# Aqueous Lubricants for Dry Mouth Applications

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# List of submitted publication during candidature

The candidate confirms that the work submitted is her own, except where work which has formed part of jointly authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

- The following submitted publication is based on Chapter 2:

**Jing Hu**, Efren Andablo-Reyes, Alan Mighell, Sue Pavitt and Anwesha Sarkar.
'Dry mouth diagnosis and saliva substitutes ─ A review from a textural perspective'
Submitted to ***Journal of Texture Studies***

*Author contribution:* Jing Hu designed the review plan, research question, selected the relevant literature, prepared the figures and study characteristics tables and wrote the review. The review was edited with feedback from Efren Andablo-Reyes, Alan Mighell, Sue Pavitt and Anwesha Sarkar.

- The following publication and the filed GB patent is based on Chapter 3:

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'Synergistic microgel-reinforced hydrogels as high-performance Lubricants'
**2020** ***ACS Macro Letters*** 1726–1731

Anwesha Sarkar, **Jing Hu**, Efren Andablo-Reyes, **GB Patent**, Application number 2007546.1 Filed in May, 2020

*Author contribution:* Jing Hu designed of the research, planned the method, did the experiments, analysed the data and wrote of the manuscript. The manuscript was edited with feedback and theoretical analysis from Efren Andablo-Reyes, Siavash Soltanahmadi and Anwesha Sarkar.

# List of accepted abstracts for conferences

The work of this research has been accepted by the following events:

- **Oral presentation** at **Food Colloid Group Symposium, Leeds, UK** – Tribology and mouthfeel

  12th Feb 2020

- **Poster Presentation** at **18th Food Colloid Conference at Lund, Sweden**– Lubrication and rheological properties of lactoferrin microgel particles

  Abstract accepted but the conference cancelled due to COVID-19

- **Poster Presentation** at **34th EFFoST (Winner of "MSc Student of the Year Award 2020")**

  Abstract accepted and invited to online conference, 10th-12th Nov 2020

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# Abstract

There has been a recent upsurge in research efforts in aqueous lubrication since the ability to create super-lubricious water-based lubricant is important for various biological and technological applications. A comprehensive literature review has identified the urgency in further developments in innovative technologies in aqueous lubricants particularly that can act as saliva substitutes offering efficient lubrication and moistening of oral mucosa for dry mouth patients. Therefore, the aim of this research was to design a novel bio-lubricant that provides effective lubrication similar to or exceeding that of real human saliva. Various methods were used to realize this aim including structural characterization with transmission electron microscopy (TEM), zeta-potential measurements, and evaluation of tribological, rheological and adsorption properties with different materials and compositional ratios. A novel non-lipidic bio-lubricant was patented, which was fabricated by reinforcing a fluid-like hydrogel with proteinaceous microgels. This aqueous lubricant demonstrates a synergistic interaction offering super-lubricity in comparison to any of the pure components alone. This two-component lubricant composed of positively-charged lactoferrin microgels dispersed in negatively-charged $\kappa$-carrageenan hydrogels, which is able to generate excellent lubricity while lubricate better than real human saliva in different oral contact mimicking conditions (*i.e.* hard, smooth, hydrophobic as well as soft, textured hydrophilic silicone surface) at certain component ratios. Such super-lubricity is attributed to the synergistic effects between mutually oppositely-charged microgels and the hydrogel, which reinforces the hydrogel, allowing friction reduction by combining the benefits of both viscosity and hydration lubrication, latter supported by adsorption onto the surface. The superlubricity mediated by the synergistic interactions of microgel-reinforced hydrogel offers a unique prospective towards the fabrication of biocompatible aqueous lubricants for dry mouth syndrome.

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# List of abbreviations

**AFM**: atomic force microscopy

**BX**: BioXtra

**COOH**: carboxylic moiety

**CMC**: carboxymethyl cellulose

**DLS**: dynamic light scattering

**HEC**: hydroxyethyl cellulose

**HEPES**: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

**HA**: hyaluronic acid

**HPC**: hydroxypropyl cellulose

**LFM**: Lactoferrin microgels

**MC**: methyl cellulose

**MTM**: mini traction machine

**OB**: Biotène Oral balance dry mouth system

**PDMS**: polydimethyl siloxane

**PTH**: parathyroid hormone

**PAA**: polyacrylic acid

**PEG**: polyethylene glycol

**pSS**: primary Sjögren's syndrome

**QCM-D**: quartz crystal microbalance with dissipation

**SPR**: surface plasmon resonance

**TEM**: Transmission electron microscopy

**VAS**: visual analogue scale

**XI**: Xerostomia Inventory

# Chapter 1

## Introduction

### 1. Motivation of the MRes project

Xerostomia which is defined as the subjective complaint of dry mouth symptoms is becoming an increasingly severe problem with aging population (Xu *et al.,* 2019). It not only has an adverse impact on the essential daily activities such as speech, nutritional intake, but also increases the risk of dental disease such as dental caries and periodontal disease and affects the overall quality of life. Although 99% of saliva is composed of water, it shows excellent lubrication behaviour which is probably due to the synergistic contribution of mucins and other low molecular weight salivary proteins (Xu *et al.*, 2020, Iorgulescu, 2009). Such excellent hydration lubrication property is lost in dry mouth patients.

To address this condition, saliva substitute is widely used as a symptomatic treatment for dry mouth patients, which generally aims at mimicking some of the functions of real human saliva such as moisturizing (Kaandorp and Michels, 1994). Existing commercial saliva substitutes in different forms such as cleansers, sprays and gels are commonly based on active agents such as mucin, modified celluloses, polysaccharide gum or polyethylene glycol (PEG) (Amerongen and Veerman, 2003). However, the effectiveness of these saliva substitutes in addressing dry mouth conditions and providing relief is often limited as these substitutes do not mimic the lubrication behaviour of real human saliva. Recent studies have demonstrated the potential of biopolymeric microgels to lubricate hydrophobic surface due to their capacity to trap water molecules providing hydration lubrication (Andablo-Reyes *et al*., 2019, Liu *et al.*, 2014). And a self-assembly of salivary proteins including positively-charged lactoferrin that electrostatically attaches to mucinous surfaces has been shown to improve lubrication behaviour similar to that of real human saliva previously (Xu *et al*., 2020). Therefore it is worthwhile to modify the technology of proteinaceous microgel fabrication and explore the ideal materials for the development of a novel bio-lubricant which is ideal for dry mouth treatment, and **this is the key motivation behind this MRes thesis**.

## 2. Aim of the project

The aim of this project is to design a novel bio-lubricant as a saliva substitute that provides effective lubrication similar to or exceeding that of real human saliva.

## 3. Experimental objectives

To achieve the aim of the project, the experimental objectives were designed as following:

- Characterize the structural properties and the zeta-potential of key materials used in this thesis including lactoferrin-based microgels, *κ*-carrageenan and the lactoferrin microgel-reinforced κ-carrageenan hydrogels to understand the interaction between different systems.
- Evaluate the tribological and adsorption behaviours of lactoferrin microgels, *κ*-carrageenan and the lactoferrin microgel-reinforced *κ*-carrageenan hydrogels using real human saliva as a control.
- Investigate the rheological properties of lactoferrin microgels, *κ*-carrageenan and the lactoferrin microgel-reinforced *κ*-carrageenan hydrogels to reveal the flow ability of these bio-lubricants and to understand the contribution of viscous forces on the lubrication performances.

## 4. Rationale behind the materials and methodology

This section introduces the key materials (*e.g. κ*-carrageenan and lactoferrin) used for the development of the substitute and methods used in the thesis, explaining the theories and principles behind the methods. While detailed experimental protocols can be found in experimental section of Chapter 3.

### 4.1. Materials

#### 4.1.1. κ-carrageenan

*κ*-Carrageenan is a linear sulphated anionic polysaccharide isolated from red algae (Rochas and Rinaudo, 1984). As shown in **Figure 1.1a**, *κ*-Carrageenan is consisting of linear repeating galactose units as backbone structure with one sulphate group per unite (Liu *et al*., 2015). *κ*-Carrageenan is only soluble in hot water (60 ℃), and presence of cations such as $K^+$ or $Ca^{2+}$ can influence the solubility and gel formation of *κ*-carrageenan (Necas and Bartosikova, 2013). Possible gelling mechanism of *κ*-carrageenan is a two-step transition

of coil to helices controlled by temperature or cations such as $K^+$ or $Ca^{2+}$ (**Figure 1.1b**) (Yuguchi *et al*., 2003). The helices in *κ*-carrageenan gel are stabilized by hydrogen bonds between the two chains, and the gel is thermo-reversible (Liu *et al*., 2015). Typical structural feature of these polysaccharides is the ability to bind a large amount of water forming hydrogel, therefore functioning as valuable thickening and texture modifiers (Venugopal, 2019). The other physicochemical features of *κ*-carrageenan such as negatively-charged groups, crosslinking ability with another polymer and hydrophilic porous network can also contribute to various promising applications of *κ*-carrageenan (Liu *et al*., 2015).

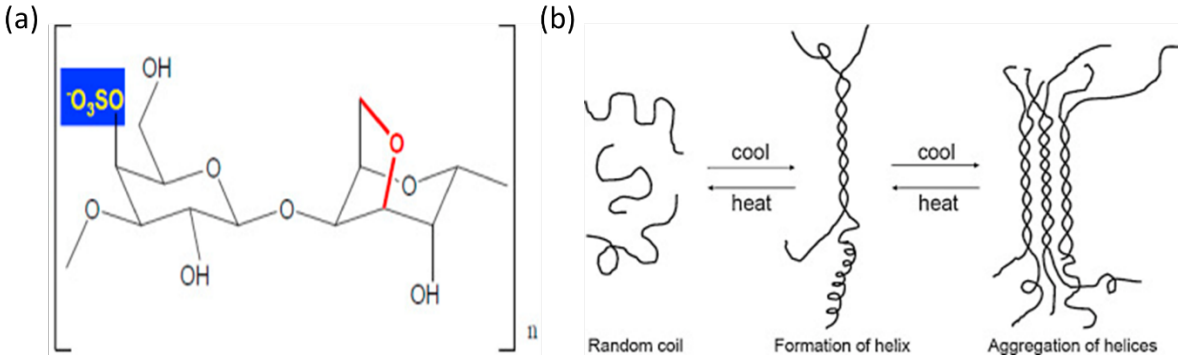


**Figure 1.1** (a) Structures of κ-carrageenan (sulfated D-galactans) (Liu *et al*., 2015) (b) Schematic model for the possible gel formation mechanism of κ-carrageenan. Adapted with permission from (Liu *et al.,* 2015).

