitosan [189], positively charged proteins can be assembled into layer-by-layer films with mucin *via* comparable adsorption mechanisms. Positively charged proteins, those at a pH lower than their IEP (such as lactoferrin at physiological pH values), strongly interact with the negatively charged glycosidic domains of mucin. For instance, proteins with a positive charge at neutral pH, such as lactoferrin and histatin, have been observed to form complexes with salivary mucin MG2 [190]. Proteins with lower IEP, like proline-rich protein 1 and statherin, have demonstrated the ability to adsorb MUC5B when pre-adsorbed on surfaces like silica and hydrophobized silica [191]. Lindh and colleagues tested the ability of lysozyme (LZM), lactoferrin (LF), lactoperoxidase (LPO) or histatin 5 (HST-5) to build up multilayered structures with mucin. They showed how adsorbed films on hydrophobic surfaces exhibited greater stability against desorption when compared to films adsorbed on hydrophilic surfaces. Both lactoferrin and lactoperoxidase showed increased adsorption to mucin, which was attributed to their larger size and positive charge at pH 7. However, only LPO had the capability to form multilayered structures with mucin; this unique ability was attributed to the higher net charge density of LPO compared to all the other proteins tested [192]. Further investigation revealed that the enzymatic activity of the adsorbed LPO experienced only minor variations [193], while increased viscous properties were observed for a mucin/LPO film associated with the highly hydrated mucin chains [194].

LF (IEP = 8.0–8.5) [195] exhibits binding affinity to both pig gastric mucins [196] and bovine submaxillary mucins [197]. In the former investigation, a noticeably higher quantity of lactoferrin demonstrated binding to mucin in comparison to β-lg, associated with the electrostatic-driven nature of the interaction [196]. In the latter study, lactoferrin was identified as playing a pivotal role in mediating interactions between mucin molecules, driven by electrostatic forces between mucin and the positively charged lactoferrin. QCM-D experiments revealed the rapid adsorption of lactoferrin to mucin, resulting in the sequential deposition of lactoferrin and mucin and the formation of a multi-layered structure. Experimental lactoferrin-mucin binding findings were corroborated by self-consistent field (SCF) calculations, which predicted the formation of a multi-layered complex [197]. All these pH-dependent mucosal interactions suggest that charge interactions tend to dominate the protein-based mucoadhesion landscape at smaller length scales (Fig. 4a).

4.2. The role of hydrophobicity

Besides the afore-mentioned electrostatic interactions, mucin biopolymers strongly interact with hydrophobic substrates in a glycosylation-dependent manner [198], and it has been demonstrated that the terminal peptide domains of mucins with a hydrophobic character play a key role when adsorbing at a PDMS hydrophobic surface [199]. Consequently, hydrophobic regions in proteinaceous materials (Fig. 5b) are expected to play a role when exposed to mucin, particularly when other forces are not the dominant driving factor. In the case of sodium caseinate's interaction with pig gastric mucin (PGM), the strength of interactions was found to be significantly influenced by the pH of the solution. Sodium caseinate, a derivative of milk protein casein, has an IEP between 3 and 5 (Fig. 5a), so that it carries overall negative charges at pH 5 and positive ones at pH 3. In the pH range between the

IEP values of PGM and sodium caseinate, attractive interactions occurred due to the negatively charged nature of mucin at pH 3, leading to the formation of complexes. Interactions at pH 1 were less pronounced, and since both materials carried a positive charge at this pH, secondary interactions such as induced dipole forces or hydrophobic interactions likely played a role in explaining the observed aggregates [200]. Such hydrophobic interactions between mucin and proteins are not specific to caseinate but have been also observed in other proteins particularly when electrostatic interactions are minimal. Specifically, at pH 7, electrostatic interactions are considerably weaker due to the repulsion between similarly charged β-lg and mucin molecules [201]. Nevertheless, hydrophobic interactions become the predominant driving force for observed association with mucin [202], with a primary contribution of enthalpy, involving the repulsion of disordered water structures by hydrophobic regions and secondary entropic contribution, linked to an increase in the disorder of water structures upon contact with hydrophobic surfaces.

When investigating the interactions between mucin and negatively charged mucoadhesive agents, it is expected that the addition of substances that screen electrostatic interactions (such as salts) will mitigate the repulsive forces between the two negatively charged surfaces; thus enhancing adhesion and allowing hydrophobic effects to be studied with a reduced influence of surface charge effects. An example of a hydrophobic-driven association appears in the study of the interactions between mucin and anionic alkyl sodium sulfates, as determined when using different hydrophobic chain lengths [203]. In the case of BSA, a protein that carries a negative charge at neutral pH, there was no discernible differences in its rheological behavior when interacting with mucin in the presence or in the absence of salt [204]. This led researchers to conclude that the primary mode of interaction between BSA and mucin is likely hydrophobic in nature [205,206]. This suggests that hydrophobic (Fig. 4a) interactions might be worth exploiting further in future studies for mucoadhesion, which tends to be largely dominated by probing electrostatic interactions.

4.3. Thiol groups

As previously noted, a significant mechanism facilitating mucoadhesion involves the formation of disulfide covalent bonds between the free thiol groups of mucin molecules (located in the cysteine-rich subdomains) and mucoadhesives (where present). Considerable research has been dedicated to thiolation of polysaccharides to enable such bonding and enhance mucoadhesion [207]; however, limited attention has been directed towards similar modification of proteins, or indeed to proteins inherently possessing free thiol groups such as β-lg. The amino acid cysteine contains a thiol functional group, and in some proteins this thiol group is free and therefore can be exposed by heating (Fig. 5a).

Once heat-treated, WPI for instance undergo structural alterations as they unfold and transform into more flexible polymeric chains, losing their secondary and tertiary structures. Due to the conformational changes, some buried groups like the thiol groups become exposed, which may affect the protein's interaction with mucins (Fig. 5a). For instance, the mucoadhesive properties of WPI in its native and denatured form were investigated as a function of pH and concentration [167]. At a lower WPI concentration (1 wt%), and at pH levels below 4.5, both denatured and native-state WPI exhibited interactions with mucin, as evidenced by turbidity and isothermal calorimetry measurements. These interactions are consistent with charge-induced mechanisms, as the two moieties are oppositely charged. However, at a higher concentration (10 wt%), both native and denatured WPI did not show significant interaction with mucin at pH 1.2. This can still be attributed to charge effects, considering the IEP of mucin and WPI (which fall between 2 and 3 and 4.5, respectively), both are positively charged, and they tend not to interact. Surprisingly, denatured WPI demonstrated attractive interactions with mucin at pH 6.8 (Table 2), where charge complementarity does not exist; in other words, charge effects cannot explain the observations. Strong interactions between denatured WPI and mucin were observed, leading to an increase in viscosity, and were attributed to two mechanisms: hydrogen bonding and the formation of disulfide bridges. The exposure of thiol groups in heat-denatured WPI enabled covalent S—S bonds to form between denatured WPI and mucins. Moreover, due to the enhanced flexibility of denatured WPI, physical interpenetration between mucin and denatured WPI was assumed to occur. Subsequent investigations revealed that in this particular scenario, the interaction strength was not dependent on the size of WPI, as attempts to create microparticles through calcium-induced aggregation did not result in increased interaction with mucin [167]. In another study, similar findings demonstrated that after heating, the viscosity, pH and charges of WPI remained unchanged, while particle size increased [208]. An additional indication of the impact of thiol groups favoring mucoadhesion was further identified by the same group, as free thiol concentration was increased after WPI denaturation, consistent with the increased mucoadhesion [209]. Another important observation was the loss of the β-barrel structure, exposing hydrophobic regions, which might have further contributed to increased mucoadhesive forces [209]. The mucoadhesive properties of hybrid protein-based system *i.e.* zein-whey protein nanoparticles can also be explained by the formation of disulfide bonds between the thiol groups of the adhesive particles and mucus [210]. These thiol groups exhibit heightened reactivity under alkaline pH conditions, which are characteristic of the small intestine [211].

Besides whey proteins, thiolated derivatives of gelatin can be synthesized through either the reaction with 2-iminothiolane, a thiolating agent interacting with the primary amine groups of gelatin, or a two-step process involving an amination reaction followed by thiolation [163]. The sequential approach, in which the carboxylate groups in gelatin are converted to amides with a side amine on their chains prior to thiolation, led to a tenfold increase in thiol content when compared to gelatin obtained through a one-step reaction [163]. While a non-thiolated sample exhibited negligible mucoadhesion, the thiolated gelatin obtained *via* the two-step reaction demonstrated adhesion to mucus for up to 24 h, mirroring the behavior observed for thiolated chitosan. Furthermore, it was observed that higher molecular weights were not as good for mucoadhesion compared to lower ones. Specifically, the gelatin sample with a molecular weight of 20–25 kDa exhibited the most favorable mucoadhesive outcomes [163]. However, the authors underscored that the correlation between mucoadhesion and molecular weight is polymer-dependent.

Certain challenges associated with thiolated materials belonging to the so-called 'first generation of mucoadhesives' – usually hydrophilic polymers with thiol modifications, include their inherent low stability at physiological pH and susceptibility to oxidation from thiol to disulfide [212]. For preactivated thiolated gelatin (synthesized through an initial reaction with 2-iminothiolane), oxidation prior to reaching a targeted site may be avoided by attaching benzylic groups (mercaptonicotinic acid) to the sulfhydryl groups. This protective group acts as an effective leaving group upon contact with mucin, facilitating enhanced mucoadhesion. Indeed, S-protected thiolated gelatin exhibited a remarkable 71-fold increase in the total work of adhesion and a 70-fold increase in residence time at porcine mucosa compared to the unthiolated control sample [164].

