Mucins as multifunctional building blocks of biomaterials†

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Mucins are large glycoproteins that are ubiquitous in the animal kingdom. Mucins coat the surfaces of many cell types and can be secreted to form mucus gels that assume important physiological roles in many animals. Our growing understanding of the structure and function of mucin molecules and their functionalities has sparked interest in investigating the use of mucins as building blocks for innovative functional biomaterials. These pioneering studies have explored how new biomaterials can benefit from the barrier properties, hydration and lubrication properties, unique chemical diversity, and bioactivities of mucins. Owing to their multifunctionality, mucins have been used in a wide variety of applications, including as antifouling coatings, as selective filters, and artificial tears and saliva, as basis for cosmetics, as drug delivery materials, and as natural detergents. In this review, we summarize the current knowledge regarding key mucin properties and survey how they have been put to use. We offer a vision for how mucins could be used in the near future and what challenges await the field before biomaterials made of mucins and mucin-mimics can be translated into commercial products.

Introduction

Mucins are a family of large glycoprotein polymers expressed both as cell membrane-tethered molecules and as a major component of the mucus gel, when secreted by the goblet cells of the epithelium. Mucins have a vital role in the offensive and defensive mechanisms of many species. The multiple and diverse physiological roles of mucins have long been of interest, but it is only more recently that some of their biophysical and biological properties have been investigated in more detail. The main properties attributed to mucins include barrier properties, dynamicity, hydration, lubrication, and bioactivity, as summarized in Table 1. In this review, we present mucin glycoproteins as a biomaterial with a strong potential for medical and chemical applications. We hypothesise that mucins could develop in a similar fashion as other biopolymers extracted from animal or plant tissues such as

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What are mucins?

A thin layer of a few hundreds of micrometers of hydrogel protects our mucosa from dehydration caused by exposure to air, from the mechanical stress of the food we swallow, and from the millions of viruses and bacteria that we encounter everyday. This feat is in large part achieved by mucins; a family of highly functional glycoproteins, of which a subcategory assembles in vivo to form mucus gels. Mucins are endowed with a rich chemical diversity, since they combine a long protein backbone with O-linked glycans terminated by a variety of sugar units (Fig. 1A). Structurally, the dense glycosylation of mucins confers a relatively extended conformation to the molecule, which can associate into large oligomers (in the tens of MDa) via disulfide bonds and other weaker interactions (Fig. 1B–D). Membrane-tethered mucins contain transmembrane domains and decorate the cell surface, extending several hundreds of nanometers outwards. Similarly to their secreted counterparts, membrane-tethered mucins contribute to the protection of the cell, while also being involved in intracellular signalling events. The chemical nature of mucins is reviewed in great detail elsewhere and thus will not be covered here. It is worth noting that the term mucin is used rather loosely. Although several mucins clearly belong to the same family of proteins due to strong amino acid sequence homologies, others possess rather unrelated protein structures and were named mucins mainly because they colocalize and share some of the functionalities with the mucin protein family. Since this review focuses on mucin functionalities, we will use a loose definition and include all glycoproteins that have been named mucins or mucin-like.

Sources of secreted mucins

In nature, mucins are ubiquitous. Their complex chemistry and diversity allows them to fulfill a variety of roles, including organism protection, locomotion, and food capture. They can be extracted from mucosal tissues either by collecting the mucus itself and extracting the mucins, or by homogenizing whole tissues. Purification is achieved by leveraging mucins’ unique physical–chemical characteristics, including their solubility profiles in solvents, large size, and strong net negative charge. Although the glycosylated fractions of mucins are relatively resistant to proteinolytic degradation, the unglycosylated sections are more easily degraded, which can alter the gelling properties of the mucins. Gel forming mucins can be purified, while preserving their structure, by using a combination of gentle water dissolution, size exclusion chromatography and cesium chloride density gradient centrifugation to remove mucin-bound impurities, including lipids and nucleotides. Mucins are commonly extracted from abundant animal sources, including pigs, cows, snails, chicken eggs, and jellyfish. The protein sequence differs based on the organism and tissue, varying in size, extent of glycosylation, and in the presence of domains such as Von Willebrand or cysteine rich regions. The glycosylation diversity, length, and structure are even more prone to variations. For instance, pig gastric mucins have longer glycan chains but with less...
However, even within the same organism, differences in inflammatory states of the epithelium and in the composition and activity of the microbiota can alter the glycan composition. The common features of the mucins referred to herein are: an extended central protein core, regions of dense O-glycosylation, and the presence of acidic sugars providing an overall negative charge to the molecule.

The challenges inherent to the extraction of natural products from animal tissues, such as batch to batch variability and risk of pathogen contamination, could be addressed by developing synthetic mucins. Recent advances in synthetic mucins have been reviewed by Authimoolam et al. and by Kiminori et al. These elegant approaches recapitulate some of the properties attributed to mucins. For instance, the Bertozzi group has been successful in generating mimics of the shorter membrane-bound mucins that bind cell receptors. However, the synthesis of complex mucins, including tens of different terminal glycan structures, a peptide core with thousands of amino acids, and the ability to auto-assemble into megadalton structures still remain elusive.

