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Lubrication by biomacromolecules: mechanisms and biomimetic strategies

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Abstract

Biomacromolecules play a key role in protecting human biointerfaces from friction and wear, and thus enable painless motion. Biomacromolecules give rise to remarkable tribological properties that researchers have been eager to emulate. In this review, we examine how molecules such as mucins, lubricin, hyaluronic acid and other components of biotribological interfaces provide a unique set of rheological and surface properties that leads to low friction and wear. We then highlight how researchers have used some of the features of biotribological contacts to create biomimetic systems. While the brush architecture of the glycosylated molecules present at biotribological interfaces has inspired some promising polymer brush systems, it is the recent advance in the understanding of synergistic interaction between biomacromolecules that is showing the most potential in producing surfaces with a high lubricating ability. Research currently suggests that no single biomacromolecule or artificial polymer successfully reproduces the tribological properties of biological contacts. However, by combining molecules, one can enhance their anchoring and lubricating capacity, thus enabling the design of surfaces for use in biomedical applications requiring low friction and wear.

Introduction

Biolubrication is an essential and ubiquitous function in the human body; it occurs during movements associated with the joints, eyes, oral cavity as well as in the digestive, respiratory, genitourinary and circulatory tracts. Impaired lubrication, as encountered in osteoarthritis, dry-eye or dry-mouth (xerostomia) syndrome, has a major impact on quality of life [1-3]. Biomacromolecules play a key role in protecting human biointerfaces from rubbing contacts and enable motion. Their multifaceted role involves creating optimal rheological conditions and surface film properties to ensure that a fluid layer is maintained between two surfaces in relative motion.

The biotribological mechanisms must be able to instantaneously respond to sudden changes in loads and speeds sometimes thousands of times each day, to enable painless motion and limit wear of the surfaces. Some examples of these situations include the sudden loading to 400-760 % bodyweight of the knee articular cartilage when jumping [4] or the rapid transition of the eyelid from immobility to 40 cm/s during a blink [5]. Additionally, the surfaces involved in biotribological contacts have very diverse mechanical, topographic and biochemical properties, which all influence how the lubricating fluids interact with the surface. The most extreme examples are found in the oral cavity: the soft, rough and epithelial nature of the tongue contrasts with the hard, smooth and mineral character of the teeth yet biolubrication operates seamlessly between these two surfaces, highlighting the requirement for versatile biolubrication mechanisms. The friction coefficients at biological interfaces are exceptionally low, even lower than some common “slippery” contacts such as sliding on ice [6]: The friction coefficient of articular cartilage against glass or cartilage lubricated by buffer or synovial fluid has been found to be in the range of 0.002-0.03 [7-12] and saliva-coated polydimethylsiloxane surfaces yield a boundary friction coefficient of order 0.01, which is two orders of magnitude lower than that of water or buffer in the same conditions [13-15]. Even more remarkably, these friction coefficients must be maintained over millions of sliding cycles during the lifetime of an individual, showing a high wear resistance through a combination of strongly anchored and replenishable lubricating layers.

The main protagonists in biolubrication are a family of large glycoproteins (0.5-20 MDa) called mucins. Mucins are present on all mucosal tissues including those lining the airways, oral cavity, digestive tract and genitourinary tracts and are also present in mucosal fluids such as saliva, nasal mucus or tears [16-17]. Although this review is limited to human/mammalian physiology, mucins or mucin-like molecules are also present in other animals and organisms such as frogs, fish, snails or even protozoan organisms [18-20] . Mucins are remarkable molecules that form films that enable the exchange of nutrients, water, and gases while being impermeable to many pathogens [17]. Mucins are considered

to be essential components in the lubrication process [21-22] and it was recently found that mucin production increases when epithelial corneal cells are subjected to friction forces [23]. Mucins are comprised of a heavily glycosylated protein core flanked by end groups with few glycosylation sites and containing von Willebrand assemblies and cysteine-rich globular domains, which are responsible for mucin polymerization through hydrogen bond interactions and the formation of disulfide bridges [24]. While mucins are absent from synovial fluid, this fluid contains the protein lubricin, which is a glycoprotein that presents a very similar structure to that of mucins (although of a smaller size), with a heavily glycosylated bottle-brush like core flanked by non-glycosylated termini containing cysteines (Figure 2B) [25-26]. Lubricin is recognised as one of the main protagonists in articular cartilage lubrication [26] and has been found to have a protective role on chondrocytes thanks to its ability to decrease boundary friction [27]. Additionally, the synovial fluid of patients with osteoarthritis was found to contain less lubricin and have a lower lubricating ability than the synovial fluid of healthy patients [28]. Interestingly, lubricin has also been detected on the ocular surface where it has been shown to play a role in reducing friction and protecting the cornea from damage [29-31]. Mucins and lubricin do not act alone in the biolubrication process and recent research has highlighted the role of complex synergistic interactions between these glycoproteins and other components of mucosal or synovial fluids [15, 32-35]. The scope of this review is to describe the mechanisms by which mucin, lubricin and other biomacromolecules provide superior lubrication and to highlight biomimetic strategies that harness these mechanisms.

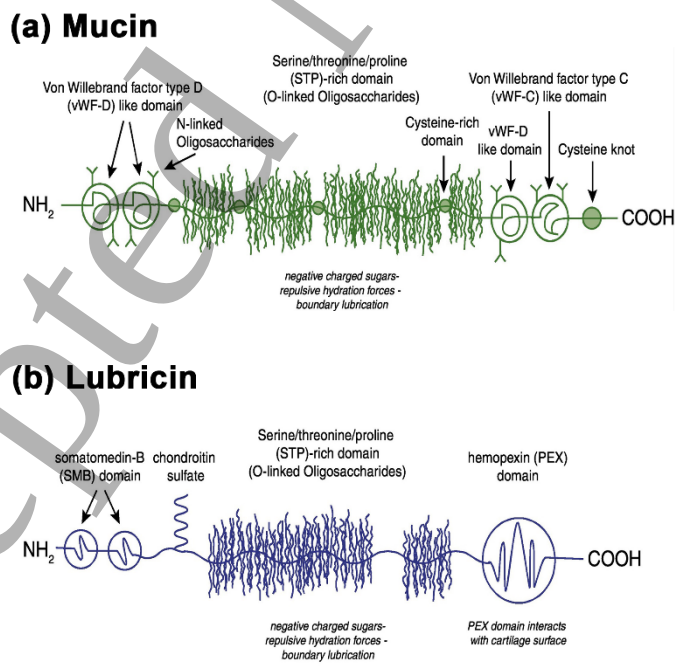

Figure 1 – Schematic representation (a) Mucin and (b) Lubricin, two main protagonists in biolubrication that share a similar structure. Adapted with permission from [36]. Copyright 2017 Elsevier.