In general, the covalent attachment of thiol groups has been proven to enhance mucoadhesive properties of various materials. Notably, some thiolated materials exhibit limited stability at pH levels exceeding 5. Consequently, in the post-2010 era, several research groups have redirected their efforts from simply attaching thiol groups to mucoadhesives towards investigating less reactive thiol ligands, such as S-protected or preactivated thiomers [213], as potential mucoadhesives. However, it is worth noting that there is currently little literature available that explores how these last modifications would impact the mucoadhesive properties of proteins other than gelatin. Moreover, it remains to be studied how exploitation of inherent thiol groups present

in protein exposed by heat/chemical treatment *vs.* externally applied thiolation to protein would impact the strength of mucoadhesion.

4.4. Multivalent interactions

So far, we have considered individual interactions isolated from other contributions. However, in many cases, multiple interactions need to be invoked to explain experimental observations. In certain cases, such as the interaction between bovine submaxillary mucin (BSM) and the plant-based lectin jacalin (JAC), multi-layered deposited structures can form, displaying resistance to a wide range of pH levels and ionic strengths [214]. Interestingly, these structures appear to form and remain stable without a predominant adhesion mechanism such as electrostatic attraction, as they persist even in the presence of salt. Another example of a mucoadhesive system associated with multivalent interactions includes the use of keratin, a fibrous protein, which exhibits distinct mucoadhesive properties depending on its reduced forms, keratose (KOS), and kerateine (KTN). In a study involving pig gastric mucin, at pH 4.5, the dominant mechanisms were electrostatic attractions (in the case of KTN) and hydrogen bonding (for both KTN and KOS). However, at pH 7.4, these mechanisms are less pronounced, and disulfide bonds become relevant in the case of KTN, while hydrophobic interactions play a key role in interactions with KOS [169] (Table 2).

In summary, charge interactions play a prominent role in the landscape of protein mucoadhesion, driven by the inherent charge carried by proteins contingent upon the pH of the surrounding medium. Generally, positive charges correlate with heightened mucoadhesive behavior (*i.e.*, at pH $<$ IEP). However, numerous proteins with a high isoelectric point remain unexplored in terms of their mucoadhesive properties. Additionally, there exists a considerable opportunity to investigate covalent interactions, either through intrinsic or extrinsic thiolation of proteinaceous materials. For instance, in copolymer-based mucoadhesives, the impact of thiolation appears to be substantial even when employing low-molecular-weight materials (20 kDa) [215]. Protein-based materials, such as thiolated BSA and gelatin (Table 2), also present promising avenues for exploration in this regard.

5. Proteins combined with polysaccharides in mucoadhesion

Recent advances have emerged from the synergistic combination of proteins and mucoadhesive polysaccharides into composite materials, resulting in a diverse spectrum of favorable outcomes, such as protection against degradation of the active ingredient and improved adhesion. Initial reports mainly focused on the delivery of peptides or proteins *via* the mucosal route, using polysaccharides as mucoadhesive materials [216,217]. Subsequently, the combination of proteins and polysaccharides as mucoadhesive materials gained attention (Table 3), exemplified by the combination of materials such as λ-carrageenan and gelatin [170] or milk/vegetal proteins with chitosan or alginate [218]. Combining proteins with polysaccharides can help in multiple interactions at short length scales (Fig. 4a) – for instance proteins focusing on electrostatic interactions whilst the glycan chain interacting with mucins *via* hydrogen bonding with improved wetting observed in the larger length scales (Fig. 4b). For instance, the ionic complexation of λ-carrageenan and gelatin yielded a mucoadhesive material with the properties of a carrier system, carrying the alkaline drug timolol maleate (an anti-glaucoma model active used in ophthalmic applications) [170]. Another bioadhesive, as described in a patent, utilized proteins of animal origin (milk proteins) and vegetal origin (pea proteins) that can be combined with a material such as chitosan or alginate [218].

In the pursuit of encapsulating and delivering insulin through the oral route, researchers undertook a comparative study involving nanoparticles of WPI and alginate, with a focus on their mucoadhesive properties. They revealed that, while there was no significant distinction in the percentage of adherence to mucosal surfaces between alginate and WPI particles, the microparticles formed by combining both alginate and WPI conferred protection to insulin against enzymatic degradation [171]. The combination of this protective effect with the mucoadhesive property of proteins and polysaccharides led to an enhancement in the oral bioavailability of insulin, and consequently in its intestinal absorption [171] (Table 3).

Similarly, when granular cold-water swelling (GCWS) corn or tapioca starches were combined with either xanthan gum (XG) or β-lg, a notable increase in adhesion with mucus was observed [219]. The work of adhesion, measured using a texture analyzer, was determined based on the force-displacement curve obtained when detaching the gelled mucoadhesives from the intestinal mucosa of a sheep's small intestine. Although the electrostatically coupled gel featuring the protein exhibited comparatively lower adhesion in contrast to xanthan gum, with a nearly 50 % lower work of adhesion, it exhibited superior stability against starch retrogradation. The diminished mucoadhesive property within the formulation containing native β-lg is likely to come from its lower molecular weight relative to XG, wherein hydrogen bonds and physical entanglements with mucin are prevalent [219]. Even so, the utilization of protein systems in tandem with polysaccharides proved to be a valuable approach given the enhanced stabilization during cold storage. As in this study only native β-lg was used [219], we suggest that the use of β-lg in its denatured form could lead to improvements in the mucoadhesivity, as previously discussed in this review.

To facilitate the delivery of tetrandrine in the context of glaucoma treatment, bovine serum albumin (BSA) nanoparticles were coated with chitosan. Rheological assessments underscored the mucoadhesivity of the system of uncoated BSA nanoparticles with the active ingredient, as a nearly 40 % increase in viscosity was observed upon incubation with mucin [220]. This enhancement was not observed for the chitosan-coated nanoparticles, but ζ-potential values measurements showed that these interact electrostatically with mucin, indicating mucoadhesivity attributed to the positive charge carried by chitosan. In this study, BSA was chosen for its role in augmenting drug solubility and suitability for ocular drug delivery. Comprehensive *in vitro* and *in vivo* evaluations demonstrated the formulation's biocompatibility as well as a desirable pharmacological response owing to the transcorneal permeation and drug release profile. A 5-fold increase in transcorneal permeation was observed upon the incorporation of a low-molecular-weight chitosan (50–190 kDa), when compared to the uncoated BSA-active nanoparticles, attributed to the polymer's characteristic chain flexibility that allows chain interpenetration with mucin [220].

In the search for nanosystems to meet specific requirements for intestinal drug delivery, a starch microgel containing lysozyme (with the starch as a protective shell) was found to exhibit heightened mucoadhesivity, particularly under conditions reflective of the intestinal pH environment, as determined by confocal imaging of the composite particles in *ex vivo* rat intestinal mucus [173] (Table 3). The inner lysozyme was assembled into positively charged micelle-like nanoparticles with a diameter around 20 nm, within which the hydrophobic active agent, quercetin (Que), was encapsulated. The oxidized starch microgels presented an average diameter of 1700 nm and a negative surface charge, allowing the electrostatic complexation of micelles into the microgels. The improved mucoadhesivity was primarily attributed to the oxidized starch shell, which served as a safeguard shielding the lysozyme from degradation when exposed to acidic gastric conditions (in the stomach). This innovative configuration also facilitated a transformation from a mucus-adhering system to a mucus-penetrating one, releasing the quercetin-lysozyme nanoparticles in response to the intestinal enzymatic and pH conditions, which then penetrated the mucus, facilitating epithelial quercetin delivery. This release could not be achieved only using lysozyme due to degradation at gastric conditions, nor using the oxidized starch microgels alone, due to poor solubility of the active [173].

Furthermore, electrostatic principles governing the propensity for or against mucoadhesion can be strategically employed to manipulate the delivery and controlled release of active compounds. For instance,

recent research has demonstrated that a chitosan core, modified by surface coverage with bovine serum albumin (BSA), can be effectively employed to encapsulate and orally deliver carvacrol, a naturally occurring antimicrobial agent [221]. QCM-D showed that the BSA corona surrounding the chitosan core reduced the mucoadhesive behavior of chitosan under gastric pH conditions, as the less-adhesive nature of BSA prevails (inverting the combined system surface charge from positive to negative). This modulation facilitates the transit of the antimicrobial agent through the gastric phase and its subsequent targeted release within the intestinal phase, thereby optimizing therapeutic efficacy [221].