**Mucin-based protective barriers**

Mucins are our first line of defence in the protection of our exposed epithelium, forming a physical barrier that prevents the passage of deleterious molecules. The pore size of mucin gels in vivo is thought to be in the hundreds of nanometers in cervical mucus, excluding larger objects such as bacteria and yeast cells. In the colon, a double layer of mucus protects the epithelium from the billions of bacteria thriving in the lumen. A top loose layer allows bacteria to interact with the mucin, while a more compact bottom layer creates a near sterile buffer zone to protect the epithelium. In addition to acting as a filter, the mucus gel acts as a barrier by affinity sorting. Objects smaller than the pore size such as viruses, also can be trapped in the mucin network by binding to the ligands present on the tip of the glycans. Once bound to the mucins, the pathogens are removed from the mucosa by an active turnover of the mucus layer.

These properties have inspired bioengineers to build mucin-based barriers to pathogens. The spontaneous formation of stable mucin coatings on a range of materials makes this approach particularly attractive to prevent microorganisms from colonizing surfaces. Purified mucins reduce the adhesion of *Streptococcus pneumoniae, Staphylococcus aureus, Staphylococcus epidermidis*, and *Candida albicans*, when adsorbed on synthetic polymers (Fig. 2D and E). Similar effects were reported with mammalian cells. For instance, neutrophils adhere poorly on mucin coatings, which was also correlated with lower activation levels compared to uncoated surfaces. In our group, we have used mucins and microfluidic devices to create anti-adhesive patterns, effectively confining fibroblasts, myoblasts, and epithelial cells for several days (Fig. 2F). The surface of poly lactic-co-glycolic acid (PLGA) nanoparticles has also been functionalized with pig gastric mucins to limit protein adsorption, shield surface antigens and enhance blood compatibility. The anti-fouling and cell
repellent properties of mucin coatings were first applied in vivo by Janairo et al. who functionalized small synthetic vascular grafts of (1 mm diameter) with mucins to improve the hemocompatibility of the device. When implanted in mice, the mucin-functionalized grafts exhibited a good patency rate and promoted cell infiltration. Mucin coatings have also been used to coat the surface of porous PDMS matrices, mimicking the affinity-based selective filtering properties of mucus gels. Due to the negative charges on mucin glycans, the mucin-functionalized filters bound to positively charged particles while allowing negatively charged particles to flow through.53

The anti-fouling effect that exists for some mucin coatings, is likely due to the specific chemical nature of the mucins and to the mucins’ arrangement at the surface. On hydrophobic surfaces, it is hypothesized that mucins extend their highly glycosylated and highly hydrated domains into the aqueous solvent, while the non-glycosylated protein domains interact with the surface. This conformation is suggested from quartz crystal microbalance with dissipation monitoring (QCM-D) that measures large frequency shifts and dissipations for mucins physisorbed on a number of surfaces, when mucin is adsorbed on the sensor. These mucin-coated surfaces are somewhat comparable to PEG brushes commonly used for anti-fouling coatings, since both form a hydration shell and cause steric hindrance preventing adsorption (Fig. 2A–C). It is worth noting that the adsorption of mucins is very much dependent on the type of mucins used, the surface properties and coating conditions. In addition, some microorganisms are equipped with membrane receptors that are able to bind mucins and overcome the repulsive effect.

The strong interaction of mucins with the surface might have denaturing effects that affect important intra- and inter-

Fig. 2 Barrier properties of mucin coatings. (A) Most surfaces are susceptible to colonization by macromolecules, cells and bacteria. (B) Mucins can adsorb to form stable coatings on a number of surface chemistries. Mucin coatings can repel bacteria, proteins, and mammalian cells. (C) Mucin coatings are hypothesised to resemble those of PEG-based antifouling surfaces. (D–E) The antifouling properties of mucin coatings as shown by microscopy observation and quantification of bacteria binding. Pseudomonas aeruginosa and other bacteria possess receptors that bind the glycan and protein domains of the mucins, allowing them to overcome the repellent effect. (F) The repellent effect of mucin coatings towards mammalian cells was utilized to patterned epithelial, fibroblast and myoblast cells on tissue culture polystyrene. (D) is reprinted from ref. 45 with permission from Wiley Materials, (E) is reprinted from ref. 47 with permission from Elsevier, and (F) is reprinted with permission from ref. 50, copyright 2018 American Chemical Society.
molecular interactions. The auto-assembly of mucins into thicker coatings by the layer-by-layer technique with other polymers and proteins provides better mimics of the intermolecular interactions found in mucus gels. Such thin films can also be combined with bioactive compounds, such as antimicrobial peptides found in intestinal mucus. However, mucins are the closest to their original conformation when presented in solutions or gels. Mucin gels and solutions have been used to investigate mucins’ protective role against microbes, viruses, and toxins (Fig. 3A). It is not entirely clear whether the protective effects of mucin gels are due to steric hindrances (i.e. physically preventing the pathogens from reaching the host cells) or rely on specific signalling, perhaps mediated by surface proteins and the mucins’ glycans or peptidic core. However, polymer solutions mimicking mucin gel rheological properties do not recapitulate the protective effect to the same extent (Fig. 3B). The importance of functional groups on mucins is also suggested by recombinant mucin-like proteins which are unable to polymerise into gels but can bind to viral adhesion proteins and toxins and by artificially poly-sialiated mucins that can counteract the cytotoxicity of extracellular histones secreted by activated neutrophils.