### 4.1.2. Lactoferrin microgels

Lactoferrin (80 kDa) is a globular protein that is positively-charged at neutral pH (isoelectric point around 8.5 (Sarkar *et al.,* 2009, Adal *et al.,* 2017)) is produced by mucosal epithelial cells (Adal et al., 2017). Lactoferrin and lysozyme are two kinds of positively-charged protein present in most biological fluids such as milk, tears and saliva (Hassoun and Sivamani, 2017). Its net positive charge and iron-binding property makes it a highly multifunctional protein, which plays an important role in several physiological process such as immune response, anti-inflammatory, antioxidant and antimicrobial processes (González-Chávez *et al.,* 2009). In addition to these biological functions, recent study also demonstrated the ability of lactoferrin functioning as “molecular glue” in mucin network, synergistically promoting the wettability and lubrication with over 72 hours’ of surface adsorption, similar to that of real human saliva (Xu *et al.,* 2020). Such study demonstrated the important role of lactoferrin in facilitating salivary properties such as lubrication,

wettability and adsorption. Therefore, lactoferrin was chosen for further studies in the application of salivary substitutes in this thesis.

Microgels are known as small gel-like particles with cross-linked networks capable of capturing a large amount of solvent, with diameters ranging from hundreds of nanometeres to tens of microns in general (McClements, 2017). Recent studies have demonstrated the excellent aqueous lubrication properties of microgel particles such as whey protein microgels, starch-based microgels and other biopolymeric microgels which have been also termed as fluid gel in the literature (Sarkar *et al*., 2017, Torres *et al*., 2018, Gabriele *et al*., 2010, Fernández Farrés and Norton, 2015, Garrec and Norton, 2013). The lubrication behaviour of whey protein microgels in presence of non-Newtonian (xanthan gum) and Newtonian (corn syrup) fluids have also been examined recently highlighting the importance of continuum in the lubrication performance (Andablo-Reyes *et al*., 2019). As one of the key components of whey protein (Gupta *et al*., 2016), lactoferrin has also showed the ability to form small particles using a top-down approach after heat-set hydrogel preparation (Sarkar *et al*., 2018). Therefore, owing to the excellent lubrication properties of lactoferrn, its positive-charge allowing it to bind to other negatively-charged species and ability to create water-swollen microgel particles by facile route of thermal gelation and homogenization, lactoferrin microgels were chosen as bio-lubricants for their potential interaction with *κ*-carrageenan in enhancing the mechanical properties of the designed saliva substitute.

## 4.2. Experimental methods

### 4.2.1. Microgel fabrication

Lactoferrin microgels (LFM) were fabricated using a top-down method through the Leeds Jet Homogenizer (University of Leeds, UK). The preparation of proteinaceous microgel involves formation of macrogel particles firstly by thermal gelation resulting in the formation of cross-linked heat-set gel. The gelation mechanism is shown in **Figure 1.2**, native protein is denatured under heating while reactive functional groups of the protein is exposed, after which interactions including a complex series of interactions such as chemical bonding (*e.g.* disulphide bond) and physical linkages (*e.g.* hydrophobic interactions, hydrgen bonding and electrostatic interactions) between these exposed functional groups happen, resulting in the gelation of the protein (Sullivan *et al*., 2009). And then the gel is broken into macrogel particles by hand blender. The resulting macrogel

particles (A) and buffer (C) are forced through the pinhole (E) of the jet homogenizer (as shown in **Figure 1.3**) (Sarkar *et al*., 2018) by the pressure of a compressed ait driven ram (D) and pistons (B) when moving down (Pravinata *et al*., 2016). The generated fluid velocities through the pinhole can be very high (>300 $m\ s^{-1}$) creating highly turbulent conditions with Reynolds number in the order of $10^5$ depending on the pressure applied (100-400 bar) (Torres *et al.,* 2017). And the Reynolds number is calculated using equation (1.1) (Torres *et al*., 2017).

$$Re = \frac{\rho v d}{\eta} \tag{1.1}$$

where, $\rho$ is the solvent density, $d$ is the diameter of nozzle, $\eta$ is the dynamic viscosity of the lactogerrin macrogel solution at 20 °C, and $v$ is the maximum fluid velocity which can be calculated using the mean velocity of a fluid in a pipe equation (1.2), where $q$ is the volumetric flow rate.

$$v = \frac{4q}{d^2\pi} \tag{1.2}$$

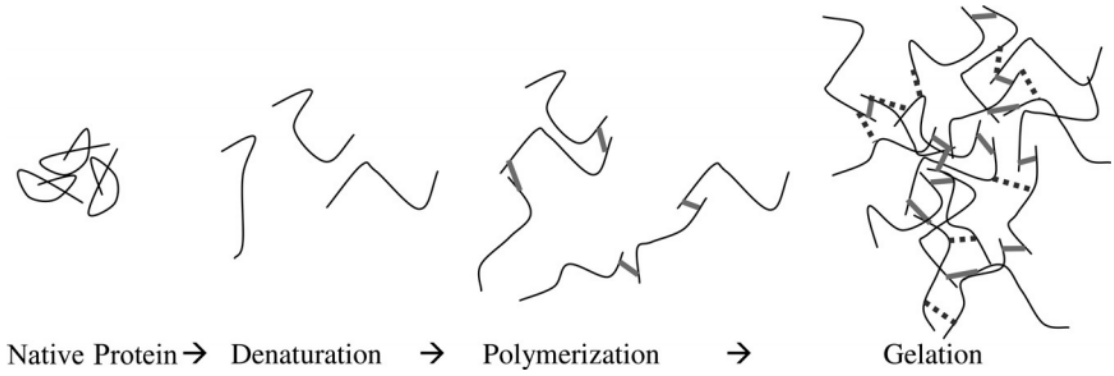


**Figure 1.2** Schematic diagram of globular protein gelation, with gray solid lines representing the chemical bonding (disulfide), dotted connections represent the physical interactions (hydrophobic interactions, hydrgen bonding and electrostatic interactions) (Sullivan *et al.,* 2009).

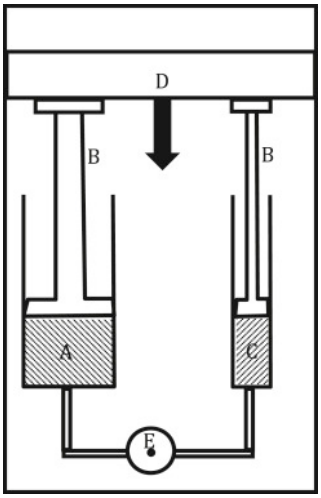


**Figure 1.3** Schematic diagram of Leeds Jet Homogenizer. A=lactoferrin macrogel, C=buffer. Adapted from (Pravinata *et al*., 2016).

### 4.2.2. Particle size and ζ-potential measurement

The particle size is measured by dynamic light scattering (DLS) in this study, where the obtained hydrodynamic diameter ($d_{\mathrm{H}}$) refers to the diameter of a sphere with same translational diffusion coefficient ($D_t$) as real particles (Stetefeld *et al*., 2016). And the $D_t$ here reflects the velocity of the Brownian motion of dispersed particles. Since larger particles move with slower Brownian motion as shown by the following Stokes-Einstein equation, the $d_{\mathrm{H}}$ can be calculated from the $D_t$ (Russel, 1981) using equation (1.3).

$$D_t = \frac{k_B T}{3\pi\eta d_H} \tag{1.3}$$

where, $D_t$ is the translational diffusion coefficient, $\eta$ is the viscosity, $k_{\mathrm{B}}$ is the Boltzmann's constant and $T$ is the absolute temperature.

The $D_t$ can be obtained by light scattering techniques, which detect the fluctuation of scattered light intensity influenced by the Brownian motion velocity of the continuously mobile particles (Bhattacharjee, 2016). Analysis of the autocorrelation function yields estimates of the true particle size distribution and the z-average value can be obtained as the intensity-weighted mean $d_{\mathrm{H}}$ of the particle. A second important number parameter that can be derived from DLS measurement is the polydispersity index, which is a measure of the width of the particle size

distribution. Polydispersity indices less than 0.1 are typically referred to as "monodisperse". The equation (1.4) for polydispersity is shown below:

$$PDI = (\frac{\sigma}{d_H})^2 \quad (1.4)$$

where $\sigma$ refers to the standard deviation.

Another important characteristic measured in this study is ζ-potential, which is the abbreviation for the electrokinetic potential difference developed when two phases are placed in contact (Hunter, 1981). As particles dispersed in liquid, there are mainly two layers surrounding the particles called the stern layer and the diffuse layer as shown in **Figure 1.4** (Williams, 2016). In the inner region of Stern layer, the opposite charged ions are strongly bound to the particles, and in the outer region of diffuse layer, both negatively- and positively-charged ions are less firmly associated (Hunter, 1981). The ions within these double layer move with the particle, while beyond the boundary the ions stay with the bulk dispersant. The electrokinetic potential at such boundary between the mobile particle and dispersant is the ζ-potential (Bhattacharjee, 2016). The electrophoretic mobility of the charged particles under applied electric field can be measured and electrophoretic mobility, which is defined as the velocity of a particle ($V_p$) in a unit electric ($E$) ($\mu_e = V_p/E$). From the obtained $\mu_e$, the ζ-potential can be calculated using the Henry's equation (1.5) (Bhattacharjee, 2016):

$$\mu_e = \frac{2\varepsilon_r \varepsilon_0 \zeta f(ka)}{3\eta} \quad (1.5)$$

where, $\varepsilon_r$ is the relative permittivity/dielectric constant, $\varepsilon_0$ is the permittivity of vacuum, ζ is the ζ-potential, *f(ka)* is the Henry's function which refers to the ratio of the particle radius ($a$) to the electrical double layer thickness ($k$) and $\eta$ the viscosity of the medium at experimental temperature in this case water at 20°C ($\eta$ = 8.9 $10^{-4}$ Pa s).