Recognition of the influence of thiol groups on mucoadhesion has led to the development of novel mucoadhesive agents combining proteins and polysaccharides. For instance, bovine serum albumin (BSA) has been chemically modified with thiol groups and subsequently combined with chitosan to create a mucoadhesive buccal patch [131]. Using this approach, the mucoadhesive properties arising from the positively charged chitosan were combined with those from the thiol groups in thiolated BSA, making a strongly mucoadhesive material. In comparison to the non-thiolated form (BSA/Chi), the thiolated patch (BSA-SH/Chi) exhibited superior swelling capacity and an enhanced work of adhesion. Importantly, both formulations demonstrated *in vitro* biocompatibility [131] (Table 3). Polysaccharides extracted from *Ophiopogon japonicus* (Mondo grass) have been effectively combined with chitosan and WPI to generate nanoparticles with size in the range of 300 to 1400 nm, depending on the chitosan molecular weight. These nanoparticles exhibited robust adhesion to porcine fresh small intestine mucus, as measured by the fluorescence intensity of labelled particles. The strong adhesion was primarily attributed to electrostatic interactions between chitosan and mucins, as well as the presence of free thiol groups in WPI, facilitating disulfide bridge formation with cysteine-rich domains in the mucus. In addition to their strong adhesion capabilities, the particles demonstrated *in vitro* protective and anti-inflammatory effects [222]. In other cases, chitosan was combined with protein peptides due to their inherent biological activities. For example, an acid-responsive hydrogel composed of *N*-acetylcysteine-grafted chitosan (CS-NAC), alginate, and tilapia collagen peptide as a bioactive component has been recently explored as a potential candidate for the treatment of gastric injuries induced by prolonged and excessive alcohol consumption. The mucoadhesivity of the CS-NAC and alginate system proved to be superior when compared to a system employing non-grafted chitosan, as determined by a colorimetric assay that measured the *in vitro* binding to mucin in simulated gastric fluid. The enhanced adhesion was attributed to disulfide bond formation, increased electrostatic interactions and hydrogen bonding [223].

Specific studies on the mucoadhesion of food-based proteins (excluding WPI) with glycans have not yet been extensively reported. Nevertheless, lactoferrin has been effectively employed in conjunction with calcium pectinate and hyaluronic acid for the targeted delivery of rhein, an active compound known for its anti-inflammatory properties [224]. The combined presence of these two carbohydrates shielded rhein against degradation within the gastrointestinal tract. Furthermore, the coexistence of lactoferrin and hyaluronic acid ligands plays a pivotal role in facilitating substantial cellular uptake, thereby alleviating inflammatory responses, and promoting mucosal repair, as shown by *in vivo* experiments [224]. Additionally, a thermo-responsive material capable of *in-situ* gel formation has been assessed for the delivery of chloramphenicol, consisting of microparticles prepared using an emulsion as the initial template. In this example, effective mucoadhesion was achieved through the combination of WPI and hydroxypropyl methylcellulose (HPMC), and the desired thermogelling behavior was imparted by the incorporation of Pluronic F127 and F68 [225].

Upon combining positively-charged lactoferrin microgels with a negatively charged κ-carrageenan hydrogel, an increase in viscosity was observed in the resulting combined system. Additionally, the formed complex exhibited effective adsorption to a hydrophobic substrate, as evidenced by QCM-D assessments, wherein higher adsorption was recorded compared to saliva. While the authors focused on measuring rheological properties and lubricity rather than direct mucoadhesion, the mechanism by which the microgel binds to the negatively charged polysaccharide reflects the electrostatic nature observed in positively charged materials adhering to mucins [226]. Further exploration could assess whether the lactoferrin microgel binds to mucus, especially considering the contribution of κ-carrageenan as a viscosity modifier, aligning with the previously discussed role of elasticity in mucoadhesive materials.

To summarize, the combination of proteins and polysaccharides offers a vast array of potential interactions, as outlined in Table 3. Similar to hydrophilic polysaccharides employed as mucoadhesives, the mechanisms governing mucoadhesion of these combined systems predominantly involves electrostatic interactions and the formation of disulfide bonds (Fig. 4a). The synergy of proteins and polysaccharides introduces distinctive features, including the potential for encapsulation of hydrophobic drugs within a core and the development of a responsive shell tailored to specific anatomical regions, such as the acidic environment of the stomach, facilitating the targeted delivery of the active ingredient to the intestine. Further exploration of how polymeric complexes containing positively charged and/or thiolated proteins interact with mucin gels holds promise for advancing the development of biocompatible and efficacious mucoadhesive materials.

6. Conclusions and future perspectives

This review provides a comprehensive exploration of the mucoadhesive characteristics exhibited by proteins and draws parallels with traditional glycan-based mucoadhesive materials. Drawing upon the existing literature, we offer a unique perspective on mucoadhesion as a process occurring across multiple length and strength scales. Notably, at smaller energy and length scales, strong electrostatic interactions and covalent disulfide bonds are highlighted as the primary mechanisms responsible for robust interactions with mucus gels. At larger length scales, the impact of viscoelasticity has been explored in a few cases, but further work is needed to elucidate underlying principles as to how this affects mucoadhesion. Meanwhile, wetting and chain interpenetration behaviors are known drivers of polymeric mucoadhesion, but have not been fully explored in proteinaceous systems.

In the field of mucoadhesion, which encompasses a range of polymers - including proteinaceous, carbohydrate-based, and synthetic polymers - there are a few challenges that remain to be addressed. One major challenge is the detailed evaluation of the mucoadhesive properties. Experimental evidence suggests that the selection of mucin significantly influences the degree of interaction with the chosen mucoadhesive polymeric system [227]. Our review highlights that the choice of mucus model is critical to the accuracy and relevance of selecting the right mucoadhesive polymer. For mucoadhesion studies, it is important to employ a mucus model that exhibits all the intrinsic characteristics outlined in this review. These characteristics include not only the chemical signature of mucins and other mucus components but also the reproduction of both linear and non-linear rheological properties. These interactions underpin the responsiveness of mucus to environmental stimuli, such as pH or ionic strength, as well as the presence of the mucoadhesive material under investigation. Additional factors that may influence the behavior of mucoadhesives require further investigation, including the effects of microbial communities, enzymes, and other minor mucosal components which are inherently present in physiology.

Regarding proteins, recently, there has been emerging research on the ability of positively charged proteins to form layers or multilayered structures when interacting with mucin [197,206]. These proteins include bovine serum albumin, lactoperoxidase, immunoglobulin G, secreted immunoglobulin A, trefoil peptides [206], and proline-rich protein 1 [191]. Charge-induced interactions with mucin have shown

to be largely dependent on the IEP of the protein, however, the mucoadhesive properties of these proteins is yet to be thoroughly investigated. Beyond charge-related interactions, our synopsis relating strength and length scales highlights the formation of disulfide bonds by thiolated materials as another strong local interaction enhancing mucosal residence times. This mechanism is well-established for polysaccharide-based mucoadhesives [228] and reports have recently begun to emerge in protein-based systems. Certain proteins, including β-lg, α-lac or BSA, contain free thiol groups that may be inaccessible due to their three-dimensional structures; denaturation of these proteins can unfold their structure and expose these free thiol groups, consequently having a significant impact on their mucoadhesivity. For proteins lacking free thiol groups, a research gap emerges in terms of breaking disulfide bonds with reducing agents such as dithiothreitol or mercaptoethanol – an approach particularly relevant for proteins containing cysteine groups within their internal structure. The potential to expose thiol groups which are concealed or bonded in the native state is a distinctive feature unique to proteins, not explored in the context of polysaccharides as these possess glycan chains instead of cysteine groups. An additional perspective arises from external thiolation and the synthesis of S-protected thiols, an approach already applied to polysaccharides such as chitosan or to gelatin, which offers further avenues for investigation and potential innovation in protein-based mucoadhesives.

Besides the need for a more thorough comprehension of how thiolation impacts proteins as mucoadhesives, we note that the aspect of elasticity remains largely unexplored and needs further attention. Understanding in this area would be highly relevant to the context of protein-based mucoadhesives, where elasticity may be imparted to varying degrees by the gelation of the proteins *via* thermal or ionic methods. Furthermore, the effects of polymer interpenetration observed in several polymeric cases, are not fully elucidated for mucoadhesive proteins, warranting further investigation. A more complete understanding of each of multiscale effects - and their interplay - is imperative to the extraction of general principles underlying mucoadhesion, which will allow informed and targeted design of the next generation of biocompatible, sustainable, and effective mucoadhesive materials.

Finally, a new challenge arises when translating fundamental knowledge into real-life application. A recent review emphasizes that exploring excipients with known biocompatibility may reduce the risk of failure in clinical trials, which are both costly and essential for the transition of basic research into therapeutic products [229]. In this context, proteins offer potential as mucoadhesives due to their inherent biocompatibility. However, the effects of protein thiolation on mucoadhesive strength and potential immunological response remain unexplored and need *in vitro*, *in vivo* and pre-clinical testing before such materials can be used in clinical settings.

CRediT authorship contribution statement

Bianca Hazt: Writing – original draft, Visualization, Methodology, Data curation, Conceptualization. **Daniel J. Read:** Writing – review & editing, Supervision, Conceptualization. **Oliver G. Harlen:** Writing – review & editing, Supervision, Conceptualization. **Wilosn C. K. Poon:** Writing – review & editing, Supervision, Conceptualization. **Adam O. Connell:** Writing – review & editing, Supervision, Conceptualization. **Anwesha Sarkar:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

None.

Data availability

No data was used for the research described in the article.