Mucins have also been suggested to form physico-chemical barriers. For instance, mucins can act as a buffer layer to protect the epithelial lining from the strong acidity and high protease activity found in the stomach. The charged terminal sialic acid sugar residues can act as a biological mask, for instance, by shielding antigens from recognition by the immune system. This strategy is employed by the Trypanosoma cruzi parasite, that secretes a coat of mucin-like molecules that shield the pathogen from the host’s immune response. Mucinous tumors also produce a physical mucin barrier that shields tumors from the immune system and limits cell-cell interactions that facilitate metastasis. Interestingly, it was standard procedure from the 1930s up to the 1970s and even occasionally more recently, to co-administer mucins along with pathogenic bacteria, viruses and fungi in small animal models, such as mice, to lower their resistance and develop pathologies. Mucins were also found to prolong the survival of bacterial cultures. The formation of a protective mucin coating around the bacteria, which was shown to prevent the entrance of Indian dye inside the cells, could explain the effect. However, one must also consider that the enhanced virulence could also be a direct effect of mucin preparation impurities on the host immune system. Although this line of research seems to have faded, the prospect of coating cells with protective macromolecules, such as mucins, is attractive and could find application in probiotic delivery or cell therapies (Fig. 3C).

Mucins could also provide protection against proteolytic activities due to the dense glycosylation on the mucin protein core that limits enzymatic access to the protein. Loomes et al., showed that replacing a glycosylated section of the surface.
human milk cholesterol esterase protein with a glycosylated section of the MUC6 mucin could confer good proteolytic resistance.82 Their results suggest that mucin fragments could be used to confer protease resistance to proteins and increase the half life of biologics used in therapies.

What lies ahead

With our improved understanding of mucins’ barrier function and properties, biomimetic approaches, consisting of the treatment of epithelial surfaces with exogeneous mucins to reinforce the mucosal barrier in the mouth, stomach, or gut, can again be considered. Indeed, studies from the 1930s suggest that pig gastric mucin solutions can be successfully used as treatment against gastric ulcers83 and a later study showed that their oral ingestion could provide some protection to rats against N-methyl-N-nitro-N-nitrosoguanidine and NaCl induced gastric carcinomas.84 To our knowledge, the molecular mechanisms behind these effects have not been thoroughly investigated. One also can hypothesise that exogeneous mucins can reconstitute a temporary protective patch over the epithelium, lowering inflammation levels, and leaving sufficient time for the epithelium to regenerate. More recently, mice overexpressing mucins or mucin fragments have shown a superior ability to be protected from pathogens in both the intestines85 and lungs.86 The exogenous delivery of mucins would be a simpler approach than the overexpression of mucins. However, the delivery of fully functional mucin molecules and their lasting presence at the site of interest remain challenges to be resolved. The barrier properties of mucins could also be used for the encapsulation and delivery of cells. For instance, a mucin encapsulation could protect probiotic bacteria from the low pH and proteolytic activity in the stomach, then release the bacteria in the intestines where the local microflora can degrade mucins.87

Exploiting the complex chemistry of mucins

The multi-functionality of mucins is closely related to their chemical complexity. The non-glycosylated domains of the glycoprotein exhibit a rich chemical diversity, including charged domains, hydrophobic domains, and thiolated groups that can engage in disulfide bridges. The mucin protein core is heavily substituted by glycan chains directly O-linked to tandem repeats of proline, threonine, and serine. A recent review by A. Corfield lists the glycan structures that make up mucin glycosylation.18 The diversity of the mucin glycan structures that have already been identified is striking, with tens of possible terminal sugars mediating interactions with cell receptors, extracellular biopolymers, lipids, and other small molecules and particles. Sulfate groups on N-acetylgalactosamine and galactose, and carboxylic groups on sialic acid sugars provide an overall negative charge to mucins in most pH conditions. Mucin glycans are rich in hydroxyl groups, which can engage in intramolecular and intermolecular hydrogen bonds. The chemical diversity of mucins has found several interesting applications.