When the thickness of the electric double layer is much smaller as compared to the particle radius (i.e. big particles > 1 μm) dispersed in the aqueous solutions of high salt concentration (10−2 M), the f(ka) is taken as 1.5, which is referred to as the Helmholtz-Smoluchowski equation (1.6):

$$\mu_e = \frac{\varepsilon_r \varepsilon_0 \zeta}{\eta} \tag{1.6}$$

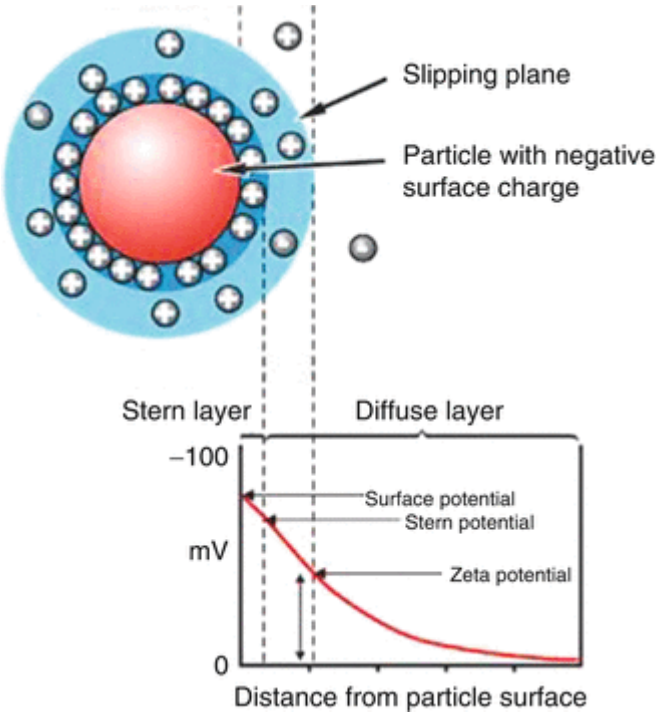


**Figure 1.4** Schematic representation of ζ-potential and different layers of a particle (Williams, 2016).

### 4.2.3. Rheological measurement

Rheological measurement is an important assessment of saliva substitute's properties, since viscosity and viscoelasticity not only correlate to sensorial thickness and firmness, respectively (Stokes *et al*., 2013), but also help to extend the duration of dry mouth relief (Partenhauser and Bernkop-Schnürch, 2016). Commonly used machine for the rheological measurement is the controlled-stress rheometer which has been used to measure shear viscosity, elastic modulus and viscous modulus in this study. The shear viscosity $\eta$ as defined in equation (1.7), indicates the material's resistance to flow deformation (Bair, 2019):

$$\eta = \frac{\tau}{\dot{\gamma}} \tag{1.7}$$

where, $\tau$ is the shear stress and $\dot{\gamma}$ is the shear rate.

The shear viscosity is commonly tested with the cone and plate set as shown in **Figure 1.5**, since it has a constant shear rate and only need a small amount of sample for testing (Macosko). As for viscoelasticity, which is defined as the property of a material to exhibit both viscous and elastic character, it can be revealed by viscous modulus $G''$, elastic modulus $G'$ and tan $\delta$ ( tan $\delta$= $G''/G'$) under dynamic oscillatory tests (Macosko).

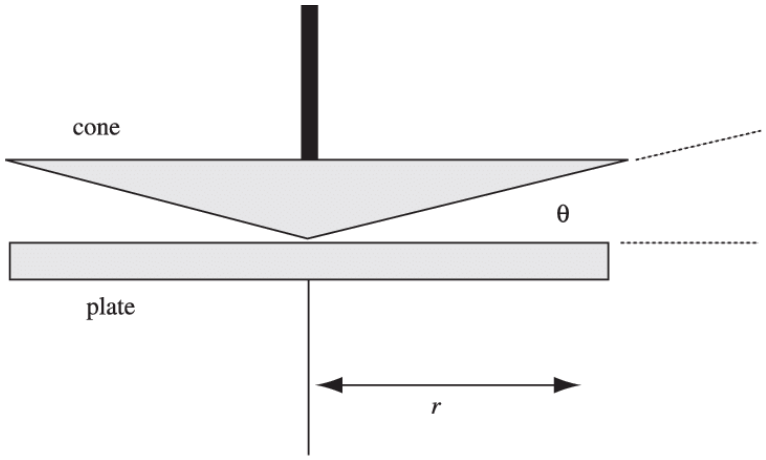


**Figure 1.5** Schematic representation of the cone and late set used in rheometer (Khan *et al.*, 2009).

## 4.2.4. Tribological measurement

Lubrication is one of the most important functions of saliva, which minimize the wear and protect the oral mucosal surfaces (Carpenter and technology, 2013). Therefore, it is essential to evaluate the tribological properties of saliva substitutes. In this study, a mini traction machine (MTM) with a ball-on-disc polydimethyl siloxane (PDMS) set mimicking tongue and palate as shown in **Figure 1.6** is used to conduct tribological measurements. The ball and disc are driven independently by electric motors and the friction force between these two tribopair pieces are measured by force transducers mounted on the ball shaft under a fixed load, thereby the friction coefficients at different speed can be tested according to the following equation (1.8) (Berthe et al., 2014):

$$\mu = \frac{F}{W} \tag{1.8}$$

where, $\mu$ is friction coefficient, $F$ is friction force and $W$ is load.

As for the choice of tribo-surface, researchers have tried different materials such as metallic (*i.e.* steel), animal tissue-based (*i.e.* pig tongue) and polymeric (*i.e.* silicone) materials to mimic the real human oral surfaces (Laguna and Sarkar, 2017). However, the surface chemistry and roughness of steel and pig tongue is difficult to control and are different from that of real human tongue and oral palate (Laguna and Sarkar, 2017). Therefore silicone including the most commonly used PDMS and a newly established polymers *i.e.* Ecoflex 00-30 with one order-of-magnitude lower modulus as compared to PDMS, surface roughness and hydrophobicity have been used as the tribo-surfaces in this study.

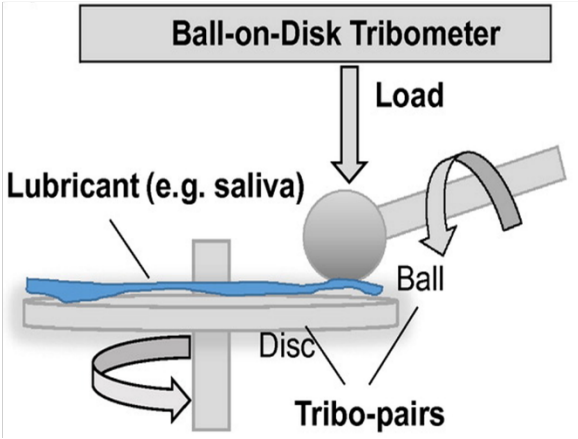


**Figure 1.6** Illustration of the typical mini traction machine (MTM) with a ball-on-disc set up (Sarkar and Krop, 2019).

Figure 1.7 shows the new oral mimetic tribological set-up adapting a rotational rheometer recently developed by Andablo-Reyes *et al.* (submitted) that is used to further test the lubrication behaviour of samples in this thesis. The 2.0 × 2.0 cm 3D-printed replica-moulded surface was glued at the rim of the top plate. Such 3D-printed replica-moulded surface is made from a softer silicone (Ecoflex 00-30) compared to previously used PDMS, which contains appropriate size and spatial distribution of fungiform and filiform papillae mimicking that of real human tongue. The rotational rheometer is equipped with a 50.0 mm diameter stainless steel plate-on-plate geometry. Experiments are performed in a controlled normal force ($F_N$) of 1.0 N with torque (M) recorded in the entrainment speeds ranging from $4 \times 10^{-5}$ to $7 \times 10^{-3}$ $m\,s^{-1}$. Therefore, the friction coefficient can be calculated

using the following equation (1.9)

$$\mu = \frac{M}{RF_N} \tag{1.9}$$

where, M is torque, $F_N$ is normal force and R is the plate radius (R=0.025 m).

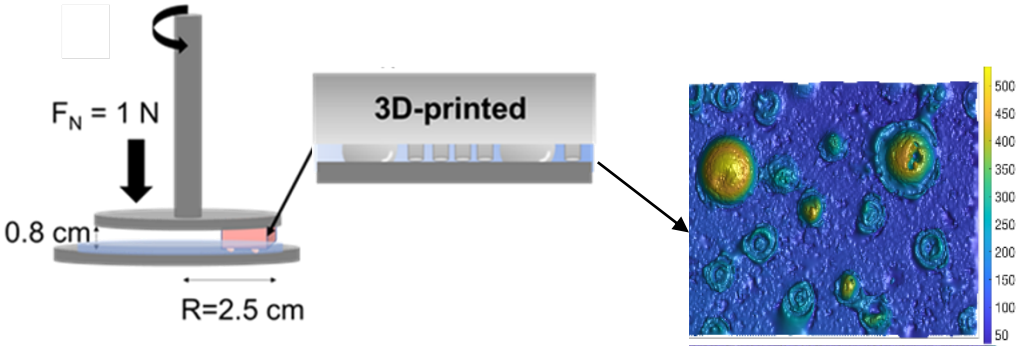


**Figure 1.7** Schematic representation of the tribological set-up adapting a rotational rheometer with 3D-printed tongue-like polymeric surface. The right picture shows the positive impressions of the 3D optical scan of the biomimetic tongue-like surface casted in soft Ecoflex$^{TM}$ 00-30 (Andablo-Reyes *et al*., 2020)

### 4.2.5. Adsorption behaviour measurement

A quartz crystal microbalance with dissipation (QCM-D) is used to measure the adsorption behaviour of κCH, LFM and LFM-reinforced κCH. Traditional quartz crystal microbalance (QCM) can analyse the mass changes on rigid crystal surfaces by monitoring the frequency change of quartz crystal with mass adsorption which initially oscillate at a specific frequency. The linear relationship between the frequency and mass change is dmonstrated by Sauerbrey in 1959 as shown in the following equation (Sauerbrey, 1959) *(1.10*(1.10):

$$\Delta m = -\frac{C}{n} \cdot \Delta f_n \tag{1.10}$$

where, $\Delta m$ is the mass deposited per unit area of crystal surface, $\Delta f_n$ is the change in resonance frequency at the *nth* hamonic (n=1,3,…), C is a constant depending on the property of the crystal. The Sauerbrey equation is valid only for evenly attributed, rigid adsorbed mass which is smaller as compared to the mass of the crystal itself. However, the interaction of crystal with flexible molecular systems such as the microgel and hydrogel used in this thesis results in the formation of soft and viscoelastic films that violate Sauerbrey's equation due to dissipation of oscillation energy. Therefore, dissipation which is defined in the following equaiton (1.11) should be taken into account when quantifying

the viscoelastic mass (Chen *et al*., 2018).

$$D = \frac{E_{dissipation}}{2\pi E_{stored}} \tag{1.11}$$

where, $E_{dissipation}$ is the energy dissipated during one oscillation cycle and $E_{stored}$ is the total energy stored in the oscillating system. Therefore, quartz crystal microbalance with dissipation moniring (QCM-D) technology allows qualititive and quantitative analysis of the adsorbed viscoelasitc molecular layer by combining frequency and dissipation measurements from multiple harmonics (Voinova *et al.,* 1999). In this thesis, a Voigt-based viscoelastic model (Figure 1.8) incorporated in Q-Sense software D-find has been used, which has been frequently used in many previous studies (Xu *et al*., 2020, Ahn *et al*., 2015, Crouzier *et al*., 2012) to estimate the hydrated mass. By fitting experimental dissipation and frequency data from more than 2 harmonics to such viscoelastic model, the hydrtated mass can be extracted to analyse the real-time adsorption behaviour of samples.