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References

[1] Smart JD. The basics and underlying mechanisms of mucoadhesion. Adv Drug Deliv Rev 2005;57:1556–68.
[2] Cui C, Mei L, Wang D, Jia P, Zhou Q, Liu W. A self-stabilized and water-responsive deliverable coenzyme-based polymer binary elastomer adhesive patch for treating oral ulcer. Nat Commun 2023;14:7707.
[3] Spleis H, Sandmeier M, Claus V, Bernkop-Schnurch A. Surface design of nanocarriers: key to more efficient oral drug delivery systems. Adv Colloid Interface Sci 2023;313:102848.
[4] Bandi SP, Bhatnagar S, Venuganti VVK. Advanced materials for drug delivery across mucosal barriers. Acta Biomater 2021;119:13–29.
[5] Sosnik A, das Neves J, Sarmento B.. Mucoadhesive polymers in the design of nano-drug delivery systems for administration by non-parenteral routes: a review. Prog Polym Sci 2014;39:2030–75.
[6] Goyal AK, Singh R, Chauhan G, Rath G. Non-invasive systemic drug delivery through mucosal routes. Artif Cells Nanomed Biotechnol 2018;46:539–51.
[7] Goubet AG, Rouanne M, Derosa L, Kroemer G, Zitvogel L. From mucosal infection to successful cancer immunotherapy. Nat Rev Urol 2023;20:682–700. https://doi.org/10.1038/s41585-023-00784-5.
[8] Kumar M, Dogra R, Mandal UK. Nanomaterial-based delivery of vaccine through nasal route: opportunities, challenges, advantages, and limitations. J Drug Deliv Sci Technol 2022;74.
[9] Peppas NA, Buri PA. Surface, interfacial and molecular aspects of polymer bioadhesion on soft tissues. J Control Release 1985;2:257–75.
[10] Wagner CE, Krupkin M, Smith-Dupont KB, Wu CM, Bustos NA, Witten J, et al. Comparison of physicochemical properties of native mucus and reconstituted mucin gels. Biomacromolecules 2023;24:628–39.
[11] Mackie AR, Goycoolea FM, Menchicchi B, Caramella CM, Saporito F, Lee S, et al. Innovative methods and applications in mucoadhesion research. Macromol Biosci 2017;17.
[12] Yeruva T, Yang S, Doski S, Duncan GA. Hydrogels for mucosal drug delivery. ACS Appl Bio Mater 2023;6:1684–700.
[13] Watchorn J, Clasky AJ, Prakash G, Johnston IAE, Chen PZ, Gu FX. Untangling mucosal drug delivery: engineering, designing, and testing nanoparticles to overcome the mucus barrier. ACS Biomater Sci Eng 2022;8:1396–426.
[14] Leal J, Smyth HDC, Ghosh D. Physicochemical properties of mucus and their impact on transmucosal drug delivery. Int J Pharm 2017;532:555–72.
[15] Schattling P, Taipaleenmaki E, Zhang Y, Stadler B. A polymer chemistry point of view on mucoadhesion and mucopenetration. Macromol Biosci 2017;17.
[16] Netsomboon K, Bernkop-Schnurch A. Mucoadhesive vs. mucopenetrating particulate drug delivery. Eur J Pharm Biopharm 2016;98:76–89.
[17] Puri V, Kaur VP, Singh A, Singh C. Recent advances on drug delivery applications of mucopenetrative/mucoadhesive particles: a review. J Drug Deliv Sci Technol 2022:75.
[18] Taipaleenmaki E, Stadler B. Recent advancements in using polymers for intestinal mucoadhesion and mucopenetration. Macromol Biosci 2020;20:e1900342.
[19] Caramella CM, Rossi S, Ferrari F, Bonferoni MC, Sandri G. Mucoadhesive and thermogelling systems for vaginal drug delivery. Adv Drug Deliv Rev 2015;92:39–52.
[20] Kumar R, Islam T, Nurunnabi M. Mucoadhesive carriers for oral drug delivery. J Control Release 2022;351:504–59.
[21] Schilling AL, Cannon E, Lee SE, Wang EW, Little SR. Advances in controlled drug delivery to the sinonasal mucosa. Biomaterials 2022;282:121430.
[22] Shipp L, Liu F, Kerai-Varsani L, Okwuosa TC. Buccal films: a review of therapeutic opportunities, formulations & relevant evaluation approaches. J Control Release 2022;352:1071–92.
[23] Ritthidej GC. Nasal delivery of peptides and proteins with chitosan and related mucoadhesive polymers. Peptide Protein Deliv 2011:47–68.
[24] Lehr C-M. Lectin-mediated drug delivery:: the second generation of bioadhesives. J Control Release 2000;65:19–29.
[25] Zhang T, Li L, Chunta S, Wu W, Chen Z, Lu Y. Enhanced oral bioavailability from food protein nanoparticles: a mini review. J Control Release 2023;354:146–54.
[26] Ha HK, Rankin SA, Lee MR, Lee WJ. Development and characterization of whey protein-based nano-delivery systems: a review. Molecules 2019:24.
[27] Lalla RV, Bensaduon R-J. Miconazole mucoadhesive tablet for oropharyngeal candidiasis. Expert Rev 2011;9:13–7.
[28] Celebioglu HY, Lee S, Chronakis IS. Interactions of salivary mucins and saliva with food proteins: a review. Crit Rev Food Sci Nutr 2020;60:64–83.
[29] Brown FN, Mackie AR, He Q, Branch A, Sarkar A. Protein-saliva interactions: a systematic review. Food Funct 2021;12:3324–51.