The rich library of mucin glycan structures are ligands to a wide range of sugar binding proteins found in viruses, bacteria, animal cells, and plant cells. For instance, mucin-associated glycans have binding affinities with many lectin-type proteins extracted from plant tissues, several of which are now industrially produced. The specificity of lectin binding has been used to probe the glyco-composition of mucins and mucus.88 Our group has exploited the multivalent binding of lectins to mucins to generate sugar-responsive thin films that are assembled layer-by-layer.89 The high avidity of the lectin–mucin interactions led to thin films surprisingly resilient to pH and ionic strength that could be selectively disassembled in the presence of a sugar solution. These assemblies were then used as sacrificial substrates to create self-standing films90 but could also be used for drug delivery, especially for insulin that could be released when triggered by high glucose concentrations.

The presence of hydrophobic protein domains and hydrophilic glycosylated domains effectively make mucins good surfactants. Mucins have been exploited as natural polymeric surfactants to host and release hydrophobic drugs. Bovine submaxillary mucins functionalized with methacrylate resulted in robust UV-crosslinked hydrogels91 (Fig. 4E). Anti-cancer drug paclitaxel and the positively charged antibiotic polymyxin B were successfully loaded and released in a sustainable manner over days from the covalently cross-linked mucin hydrogels (Fig. 4F), while lidocaine was shown to be released over hours from mixed bovine submaxillary mucin (BSM)-gelatin hydrogels.92 The sustained release of the drugs could be attributed to interactions with mucins, since the high molar mass but poorly mucoadhesive molecules released faster than the two small model drugs. The hydrophilicity and negative charge of mucins were also used by Nowald et al. who mixed pig gastric mucins with methylcellulose to create thermo-sensitive self-standing hydrogels that bound to positively charged molecules.93 By removing some of the pig gastric mucin glycosylation, Fukui et al. were able to generate nanoparticles that could incorporate positively charged antibacterial lysozyme enzymes.94 The particles degraded over time and released their content, due to the proteolytic action of the encapsulated lysozyme.

The amphiphilicity of mucins has also been used to generate water-soluble carbon nanomaterials, a prerequisite to apply carbon nanotubes in biomedical applications. This was achieved by using both a mimic of membrane tethered mucins, consisting of a long C18 lipid hydrophobic anchor,42 and the full bovine submaxillary mucins.85 In related work, Gozin and his group have thoroughly investigated the solubilization of hydrophobic nanoparticles by mucins. In particular, they investigated the ability of mucins to promote the cellular uptake of two hydrocarbons: anthracene and coronene96 (Fig. 4A and B). The same group also showed that BSM can bind and solubilize these hydrocarbons, which enhanced their uptake by the epithelial Caco-2 cell line (Fig. 4C and D).
Deglycosylated BSM had no effect on the hydrocarbon uptake, which suggested that mucin-associated glycans are essential for the effect to occur. Similar results were also reported for pig gastric mucins (PGM).95,97 Although these studies point to the potential dangers of mucin-mediated cellular uptake of hydrocarbons, they also suggest that mucins could help to efficiently deliver hydrophobic therapeutics to cells. Similarly, the capacity of jellyfish mucus to strongly interact with gold nanoparticles implies that mucus could mediate the toxicity of nanoparticles but also points to the use of mucin-based materials for decontamination purposes.98 Other applications of mucins’ promiscuous interaction properties include the separation of dyes to create white luminescent devices,99 the promotion of organic reactions,100 and the synthesis of chiral silver nanoparticles.101

What lies ahead

The diverse repertoire of glycan structures that decorate mucins holds great potential for various applications. Milk-derived mucin glycans have been suggested to be used to modulate bacterial population of the gut.102 One can also foresee that materials functionalized with mucin glycans could capture viruses, bacteria, and toxins that contain glycan-binding proteins and be used as the basis for filtration systems and sensors for diagnostic purposes. Recent academic work has shown that mucins can be used to carry and deliver therapeutic molecules and several patents mention the use of mucins to deliver proteins and peptides.103,104 Mucins’ surfactant properties could also find new uses. Surfactants are widely used in industrial processes as foaming agents, pigment dispersants, wetting agents, solubilizers, and emulsifiers. Natural surfactants like mucins and mucin-like proteins are good candidates to replace their synthetic counterparts that are increasingly disfavored over greener alternatives. Indeed, salivary mucins are known to affect emulsions in the mouth105 and ovomucins extracted from egg white are used for emulsion and as a stabilizing agent of foams.106 These properties could be exploited to create new foods. Böni et al. recently investigated mixing hagfish slime containing mucins with soy emulsions and soy protein isolate suspension, to form a cohesive hydrogel.107 Once cooked, this mixture resembled cooked tofu in many respects.