Mechanisms of lubrication by biomacromolecules

Importance of rheology in maintaining a fluid film between the surfaces.

The most efficient way to limit friction and wear damage between surfaces is to keep the surfaces well separated. This is achieved by either operating at lubricant entrainment speeds that produce sufficient hydrodynamic lift forces to support the load, and/or using lubricants of sufficient viscosity to provide resistance to squeeze flow forces so that there is a so-called 'full-film' between the substrates. In engineering tribology involving deformable (soft) substrates, this regime is referred to as elasto-hydrodynamic (EHL) lubrication, with the lubricant rheology being a key design factor. Rheology plays a key role in biotribological contacts and it is observed that biological lubricants usually possess a very complex set of rheological properties due to the presence of biomacromolecules. Saliva is an example of biolubricating fluid whose functional properties have a mucosal origin. Saliva is shear-thinning with a viscosity decreasing from around 10 mPa.s at low shear rates to 1 mPa.s at high shear rates (>100 s^{-1}) [37-39], has a high extensional viscosity [40-41] and exhibits a remarkably high normal stress ratio (ratio of primary normal stress differences and shear stress), which is ca. 10 and 100 for mechanical and acid-stimulated stimulated saliva, respectively (Figure 2). Collectively, these properties indicate that elastic stresses dominate over viscous stresses during salivary flow [37, 42]. Mucin glycoproteins are the main actor in the rheology of mucosal fluids. In saliva, high molecular weight mucins are thought to form super-macromolecules by aggregating end-to-end, which uniquely leads to its low viscosity yet extremely high elasticity [24, 37, 43]. Gastric and intestinal mucins have been shown to form gels at low pH through a combination of disulphide bridges, hydrogen bonding and Ca^{2+} mediated links between mucins and other non-mucin proteins [44-46]. Reduction of the disulphide bonds with dithiothreitol, disruption of the hydrogen bonds with chaotropic agents and addition of calcium chelating agents cause the mucin network to disassemble [45-46]. Conversely, oxidation of airway mucus leads to an increase in elasticity due to the formation of disulphide bridges between mucins, which could be a cause for the high elasticity found in the mucus of patients suffering from cystic fibrosis [47]. Different mucins are thought to be playing specific roles in the viscoelastic properties of lubricating biofluids: saliva samples with higher levels of MUC5B have been linked to more viscous saliva while higher levels of MUC7 have been linked to saliva samples with higher extensional viscosity [48]. These findings suggest that mucosal cells can modulate the nature of mucus by varying the relative concentrations of individual mucins, perhaps in response to changing environmental conditions.

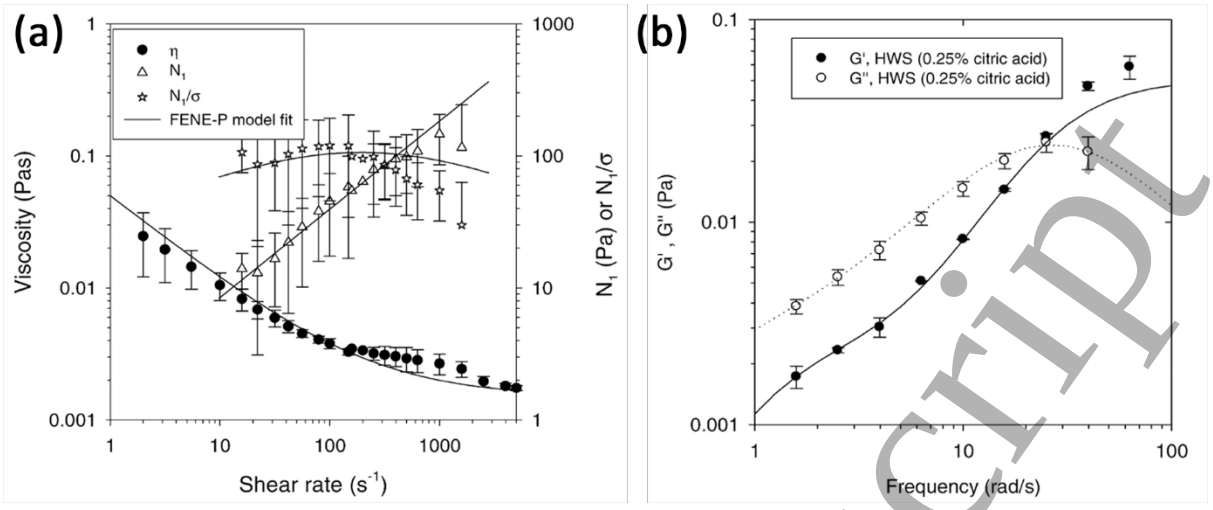

Figure 2 – Rheology of human whole mouth saliva obtained following stimulation using 0.25% citric acid. (a) Steady-state shear rheological properties where lines represent predictions using the FENE-P dumbbell model. (b) Dynamic rheological properties as a function of frequency at a strain of 5, where the lines indicate a fit to the data using a multi-mode Maxwell model. Reproduced with permission from [37]. Copyright 2007 IOS Press.