**Figure 1.8** Schematic representation of Voigt based viscoelastic film model, with a viscoelastic flim on a quartz in contact with a Newtonian bulk liquid labled with the seven physical parameters that fully define the system (McNamara and Blanford, 2016)

### 4.2.6. Microscopy measurement

Transmission electron microscopy (TEM) is a commonly applied structural analysis tool for soft-matter, which provides images with spatial resolution at the level of atomic scale (Kumar *et al*., 2019). Although both TEM and scanning electron microscopy can provide valuable information of nanomaterials such as size and degree of aggregation, TEM offers high-quality spatial resolution and details regarding nanoparticles (Kumar *et al*., 2019). The working principle of TEM is shown in **Figure 1.9**, which is similar to the principle of optical microscopy, while electrons, electromagnetic lenses and screen replaced photons, glass lenses and eyepiece respectively (Kumar *et al*., 2019). According to the concept of wave-particle duality, electrons are like light which can interact with matter by scattering

and then the resulting image can be magnified by a series of electromagnetic lenses (Franken *et al.*, 2020). The interaction between the electrons and atoms from the samples can be classified into two types called elastic scattering and inelastic scattering, while radiation damage happened in inelastic scattering processes resulting in differences in focal lengths and low resolution information (Franken *et al*., 2020). Therefore, to change the ratio of elastic and inelastic scattering and increase the interaction of elections and atom, heavy metals are normally added to sample especially those contain mainly carbons, which is called staining (Franken *et al*., 2020). In this MRes thesis, the samples were negatively stained by uranyl acetate.

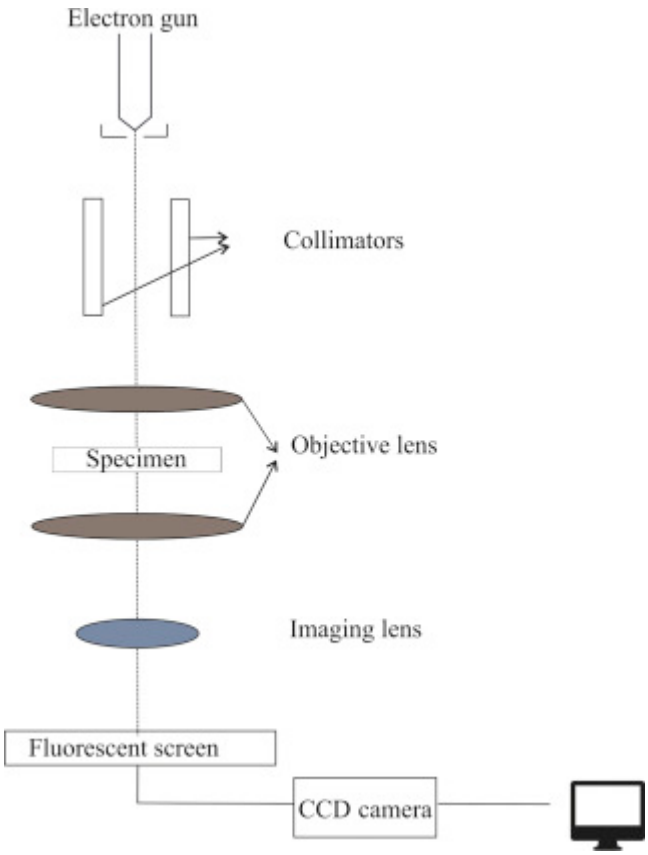


**Figure 1.9** Illustration of the working principle of transmission electron microscopy (TEM) (Kumar *et al*., 2019).

## 5. Structure of MRes thesis

The thesis is divided into four chapters and an illustration framework of the thesis is shown in **Figure 1.10**.

Chapter 1 (Introduction): This chapter introduces the motivation, aim, experimental objectives and rationale behind the selection of the materials and methodology used in this thesis.

Chapter 2 (Literature review): This chapter presents a comprehensive review of the literatures covering the diagnosis as well as symptomatic treatment of dry mouth conditions (salivary substitutes) with a clear focus on textural perspective, identifying the urgency in further developments in innovative technologies for novel saliva substitutes that might offer efficient lubrication and moistening of oral mucosa**.** This literature review is submitted to ***Journal of Texture Studies.***

Chapter 3 (Synergistic microgel-reinforced hydrogels as high-performance lubricants): This chapter presents the experimental approach and the main results obtained in this thesis. A fluid-like hydrogel composed of biopolymeric nanofibrils with proteinaceous microgels is fabricated, which synergistically provide super-lubricity, advanced mechanical and adsorption property, in comparison to any of the pure components alone. The favourable electrostatic attraction between mutually oppositely-charged microgels and the hydrogel reinforces the mechanical properties of the hydrogel, resulting in high lubricating performance exceeds that of real human saliva in oral contact mimicking conditions. The key finding of this research is filed in a patent in May 2020 (GB Patent Application number 2007546.1), and the revised manuscript based on this chapter is also submitted to ***ACS Macro Letters***.

Chapter 4 (Concluding and future directions): In this chapter, the major findings, practical implications and future perspectives are summarised.

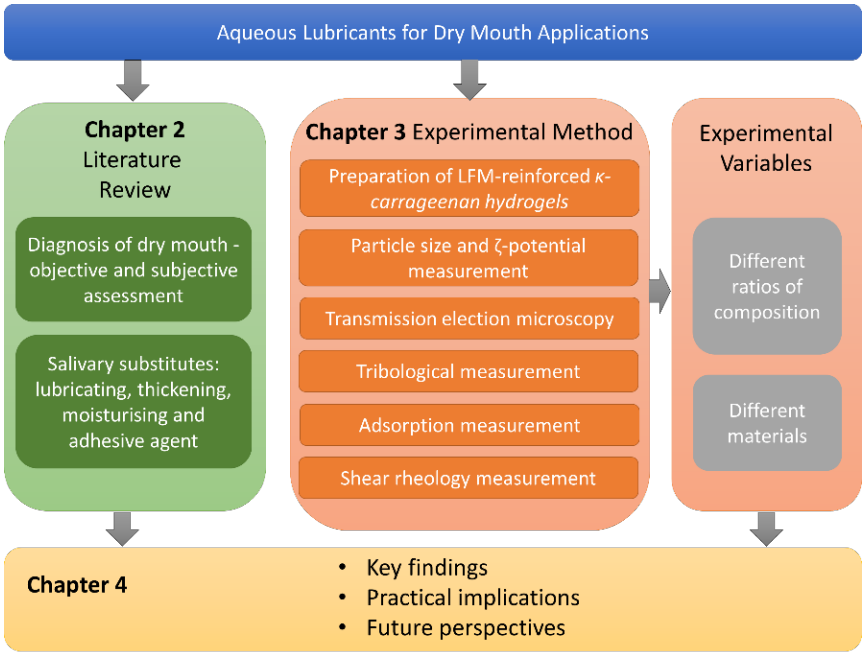


**Figure 1.10** Graphical framework of this MRes thesis.

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# Chapter 2

## Literature review [1]


### Abstract

The aim of this review is to assess the objective and subjective diagnosis, as well as symptomatic topical treatment of dry mouth conditions with a clear focus on textural perspective. We critically examine both the current practices as well as outline emerging possibilities in dry mouth diagnosis and treatment, including a patent scan for saliva substitutes. For diagnosis, salivary flow rates and patient-completed questionnaires have proven to be useful tools in clinical practice. To date, objective measurements of changes in mechanical properties of saliva *via* rheological, adsorption and tribological measurements and biochemical properties of saliva such as assessing protein, mucins (MUC5B) are seldom incorporated into clinical diagnostics; these robust diagnostic tools have been largely restricted to application in non-clinical settings. As for symptomatic treatments of dry mouth, four key agents including lubricating, thickening, adhesive and moisturizing agents have been identified covering the overall landscape of commercial saliva substitutes. Although thickening agents such as modified celluloses, polysaccharide gum, polyethylene glycol (PEG) *etc.* are most commonly employed saliva substitutes, they offer short-lived relief from dry mouth and generally do not provide boundary lubrication properties of real human saliva. Innovative technologies such as self-assembly, emulsion, liposomes, microgels are emerging as novel saliva substitutes that hold promise for alternative approaches for efficient moistening and lubrication of the oral mucosa. Their adoption into clinical practice will be dependent on their efficacies, duration of relief, ease of application by the practitioners and patient compliance.