Exploiting the hydration and lubrication properties of mucins

Another core property of secreted mucins is their ability to hydrate and lubricate the mucosal epithelium.12 On synthetic
surfaces, even from dilute solutions, mucins can form highly hydrated coatings containing more than 95% water that can efficiently lubricate (Fig. 5A). Mucins’ exceptional lubricity is attributed to aqueous boundary lubrication, a mechanism reviewed elsewhere by Coles et al. The hydration and lubrication role of mucins is perhaps most obvious in saliva, which contains MUC5B and MUC7 mucins and which is essential with anti-lubricin antibody. In addition to lubricity, mucins and lubricins are able to limit wear of surfaces in friction. In a steel on cartilage model system, the surface topography of the cartilage was analysed for the effect of wear. (D) The cartilage lubricated with 0.1% (w/v) hyaluronic acid (HA) or lubricin solutions led to micropitting effects. (E) Lubrication by 0.1% (w/v) HA led to the formation of tracks in the cartilage that decreased surface isotropy compared to samples lubricated with mucins at a similar concentration. The whiskers denote the 1.5 IQR (n = 9). A is reprinted with permission from ref. 130 copyright 2018 American Chemical Society. (B and C) are reprinted from ref. 122 with permission from Wiley Materials, and (D and E) are reprinted from ref. 123 with permission from Elsevier.

Fig. 5 Hydration and lubrication of mucins. (A) Stribeck curve of pig gastric mucins dissolved in pure water lubricating smooth polydimethylsiloxane (PDMS) surfaces. (B) Boundary friction coefficient measured on cartilage explants lubricated with lubricin. (C) Histology images revealing the beneficial effect of a lubricin (LUB:1) injection in the knee compared to control (PBS) in a rat model of osteoarthritis. The sections were immunostained with anti-lubricin antibody. In addition to lubricity, mucins and lubricins are able to limit wear of surfaces in friction. In a steel on cartilage model system, the surface topography of the cartilage was analysed for the effect of wear. (D) The cartilage lubricated with 0.1% (w/v) hyaluronic acid (HA) or lubricin solutions led to micropitting effects. (E) Lubrication by 0.1% (w/v) HA led to the formation of tracks in the cartilage that decreased surface isotropy compared to samples lubricated with mucins at a similar concentration. The whiskers denote the 1.5 IQR (n = 9). A is reprinted with permission from ref. 130 copyright 2018 American Chemical Society. (B and C) are reprinted from ref. 122 with permission from Wiley Materials, and (D and E) are reprinted from ref. 123 with permission from Elsevier.
Mucins are efficient boundary lubricators for a surprisingly large number of materials.\textsuperscript{112} They are, therefore, good candidates as biolubricants to be used in the prevention of implant wear or treatment of osteoarthritis. One study used qniumucin extracted from the Aurelia aurita jellyfish injected intra-articularly in a rabbit osteoarthritis model either alone or mixed with hyaluronic acid.\textsuperscript{117} The combination of hyaluronic acid and mucins showed a significant reduction in cartilage degeneration. Mucins extracted from porcine stomachs were also used to lubricate contact lenses and tested in contact with corneal tissue explants. Although mucins were not better at lubricating than hyaluronic acid, the careful analysis of the corneal tissue surface showed that the mucins were better at preventing tissue damage.\textsuperscript{118} Although such reports on the use of mucins for their lubricity are still limited, there is a relatively large body of literature reporting the use of lubricin, a mucin-like glycoprotein which is naturally present in the joints.\textsuperscript{119,120} A recombinantly expressed version of this mucin-like protein was shown to lubricate explanted bovine cartilage\textsuperscript{121} and prevent cartilage degeneration in a rat osteoarthritis model\textsuperscript{122} (Fig. 5B and C). However, also on explanted cartilage, mucins were shown to outperform recombinant lubricin in wear reduction\textsuperscript{123} (Fig. 5D and E).

In addition to its role in lubrication, the mucus layer also ensures proper hydration of the mucosal surfaces exposed to air. This constant struggle against dehydration is carried by mucins thanks to their exceptional hygroscopic properties.\textsuperscript{124} Mucins’ ability to bind large amounts of water could make them interesting alternatives to the synthetic and natural polymers commonly used in cosmetics to maintain skin hydration. This particular aspect was investigated for bovine maxillary mucin (BSM)\textsuperscript{125} and jellyfish mucins which seemed to perform better than hyaluronic acid.\textsuperscript{126} Skin cosmetics, based on snail mucins, date back to the 18th century when snail and slug mucus-based concoctions were used for treating skin wounds and inflammations\textsuperscript{127} and are re-gaining their popularity. In addition to mucins, snail and slug mucus contains minerals, vitamins, and bioactive peptides that are likely to play a role in skin rejuvenation and wound healing reported in several studies.\textsuperscript{128,129}

**What lies ahead**

Mucin-rich materials are obviously good hydrators and lubricants and, therefore, have been used for centuries for lubrication, such as when slugs were used to lubricate the wooden axle-trees.\textsuperscript{131} Today, mucins are investigated as a way to recapitulate the lubrication and hydration of ocular and oral surfaces, which are the results of properties and complex interplay between the components of the saliva or the tear film. Although our limited understanding of these phenomena hampers progress towards effective saliva and tear film substitutes, mucins are good candidates to be used in mucus moisturizing products.\textsuperscript{132} Mucins could also be used to functionalize medical devices that encounter potentially damaging friction with soft tissues, including contact lenses, catheters,\textsuperscript{133} and endoscopes. However, these applications will depend on the availability of purified, full-length, and non-degraded mucin molecules that retained their original properties.