Synovial fluid does not have a mucosal origin and is a plasma dialysate modified by constituents secreted by the joint tissues. It is highly shear thinning compared to saliva with a viscosity decreasing from about 10,000 mPa.s at low shear rates to 10 mPa.s at high shear rates [49-50] , and displays a viscoelastic behaviour [51-52] (Figure 3). The viscosity of synovial fluid is observed to be lower in patients with osteoarthritis or rheumatoid arthritis [49, 53]. This loss of viscosity may decrease the ability of the joint to maintain a fluid film and thus cause the cartilage surfaces to come into contact more easily, which increases the potential for surface wear. The viscoelasticity of synovial fluid is highly dependent on the concentration and molecular weight of hyaluronic acid (HA), an anionic glycosaminoglycan present at high concentrations in synovial fluid. HA forms entangled networks that are thought to be responsible for the viscoelastic behaviour of synovial fluid [49, 54-55]. Additionally, entanglements between HA and lubricin have also been shown to contribute to the elastic response of synovial fluid [51].

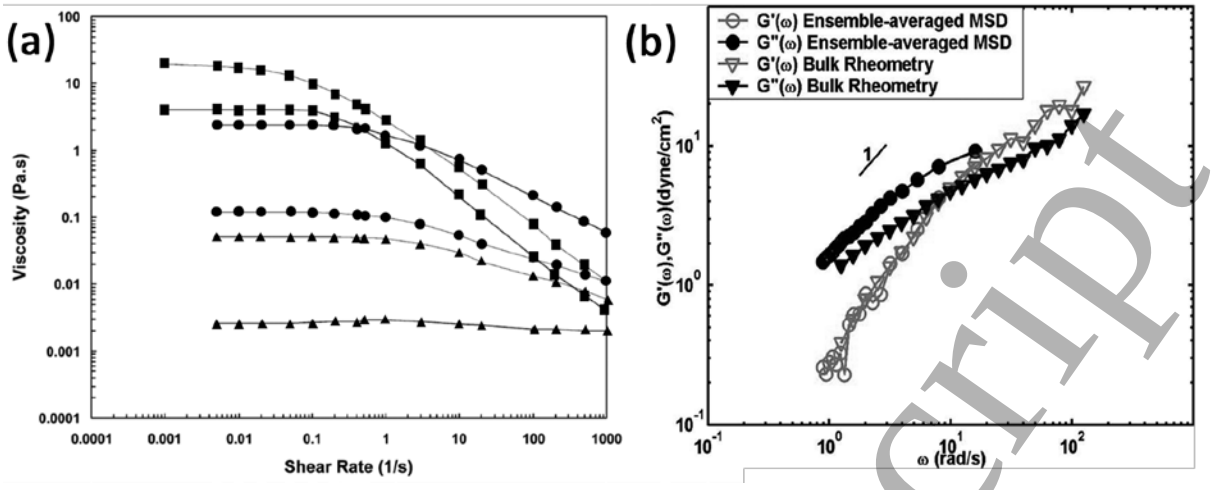

Figure 3 – (a) Viscosity ranges for healthy, degenerative, and inflammatory synovial fluids. The two lines of squares, circles and triangles show the upper and lower viscosity boundaries of healthy, degenerative, and inflammatory synovial fluids, respectively. Reproduced with permission from [49]. Copyright 2007 IOS Press. (b) Storage (G') and loss (G") moduli of bovine synovial fluid as a function of frequency measured by multiple particle-tracking micro-rheology (circles) and macro-rheology (triangles). Reproduced with permission from [51]. Copyright 2007 National Academy of Sciences.

The shared characteristics observed in lubricating fluids such as the shear thinning behaviour and elasticity play an important role in keeping opposing surfaces separated. The shear thinning behaviour of the lubricating fluids is an advantageous feature in the EHL regime because it dampens the increase of friction due to viscous losses normally observed at high entrainment speeds. The synovial fluid of rheumatoid arthritis patients has been found to lose its shear thinning behaviour and therefore may lose its EHL friction dampening property [49, 53, 56]. Fluid elasticity is observed as an anisotropic response under shear flow that is characterised as non-zero normal stress differences, and an added resistance within extensional flows (i.e. extensional viscosity) that goes beyond that expected from shear-viscosity. Elasticity is suggested as contributing to the shock absorbing properties of synovial fluid [57] and it has been observed that the pathological synovial fluid or synovial fluid from elderly people tends to lose its elastic character [49, 58-59]. The elasticity of saliva has been speculated to contribute to the adhesion of the salivary film to the surfaces of the mouth and the food bolus [40-41]. The saliva of dry mouth patients has been found to have a lower extensional viscosity than normal saliva, which may be linked to changes in the glycosylation pattern of the salivary mucins [60]. These changes could impact the ability of saliva to coat the oral surfaces. It is also predicted that a large normal stress ratio contributes to load-bearing properties of the lubricant [37, 61-62]. All these properties participate in the maintenance of a fluid film at the interface.

The rheological properties of synovial fluid are also very important for a lubrication mechanism thought to be specific to cartilage, called weeping lubrication, which participates in maintaining a fluid film. In weeping lubrication, the pores of the cartilage, which has a poroelastic nature, are filled with interstitial fluid that can be released to the cartilage surface under the action of a load. The release of this fluid helps to enhance the EHL by maintaining a layer of fluid at the cartilage/cartilage contact point [63-64]. Recently, a mechanism called "tribological rehydration", whereby the cartilage rehydrates during the unloaded sliding motion has been proposed to explain the fluid recovery of cartilage [9, 65]. Both the weeping and rehydration mechanisms heavily depend on the rheological properties of the fluid, therefore it is likely that any disease or age related rheological change will affect these aspects of joint lubrication although this effect has not been specifically studied.