[30] Ritzoulis C. Interactions between food macromolecules and mucin: relevance to oral processing and personalized nutrition, focused on xerostomia and dysphagia. Food Hydrocoll 2025;158.
[31] Corfield AP. Mucins: a biologically relevant glycan barrier in mucosal protection. Biochim Biophys Acta 2015;1850:236–52.
[32] Wang BX, Wu CM, Ribbeck K. Home, sweet home: how mucus accommodates our microbiota. FEBS J 2021;288:1789–99.
[33] McShane A, Bath J, Jaramillo AM, Ridley C, Walsh AA, Evans CM, et al. Mucus. Curr Biol 2021;31. R938-R45.
[34] HUGO Gene Nomenclature Committee. Available at: https://www.genenames.org/ (last accessed June 1, 2023).
[35] Voynow JA. Mucins. In: Janes SM, editor. Encyclopedia of respiratory medicine. 2nd ed. Oxford: Academic Press; 2022. p. 77–85.
[36] Wu AM, Csako G, Herp A. Structure, biosynthesis, and function of salivary mucins. Mol Cell Biochem 1994;137:39–55.
[37] Dinu V, Gadon A, Hurst K, Lim M, Ayed C, Gillis RB, et al. An enzymatically controlled mucoadhesive system for enhancing flavour during food oral processing. NPJ Sci Food 2019;3:11.
[38] Demouveaux B, Gouyer V, Gottrand F, Narita T, Desseyn JL. Gel-forming mucin interactome drives mucus viscoelasticity. Adv Colloid Interface Sci 2018;252:69–82.
[39] Bansil R, Turner BS. Mucin structure, aggregation, physiological functions and biomedical applications. Curr Opin Colloid Interface Sci 2006;11:164–70.
[40] Harding SE. Mucoadhesive interactions. Biochem Soc Trans 2003;31:1036–41.
[41] Harding SE. Trends in mucoadhesive analysis. Trends Food Sci Technol 2006;17:255–62.
[42] Button B, Cai L-H, Ehre C, Kesimer M, Hill DB, Sheehan JK, et al. A periciliary brush promotes the lung health by separating the mucus layer from airway epithelia. Science 2012;337:937–41.
[43] Hill DB, Button B, Rubinstein M, Boucher RC. Physiology and pathophysiology of human airway mucus. Physiol Rev 2022;102:1757–836.
[44] Biering SB, Sarnik SA, Wang E, Zengel JR, Leist SR, Schafer A, et al. Genome-wide bidirectional CRISPR screens identify mucins as host factors modulating SARS-CoV-2 infection. Nat Genet 2022;54:1078–89.
[45] Cone RA. Barrier properties of mucus. Adv Drug Deliv Rev 2009;61:75–85.
[46] Popov A. Mucus-penetrating particles and the role of ocular mucus as a barrier to micro- and nanosuspensions. J Ocul Pharmacol Ther 2020;36:366–75.
[47] Guyton AC. Text book of medical physiology. China. 2006.
[48] Derrien M, van Passel MW, van de Bovenkamp JH, Schipper RG, de Vos WM, Dekker J. Mucin-bacterial interactions in the human oral cavity and digestive tract. Gut Microbes 2010;1:254–68.
[49] Paone P, Cani PD. Mucus barrier, mucins and gut microbiota: the expected slimy partners? Gut 2020;69:2232–43.
[50] Atuma C, Strugala V, Allen A, Holm L. The adherent gastrointestinal mucus gel layer: thickness and physical state in vivo. Am J Physiol Gastrointest Liver Physiol 2001;280. G922-G9.
[51] Lai SK, Wang YY, Wirtz D, Hanes J. Micro- and macrorheology of mucus. Adv Drug Deliv Rev 2009;61:86–100.
[52] Meldrum OW, Yakubov GE, Bonilla MR, Deshmukh O, McGuckin MA, Gidley MJ. Mucin gel assembly is controlled by a collective action of non-mucin proteins, disulfide bridges, Ca(2+)-mediated links, and hydrogen bonding. Sci Rep 2018;8:5802.
[53] Philippe A-M, Cipelletti L, Larobina D. Mucus as an arrested phase separation gel. Macromolecules 2017;50:8221–30.
[54] Dawson M, Wirtz D, Hanes J. Enhanced viscoelasticity of human cystic fibrotic sputum correlates with increasing microheterogeneity in particle transport. J Biol Chem 2003;278:50393–401.
[55] Radtke T, Boni L, Bohnacker P, Fischer P, Benden C, Dressel H. The many ways sputum flows - dealing with high within-subject variability in cystic fibrosis sputum rheology. Respir Physiol Neurobiol 2018;254:36–9.
[56] Majima Y, Harada T, Shimizu T, Takeuchi K, Sakakura Y, Yasuoka S, et al. Effect of biochemical components on Rheologic properties of nasal mucus in chronic sinusitis. Am J Respir Crit Care Med 1999;160:421–6.
[57] Majima Y, Hirata K, Takeuchi K, Hattori M, Sakakura Y. Effects of orally administered drugs on dynamic viscoelasticity of human nasal mucus. Am Rev Respir Dis 1990;141:79–83.
[58] Nordgard CT, Draget KI. Oligosaccharides as modulators of rheology in complex mucous systems. Biomacromolecules 2011;12:3084–90.
[59] Rubin BK, Druce H, Ramirez OE, Palmer R. Effect of clarithromycin on nasal mucus properties in healthy subjects and in patients with purulent rhinitis. Am J Respir Crit Care Med 1997;155:2018–23.
[60] Girod S, Zahm JM, Plotkowski C, Beck G, Puchelle E. Role of the physicochemical properties of mucus in the protection of the respiratory epithelium. Eur Respir J 1992;5:477–87.
[61] Bucher S, Schmid-Grendelmeier P, Soyka MB. Altered viscosity of nasal secretions in postnasal drip. Chest 2019;156:659–66.
[62] Lu W, Liu X, Wang T, Liu F, Zhu A, Lin Y, et al. Elevated MUC1 and MUC5AC mucin protein levels in airway mucus of critical ill COVID-19 patients. J Med Virol 2021;93:582–4.
[63] Kavishvar D, Ramachandran A. The yielding behaviour of human mucus. Adv Colloid Interface Sci 2023;322:103049.
[64] Taylor C, Allen A, Dettmar PW, Pearson JP. The gel matrix of gastric mucus is maintained by a complex interplay of transient and nontransient associations. Biomacromolecules 2003;4:922–7.
[65] Wu P, Dhital S, Williams BA, Chen XD, Gidley MJ. Rheological and microstructural properties of porcine gastric digesta and diets containing pectin or mango powder. Carbohydr Polym 2016;148:216–26.
[66] Kočevar-Nared J, Kristl J, Šmid-Korbar J. Comparative rheological investigation of crude gastric mucin and natural gastric mucus. Biomaterials 1997;18:677–81.
[67] Bej R, Haag R. Mucus-inspired dynamic hydrogels: synthesis and future perspectives. J Am Chem Soc 2022;144:20137–52.
[68] Cook MT, Khutoryanskiy VV. Mucoadhesion and mucosa-mimetic materials–A mini-review. Int J Pharm 2015;495:991–8.
[69] Navarro LA, French DL, Zauscher S. Advances in mucin mimic synthesis and applications in surface science. Curr Opin Colloid Interface Sci 2018;38:122–34.
[70] Hamed R, Fiegel J. Synthetic tracheal mucus with native rheological and surface tension properties. J Biomed Mater Res A 2014;102:1788–98.
[71] Yan H, Seignez C, Hjorth M, Winkeljann B, Blakeley M, Lieleg O, et al. Immune-informed mucin hydrogels evade fibrotic foreign body response in vivo. Adv Funct Mater 2019;29.
[72] Jiang K, Yan H, Rickert C, Marczynski M, Sixtensson K, Vilaplana F, et al. Modulating the bioactivity of mucin hydrogels with crosslinking architecture. Adv Funct Mater 2021;31.
[73] Song D, Iverson E, Kaler L, Bader S, Scull MA, Duncan GA. Modeling airway dysfunction in asthma using synthetic mucus biomaterials. ACS Biomater Sci Eng 2021;7:2723–33.
[74] Furst EM, Squires TM. Microrheology. 1st ed. Oxford: Oxford University Press; 2017.
[75] Abrami M, Maschio M, Conese M, Confalonieri M, Gerin F, Dapas B, et al. Combined use of rheology and portable low-field NMR in cystic fibrosis patients. Respir Med 2021;189:106623.
[76] Duncan GA, Jung J, Joseph A, Thaxton AL, West NE, Boyle MP, et al. Microstructural alterations of sputum in cystic fibrosis lung disease. JCI Insight 2016;1:e88198.
[77] Pangeni R, Meng T, Poudel S, Sharma D, Hutsell H, Ma J, et al. Airway mucus in pulmonary diseases: Muco-adhesive and muco-penetrating particles to overcome the airway mucus barriers. Int J Pharm 2023;634:122661.
[78] Duncan GA, Jung J, Hanes J, Suk JS. The mucus barrier to inhaled gene therapy. Mol Ther 2016;24:2043–53.
[79] Howard RL, Markovetz M, Wang Y, Ehre C, Sheikh SZ, Allbritton NL, et al. Biochemical and rheological analysis of human colonic culture mucus reveals similarity to gut mucus. Biophys J 2021;120:5384–94.
[80] Witten J, Samad T, Ribbeck K. Selective permeability of mucus barriers. Curr Opin Biotechnol 2018;52:124–33.
[81] Olmsted SS, Padgett JL, Yudin AI, Whaley KJ, Moench TR, Cone RA. Diffusion of macromolecules and virus-like particles in human cervical mucus. Biophys J 2001;81:1930–7.
[82] Saltzman WM, Radomsky ML, Whaley KJ, Cone RA. Antibody diffusion in human cervical mucus. Biophys J 1994;66:508–15.
[83] Lai SK, Wang YY, Hanes J. Mucus-penetrating nanoparticles for drug and gene delivery to mucosal tissues. Adv Drug Deliv Rev 2009;61:158–71.
[84] Lai SK, O'Hanlon DE, Harrold S, Man ST, Wang Y-Y, Cone R, et al. Rapid transport of large polymeric nanoparticles in fresh undiluted human mucus. Proc Natl Acad Sci 2007;104:1482–7.
[85] Huckaby JT, Lai SK. PEGylation for enhancing nanoparticle diffusion in mucus. Adv Drug Deliv Rev 2018;124:125–39.
[86] Tang BC, Dawson M, Lai SK, Wang Y-Y, Suk JS, Yang M, et al. Biodegradable polymer nanoparticles that rapidly penetrate the human mucus barrier. Proc Natl Acad Sci 2009;106:19268–73.
[87] Yang M, Lai SK, Wang YY, Zhong W, Happe C, Zhang M, et al. Biodegradable nanoparticles composed entirely of safe materials that rapidly penetrate human mucus. Angew Chem Int Ed Engl 2011;50:2597–600.
[88] Chen D, Xia D, Li X, Zhu Q, Yu H, Zhu C, et al. Comparative study of Pluronic((R)) F127-modified liposomes and chitosan-modified liposomes for mucus penetration and oral absorption of cyclosporine A in rats. Int J Pharm 2013;449:1–9.
[89] Müller C, Leithner K, Hauptstein S, Hintzen F, Salvenmoser W, Bernkop-Schnürch A. Preparation and characterization of mucus-penetrating papain/poly (acrylic acid) nanoparticles for oral drug delivery applications. J Nanopart Res 2012;15.
[90] Sanders NN, Smedt SCD, Rompaey EV, Simoens P, Baets FD, Demeester J. Cystic fibrosis sputum. Am J Respir Crit Care Med 2000;162:1905–11.
[91] Lai SK, Wang YY, Cone R, Wirtz D, Hanes J. Altering mucus rheology to "solidify" human mucus at the nanoscale. PloS One 2009;4:e4294.
[92] Tomaiuolo G, Rusciano G, Caserta S, Carciati A, Carnovale V, Abete P, et al. A new method to improve the clinical evaluation of cystic fibrosis patients by mucus viscoelastic properties. PloS One 2014;9:e82297.
[93] Esteban Enjuto L, Robert de Saint Vincent M, Maurin M, Degano B, Bodiguel H. Sputum handling for rheology. Sci Rep 2023;13:7695.
[94] Jory M, Donnarumma D, Blanc C, Bellouma K, Fort A, Vachier I, et al. Mucus from human bronchial epithelial cultures: rheology and adhesion across length scales. Interface Focus 2022;12:20220028.
[95] Lu Q, Solomon MJ. Probe size effects on the microrheology of associating polymer solutions. Phys Rev E Stat Nonlin Soft Matter Phys 2002;66:061504.
[96] Weigand WJ, Messmore A, Tu J, Morales-Sanz A, Blair DL, Deheyn DD, et al. Active microrheology determines scale-dependent material properties of Chaetopterus mucus. PloS One 2017;12:e0176732.
[97] Schuster BS, Suk JS, Woodworth GF, Hanes J. Nanoparticle diffusion in respiratory mucus from humans without lung disease. Biomaterials 2013;34:3439–46.