In cosmetics, mucins could be investigated as dermal fillers. Similarly to hyaluronic acid and collagen, which are currently used as dermal fillers, mucins have a high molecular weight and hydrated molecules that can be injected subcutaneously to stretch the dermis. However, in contrast to hyaluronic acid and collagen, mucins are more resistant to enzymatic degradation\textsuperscript{31,82,134} and could, therefore, persist longer without the use of chemical crosslinking. Another impressive feat of secreted mucins is their rapid hydration and swelling, when expelled from the secretory granules of epithelial cells. An acidic and calcium-rich environment keeps the mucins extremely compacted. The fusion of the vesicles with the cell membrane results in ionic exchanges and a sudden expansion of mucins into a hydrogel.\textsuperscript{6,135} Although not fully understood for mucins, the basic mechanisms were successfully reproduced in vitro using synthetic anionic polymers.\textsuperscript{136} The delivery of macromolecules in such a compacted format, could protect the molecules from degradation, while keeping the viscosity of the solutions low. This type of system could find useful applications in drug delivery, tissue engineering, and cosmetics.

**Exploiting mucins for their bioactivity**

A recent increase in interest about mucins’ biological and bio-physical properties is leading to a paradigm shift in our perception of mucins. Mucins are not only a passive protective coat on our epithelium but are also bioactive molecules regulating biological processes. In many ways, this shift is similar to the realization that the extracellular matrix in animal tissues acts as a reservoir for biochemical signals that determine, to a large extent, the fate of the cells it embeds. Mucins are key for the bioactivity of the mucus matrix. Mucins can illicit biological responses by binding to bacterial and mammalian cell receptors,\textsuperscript{137} but can also sequester bioactive molecules such as proteins\textsuperscript{138} and peptides,\textsuperscript{139} thus mediating their bioavailability and bioactivity (Fig. 6).

The non-glycosylated protein domains of mucins contribute to the intrinsic bioactivity of the mucin molecules. For instance, the mucin epidermal growth factor-like domain (EGF-like) is a cysteine-rich region found in several membrane-tethered mucins. The domain can bind to EGF receptors that are involved in modulating growth, migration and differentiation of cells.\textsuperscript{140} For instance, the EGF-like region of MUC13 was found to stimulate cell migration, inhibit apoptosis, and accelerate wound healing in the colon.\textsuperscript{141} The membrane-bound MUC13 was also shown to protect the colonic epithelium by inhibiting toxin-induced apoptosis, perhaps through signaling of the intracellular domain of the mucin.\textsuperscript{142} The mucins bearing EGF-like domains are membrane-bound, but can also be found in the mucus layer because they are constitutively released from the cells.\textsuperscript{143} The rich library of glycan...
structures decorating the mucin molecules are also bioactive. For instance, Kawakubo et al. showed that the terminal alpha 1,4-linked N-acetylglucosamines in gastric mucins inhibit the biosynthesis of cholesteryl-alpha-D-glucopyranoside in Helicobacter pylori, a causative agent for gastric ulcers and cancer. A particularly active role of mucin glycans in the regulation of the immune response was recently suggested by a study suggesting that intestinal mucin (MUC2) glycans can induce immune tolerance in dendritic cells by binding their galectin-3 receptor proteins. Other sugar residues, such as the terminal sialic acids, modulate both the innate and adaptive immune systems through their interactions with the siglec receptor family. For instance, siglec receptors binding to sialic acid regulate the B cell tolerance signaling, by mediating their recognition of "self", with siglec knockout mice developing autoimmune phenotypes. The sialic acid-siglec systems is, for instance, exploited by parasites, bacteria, viruses and by cancer cells to depend and evade the immune clearance.

In addition to mucin intrinsic signaling, mucins interact with a variety of bioactive proteins and peptides. Several examples already suggest that mucins' ability to act as reservoirs for bioactive molecules is essential for maintaining an effective mucus barrier. An example is the association of the intestinal mucus layer with antimicrobial peptides (AMPs) to constitute the body's first line of defence against bacterial infections. The antimicrobial spectrum of AMPs is broad, ranging from antifungal and antibacterial, to antiviral activity. AMPs are strongly colocalized with mucus, with tens of antimicrobial peptides found in the colonic mucus extracts of healthy individuals and mice. At neutral pH, many antimicrobial peptides are positively charged, which favors their interactions with the negatively charged membranes of microbes, but also with the negatively charged sulfate and sialic acid residues of mucins. The cathelicidin LL-37 AMP has been shown to electrostatically interact with human airway mucus as well as with porcine gastric mucus and bovine submaxillary mucins, which decreased its antimicrobial activity. In contrast, human beta-Defensin-3 and the CSA-13 synthetic antimicrobial agent maintained a high level of bactericidal activity in mucin even though their antimicrobial action also relies on their positive charges. Similarly, the intestinal MUC2 mucin suppressed the antimicrobial activity of beta-defensin 2 against nonpathogenic and enteropathogenic Escherichia coli. However, in vivo, Muc2+/− mice demonstrated reduced expression of beta-defensin 2 in the colon, compared with Muc2+/− and Muc2+/-, suggesting that MUC2 regulates the expression and antimicrobial activity of beta-defensin 2.