Maintaining hydration in boundary films through glycosylated brushes and multilayered architecture

When the relative speed between the surfaces decreases and/or when the load increases, it is not always possible to maintain a fluid film between the surfaces. To avoid a damaging increase in the friction caused by the contact between the surfaces, intricate boundary films composed mainly of glycosylated molecules enable the surfaces to remain separated by a highly hydrated layer that can withstand high contact pressures while maintaining a low friction coefficient. Glycosylation is a key element in the boundary lubrication process and glycosylated molecules are ubiquitous in biotribological contacts: mucins are found in mucosal fluids such as saliva, nasal mucus or tears and the surface of all mucosal epithelial cells is highly decorated with membrane bound mucins. Synovial fluid contains other heavily glycosylated molecules such as lubricin and aggrecan. Crouzier et al. have shown that partial and complete deglycosylation of pig gastric mucins resulted in an increase in their boundary friction by two orders of magnitude compared to the native mucins [66]. Similarly, de-glycosylation of lubricin yielded a significant increase in its boundary friction coefficient [67]. The effect of glycosylation on boundary lubrication is two-fold. Glycosylation enables a high level of hydration thanks to the presence of numerous hydroxyl groups that engage in hydrogen bonding with water. The strong interactions between the sugar moieties and the water molecules enable the water to remain "trapped" in the contact, rather than be squeezed out when the surfaces are pushed towards each other, thus ensuring that a hydrated layer is maintained in the contact. Additionally, glycosylated molecules have a brush-like architecture, where the protein backbone is decorated with sugar chains. Brushes are a very powerful way to reduce friction in the boundary regime: when two surfaces covered

with neutral polymer brushes come in contact at low to moderate compression, it is entropically more favourable for the brushes to compress within themselves than to interpenetrate with the brushes on the opposite surface, thus decreasing the interaction between the surfaces [68] . When the brushes are charged, the additional effects of the osmotic pressure created by trapped counterions and the lubricating effect of the hydration shells around the charged monomers, called the hydration lubrication effect, improve the lubrication properties of the brushes at high loads [69-71]. This effect is applicable to mucins, lubricin and aggrecan which possess negatively charged brushes thanks to the presence of sialic acid and sulphates on the oligosaccharide side chains.

The positive effect of the glycosylated molecules on lubrication can only occur if they remain in the contact and adopt a conformation that maximises their lubricating ability. Synergies between the components of the boundary films give rise to a multilayered architecture that ensures that the surface layer is well anchored and that the brush-like domains of the glycosylated molecules protrude away from the surface to create a thick hydrated layer. The salivary and synovial films are good illustrations of this process. Saliva forms a supramolecular film on the various surfaces of the mouth. Although the composition of the salivary pellicle varies substantially depending on the location in the oral cavity, the nature of the substrate as well as environmental effects [72] the general structure of the salivary film is made up of two layers: a dense base layer formed by small proteins such as proline rich proteins (PRPs), cystatin, statherin, histatin, mucin MUC7 or immunoglobulin A [73-75] and a sparser top layer composed mainly of the larger mucin MUC5B [76] (Figure 4A). The assembly is reinforced by interactions of MUC5B with membrane bound mucin MUC1 [73, 77] as well as crosslinking of the base layer by transglutaminase [78-79]. Tribology and adsorption studies have given rise to the hypothesis that MUC5B is in a loop conformation, whereby the non-glycosylated domains of MUC5B interact with the surface and the other proteins in the base layer, while its central glycosylated domain interacts with the liquid layer, thus forming “hairy” hydrated loops that protrude away from the surface and can support high loads [14,80]. Removal of mucin end groups prevents its adsorption onto hydrophobic surfaces and increases the boundary friction coefficient by two orders of magnitude [81]. Although mucins have been put forward as the key element in salivary boundary lubrication, most studies using purified mucins have not managed to reproduce the friction coefficient of saliva, especially at high contact pressures [21-22, 82]. Recent studies that have obtained a friction coefficient comparable to saliva using non-denatured purified human salivary mucins and purified pig gastric mucins [66, 81, 83]. These studies may highlight the important role of mucin glycosylation, meaning that different methods of purification could lead to isolating mucin fractions with different glycosylation types [84] with varying lubrication enhancement properties. In addition, it is important to note the possibility of the presence of small mucin-bound proteins that may have remained associated with mucins during the non-

denaturing purification steps [45]. These small proteins could alter the lubrication performance of the mucins by changing their adsorption or assembly properties [15, 45]. The difficulty to reproduce saliva's lubricating properties using mucins alone indicates that both the base layer and the mucin layer are critical in enabling the low friction properties of saliva. To prove this, Yakubov et al. separated salivary proteins in several fractions and showed that individual fractions could not reproduce the lubricating properties of saliva but the synergistic combination of the mucin rich fraction with the PRP rich fraction did lead to friction coefficients comparable to those of saliva [15]. This indicates that the small salivary proteins are necessary to anchor the mucin layer and "force" the mucin molecules to adopt a lubricating loop conformation.

On the articular surface, a complex arrangement of polysaccharides, proteins and phospholipids coexists. Although the exact structure is not fully understood, it is thought that HA molecules protrude from the cartilage surface, where they interact with aggrecan or lubricin to form a highly hydrated, glycosylated brush-like structure (Figure 4B) [85-86]. This structure is strikingly similar to the one proposed for saliva and yields a highly hydrated layer of "hairy" loops and brushes. To account for the presence of HA at the surface at high pressures and despite the fact that HA is not covalently attached to the surface, Greene et al. have proposed a "trapping" mechanism for HA molecules whereby they become entangled in the collagen network (present in the cartilage) as it is compressed [87]. This mechanism could explain cartilage resilience to wear and the remarkable lubrication properties of the articular surface even at high loads. In a similar fashion to saliva, synergies between the individual components of synovial fluid are necessary to create the friction and wear properties of the articular cartilage contact and the individual components are insufficient to explain the exceptional properties of articular cartilage [32]. Synergistic interactions of HA grafted on mica surfaces with lubricin provide both a lower friction coefficient (down from 0.5 to 0.09) and a better resistance to wear, with the pressure at which wear initiates increasing from 2 MPa for HA alone to 4 MPa when lubricin is present [33, 88]. Proposed mechanisms suggest that the combination of lubricin and HA helps anchor the lubricating film on the surface as well as creating a viscous layer that shifts the surfaces away from boundary lubrication [34-35, 87]. Proteins found in synovial fluid have also been shown to participate in synergistic interactions with lubricin. Fibronectin [89], collagen type II [34, 90], cartilage oligomeric matrix protein [91] or the galectin-3 protein [92] have all been shown to enhance the lubrication and/or the wear resistance of lubricin. It is likely that HA and these proteins are to lubricin as what the small salivary proteins are for mucin: they mediate the anchoring of lubricin in a favourable lubricating conformation. Finally, surface active phospholipids, mostly composed of zwitterionic phosphatidylcholines have also been put forward as a key element in cartilage boundary lubrication, in synergy with HA and lubricin [93]. Although the mechanisms are still not fully understood, it is thought

that hydration lubrication involving the charges on the phosphatidylcholine groups participates in reducing the friction [94].