[98] Huck BC, Hartwig O, Biehl A, Schwarzkopf K, Wagner C, Loretz B, et al. Macro- and microrheological properties of mucus surrogates in comparison to native intestinal and pulmonary mucus. Biomacromolecules 2019;20:3504–12.
[99] Meziu E, Koch M, Fleddermann J, Schwarzkopf K, Schneider M, Kraegeloh A. Visualization of the structure of native human pulmonary mucus. Int J Pharm 2021;597:120238.
[100] Wagner CE, Wheeler KM, Ribbeck K. Mucins and their role in shaping the functions of mucus barriers. Annu Rev Cell Dev Biol 2018;34:189–215.
[101] Kesimer M, Makhov AM, Griffith JD, Verdugo P, Sheehan JK. Unpacking a gel-forming mucin: a view of MUC5B organization after granular release. Am J Physiol Lung Cell Mol Physiol 2010;298:L15–22.
[102] Pearson JP, Chater PI, Wilcox MD. The properties of the mucus barrier, a unique gel – how can nanoparticles cross it? Ther Deliv 2016;7:229–44.
[103] Ambort D, Johansson ME, Gustafsson JK, Nilsson HE, Ermund A, Johansson BR, et al. Calcium and pH-dependent packing and release of the gel-forming MUC2 mucin. Proc Natl Acad Sci U S A 2012;109:5645–50.
[104] Allen A, Flemstrom G. Gastroduodenal mucus bicarbonate barrier: protection against acid and pepsin. Am J Physiol Cell Physiol 2005;288:C1–19.
[105] Raynal BD, Hardingham TE, Sheehan JK, Thornton DJ. Calcium-dependent protein interactions in MUC5B provide reversible cross-links in salivary mucus. J Biol Chem 2003;278:28703–10.
[106] Celli JP, Turner BS, Afdhal NH, Ewoldt RH, McKinley GH, Bansil R, et al. Rheology of gastric mucin exhibits a pH-dependent sol−gel transition. Biomacromolecules 2007;8:1580–6.
[107] Guo Y, Ma Y, Chen X, Li M, Ma X, Cheng G, et al. Mucus penetration of surface-engineered nanoparticles in various pH microenvironments. ACS Nano 2023;17:2813–28.
[108] Curnutt A, Smith K, Darrow E, Walters KB, Vasquez ES, Kundu S. Physicochemical characterization of mammalian mucus and mucin solutions in response to pH and [Ca2+]. In: ASME-JSME-KSME 2019 8th joint fluids engineering conference. vol. volume 4: Fluid measurement and instrumentation; micro and nano fluid dynamics; 2019.
[109] Celli JP, Turner BS, Afdhal NH, Keates S, Ghiran I, Kelly CP, et al. Helicobacter pylori moves through mucus by reducing mucin viscoelasticity. Proc Natl Acad Sci 2009;106:14321–6.
[110] Parlato RM, Greco F, Maffettone PL, Larobina D. Effect of pH on the viscoelastic properties of pig gastric mucus. J Mech Behav Biomed Mater 2019;98:195–9.
[111] Hill DB, Long RF, Kissner WJ, Atieh E, Garbarine IC, Markovetz MR, et al. Pathological mucus and impaired mucus clearance in cystic fibrosis patients result from increased concentration, not altered pH. Eur Respir J 2018;52.
[112] Wang YY, Lai SK, Ensign LM, Zhong W, Cone R, Hanes J. The microstructure and bulk rheology of human cervicovaginal mucus are remarkably resistant to changes in pH. Biomacromolecules 2013;14:4429–35.
[113] Glantz PO, Arnebrant T, Nylander T, Baier RE. Bioadhesion–a phenomenon with multiple dimensions. Acta Odontol Scand 1999;57:238–41.
[114] Sogias IA, Williams AC, Khutoryanskiy VV. Why is chitosan mucoadhesive? Biomacromolecules 2008;9:1837–42.
[115] Taylor C, Pearson J, Draget K, Dettmar P, Smidsrod O. Rheological characterisation of mixed gels of mucin and alginate. Carbohydr Polym 2005;59:189–95.
[116] Lieleg O, Vladescu I, Ribbeck K. Characterization of particle translocation through mucin hydrogels. Biophys J 2010;98:1782–9.
[117] Joergensen L, Klosgen B, Simonsen AC, Borch J, Hagesaether E. New insights into the mucoadhesion of pectins by AFM roughness parameters in combination with SPR. Int J Pharm 2011;411:162–8.
[118] Cook SL, Bull SP, Methven L, Parker JK, Khutoryanskiy VV. Mucoadhesion: a food perspective. Food Hydrocoll 2017;72:281–96.
[119] Sumarokova M, Iturri J, Weber A, Maares M, Keil C, Haase H, et al. Influencing the adhesion properties and wettability of mucin protein films by variation of the environmental pH. Sci Rep 2018;8:9660.
[120] Lee S, Müller M, Rezwan K, Spencer ND. Porcine gastric mucin (PGM) at the water/poly(dimethylsiloxane) (PDMS) interface: influence of pH and ionic strength on its conformation, adsorption, and aqueous lubrication properties. Langmuir 2005;21:8344–53.
[121] Bernkop-Schnurch A, Schwarz V, Steininger S. Polymers with thiol groups: a new generation of mucoadhesive polymers? Pharm Res 1999;16:876–81.
[122] Bernkop-Schnurch A, Thaler SC. Polycarbophil-cysteine conjugates as platforms for oral polypeptide delivery systems. J Pharm Sci 2000;89:901–9.
[123] Bernkop-Schnurch A, Steininger S. Synthesis and characterisation of mucoadhesive thiolated polymers. Int J Pharm 2000;194:239–47.
[124] Bernkop-Schnurch A, Kast CE, Richter MF. Improvement in the mucoadhesive properties of alginate by the covalent attachment of cysteine. J Control Release 2001;71:277–85.
[125] Leitner VM, Walker GF, Bernkop-Schnurch A. Thiolated polymers: evidence for the formation of disulphide bonds with mucus glycoproteins. Eur J Pharm Biopharm 2003;56:207–14.
[126] Bernkop-Schnurch A, Leitner V, Moser V. Synthesis and in vitro characterization of a poly(acrylic acid)-homocysteine conjugate. Drug Dev Ind Pharm 2004;30:1–8.
[127] Bernkop-Schnurch A, Konig V, Leitner VM, Krauland AH, Brodnik I. Preparation and characterisation of thiolated poly(methacrylic acid)-starch compositions. Eur J Pharm Biopharm 2004;57:219–24.
[128] Bernkop-Schnurch A, Hornof M, Zoidl T. Thiolated polymers-thiomers: synthesis and in vitro evaluation of chitosan-2-iminothiolane conjugates. Int J Pharm 2003;260:229–37.
[129] Kast CE, Bernkop-Schnurch A. Thiolated polymers — thiomers: development and in vitro evaluation of chitosan–thioglycolic acid conjugates. Biomaterials 2001;22:2345–52.
[130] Kafedjiiski K, Foger F, Werle M, Bernkop-Schnurch A. Synthesis and in vitro evaluation of a novel chitosan-glutathione conjugate. Pharm Res 2005;22:1480–8.
[131] Bal-Öztürk A, Torkay G, Alarçin E, Özbaş Z, Özkahraman B. The effect of thiol functional groups on bovine serum albumin/chitosan buccal mucoadhesive patches. J Drug Deliv Sci Technol 2022;74.
[132] Zhang S, Asghar S, Yu F, Chen Z, Hu Z, Ping Q, et al. BSA nanoparticles modified with N-acetylcysteine for improving the stability and mucoadhesion of curcumin in the gastrointestinal tract. J Agric Food Chem 2019;67:9371–81.
[133] Bernkop-Schnurch A. Thiomers: a new generation of mucoadhesive polymers. Adv Drug Deliv Rev 2005;57:1569–82.
[134] Gu JM, Robinson JR, Leung SH. Binding of acrylic polymers to mucin/epithelial surfaces: structure-property relationships. Crit Rev Ther Drug Carrier Syst 1988;5:21–67.
[135] Lehr C-M, Bouwstra JA, Boddé HE, Junginger HE. A surface energy analysis of mucoadhesion: contact angle measurements on polycarbophil and pig intestinal mucosa in physiologically relevant fluids. Pharm Res 1992;9:70–5.
[136] Andrews GP, Laverty TP, Jones DS. Mucoadhesive polymeric platforms for controlled drug delivery. Eur J Pharm Biopharm 2009;71:505–18.
[137] Rojewska M, Olejniczak-Rabinek M, Bartkowiak A, Snela A, Prochaska K, Lulek J. The wettability and swelling of selected mucoadhesive polymers in simulated saliva and vaginal fluids. Colloids Surf B Biointerfaces 2017;156:366–74.
[138] Peppas NA, Sahlin JJ. Hydrogels as mucoadhesive and bioadhesive materials: a review. Biomaterials 1996;17:1553–61.
[139] Khutoryanskiy VV. Advances in mucoadhesion and mucoadhesive polymers. Macromol Biosci 2011;11:748–64.
[140] Sriamornsak P, Wattanakorn N, Nunthanid J, Puttipipatkhachorn S. Mucoadhesion of pectin as evidence by wettability and chain interpenetration. Carbohydr Polym 2008;74:458–67.
[141] Maisel K, Reddy M, Xu Q, Chattopadhyay S, Cone R, Ensign LM, et al. Nanoparticles coated with high molecular weight PEG penetrate mucus and provide uniform vaginal and colorectal distribution in vivo. Nanomedicine 2016;11:1337–43.
[142] Marschütz MK, Bernkop-Schnürch A. Thiolated polymers: self-crosslinking properties of thiolated 450 kDa poly(acrylic acid) and their influence on mucoadhesion. Eur J Pharm Sci 2002;15:387–94.
[143] Salamat-Miller N, Chittchang M, Johnston TP. The use of mucoadhesive polymers in buccal drug delivery. Adv Drug Deliv Rev 2005;57:1666–91.
[144] Chaturvedi M, Kumar M, Pathak K. A review on mucoadhesive polymer used in nasal drug delivery system. J Adv Pharm Technol Res 2011;2:215–22.
[145] Guo J-H. Bioadhesive polymer buccal patches for buprenorphine controlled delivery: formulation, in-vitro adhesion and release properties. Drug Dev Ind Pharm 1994;20:2809–21.
[146] McGhee EO, Hart SM, Uruena JM, Sawyer WG. Hydration control of gel-adhesion and muco-adhesion. Langmuir 2019;35:15769–75.
[147] Wu SJ, Zhao X. Bioadhesive technology platforms. Chem Rev 2023;123:14084–118.
[148] Baljon ARC, Robbins MO. Energy dissipation during rupture of adhesive bonds. Science 1996;271:482–4.
[149] Zhu Z, Yang Z, Xia Y, Jiang H. A review of debonding behavior of soft material adhesive systems. Mech Soft Mater 2022:4.
[150] Israelachvili J, Berman A. Irreversibility, energy dissipation, and time effects in intermolecular and surface interactions. Israel J Chem 1995;35:85–91.
[151] Subramanian DA, Langer R, Traverso G. Mucus interaction to improve gastrointestinal retention and pharmacokinetics of orally administered nano-drug delivery systems. J Nanobiotechnol 2022;20:362.
[152] Bonfim AP, Sakita KM, Faria DR, Arita GS, Vendramini F, Capoci IRG, et al. Preclinical approaches in vulvovaginal candidiasis treatment with mucoadhesive thermoresponsive systems containing propolis. PloS One 2020;15:e0243197.
[153] Laffleur F, Netsomboon K, Bernkop-Schnürch A, Westmeier D, Stauber RH, Docter D. Comprehensive mucoadhesive study of anionic polymers and their derivate. Eur Polym J 2017;93:314–22.
[154] Kovalainen M, Monkare J, Riikonen J, Pesonen U, Vlasova M, Salonen J, et al. Novel delivery systems for improving the clinical use of peptides. Pharmacol Rev 2015;67:541–61.
[155] Schneider CS, Xu Q, Boylan NJ, Chisholm J, Tang BC, Schuster BS, et al. Nanoparticles that do not adhere to mucus provide uniform and long-lasting drug delivery to airways following inhalation. Sci Adv 2017;3:e1601556.
[156] Yarragudi SB, Richter R, Lee H, Walker GF, Clarkson AN, Kumar H, et al. Formulation of olfactory-targeted microparticles with tamarind seed polysaccharide to improve nose-to-brain transport of drugs. Carbohydr Polym 2017;163:216–26.
[157] Watchorn J, Stuart S, Burns DC, Gu FX. Mechanistic influence of polymer species, molecular weight, and functionalization on mucin–polymer binding interactions. ACS Appl Polymer Mater 2022;4:7537–46.
[158] Edsman K, Carlfors J, Harju K. Rheological evaluation and ocular contact time of some carbomer gels for ophthalmic use. Int J Pharm 1996;137:233–41.
[159] Bayer IS. Recent advances in mucoadhesive interface materials, mucoadhesion characterization, and technologies. Adv Mater Interfaces 2022:9.
[160] Jones DS, Laverty TP, Morris C, Andrews GP. Statistical modelling of the rheological and mucoadhesive properties of aqueous poly(methylvinylether-co-maleic acid) networks: redefining biomedical applications and the relationship