[1] This chapter has been submitted as: J. Hu, E. Andablo-Reyes, A. Mighell, S. Pavitt, and A. Sarkar (2020), Dry mouth diagnosis and saliva substitutes – A review from a textural perspective. *Journal of Texture Studies* (Accepted)

# 1. Introduction

Xerostomia, clinically defined as the subjective complaint of "dry mouth" has an estimated prevalence of approximately 20% in the general population. The prevalence increases to 46% in older people aged >75 years, attributable in part to co-morbidity conditions and polymedication/ polypharmacy (Orellana *et al.*, 2006). Other causes, include, but not limited to, autoimmune exocrinopathy (*e.g.* primary Sjögren's syndrome (pSS), see Figure 2.1), radiotherapy, sarcoidosis, HIV, hepatitis C and poorly-controlled diabetes mellitus (Mortazavi *et al.*, 2014). Xerostomia has a detrimental impact on quality of life affecting the most essential activities such as speaking and eating, with dysphagia inhibiting easy entrance of nutrients and increases the risks of malnutrition (Vainshtein *et al.*, 2016). Furthermore, it increases the risk of dental complications such as, caries, periodontal disease, candidiasis, and oral ulceration (Hopcraft and Tan, 2010). Xerostomia patients may have both hyposalivation and also alteration in salivary composition (Jellema *et al.*, 2005; Mortazavi *et al.*, 2014; Villa and Avati, 2011). It is also worth noting that xerostomia patients may or may not have hyposalivation, which is a sign of abnormally lower salivary flow rate. For example, besides hyposalivation, dehydration (in elderly or dialysis patients) could also result in xerostomia (Mortazavi *et al.*, 2014). Xerostomia represents an enormous and growing health burden resulting from an increase in the global aging population and highlights the need for more effective topical dry mouth therapies (Ship *et al.*, 2002; Guggenheimer and Moore, 2003).

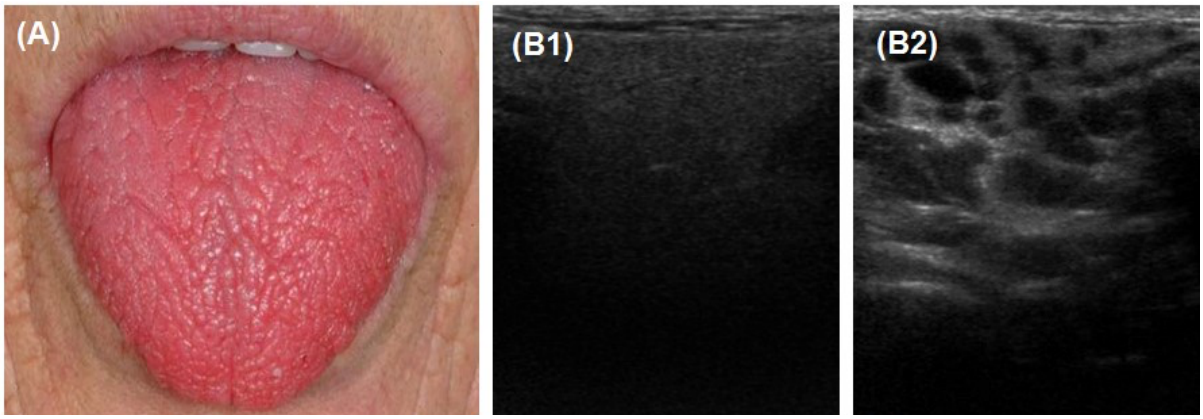


**Figure 2.1** Diagnosis of dry mouth conditions by visual imaging of (A) an extreme dry mouth condition due to primary Sjögren's syndrome (pSS) and ultrasound images of the partotid gland in a healthy people (B1) and in a pSS patient (B2) where multi-hypoechoic areas reflect salivary gland damage. Images have been captured by co-author Dr. Alan Mighell in Leeds Teaching Hospitals NHS Trust, UK.

Hyposalivation may lead to impairment in both the quantity and quality of saliva. Saliva, which is constituted mainly of water (99%), ions and proteinaceous compounds such as mucins, amylases and others low molecular weight proteins (Sarkar *et al.*, 2019b), plays an important role in assuring the general and oral health as well as oral processing of food. It is generally the proteins that render saliva its rheological (viscosity, elasticity, stickiness), unique water-holding and lubrication properties (Alliende *et al.*, 2008; Tanasiewicz *et al.*, 2016; Sarkar *et al.*, 2017). Various functions of saliva can be classified into two aspects: 1) *protection of the oral tissues* including lubrication, dilution, antimicrobial activity, cleansing activity, buffering action, remineralisation and tissue repair, and 2) *facilitating speech and oral processing* including food disintegration and digestion, bolus formation and swallowing, medium for flavour and aroma compounds diffusion (Carpenter, 2013; Dodds *et al.*, 2015).

To address dry mouth conditions, various topical therapies are employed. Typical therapies for dry mouth can be classified into three main groups: 1) salivary stimulants, 2) symptomatic treatments and 3) emerging regenerative and gene therapies (Salum *et al.*, 2018).

**Salivary stimulants** are most commonly used but require some the salivary gland tissue to be functional. There are broadly three ways to stimulate the salivary secretion: acid, pharmaceutical and mechanical approaches. Citric and malic acids are the most commonly used as plant acids to stimulate the salivary secretion, the mechanism is that the topical acidification of the oral environment generates stimulation of salivary secretion to dilute the acid concentration (Han *et al.*, 2015; Salum *et al.*, 2018). Although improvement in dry mouth condition is shown by acid-based salivary stimulants, application of acid may increase the risk of dental erosion and hypersensitivity (da Mata *et al.*, 2009). Besides citric acid, umami taste substance like monosodium glutamate has been also found to stimulate salivation (Sasano *et al*., 2015).

Pilocarpine is the most commonly used pharmacological systemic medication given in a tablet form typically for relieving the symptoms of radiotherapy-induced xerostomia; it functions as muscarinic receptor agonists stimulating the secretion of saliva (Gil-Montoya *et al.*, 2016). However, based on a recent meta-analysis carried out using 39 studies that randomised 3520 participants (Riley *et al*., 2017), it can be inferred that insufficient evidence exist to determine whether or not pilocarpine performed better or worse than a

placebo in terms of treatment of xerostomia, salivary flow rate, survival, and quality of life. Thus, the pharmacological proposed benefits of pilocarpine can be questioned. In addition, pilocarpine, as a parasympathomimetic drug can lead to adverse pulmonary and cardiovascular side effects (Bernardi *et al.*, 2002) Mechanical salivary stimulation on the other hand includes use of chewing gums, acupuncture, and electrostimulation, among which sugar-free chewing gum is widely used because it is an easy way to mechanically stimulate salivary secretion without side effects (Davies, 2000; Han *et al.*, 2015; Łysik *et al.*, 2019).

**Symptomatic treatments** of dry mouth aim to moisten the oral mucosa (Narhi *et al.*, 1999). The most frequently used symptomatic therapies include some form of water intake or hydrating materials and commercial saliva substitutes (Salum *et al.*, 2018). Although fluid intake can be useful for temporary relief of dry mouth symptoms (Łysik *et al.*, 2019), other functions of saliva such as coating and lubrication cannot be achieved by this approach. Existing commercial saliva substitutes in different forms like cleansers, sprays and gels are commonly based on thickening agent and moisturizing agent such as cellulose-based polymers (*e.g.* carboxymethyl cellulose (CMC)) and water-soluble polymers such as xanthan gum, glycerine and carbomer (Nieuw Amerongen and Veerman, 2003; Oh *et al.*, 2008; Han *et al.*, 2015). It is thus important to understand how far these polymers are successful in mimicking the techno-functionalities of real human saliva.

**Experimental regenerative and gene therapies** to ameliorate dry mouth conditions are currently under development. Regenerative therapies aim to attenuate salivary gland dysfunction, whereas stem cell and gene therapies aim to repair or prevent the salivary glands damage by gene transfer (Lombaert *et al.*, 2008; Samuni and Baum, 2011).

With this overview in mind, the aim of this review is to examine the measurable symptoms of dry mouth and saliva properties as well as critically examine the saliva substitutes focussing on textural aspects, such as lubrication and adsorption properties. In particular, a key objective is to provide a concise overview on several challenges associated with dry mouth diagnosis and therapy and discuss how the food textural research community might contibute to overcome them. Firstly, we discuss the various approaches for diagnosis of dry mouth to identify the objective versus subjective assessment of dry mouth conditions clearly highlighting the type of dry mouth therapies needed for most patients. We also

highlight what kind of diagnostic tools can be used in the future to estimate the objective changes in biochemical, rheological, adsorption and tribological quality of saliva in dry mouth patients. Then, we critically analyse the common formulation agents of salivary substitutes highlighting the importance of tribological (*i.e.* friction, wear and lubrication) and adhesive properties. We also evaluate the patents over the last two decades to clearly pinpoint the latest advancements in development and highlight the development needed for salivary substitutes. Specifically, our focus is on salivary substitutes for symptomatic treatments. Formulations with active stimulants or medicines are beyond the scope of this review. Complementary reviews that focus on therapeutic trials of salivary substitutes are available (Brennan *et al.*, 2002; Furness *et al.*, 2011; Salum *et al.*, 2018; Assery, 2019; See *et al.*, 2019). Abbreviations used throughout this review article are shown in Table 2.1.

**Table 2.1** A list of abbreviation used in this review article.

| Abbreviation | Meaning |
|---|---|
| BX | BioXtra |
| COOH | carboxylic moiety |
| CMC | carboxymethyl cellulose |
| HEC | hydroxyethyl cellulose |
| HA | hyaluronic acid |
| HPC | hydroxypropyl cellulose |
| MC | methyl cellulose |
| MTM | mini traction machine |
| OB | Biotène Oral balance dry mouth system |
| PTH | parathyroid hormone |
| PAA | polyacrylic acid |
| PEG | polyethylene glycol |
| pSS | primary Sjögren's syndrome |
| QCM-D | quartz crystal microbalance with dissipation |
| VAS | visual analogue scale |
| XI | Xerostomia Inventory |

## 2. Diagnosis of dry mouth — objective and subjective assessment

Generally, diagnosis of xerostomia starts with a thorough evaluation of medical history, focusing on the illness and past medical history of the patients in a clinical setting (Kho, 2014). The key diagnosis method that have been used are generally subjective in nature such as questionnaires with rating scales for patients to fill and complementary objective assessment such as salivary secretion tests (Fox *et al.*, 1987). Although other tests deploying different imaging techniques (*e.g.* sialography and scintigraphy) were reported

for dry mouth diagnosis, their usage is limited by the invasive character or high cost. In some medical settings, ultrasound is gaining interest as a useful diagnostic tool (Martire *et al.*, 2018). Other measurements that have been primarily used in research settings to assess salivary properties (*e.g.* salivary biochemical composition, adsorption, rheological and tribological tests) might also be utilized for aiding the diagnosis of dry mouth in the future and are discussed in the following sections.