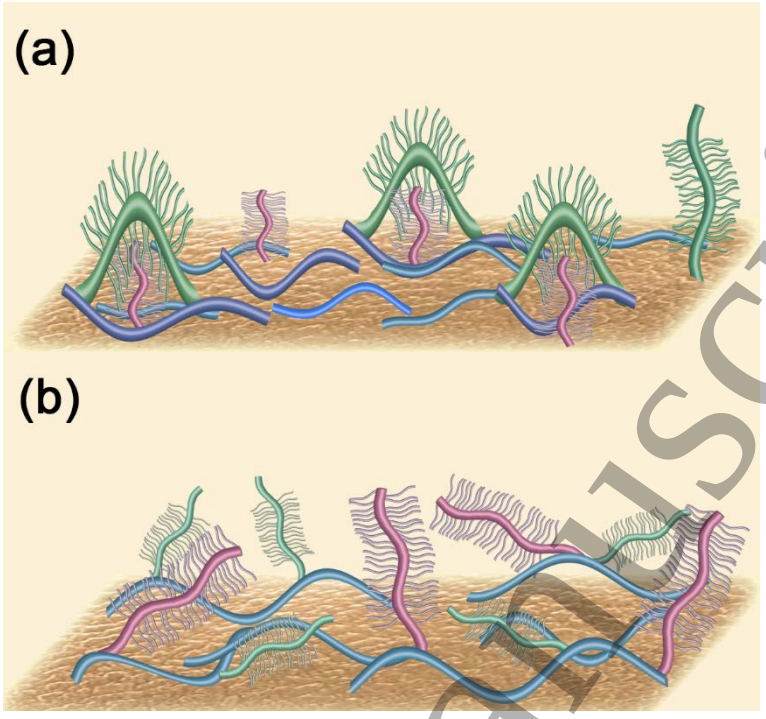

Figure 4 - Structure similarities of aqueous lubricating films in the body. (a) Proposed structure of the salivary film. Blue: small proteins (PRP, statherin, histatins, cystatins…) forming the tight baselayer. Red: membrane bound mucins forming the anchor for secreted mucins. Green: Secreted mucins forming a highly hydrated loose layer, either by forming loops or linear structures. Adapted from [72]. (b) Proposed structure for the articular lubricating film. Blue: Hyaluronic acid forming the baselayer. Green: Lubricin and Red: Aggrecan, forming a highly hydrated loose layer. Adapted from [86].

The various mechanisms described here contribute to creating a tribological environment where the substrate, surface and fluid act in synergy to respond to varied and demanding ranges of motion and ensure that a low friction coefficient is maintained under all conditions. A common feature in biotribological contacts is the presence of a fluid that has the following features: (i) complex rheology (including viscoelasticity) that contributes to the maintenance of a fluid film between biosurfaces over a wide range of movements and loads; and (ii) contains macromolecules that strongly adsorb or bind to biological substrates to form a highly hydrated surface layer that is resistant to wear and/or is naturally replenished.

Biomimetic strategies

Mimicking the rheological properties of biolubricating fluids: an incomplete solution

Current formulations for dry eye syndrome, dry mouth syndrome or synovial fluid replacement mostly focus on matching the rheological properties and therefore the EHL properties of the biological fluid they aim to replace. HA has been the prime candidate for visco-supplementation in osteoarthritis and has shown good results for pain reduction in clinical trials [95]. *In vitro*, high molecular weight and crosslinked HA is more efficient at restoring the rheological properties of osteoarthritic synovial fluid [96-98], however no consensus has been reached about the effect of molecular weight or crosslinked status of HA *in vivo* [99-100] showing that creating an effective lubricant is not as simple as emulating the rheological properties of healthy synovial fluid.

Mimicking the rheology of saliva is an admirable challenge, but clever strategies are needed to replicate saliva's very unusual rheology when compared to standard polymer solutions. To put in perspective, Newtonian fluids are inelastic such that they have normal stress ratio of zero, while values of < 10 are typically found for high molecular weight (> 1 million) polymer solutions that are considered to be highly elastic. With normal stress ratios ranging between 10 and 100, saliva substantially surpasses the elasticity of polymer solutions of comparable viscosity. Mucin solutions are an obvious candidate in attempting to replicate the rheological properties of saliva, however, the viscoelastic properties of aqueous solutions containing extracted mucin are highly dependent on the purification method used. Commercial preparations of pig gastric mucin (Sigma) or other purified mucins ("Orthana" mucin, similar to human MUC6) have been partially denatured and the non-mucin proteins have at least partially been removed, thus lowering the viscosity compared to native mucus and disrupting the formation of gels [45, 101-102]. Such solutions are found to be viscous with no apparent elasticity, and thus it has not been possible to replicate saliva's unique rheology using purified mucins. Other formulations based on carboxymethylcellulose, hydroxyethylcellulose, polyethylene oxide or xanthan gum have been investigated but failed to reproduce saliva's rheology [103-105]. The low elasticity and surface tension of commercial artificial formulations prevents them from forming films on the oral surfaces. In some cases, formulations can even interact with and disrupt the salivary film already present in the mouth, which causes them to fail to produce a lasting beneficial effect to dry mouth symptoms [103, 106].