between viscoelasticity and mucoadhesion. Colloids Surf B Biointerfaces 2016; 144:125–34.
[161] Jones DS, Yu T, Li S, Pick L, Andrews GP. A new mathematical model derived from transient (creep) analysis to estimate the vaginal retention of semi-solid dosage forms. Int J Pharm 2023;632:122521.
[162] Morimoto K, Katsumata H, Yabuta T, Iwanaga K, Kakemi M, Tabata Y, et al. Evaluation of gelatin microspheres for nasal and intramuscular administrations of salmon calcitonin. Eur J Pharm Sci 2001;13:179–85.
[163] Duggan S, O'Donovan O, Owens E, Cummins W, Hughes H. Synthesis of mucoadhesive thiolated gelatin using a two-step reaction process. Eur J Pharm Biopharm 2015;91:75–81.
[164] Rohrer J, Zupančič O, Suchaoin W, Netsomboon K, Laffleur F, Oh S, et al. Synthesis and in vitro characterisation of preactivated thiolated gelatin. Eur Polym J 2015;73:268–77.
[165] Teng Z, Li Y, Luo Y, Zhang B, Wang Q. Cationic beta-lactoglobulin nanoparticles as a bioavailability enhancer: protein characterization and particle formation. Biomacromolecules 2013;14:2848–56.
[166] Teng Z, Li Y, Niu Y, Xu Y, Yu L, Wang Q. Cationic beta-lactoglobulin nanoparticles as a bioavailability enhancer: comparison between ethylenediamine and polyethyleneimine as cationizers. Food Chem 2014;159:333–42.
[167] Hsein H, Garrait G, Beyssac E, Hoffart V. Whey protein mucoadhesive properties for oral drug delivery: mucin-whey protein interaction and mucoadhesive bond strength. Colloids Surf B Biointerfaces 2015;136:799–808.
[168] Hsein H, Garrait G, Tamani F, Beyssac E, Hoffart V. Denatured whey protein powder as a new matrix excipient: design and evaluation of mucoadhesive tablets for sustained drug release applications. Pharm Res 2017;34:365–77.
[169] Cheng Z, Chen X, Zhai D, Gao F, Guo T, Li W, et al. Development of keratin nanoparticles for controlled gastric mucoadhesion and drug release. J Nanobiotechnol 2018;16:24.
[170] Bonferoni MC, Chetoni P, Giunchedi P, Rossi S, Ferrari F, Burgalassi S, et al. Carrageenan-gelatin mucoadhesive systems for ion-exchange based ophthalmic delivery: in vitro and preliminary in vivo studies. Eur J Pharm Biopharm 2004;57: 465–72.
[171] Deat-Laine E, Hoffart V, Cardot JM, Subirade M, Beyssac E. Development and in vitro characterization of insulin loaded whey protein and alginate microparticles. Int J Pharm 2012;439:136–44.
[172] Davoudi Z, Rabiee M, Houshmand B, Eslahi N, Khoshroo K, Rasoulianboroujeni M, et al. Development of chitosan/gelatin/keratin composite containing hydrocortisone sodium succinate as a buccal mucoadhesive patch to treat desquamative gingivitis. Drug Dev Ind Pharm 2018;44:40–55.
[173] Li D, Liu A, Liu M, Li X, Guo H, Zuo C, et al. The intestine-responsive lysozyme nanoparticles-in-oxidized starch microgels with mucoadhesive and penetrating properties for improved epithelium absorption of quercetin. Food Hydrocoll 2020;99.
[174] Butnarasu C, Petrini P, Bracotti F, Visai L, Guagliano G, Fiorio Pla A, et al. Mucosomes: intrinsically mucoadhesive glycosylated mucin nanoparticles as multi-drug delivery platform. Adv Healthc Mater 2022;11:e2200340.
[175] Fu M, Filippov SK, Williams AC, Khutoryanskiy VV. On the mucoadhesive properties of synthetic and natural polyampholytes. J Colloid Interface Sci 2024; 659:849–58.
[176] Ye A, Zheng T, Ye JZ, Singh H. Potential role of the binding of whey proteins to human buccal cells on the perception of astringency in whey protein beverages. Physiol Behav 2012;106:645–50.
[177] Perez-Jimenez R, Godoy-Ruiz R, Ibarra-Molero B, Sanchez-Ruiz JM. The efficiency of different salts to screen charge interactions in proteins: a hofmeister effect? Biophys J 2004;86:2414–29.
[178] Boland M. Whey proteins. In: Handbook of food proteins; 2011. p. 30–55.
[179] Pelegrine DHG, Gasparetto CA. Whey proteins solubility as function of temperature and pH. LWT- Food Sci Technol 2005;38:77–80.
[180] Beecher JW, Drake MA, Luck PJ, Foegeding EA. Factors regulating astringency of whey protein beverages. J Dairy Sci 2008;91:2553–60.
[181] Ye A, Streicher C, Singh H. Interactions between whey proteins and salivary proteins as related to astringency of whey protein beverages at low pH. J Dairy Sci 2011;94:5842–50.
[182] Kakati N, Parashar CK, Thakur S, Deshmukh OS, Bandyopadhyay D. Microrheology of mucin-albumin assembly using diffusing wave spectroscopy. ACS Appl Bio Mater 2022;5(9):4118–27. https://doi.org/10.1021/acsabm.2c00098.
[183] Teng Z, Luo Y, Li Y, Wang Q. Cationic beta-lactoglobulin nanoparticles as a bioavailability enhancer: effect of surface properties and size on the transport and delivery in vitro. Food Chem 2016;204:391–9.
[184] Mosquera J, Garcia I, Liz-Marzan LM. Cellular uptake of nanoparticles versus small molecules: a matter of size. Acc Chem Res 2018;51:2305–13.
[185] Withers CA, Cook MT, Methven L, Gosney MA, Khutoryanskiy VV. Investigation of milk proteins binding to the oral mucosa. Food Funct 2013;4:1668–74.
[186] Hernandez-Ledesma B, Recio I, Amigo L. Beta-lactoglobulin as source of bioactive peptides. Amino Acids 2008;35:257–65.
[187] Oh J, Kim B. Mucoadhesive and pH-responsive behavior of gelatin containing hydrogels for protein drug delivery applications. Korea-Aust Rheol J 2020;32: 41–6.
[188] Filatova L, Emelianov G, Balabushevich N, Klyachko N. Supramolecular assemblies of mucin and lysozyme: formation and physicochemical characterization. Process Biochem 2022;113:97–106.
[189] Svensson O, Lindh L, Cardenas M, Arnebrant T. Layer-by-layer assembly of mucin and chitosan–influence of surface properties, concentration and type of mucin. J Colloid Interface Sci 2006;299:608–16.
[190] Soares RV, Siqueira CC, Bruno LS, Oppenheim FG, Offner GD, Troxler RF. MG2 and lactoferrin form a heterotypic complex in salivary secretions. J Dent Res 2003;82:471–5.
[191] Svendsen IE, Lindh L, Elofsson U, Arnebrant T. Studies on the exchange of early pellicle proteins by mucin and whole saliva. J Colloid Interface Sci 2008;321: 52–9.
[192] Lindh L, Svendsen IE, Svensson O, Cardenas M, Arnebrant T. The salivary mucin MUC5B and lactoperoxidase can be used for layer-by-layer film formation. J Colloid Interface Sci 2007;310:74–82.
[193] Haberska K, Svensson O, Shleev S, Lindh L, Arnebrant T, Ruzgas T. Activity of lactoperoxidase when adsorbed on protein layers. Talanta 2008;76:1159–64.
[194] Halthur TJ, Arnebrant T, Macakova L, Feiler A. Sequential adsorption of bovine mucin and lactoperoxidase to various substrates studied with quartz crystal microbalance with dissipation. Langmuir 2010;26:4901–8.
[195] Elzoghby AO, Abdelmoneem MA, Hassanin IA, Abd Elwakil MM, Elnaggar MA, Mokhtar S, et al. Lactoferrin, a multi-functional glycoprotein: active therapeutic, drug nanocarrier & targeting ligand. Biomaterials 2020;263:120355.
[196] Sarkar A, Goh KKT, Singh H. Colloidal stability and interactions of milk-protein-stabilized emulsions in an artificial saliva. Food Hydrocoll 2009;23:1270–8.
[197] Xu F, Liamas E, Bryant M, Adedeji AF, Andablo-Reyes E, Castronovo M, et al. A self-assembled binary protein model explains high-performance salivary lubrication from macro to nanoscale. Adv Mater Interfaces 2019:7.
[198] Crouzier T, Jang H, Ahn J, Stocker R, Ribbeck K. Cell patterning with mucin biopolymers. Biomacromolecules 2013;14:3010–6.
[199] Kasdorf BT, Weber F, Petrou G, Srivastava V, Crouzier T, Lieleg O. Mucin-inspired lubrication on hydrophobic surfaces. Biomacromolecules 2017;18:2454–62.
[200] Ritzoulis C, Siasios S, Melikidou KD, Koukiotis C, Vasiliadou C, Lolakos S. Interactions between pig gastric mucin and sodium caseinate in solutions and in emulsions. Food Hydrocoll 2012;29:382–8.
[201] Ahmad M, Ritzoulis C, Bushra R, Meigui H, Zhang X, Chen J, et al. Mapping of β-lactoglobulin – mucin interactions in an in vitro astringency model: phase compatibility, adsorption mechanism and thermodynamic analysis. Food Hydrocoll 2022;129.
[202] Ahmad M, Ritzoulis C, Pan W, Chen J. Chemical physics of whey protein isolate in the presence of mucin: from macromolecular interactions to functionality. Int J Biol Macromol 2020;143:573–81.
[203] Bastardo L, Claesson P, Brown W. Interactions between mucin and alkyl sodium sulfates in solution. A light scattering study. Langmuir 2002;18:3848–53.
[204] List SJ, Findlay BP, Forstner GG, Forstner JF. Enhancement of the viscosity of mucin by serum albumin. Biochem J 1978;175:565–71.
[205] Feiler AA, Sahlholm A, Sandberg T, Caldwell KD. Adsorption and viscoelastic properties of fractionated mucin (BSM) and bovine serum albumin (BSA) studied with quartz crystal microbalance (QCM-D). J Colloid Interface Sci 2007;315: 475–81.
[206] Svensson O, Arnebrant T. Mucin layers and multilayers — physicochemical properties and applications. Curr Opin Colloid Interface Sci 2010;15:395–405.
[207] Leichner C, Jelkmann M, Bernkop-Schnurch A. Thiolated polymers: bioinspired polymers utilizing one of the most important bridging structures in nature. Adv Drug Deliv Rev 2019;151-152:191–221.
[208] Bull SP, Hong Y, Khutoryanskiy VV, Parker JK, Faka M, Methven L. Whey protein mouth drying influenced by thermal denaturation. Food Qual Prefer 2017;56: 233–40.
[209] Bull SP, Khutoryanskiy VV, Parker JK, Faka M, Methven L. Oral retention of thermally denatured whey protein: in vivo measurement and structural observations by CD and NMR. Food Chem 2022;374:131650.
[210] Islam MS, Reineke J, Kaushik R, Woyengo T, Baride A, Alqahtani MS, et al. Bioadhesive food protein nanoparticles as pediatric oral drug delivery system. ACS Appl Mater Interfaces 2019;11:18062–73.
[211] Kollner S, Dunnhaupt S, Waldner C, Hauptstein S, Pereira de Sousa I, Bernkop-Schnurch A. Mucus permeating thiomer nanoparticles. Eur J Pharm Biopharm 2015;97:265–72.
[212] Sanchez Armengol E, Laffleur F. The progress on sulfhydryl modified polymers with regard to synthesis, characterization and mucoadhesion. Int J Pharm 2021; 592:120016.
[213] Ijaz M, Bernkop-Schnurch A. Preactivated thiomers: their role in drug delivery. Expert Opin Drug Deliv 2015;12:1269–81.
[214] Polak R, Crouzier T, Lim RM, Ribbeck K, Beppu MM, Pitombo RNM, et al. Sugar-mediated disassembly of mucin/lectin multilayers and their use as pH-tolerant, on-demand sacrificial layers. Biomacromolecules 2014;15:3093–8.
[215] Mahmood A, Bonengel S, Laffleur F, Ijaz M, Idrees MA, Hussain S, et al. Can thiolation render a low molecular weight polymer of just 20-kDa mucoadhesive? Drug Dev Ind Pharm 2015;42:686–93.
[216] Lehr CM. Bioadhesion technologies for the delivery of peptide and protein drugs to the gastrointestinal tract. Crit Rev Ther Drug Carrier Syst 1994;11:119–60.
[217] Boddé HE, De Vries ME, Junginger HE. Mucoadhesive polymers for the buccal delivery of peptides, structure-adhesiveness relationships. J Control Release 1990; 13:225–31.
[218] Attali P, Lemarchand C, Roulet V, Scheuir C, Zakin L. Treating inflammation and inflammatory pain in mucosa using mucosal prolonged release bioadhesive therapeutic carriers. 2015. p. 19.
[219] Kaveh Z, Azadmard-Damirchi S, Yousefi G, Hashem Hosseini SM. A new approach in improving granular cold water swelling starch properties using xanthan gum and β-lactoglobulin/xanthan gum electrostatic coupled gel. Food Hydrocoll 2021; 113.
[220] Radwan SE, El-Moslemany RM, Mehanna RA, Thabet EH, Abdelfattah EA, El-Kamel A. Chitosan-coated bovine serum albumin nanoparticles for topical

