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Insufficient biolubrication represents a major healthcare burden that is facing greater pressure and impact on the quality of life with increasing age and life expectancy. Insufficient biolubrication can yield severe discomfort, and rather frequently occurs in the elderly, in patients using drugs or subjected to head-neck radiotherapy and in patients with Sjögren’s syndrome, a syndrome which includes dryness of the mouth impeding proper speech and mastication, dry and irritated eyes, vaginal dryness and, in its secondary form, excessive friction and wear of articulating cartilage surfaces in hips and knees. Currently, our understanding of biolubrication is insufficient to design effective therapeutics to restore biolubrication in the elderly and diseased.

First, we decided to select a model system for our biolubrication research. As described in chapter 1, we selected the oral cavity as a model system, mainly due to its ease of accessibility and availability of its lubricating fluid, i.e. saliva. Adsorbed salivary conditioning films (SCFs) in the oral cavity are known to provide boundary lubrication, which can be permanently hampered due to disease but also temporarily perturbed by the use of oral hygiene products. Using the simple daily dynamics of perturbation of SCFs, we have tried to provide a comprehensive analysis of biolubrication in the oral cavity at a molecular level and to identify the role of SCFs in lubrication and oral tactile perception. Influences of chemical and mechanical perturbation of SCFs on biolubrication were analyzed and effects of recombinant proteins adsorbed into adsorbed SCFs on biolubrication were determined to provide a clue to improve current saliva substitutes.

SCFs are formed on all oral surfaces exposed to saliva and protect the oral surfaces against its often hostile environment. Oral hygiene products, including toothpastes, are mainly designed for biofilm control, but their detergents and other active ingredients also affect the general properties of adsorbed SCFs. In
chapter 2, the kinetics of SCF formation, its hydrated thickness and visco-elasticity are determined using a Quartz Crystal Microbalance with Dissipation (QCM-D). Two hour old in vitro adsorbed SCFs were 43.5 nm thick and its characteristic frequency was 9.4 MHz, whereas the dehydrated thickness, measured using X-ray photoelectron spectroscopy, was 2.4 nm. Treatment with toothpaste slurries decreased the film thickness depending on fluoride-detergent combination involved. Secondary exposure to saliva replenished the perturbed SCFs and increased the film thickness to much of its original thickness, although no relation existed between hydrated and dehydrated film thicknesses indicating differences in film structure. Treatment with SnF₂-SLS containing toothpaste slurries yielded a strong, immediate two-fold increase in characteristic film frequency with respect to untreated films, indicating cross-linking in adsorbed salivary-protein-films by Sn²⁺ that was absent when SLS was replaced by NaHMP. Secondary exposure to saliva of SCFs treated with SnF₂ caused a strong six-fold increase in characteristic frequency compared with primary salivary-protein-films, regardless whether SLS or NaHMP was the detergent. This suggests that ionized stannous, is not directly available for cross-linking in combination with highly negatively charged NaHMP, but becomes slowly available after initial treatment to cause cross-linking during secondary exposure to saliva.

Detergents like SLS and NaHMP in toothpastes not only influence the structure and composition of the SCFs, but also affect the lubrication by SCFs and sensory perception in the volunteers, as determined in chapter 3. Using different surface analytical techniques like atomic force microscopy (AFM), QCM-D, X-ray photoelectron spectroscopy (XPS) and contact angle measurements we demonstrated that adsorbed SCFs in vitro are more lubricious when their hydrophilicity and degree of glycosylation increases, meanwhile decreasing their
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structural softness. High-molecular-weight, glycosylated proteins adsorbing in loops and trains, are described as necessary scaffolds impeding removal of water during loading of articulating surfaces. Comparing in vitro and in vivo water contact angles measured intra-orally, the sensory-perception in human volunteers could be related with structural softness and glycosylation of adsorbed protein films on tooth surfaces.