Polymer brushes and other glycoprotein mimics

Solely mimicking the rheological properties of biological fluids is insufficient to emulate the lubricating behaviour of biotribological contacts. The main challenge lies in mimicking their boundary lubrication and wear resistance properties. Owing to the glycosylated nature of the molecules present in biotribological contacts, research has mostly focused on using polymer brushes to reproduce the boundary lubrication properties observed *in vivo*. When choosing polymer brush systems, several options are available. First, brushes can be either adsorbed or covalently grafted onto the surface. Generally, covalently grafted brushes present the advantage of withstanding repeated or higher loads than adsorbed layers. However, adsorbed films can more readily "heal" after being worn away, provided that the desorbed molecules are still available near the surface and that the kinetics of adsorption are adequate for the tribological conditions tested [107]. Additionally, grafted brushes are only suitable for applications where material synthesis and coating can be made *ex vivo* (e.g. hip implants or contact lenses) whereas adsorbed polymers can be useful for applications where they are injected or applied *in vivo* (e.g. saliva or tear replacements). Another important property for polymer brushes is their charge. In general, charged polymers provide better lubrication thanks to the hydration lubrication phenomenon. An example of an adsorbed polymer that forms uncharged brushes is a copolymer composed of a poly(L-lysine) (PLL) backbone and poly(ethylene glycol) (PEG) or dextran side chains. Thanks to its positive charge, PLL adsorbs on negatively charged surfaces, such as silicon dioxide, with a high enough chain density to force the hydrophilic PEG or dextran chains into a brush conformation (Figure 5A) [108-114]. Adsorption has also been shown to occur on non-polar hydrophobic PDMS surfaces through hydrophobic interactions [108]. Although the lubricating properties of this system do not match the ones of biological contacts, it enabled the elucidation of the effect of chain length and density, pH, salts and solvent viscosity on the lubrication properties of PEG brushes. Charged polymer brushes made of grafted polyzwitterion poly[2-(methacryloyloxy)ethyl phosphorylcholine)] (PMPC) have shown promising results, yielding a friction coefficient of $\mu \sim 10^{-4}$ up to 15 MPa pressure [115-116] (Figure 5B). These brushes have been shown to have a high amount of strongly associated water, which gives rise to the hydration lubrication mechanism [70], and are a good candidate for artificial biolubrication.

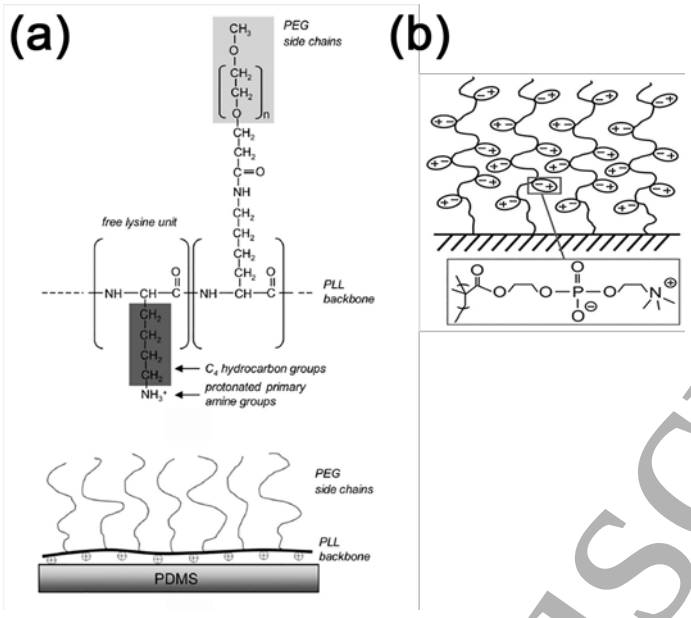

Figure 5 – Examples of polymer brushes that have been used as boundary lubricants. (a) The poly(L-lysine) (PLL) - poly(ethylene glycol) (PEG) system, where the PLL backbone adsorbs onto surfaces and forces the PEG side chains into a brush conformation. Reproduced with permission from [108]. Copyright 2008 American Chemical Society. (b) Brushes made of Poly[2-(methacryloyloxy)ethyl phosphorylcholine)] (PMPC). From [115]. Reprinted with permission from AAAS.

Architectures other than brushes have been used to emulate biolubrication. A recent study has investigated the lubrication of polyethylene glycol loops anchored to the surface via catechol groups, which provide a strong adhesion between the polymer and the surface (Figure 6A) [117]. The loop architecture, which mimics the proposed loop architecture of mucins in a biolubricating film [14, 80], resulted in better lubrication than brushes and the use of anchoring groups with a strong adhesion to the surface enabled better resistance to wear upon exposure to high contact pressures.

Inspired by the structure of lubricin, several bottle brush mimics have been designed as artificial boundary lubricants. Lubricin mimics are usually designed to have a branched core flanked by end groups that show affinity for the substrate. In a series of experiments, a bottle brush composed of PMPC side chains with or without cationic end groups was used (Figure 6B) [118-121]. It was shown that having cationic end groups on both ends of the polymer does not change the friction coefficient but enables the bottle brushes to withstand higher contact pressures before wear initiation than when only one or no end groups are present [120]. Other mimics composed of PEG brushes on a polyacrylic acid backbone [122-123] or using HA binding peptides on a chondroitin sulfate backbone [124]. However, despite showing some lubrication ability, none of these systems matches the superior lubricating properties of articular cartilage, highlighting the need for a more comprehensive approach.