It, therefore, appears that the physico-chemical properties of the AMPs mediate their interactions with mucins and other extracellular macromolecules and their bioactivity. It is still unknown how the mucus reservoir of AMPs performs under such inhibitory conditions. Nevertheless, their role as bactericidal molecules in mucus could be explained by sudden bursts of AMP secretion that could overcome mucin binding, or the local degradation and release of the AMP by incoming pathogens. In both cases, the mucins would be an essential component of the AMPs' function.

Trefoil factors (7–12 kDa) are protease-resistant secretory peptides characterized by one (TFF1 and TFF3) or two trefoil domains (TFF2) expressed by mucosal tissues. TFFs are produced by goblet cells in the gastrointestinal mucosa and are essential for the mucosal healing through signal transduction events, such as the induction of the EGF receptor and the activation of the ERK/MAPK pathway and subsequent prevention of apoptosis. The co-production, co-secretion and co-localization of trefoil factors with mucus and mucins imply that they may also be co-regulated and potentially assist each other's functions, jointly promoting the protection and repair of the gastrointestinal mucosa. Although the detailed mechanism has not been elucidated, TFFs have been shown to affect the viscoelastic properties of mucus gels. Thim et al. have shown that the addition of TFF2 to pig gastric mucin results in more than a 10-fold increase in viscosity and elasticity. The TFF3 dimer increased the viscosity of the mucin solution, while TFF1 and TFF3 monomers had very little effect. Moreover, in the ulcer-associated cell lineage as well as in the gastrointestinal mucosa, each TFF seems to be co-localized with a specific mucin type, hinting that TFFs could act differently when coupled with different mucins. Similarly to AMPs, the strong interactions and systematic co-localization could also imply functional synergies between mucins and the TFF molecules.

Growth factors are also found co-localized with mucins, especially in the gut. EGF has been of particular interest, since it was shown to upregulate the expression of MUC2 and MUC5AC at the transcriptional and protein level and is involved in mucosal regeneration. Although our knowledge on how EGF affects the expression of mucins and cell proliferation is well-established, details regarding the potential inter-
actions between mucins and EGF are scarce. EGF and mucins are co-localized in saliva,\textsuperscript{166} milk, and in the digestive system.\textsuperscript{166} In the gut, EGF is thought to act as a surveillance peptide, stimulating mucosal repair, only when the altered epithelial barrier allows access to the basolateral side where the receptors have been localized.\textsuperscript{172} In this sense, the maintenance of the EGF concentration in the mucus is important, and the mucins–EGF interaction could play a role by decreasing proteolytic activity and helping to maintain a concentration gradient of EGF at the epithelial interface. Thus, the mucus matrix would play a role similar to that of the extracellular matrix (ECM), which mediates cellular processes through the sequesteration of growth factors.\textsuperscript{173}

Our understanding of the role of mucins in the modulation of cellular behaviors is still limited, which is why there has been a limited number of attempts at re-creating these effects in engineered systems. Early results obtained with coatings of mucin from commercial sources exposed to neutrophils, did suggest an immunomodulatory effect of mucins. The mucin coatings were found to reduce the adhesion of neutrophils and their secretion of inflammatory markers, such as reactive oxygen species.\textsuperscript{48,49} However, it is unknown if the decrease was due to the interaction between neutrophils and mucin-bound ligands, or due to differences in adhesion, which is known to be an important regulator of neutrophil activation.\textsuperscript{174} Originating from a long tradition of using snail mucus for healing purposes,\textsuperscript{127} mucins extracted from \textit{Archachatina magnnata} snail mucus were tested as a component of drug delivery materials\textsuperscript{175} and as injectable matrices for treating knee osteoarthritits.\textsuperscript{176} In this latter study, intramuscular injections of snail mucins seemed to lower the levels of IL-6 and MMP-3 osteoarthritis markers in an experimental model of knee osteoarthritis in dogs. Some inflammation was noticed after prolonged injection, which could be attributed to the relatively crude mucin extraction protocol used. Ovomucins are known to have anti-viral, anti-tumor and immune stimulating properties.\textsuperscript{106} A recent in \textit{vivo} study demonstrated the suitability of using ovomucins as part of implantable materials by preparing an ovomucin–gelatin mix system, stabilized by crosslinking with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)\textsuperscript{177} and implanting it in mice. The \textit{in vivo} studies showed good infiltration of fibroblasts cells in the hydrogels, most likely due to the gelatin components. Interestingly, the subcutaneous implantation of the gel in rats favored the infiltration of alternatively activated macrophage cells that are favorable for tissue repair and did not trigger any allergic response measured by IgE levels in rat serum. Unfortunately, the study did not compare the mucin-gelatin to gelatin-only materials, which makes it difficult to isolate the contribution of ovomucins to these effects.