## 2.1. Questionnaires

Questionnaires have played an important role in the evaluation of xerostomia. Since xerostomia is a subjective complaint, questionnaires on dry mouth do not always reflect the true hyposalivation. However, it is useful to identify certain questions that may predict true salivary dysfunction. For instance, evaluation of the relationship between subjective symptoms and objective salivary flow often helps in more efficient diagnosis of hyposalivation than using questionnaires alone (van der Putten *et al.*, 2011). Table 2.2 summaries the major xerostomia questionnaires developed from 1987 to 2007, where a relationship with objective salivary flow rates has been established, with three classical evaluation systems being included *i.e.* binary scale, categorical scoring scale and visual analogue scale (VAS).

Fox *et al.* (1987) employed useful questions in identifying salivary gland output dysfunction. They found that the responses to eating-dryness related questions (question 6-8) (Table 2.2) and saliva quantity question (question 9) were highly indicative of true salivary output deficiency reflected by stimulated and unstimulated flow, while questions concerning the presence or relief behaviour of mouth dryness (question 1-5) were not correlated significantly with the salivary hypofunction. The Xerostomia Inventory (XI) (Thomson *et al.*, 1999) was developed acting as a multi-item instrument estimating the severity of xerostomia symptoms with a continuous scale. Eleven items covering both experiential (experiences of awareness of dry mouth conditions *e.g.* “my mouth feels dry when eating a meal”) and behavioural (consequent behaviours *e.g.* “I sip liquids to aid in swallowing food”) aspects of patients’ experiences of dry mouth, and the responses to these items were summated to give a single XI scale score. Although the resulting score had a very low correlation with resting saliva flow rate, it had a positive and much stronger correlation than the standard single dry-mouth question responses, and the XI itself showed

concurrent validity (Thomson and Williams, 2000).

Pai, Ghezzi, & Ship (2001) developed an eight-item VAS questionnaire for hyposalivation diagnosis. Seven items (Table 2.2) showed significant reliability, while only one question ("rate how much saliva is in your mouth") regarding the quantity of saliva in mouth was not significantly correlated. Five items (1, 2, 3, 5 and 6) show significant validity with unstimulated submandibular saliva flow rates. Only item 1 and 6 were significantly correlated for stimulated submandibular flow rates, while only item 2 was significantly correlated for stimulated parotid flow rates.

Suh *et al.* (2007) developed a questionnaire with a combination of a binary scale, categorical scoring scale and VAS to evaluate the relationship between subjective dry mouth symptoms and salivary flow rate. They reported that the duration and frequency of oral dryness or usage of chewing gum are not significantly associated with salivary flow rate, while dry mouth-related symptoms and behaviours like awakening from sleep at night because of oral dryness were significantly associated with whole salivary flow rate. Comparing all these four questionnaires (Table 2.2) and their relationship with salivary flow rate indicates that the questions regarding the behaviour to relieve dry mouth like chewing gum and candy intake are less related to salivary flow rate, while dry mouth symptoms and eating behaviour related questions are more predictive for diagnosis of salivary dysfunction.

**Table 2.2** Questionnaires for subjective diagnosis of dry mouth and their relationship with salivary flow rates.

| **Questions/statements** | **Rating scales/ Scores** | **Correlation with salivary flow rate** | **Reference** |
|---|---|---|---|
| Fox et al Questionnaire | | | |
| 1. Does your mouth feel dry at night or on awakening?<br>2. Does your mouth feel dry at other times of the day?<br>3. Do you keep a glass of water by your bed?<br>4. Do you chew gum daily to relieve oral dryness?<br>5. Do you use hard candies or mints daily to relieve oral dryness?<br>6. Do you sip liquids to aid in swallowing dry foods? | Binary scale (Positive or negative answer) | Question 1-5 were not indicative of a decreased salivary output (stimulated and unstimulated salivary flow), while the responses to questions 6-9 were highly indicative of diminished salivary output. | (Fox *et al.*, 1987) |

| | | | |
|---|---|---|---|
| 7. Does your mouth feel dry when eating a meal?<br>8. Do you have difficulties swallowing any foods?<br>9. Does the amount of saliva in your mouth seem to be too little, too much, or you don't notice it? | | | |
| *The Xerostomia Inventory* | | | |
| 1. I sip liquids to help swallow food<br>2. My mouth feels dry when eating a meal<br>3. I get up at night to drink<br>4. My mouth feels dry<br>5. I have difficulty in eating dry food<br>6. I suck sweets or cough lollies to relieve dry mouth<br>7. I have difficulties swallowing certain foods<br>8. The skin of my face feels dry<br>9. My eyes feel dry<br>10. My lips feel dry<br>11. The inside of my nose feels dry | Categorical scoring scale<br>Never, hardly, occasionally, fairly often and very often (scoring 1-5, respectively) | The single Xerostomia Inventory (XI) scale score has a very low correlation with resting salivary flow rate but a much stronger correlation with the standard dry mouth question responses. | (Thomson *et al.*, 1999) |
| *Visual Analogue Scale questionnaire for subjective assessment of salivary dysfunction* | | | |
| 1. Rate the difficulty you experience in speaking due to dryness<br>2. Rate the difficulty you experience in swallowing due to dryness<br>3. Rate how much saliva is in your mouth<br>4. Rate the dryness of your mouth<br>5. Rate the dryness of your throat<br>6. Rate the dryness of your lips<br>7. Rate the dryness of your tongue<br>8. Rate the level of your thirst | Visual Analog Scale (100-mm horizontal scale) | Significant reliability for 7 VAS items (excluding item 3). Five items (1, 2, 3, 5 and 6) show significant validity with unstimulated submandibular saliva flow rates. Two items (1 and 6) show significant validity with stimulated submandibular flow rates. Only item 2 was significantly correlated for stimulated parotid flow rates. | (Pai *et al.*, 2001) |
| *Combination Questionnaire* | | | |
| 1. Duration of oral dryness<br>2. Frequency of oral dryness<br>3a. Oral dryness at night or on awakening<br>3b. Oral dryness at other times of the day<br>3c. Oral dryness during eating<br>3d. Difficulty in swallowing foods<br>3e. Amount of saliva in usual, everyday life<br>3f. Effect of oral dryness on daily life<br>4. Awakening from sleep at night because of oral dryness<br>5. Taking a water to bed | Combination of binary scale, categorical and VAS:<br>1. Recently, Several months, Several years<br><br>2. Occasionally, Frequently, Always<br><br>3. Visual Analog Scale (0-10, 10 means worst possible)<br><br>4 and 5. Never, 1-2 per week, 3-4 per week, 5-6 per week, Everyday | Dry mouth-related symptoms and behaviours (question 3a-3f) are significantly associated with whole salivary flow rate. While question 1, 2 and 7 are not significantly associated with salivary flow rate. | (Suh *et al.*, 2007) |

| | |
|---|---|
| 6. Sipping liquids to aid in swallowing dry foods<br>7. Using candy or chewing gum because of oral dryness | 6 and 7. Never, Occasionally, Frequently, Always |
| 8.Dry mouth-associated complaints (sensation of burning mouth, taste disturbances and oral malodour) | 8. Yes/No |

## 2.2. Salivary secretion test

Salivary secretion test is the most advocated clinical method for diagnosis of salivary dysfunction, which is typically defined by an unstimulated whole saliva flow rate *i.e.* less than 0.1 mL/min or a stimulated whole saliva flow rate *i.e.* less than 0.5-0.7 mL/min (Löfgren *et al.*, 2012). Accurate and standardized method for measurement of salivary secretion is essential since the quality and quantity of saliva are significantly affected by the sources and methods used for saliva collection (Navazesh and Kumar, 2008). Different sources for saliva collection are from mixed or individual glands corresponding to whole saliva and individual gland saliva, respectively. While the unstimulated saliva is mainly secreted by submandibular glands, the stimulated saliva is mainly contributed by parotid glands (Navazesh and Kumar, 2008). Methods of whole saliva collection include draining method, spitting method, suction method and swab method (Navazesh, 1993). Among them, draining and spitting methods by dripping saliva off the lower lip or spitting the saliva from the floor of the mouth are reproducible and reliable for unstimulated whole saliva collection (Navazesh and Christensen, 1982). While the suction method and swab method by saliva ejector or pre-weighed saliva adsorption swab were found to be less reliable with some degree of variability, and thus were not recommended.

To stimulate whole saliva secretion, standard-sized gum base, paraffin wax, rubber bands and citric acid are commonly used, and spitting method is suitable for stimulated whole saliva collection (Navazesh and Kumar, 2008). As for individual gland saliva collection, custom-made collection devices are commonly required. For example, the parotid gland saliva is typically collected by the Lashley cup or Carlson–Crittenden collector, the submandibular and sublingual glands saliva is commonly collected through Wharton duct, and the minor salivary gland secretions can be collected by filter paper (Lashley, 1916; Eliasson and Carlén, 2010). By using the afore-mentioned collection methods, the salivary flow rate can be calculated as weight or volume of collected saliva divided by collection period time (Navazesh and Kumar, 2008). Saliva collection from individual gland is more

reliable compared with whole saliva collection which, is a mixture of saliva, fluids, debris and oral mucosal cells. The flow rate of unstimulated parotid saliva was reported as 0.04 and 0.00 mL/min/gland for healthy controls and pSS patients, respectively (Pedersen *et al.*, 2005). Therefore, the techniques for individual glands are tedious and impractical with extremely limited salivary flow rate (Navazesh and Kumar, 2008)

## 2.3. Potential diagnostic tests for use in future

Salivary quantity and flow rate vary dramatically within and between individuals. In addition, accurate assessment of dry mouth according to the quantity of saliva is difficult. Therefore, biochemical and mechanical measurements offer promise to support diagnostic tests for dry mouth. Saliva quality in terms of its compositional feature and mechanical properties such as adsorption, rheological and tribological properties have been studied in research setting over the last decade. These tests can be employed to understand the changes in salivary quality in mechanical terms in dry mouth patients, which is discusesd in the following subsections.

### 2.3.1 Biochemical composition measurements

One obvious change in the saliva of dry mouth patients is the alteration in biochemical composition, while detailed changes in saliva depends on the particular cause of hyposalivation. For example, increased $Na^+$, $K^+$, $Cl^-$, $Ca^{2+}$, immunoglobulin A (IgA) and amylase were found in patients with oral sensorial complaints who were not having any psychiatric disorders or any major diseases such as cancer or sepsis (Granot and Nagler, 2005). Increased calcium, parathyroid hormone (PTH) and cortisol concentrations, in contrast to decreased oestrogen and progesterone concentrations were found in menopausal women with xerostomia (Agha-Hosseini and Moosavi, 2013). Reduced sulfation of mucin was found in pSS patients with xerostomia (Alliende *et al.*, 2008).