Lubrication by SCFs and sensory perception in volunteers are not only affected by detergents, but also by the different modes of mechanical brushing, as shown in chapter 4. Boundary lubrication by SCFs was influenced by different modes of tooth brushing, which corresponds to changes in SCFs roughness, dehydrated layer thickness and degree of glycosylation. Coefficient of frictions (COFs) on 16 hours old SCFs after manual, rotary-oscillatory and sonically-driven brushing were measured using colloidal probe AFM. AFM was also used to assess the roughness of SCFs prior to and after brushing. Dehydrated layer thicknesses and glycosylation of the SCFs were determined using XPS. Mouthfeel after manual and rotary-oscillatory and sonically-driven brushing was evaluated employing a split-mouth design. Compared with unbrushed and manually or sonically-driven brushed SCFs, powered rotary-oscillatory brushing lead to deglycosylation of the SCF, loss of thickness and a rougher protein film. Concurrently, due to deglycosylation and its increased roughness, the COF of a powered rotary-oscillatory brushed SCF increased strongly by a factor of ten with respect to an unbrushed SCF. Volunteers reported a slightly preferred mouthfeel after sonic-brushing as compared to powered rotating-oscillating brushing. Overall, powered rotary-oscillatory brushing can deglycosylate a SCF, leading to a rougher protein film as compared with manual and sonic-brushing, therefore decreasing the
lubricative function of the SCF. This is consistent with clinical mouthfeel evaluation after different modes of brushing.

We have shown that biolubrication is influenced by the structure and glycosylation of adsorbed protein films, providing an important clue to design effective therapeutics to restore biolubrication in patients with insufficient biolubrication. In chapter 5, we apply recombinant supercharged unfolded proteins (SUPs) with 36 (K36) and 72 (K72) positive charges based on elastin-like polypeptides to improve lubrication of adsorbed SCF. Adsorbed K36 and K72 interact with glycosylated mucins in SCFs to form a rigid film, which increases with the number of positive charges. Renewed exposure to saliva after adsorption of cationic SUPs recruits additional negatively charged glycosylated mucins to create a soft, hydrated film, especially when K72 is involved. These hydrated and rigid films improve lubrication and maintain their structural integrity upon high contact pressures. Current generations of artificial salivas are inadequate to restore oral lubrication on a lasting basis. Therefore, cationic SUPs represent a potential novel therapeutic modality to restore lubrication when availability of naturally occurring proteins is reduced.

In healthy persons, adsorbed SCFs are known for protecting the tooth surfaces against abrasion and also erosion. The structure and glycosylation of the SCFs influencing the lubrication behaviour or abrasion resistance can be also expected to influence the erosion protection by the SCFs. As shown in chapter 6, we use a SnF2 containing mouthrinse to demonstrate the importance of structural and glycosylation changes in SCFs, as induced by Sn2+ ions in the protection of enamel surfaces against erosion and abrasion. QCM-D showed that SCFs became rigid after exposure to a SnF2 containing mouthrinse, which we attributed to cross-
linking of adsorbed proteins by Sn$^{2+}$ ions. During renewed exposure to saliva, the SnF$_2$ treated SCF recruited more salivary proteins, thereby increasing the adsorbed mass and degree of glycosylation in the SCF, as determined from QCM-D and XPS, respectively. The renewed adsorbed film on a SnF$_2$ treated SCF provided a lower friction than when formed on an untreated SCF. Moreover, such rigid, more heavily glycosylated and lubricious SCFs yielded a lower calcium loss during exposure to a citric acid solution than untreated SCFs. Therewith, this is the first study to demonstrate physical changes in SCFs due to Sn$^{2+}$ adsorption that can be related to the control of erosion and abrasion of enamel surfaces in vitro.

In chapter 7, we emphasize the advantages of using the oral cavity as a model system for biolubrication studies. Also, we highlight the role of biolubrication in tactile perception which can be of benefit for consumer based design of oral health care products. In the end, we provide details regarding naturally occurring SCFs lubricous architectures which can be important for biomimetic lubrication research to develop artificial lubricants that can provide better wetting of oral surfaces, reducing the sensation of pain due to oral dryness and improving the oral function like chewing, swallowing and speech.