What lies ahead

The mucus lining the gastrointestinal tract hosts the billions of bacteria of our gut microbiota. Our understanding of the reciprocal interactions between bacteria and mucus is evolving, with mucins being increasingly recognized as essential modulators of the homeostatic state of these tissues. Therefore, very much like probiotics are administered in the hope of modulating the composition of the microbiota, mucins supplements could help control the composition of bacterial populations in the gut. The addition of mucins or mucin mimics could provide a competitive advantage to certain bacterial species and help modulate the gut microbiome composition and activity.\textsuperscript{178} As seen above, the co-localization of mucin gels and bioactive proteins and peptides, such as antimicrobial peptides,\textsuperscript{136} trefoil factors,\textsuperscript{160} antibodies, and growth factors\textsuperscript{167} could also point to the existence of interactions and functional synergies between such bioactive components and mucus. Given mucins’ immunomodulating properties, mucins are natural candidates to modify the surface of implantable materials in the hope of improving their biointegration. Although several approaches to surface modifications with mucins have already been reported,\textsuperscript{48,179,180} more work is needed to generate mucin-based materials that can retain their bioactivity \textit{in vivo}.

Challenges and opportunities

The development of biopolymer blockbusters has typically started with their discovery in plant or animal tissues, followed by the determination of their structural, physical, chemical, and biological properties. Throughout the process, purification methods are refined, yielding purer and more defined products. In some cases, the combination of high applicative potential, low production costs, and regulatory approval lead to successful commercial products. Examples include hyaluronic acid, first extracted from rooster combs and now used in cosmetic and biomedical products.\textsuperscript{181,182} Heparin, which is used as anticoagulant, was first isolated from liver tissues while searching for anticoagulant factors in animal tissues.\textsuperscript{183} Alginates, extracted from algae, have large scale applications as food texturing agents,\textsuperscript{184} drug encapsulation material,\textsuperscript{185} and in other niche biomedical applications.\textsuperscript{186} Chitosans, extracted from the shells of shrimps and calamari, are used as antifungals in agriculture and show promise as scaffold materials for tissue engineering.\textsuperscript{187}

Although the technological potential of mucins seems clear from a functional point of view, some obstacles need to be overcome before translational work can begin. First, mucin research heavily relies on the commercial sources of mucins. The batch to batch variability, the uncertainty as to what protocols are used for purification, and the presence of impurities are challenges that need to be addressed. The development of protocols to further purify commercial mucins,\textsuperscript{188} protocols to produce native-like mucin on a larger scale,\textsuperscript{35} and protocols for the quality control of the molecular\textsuperscript{189,190} and physical properties\textsuperscript{191} of the mucins will be essential. Challenges around sterilization and conservation of mucin containing products have started to be addressed,\textsuperscript{115} but could face more challenges if the products are to rely on un-altered protein and glycan structures of the mucins.
Other strategies to produce highly-reproducible mucin materials have been suggested. For instance, one approach is to first simplify the protein or glycan sections of mucins through enzymatic treatments, and then to use this natural backbone to reconstruct the mucins or mucin-mimic in a controlled manner. The complementary approach could also be viable, where glycan libraries are first extracted from mucins, then used to decorate a synthetic polymer backbone. This would result in mucin mimics incorporating the unique glycan ligand diversity of native mucins.

There is now a significant body of research focused on the study and engineering of the chemical and physical functionalities of mucins. Scarcer, however, are studies investigating the bioactivity of mucin-materials either in vitro or in vivo. We found seven studies reporting on in vivo testing of mucin materials, providing only initial indications that valuable functionalities could result, but also clearly showing the very early stages of mucin materials for biomedical applications (Table 2). Indeed, most of these early studies do not describe the purification protocols used to obtain mucins. Contaminants co-purified with the mucins can then lead to unwanted side effects. Two more recent experiments performed with purified mucins and implanted in rats and sheep did show encouraging results. In both cases, the investigators found the animals had a low immune reaction to the allogeneic mucin implementation.

### Conclusions

As we better understand the properties of the mucin molecules, it is clear that the mucins are gaining interest as materials. The field, however, is still limited, with 73 articles and patents referenced in this review that investigate the properties and use of mucins and mucin-based materials. This number excludes the growing body of literature dealing with mucin mimics and synthetic mucins. Out of the 73 references, a majority have exploited the barrier properties of mucins (42%), followed by the hydration and lubrication and the chemical properties of mucins (Fig. 7A). Finally, the potential of utilizing the bioactivity of mucin as implantable materials is only in its infancy, with 9% of the referenced publications falling in that category. Although several references cited here are more than 30, even 90 years old, most of the publications are less than 10 years old (Fig. 7B), most likely stimulated by recent improvements in our understanding of mucins and their properties. Further research in mucin-based technologies will contribute to advance our understanding of the mucin molecules, while also leading to new biomimetic approaches to solve important biomedical and chemical problems.
Conflicts of interest

There are no conflicts to declare.

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