Mucin plays an important role in the rheological, tribological and surface adsorption properties of saliva, mainly because of their highly hydrated oligosaccharide side-chains, "bottlebrush" configuration *i.e.* oligosaccharide chains like "brushes" are attached to protein backbone of mucin and negatively charged sialic acid residues (Coles *et al.*, 2010; Xu *et al.*, 2019). MUC5B (~1 to 20 MDa) and MUC7 (~150 kDa) are two major physically distinct salivary mucins that are rich in *O*-glycosylation with an extended linear structure and a high degree of sialylation (Thomsson *et al.*, 2002; Morzel *et al.*, 2014). Structural

changes of these two mucins have been found in dry mouth patients (Alliende *et al.*, 2008; Dijkema *et al.*, 2012; Chaudhury *et al.*, 2015; Chaudhury *et al.*, 2016). For example, relative levels of sulfo-MUC5B were found to be substantially decreased in gland extracts from patients with Sjögren syndrome and dry mouth (n=10) as compared with the healthy control group (n=9), indicating a notable reduction of MUC5B sulfation level in the former group (Figure 2.2a) (Alliende *et al.*, 2008). Reduced MUC5B and MUC7 glycosylation were also found in patients with Sjögren syndrome associated oral dryness, although the mucin concentrations were found to be similar between the patients and the control group (Chaudhury *et al.*, 2016). These findings indicate that changes in mucin quality are indicative of dry mouth symptoms and could be a potential objective diagnostic tool for xerostomia patients with pSS.

### 2.3.2 Rheological measurements

Researchers have demonstrated that rheological properties of saliva alter in dry mouth patients (Chaudhury *et al.*, 2015) or with growing age (Pushpass *et al.*, 2019a). Figure 2.2b shows that patients complaining of xerostomia (n=34) exhibited significantly lower saliva spinnbarkeit (*i.e*. extensional viscosity) in comparison to healthy control subjects (n=30) (Chaudhury *et al.*, 2015). Such statistically significantly reduction ($p < 0.05$) in saliva spinnbarkeit is also shown in another study with Sjögren's patients (n=21) as compared to healthy controls (n=30) (Chaudhury *et al.*, 2016).

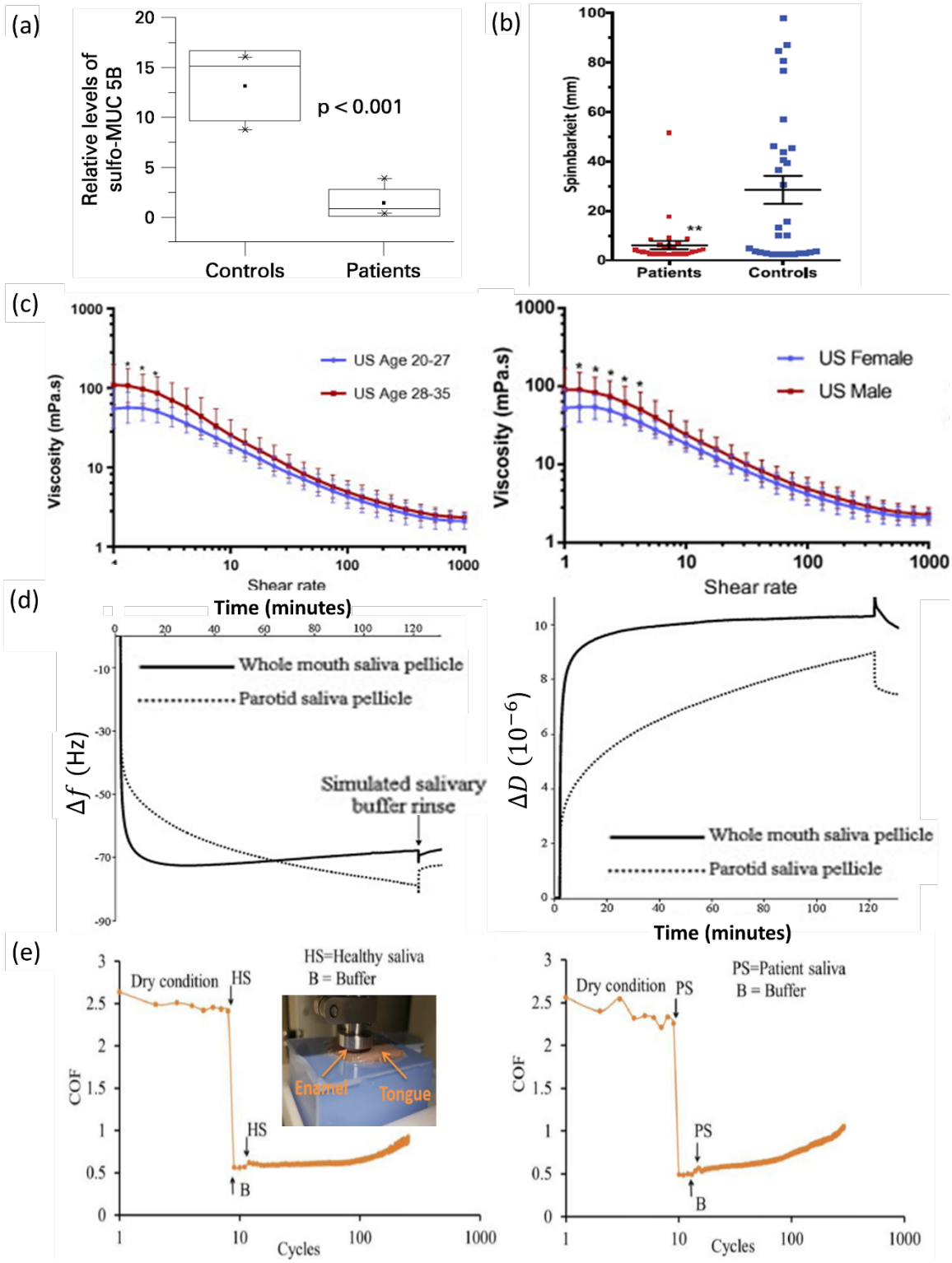


**Figure 2.2** Potential dry mouth diagnostic tests of saliva. (a) relative levels of sulfo-MUC5B in labial salivary glands from Sjögren syndrome patients and control group (Alliende *et al.*, 2008) (Reproduced with permission from BMJ Publishing Group Ltd. & European League Against Rheumatism), (b) Spinnbarkeit measurement of saliva in the groups of patients with dry mouth patients and healthy controls (Chaudhury *et al.*, 2015) (Reproduced with permission from SAGE Publications), (c) viscosity of unstimulated saliva (US) in different age (age 20-27 versus 28-35) and gender (female versus male) group as a function of shear rates (Gittings *et al.*, 2015) (Reproduced with permission from

Elsevier), (d) adsorption profile of whole mouth saliva and parotid saliva measured at 3rd overtone by quartz crystal microbalance with dissipation monitoring (QCM-D) on hydroxyapatite-coated sensors (Ash *et al.*, 2014) (Reproduced with permission from Elsevier), and (e) friction coefficient of healthy saliva and Sjögren syndrome patients' saliva at different sliding cycles in an *ex-vivo* tongue-enamel tribological system (Wan *et al.*, 2020) (Reproduced with permission from SAGE Publications).

Viscosity is another important rheological property which is usually used as an essential objective assessment of mechanical properties of both saliva and salivary substitutes. Viscosity changes of unstimulated human saliva in different age and gender groups have been reported (Gittings *et al.*, 2015). As shown in Figure 2.2c, the viscosity of unstimulated saliva for the age group 28-35 (n=8) was significantly higher than that of 20-27 (n=22) at low shear rate region. The viscosities of unstimulated saliva in males (n=13) were reported to be higher than those in females (n=17). In another study, a slightly higher viscosity was found for unstimulated whole saliva of younger groups (1.73$\pm$ 0.2 mPa·s, 18-30 years old, n=30) compared with older groups (1.55 $\pm$ 0.2 mPa·s, 60+ years old, n=24) (Pushpass *et al.*, 2019b). To the best of authors' knowledge, no study exist comparing the salivary shear viscosity of dry mouth patients versus healthy controls. Nevertheless, these aforementioned salivary viscosity measurements suggest potential use of flow curve as a reproducible tool to indicate age-dependent alteration of salivary viscosity in dry mouth patients in the future (Schein *et al.*, 1999).

### 2.3.3 Adsorption measurements.

Both MUC5B and MUC7 are major constituents of the mucosal pellicle which coats and protects the oral surface (Thomsson *et al.*, 2002; Morzel *et al.*, 2014). Therefore, changes of these two mucins can lead to an alteration of pellicle properties. One quantitative approach to measure the adsorption properties of salivary pellicles is quartz crystal microbalance with dissipation monitoring (QCM-D), which is a real-time, surface sensitive technique for analysis of layer properties, surface phenomena, and to derive quantitative information on thin film formation on a substrate (Veeregowda *et al.*, 2012; Ash *et al.*, 2014). For example, the real-time dissipation and frequency profiles of whole mouth saliva (n=10) pellicle and parotid saliva (n=10) pellicle adsorbed onto hydroxyapatite (main component of enamel) surfaces are shown in Figure 2.2d (Ash *et al.*, 2014). A rapid decrease in frequency of both whole mouth saliva and parotid saliva pellicle is observed. In comparison to a plateau reached after 20 minutes of whole mouth saliva addition, the

frequency of parotid saliva keeps a decreasing trend in the overall 120 minutes time period, indicating a continuous saliva pellicle adsorption. A slower increase in dissipation of parotid saliva pellicle compared to whole saliva was observed, indicating a more rigid layer being formed by parotid saliva. Flow rate changes of whole saliva and parotid saliva with age were also found to be different, with a significant lower whole salivary flow in 80+ individuals in compared with no age-related decline for parotid saliva (Percival *et al.*, 1994). In this way, differences in rate and degree of adsorption between whole and parotid saliva can be used as a suitable analytical tool to evaluate the changes in the saliva pellicle properties of dry mouth patients, which has received limited attention so far in dry mouth diagnosis.