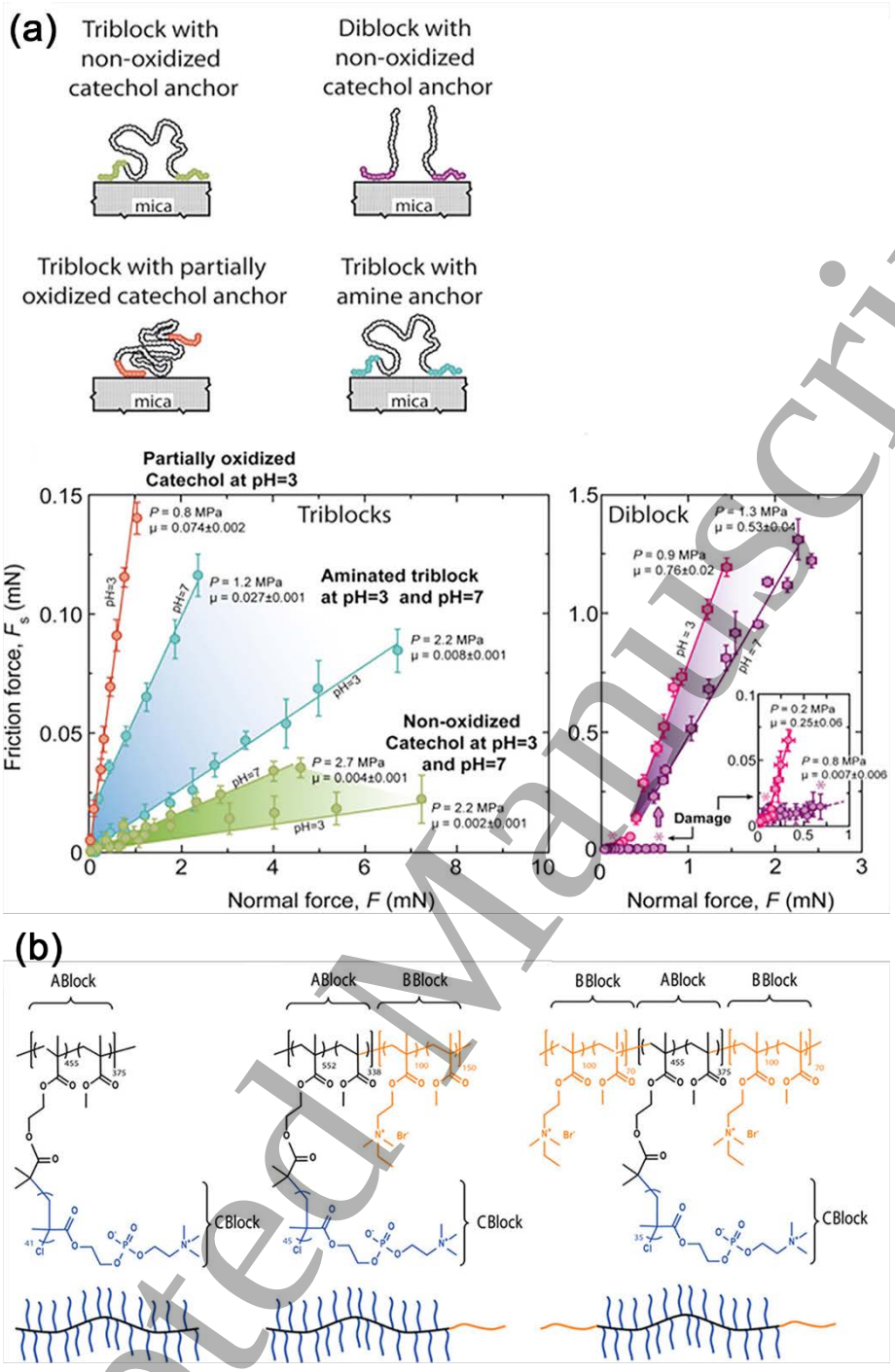

Figure 6 – Examples of biomimetic lubricating systems. (a) Polymer loops that mimic the loop conformation of mucins on surfaces. The graphs show the tribological testing of the triblock and diblock polymers and the impact of the conformation on polymer performance. The triblock with the strong anchoring groups has the lowest friction and withstands the highest loads. Reproduced with permission from [117]. Copyright 2016 American Chemical Society. (b) Bottle brush polymers with various architectures. The B-block provides adhesion to the substrate. Having two adhesive blocks increases the resistance to wear of the bottlebrush. Reproduced with permission from [120]. Copyright 2018 American Chemical Society.

Harnessing synergistic interactions

As described earlier, biotribological research has recently uncovered numerous synergistic interactions between biomacromolecules that contribute to lowering the friction coefficient or enhancing the wear properties of biological contacts. With this new knowledge, researchers are turning their efforts towards designing biomimetic lubricants that harness these synergistic effects. Faivre et al. have designed wear resistant surfaces comprised of HA and a synthetic bottle brush polymer that acts as a lubricin mimic [119]. They found that films formed by the combined polymers in water or saline solution could withstand higher pressures than HA-only films before the onset of wear damage. They attributed this effect to the trapping effect of HA by the bottlebrush polymer inside the contact through chain entanglement, which enables the polymer film to be maintained for higher contact pressures (Figure 7A). In follow up work, adhesive end groups were added to the bottle brushes, which improved their wear properties, in particular, in the presence of HA the bottle brush/ HA films yielded a friction coefficient of ~ 0.02 and could withstand pressures up to 14 MPa before sustaining wear [120].

Utilising the interactions between surface active phospholipids and HA, Seror et al. recently obtained low friction coefficients ($\mu \sim 10^{-3}$), even at high pressure (10 MPa) between mica surfaces functionalised with HA and interacting with phosphatidylcholines [125]. In comparison, in the absence of phosphatidylcholines, the friction coefficient between the HA modified surfaces was around 0.3. It was hypothesised that the lipids interact with HA in a way that exposes their zwitterionic head groups to the surrounding environment, thus allowing water to interact with the charged groups and enhancing the hydration lubrication effect.

Biomimetic systems can also be designed to enhance synergies between the substrate and biomacromolecules present in the fluid. Singh et al. designed a system that comprises a PEG chain with a collagen binding peptide on one end to bind onto cartilage and a HA binding peptide to attract HA molecules from the surrounding media and trap them in the contact [126]. They found that HA bound to cartilage through the PEG/peptide system in the absence of an exogenous lubricant could reproduce the friction coefficients of lubricants containing high concentrations of HA on unmodified cartilage. Morgese et al. proposed a biolubricating system composed of three polymeric elements that are combined to provide interactions with the cartilage surface and lubrication: a polyglutamic acid backbone (PGA) is coupled to brush-forming, charged poly-2-methyl-2-oxazoline (PMOXA) side chains to provide lubricity to the surface, and to aldehyde-bearing groups, that can anchor on damaged cartilage via Schiff bases (Figure 7B) [127]. These polymers could restore the friction coefficient of damaged cartilage in synovial fluid and in some cases surpass the friction properties of native

cartilage tested in the same experimental conditions. A further improvement to the polymer design was recently proposed whereby the PMOXA moieties formed loops rather than linear chains. For most values of side chain densities, the loops provided better lubrication than the linear polymers, possibly thanks to less polymer interpenetrations between opposing sides [128]. The systems described above focus on finding synergies that enhance the boundary friction and/or wear resistance of the surfaces. Future improvements could include finding synergies that also result in enhanced rheological behaviour of the fluid and the surface film in order to provide a biotribological system able to function at a wide range of speeds and loads.