tetrandrine delivery in glaucoma: in vitro and in vivo assessment. Drug Deliv 2022;29:1150–63.
[221] Niaz T, Sarkar A, Mackie A, Imran M. Impact of albumin corona on mucoadhesion and antimicrobial activity of carvacrol loaded chitosan nano-delivery systems under simulated gastro-intestinal conditions. Int J Biol Macromol 2021;169:171–82.
[222] Lin C, Kuo TC, Lin JC, Ho YC, Mi FL. Delivery of polysaccharides from Ophiopogon japonicus (OJPs) using OJPs/chitosan/whey protein co-assembled nanoparticles to treat defective intestinal epithelial tight junction barrier. Int J Biol Macromol 2020;160:558–70.
[223] Lu S, Kong S, Wang Y, Hu Z, Zhang L, Liao M. Gastric acid-response chitosan/alginate/tilapia collagen peptide composite hydrogel: protection effects on alcohol-induced gastric mucosal injury. Carbohydr Polym 2022;277:118816.
[224] Luo R, Lin M, Fu C, Zhang J, Chen Q, Zhang C, et al. Calcium pectinate and hyaluronic acid modified lactoferrin nanoparticles loaded rhein with dual-targeting for ulcerative colitis treatment. Carbohydr Polym 2021;263:117998.
[225] Nurul Fitri Marzaman A, Sartini Mudjahid M, Puspita Roska T, Sam A, Permana AD. Development of chloramphenicol whey protein-based microparticles incorporated into thermoresponsive in situ hydrogels for improved wound healing treatment. Int J Pharm 2022;628:122323.
[226] Hu J, Andablo-Reyes E, Soltanahmadi S, Sarkar A. Synergistic microgel-reinforced hydrogels as high-performance lubricants. ACS Macro Lett 2020;9:1726–31.
[227] Stie MB, Cunha C, Huang Z, Kirkensgaard JJK, Tuelung PS, Wan F, et al. A head-to-head comparison of polymer interaction with mucin from porcine stomach and bovine submaxillary glands. Sci Rep 2024;14:21350.
[228] Bonengel S, Bernkop-Schnurch A. Thiomers–from bench to market. J Control Release 2014;195:120–9.
[229] Michael T, Cook DS. Reconceptualising mucoadhesion for future medicines. RSC Pharm 2024. https://doi.org/10.1039/D4PM00149D. Advance Article.