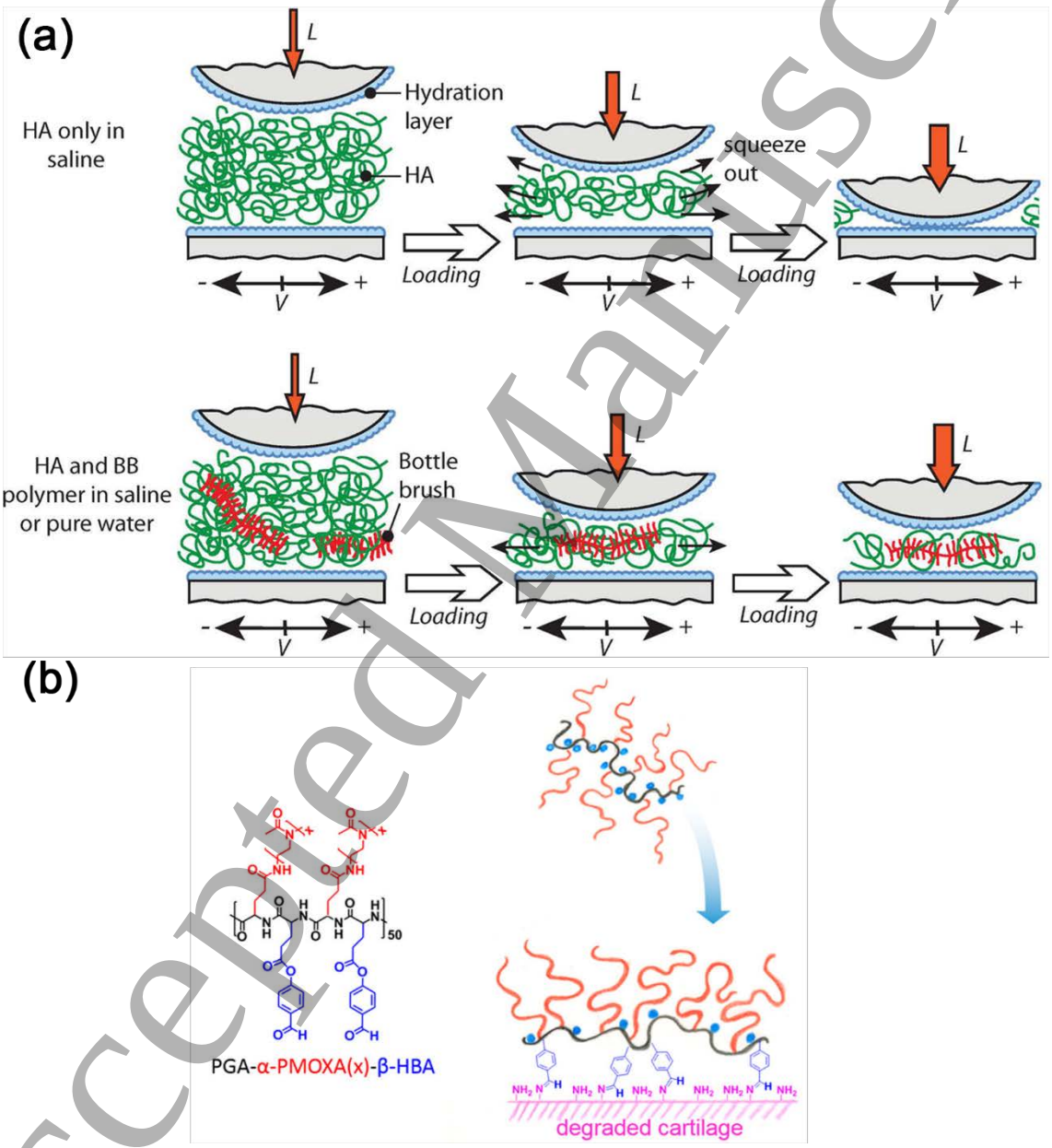

Figure 7 – (a) Synergistic interactions between a bottle brush (BB) polymer (lubricin mimic) and HA prevent HA from being squeezed out of the contact area during loading. Reproduced with permission from [119]. Copyright 2017 American Chemical Society. (b) Copolymer with charged

lubricating side chains and anchoring groups that interact with damaged cartilage. Reproduced with permission from [127]. Copyright 2017 American Chemical Society.

Conclusion

Biomacromolecules are essential ingredients in the maintenance of healthy biological interfaces by preventing direct rubbing between surfaces. Mucins, lubricin and other biomolecules such as hyaluronic acid or small salivary proteins act in synergy to provide a unique set of rheological and surface properties that ensures the presence of a full fluid film or of a highly hydrated boundary film between the two opposing surfaces. As the knowledge about the biomacromolecules and mechanisms involved in lubrication increases, better biomimetic solutions are being developed. Polymer brushes, molecules that enhance the hydration lubrication phenomenon or glycoprotein mimics have shown promising results despite the complexities of the natural fluids. However, it is the recent advances in the understanding of synergies between biomacromolecules that may hold the key to the development of highly lubricating and wear resistant biotribological contacts. By combining molecules, one can enhance their anchoring capacity and lubricating ability. Further improvement could come from more comprehensive system approaches that combine and optimise the properties of the fluid, surface film and substrate to provide tribological solutions able to operate under the demanding conditions encountered in the body.

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