### 2.3.4 Tribological measurements

Poor lubrication performance is a key complaint in dry mouth conditions and therefore tribological analysis *i.e.* measuring the frictional properties could be an important diagnostic tool. The comparison of dry mouth patient (n=4) and healthy individuals (n=4) salivary lubrication has been once implemented in a tongue-enamel friction system (an *ex vivo* laboratory-based friction tester) with the tooth enamel sliding against the porcine tongue for 10 cycles mimicking dry mouth (Figure 2.2e) (Wan *et al.*, 2020). Then, a drop of stimulated whole saliva from healthy controls or Sjögren syndrome patients was placed and spread for 4 cycles, followed by another drop of buffer for 4 cycles and finally another drop of healthy or patient saliva. A sharp decrease in friction coefficient from around 2.5 in dry mouth condition to 0.5 was observed after the addition of healthy or patient saliva, representing the relief feeling after rinsing the mouth with a particular lubricant in dry mouth patients. The upcoming duration period with remaining low friction coefficient under continuous sliding was called ‘relief period’. As shown in Figure 2.2e, healthy saliva resulted in a longer ‘relief period’ compared to that of patient saliva, indicating the relatively weak lubrication performance of dry mouth patients’ saliva.

To further promote the usage of these emerging mechanical, chemical and adsorption tests, there are still some aspects that need improved. For instance, reduction in the volume of saliva samples needed for measurements, decreasing the time of testing and the cost of measurements will be the obvious way forward to make these tests suitable in a clinical setting.

## 3. Salivary substitutes

Salivary substitutes are frequently used as symptomatic treatments for patients with decreased salivary flow rate or poor salivary quality. Commercial salivary substitutes can be categorized into eight platform technologies according to their functions (Figure 2.3). Four key functions of saliva substitutes *i.e.* lubricating, thickening, adhesive and moisturizing are discussed in this review. These functions are related directly to the wear and dryness of oral surfaces. Buffering functions are needed to neutralize product pH and protect dental health, while optional agent such as sweetener, surfactant, colorant and preservative are usually added to further improve patient's acceptance and adherence (Scott *et al.*, 2010). Although saliva stimulant agent is also included in some artificial saliva to stimulate the salivary flow (Furness *et al.*, 2011), such stimulants do not mimic any salivary functions and thus not discussed in this review.

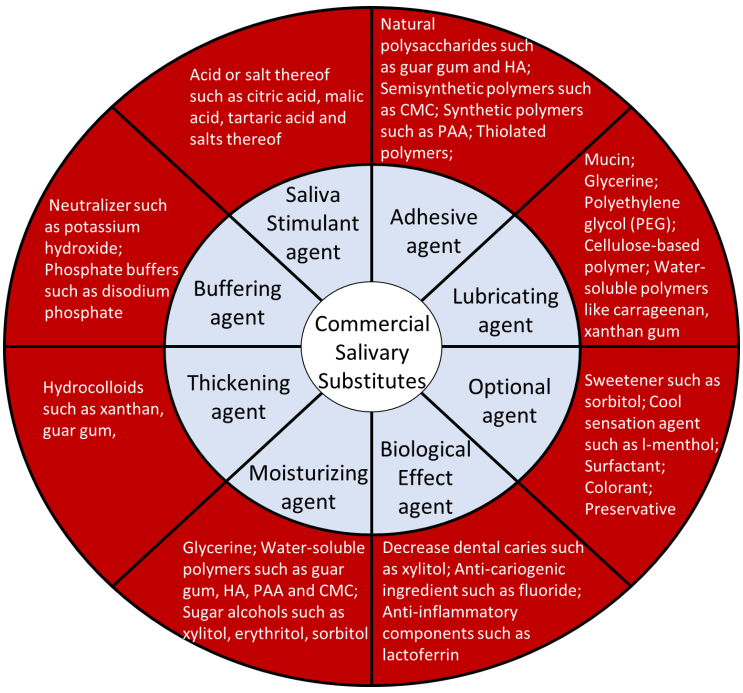


**Figure 2.3** Common ingredients used in commercial salivary substitutes and the rationale behind their use.

### 3.1. Thickening and lubricating agents

Hydrocolloids with a large number of hydroxyl (-OH) groups such as xanthan, guar gum, starch, alginate, pectin, gellan, agar, carrageenan and cellulose derivatives are commonly used as thickening agents not only in food but also in saliva substitutes (Van der Reijden *et al.*, 1994; Saha and Bhattacharya, 2010). Thickening agent is usually added to increase

the viscosity of commercial salivary substitute products, such as high-viscosity saliva substitutes or gels with an objective to extend the duration of dry mouth relief (Partenhauser and Bernkop-Schnürch, 2016). For instance, hydroxyethyl cellulose- (HEC) based Biotène Oral balance dry mouth system (OB) and BioXtra (BX) gel have similar composition, while BX is more viscous than OB (23.0 vs 16.8 Pa s) (Shahdad *et al.*, 2005). A small double-blind, crossover study (n=20 xerostomia patients) found that the moisturizing effect of OB gel lasted no more than 2 hours. However, nine patients reported the effect of BX gel lasting for more than 2 hours. This supports the beneficial effects of thickening agents in enhancing the relief period.

One of the most important function of saliva is lubrication, which minimize the wear of mucosal surfaces and therefore supports food oral processing (Carpenter, 2013). Therefore, it is crucial for salivary substitutes to exhibit similar or even better lubrication properties as compared to healthy human saliva. Typical manifestation of lubrication properties is Stribeck curve with friction coefficient plotted as a function of film thickness *i.e.* entrainment speed (speed at which the lubricant is entrained into the contact) multiplied by the lubricant viscosity and divided by the normal force (Sarkar *et al.*, 2019a). According to the adsorbed film thickness between two moving surfaces, the Stribeck curve can be divided into three regimes: boundary, mixed and hydrodynamic lubrication regime. Boundary lubrication regime occurs at low entrainment speeds where the moving surfaces are almost in full contact. In this regime, the surface characteristics account for the friction coefficient. So, a tightly adhered lubricant of thickness of few molecules to the moving surfaces can facilitate boundary lubrication (Coles *et al.*, 2010). As the entrainment speed increases, the hydrodynamic forces of fluid rise causing a reduction in friction coefficient. Then, in hydrodynamic lubrication regime, the surfaces are fully separated by fluid where viscosity plays an important role (Sarkar *et al.*, 2019a). Whole unstimulated saliva shows excellent lubricating behaviour in all the three regimes, which is probably due to the presence of salivary proteins that contribute to hydration lubrication (Xu *et al.*, 2020). Highly glycosylated mucins (MUC5B) and other low molecular weight proteins such as lactoferrin in synergy contributes to both boundary and fluid film lubrication of salivary pellicle (Xu *et al.*, 2020). Especially the aforementioned MUC5B, which is dysregulated in dry mouth patients, is a major gel-forming mucin in human saliva (Wickström *et al.*, 1998). Therefore, mucin-based salivary substitutes have been also developed. Saliva Orthana® is the only saliva substitute containing an animal-derived mucin currently on the market,

probably due to the risk of transmissible spongiform encephalopathy (Kelly *et al.*, 2004; Partenhauser and Bernkop-Schnürch, 2016).

In addition to mucin, other commonly used lubricating agents that act in the hydrodynamic regime include glycerine, polyethylene glycol (PEG), cellulose-based polymer such as HEC and carboxymethyl cellulose (CMC), and water-soluble polymers such as carrageenan and xanthan gum (van der Reijden *et al.*, 1996; Vinke *et al.*, 2020). However, unlike saliva, the afore-mentioned substitutes do not offer any boundary lubrication *i.e.* lubrication in the low speeds, which is more relevant in oral conditions. Glycerine and water-soluble polymers also work as thickening agents with high shear viscosity at low concentrations (de Vicente *et al.*, 2005). Glycerine-based salivary substitutes were found to be less effective in boundary lubrication in comparison to mucin-based ones, despite an approximately 300 times greater viscosity than other fluid samples (Aguirre *et al.*, 1989).

Hydrodynamic lubrication behaviour of mucin and CMC-based salivary substitutes have been widely studied (Vissink *et al.*, 1983; Hatton *et al.*, 1987; Christersson *et al.*, 2000), saliva substitutes based on mucin has been proven to provide better lubrication than CMC in biocompatible hard interface (tooth-glass interface) with relative lubrication values (77 ± 6% of the positive control) comparable to those of whole human saliva (63 ± 7% of the positive control) (Hatton *et al.*, 1987). Clinical studies (n=137 dry mouth patients) (Vissink *et al.*, 1983) have also found higher patient preference for mucin-containing saliva substitute over the CMC ones. Such performance may result from more similarity of mucin-containing artificial saliva and real human saliva as compared to CMC counterparts. On the other hand, a recent oral lubrication study of various commercially available saliva substitutes containing active ingredients such as mucin, HEC, PEG-hydrogenated castor oil, xanthan gum, CMC, plant polysaccharide and oxidized glycerol triesters found that all those saliva substitutes lack optimum lubricating properties (Vinke *et al.*, 2020). Therefore, more effective combination of thickening and lubricating agents and standardised subjective and objective clinical test to understand the effect of the salivary substitutes are needed for development of effective saliva substitutes that mimic real salivary lubrication.

## 3.2. Adhesive and moisturizing agent

Adhesive agent is often added to saliva substitutes facilitating the formation of a coating, which provides sufficient barrier for oral tissues from external irritation. Mucoadhesive materials are ideal adhesive agents, which demonstrate attractive interactions with mucosal surface (Partenhauser and Bernkop-Schnürch, 2016). Such mucoadhesive materials usually possess good wettability properties with numerous hydrogen bond forming groups (Ben-Zion and Nussinovitch, 1997), therefore can also act as moisturizing agent in saliva substitutes. Effective mucoadhesive materials can spread over and diffuse into substrate increasing the surface area of contact, through dominant attractive forces such as covalent force, hydrogen bond or electrostatic interaction (Lee *et al.*, 2000). According to the origin, mucoadhesive materials can be classified into four types (Partenhauser and Bernkop-Schnürch, 2016): 1) natural mucoadhesive materials, such as guar gum, xanthan gum, starch, pectin and gellan gum, chitosan, natural glycosaminoglycans such as hyaluronic acid (HA), and natural polypeptides such as gelatine; 2) semi-synthetic mucoadhesive materials, such as cellulose ethers *e.g.* hydroxypropyl cellulose (HPC) and methyl cellulose (MC), HEC and CMC; 3) synthetic mucoadhesive materials, such as PEG and polyacrylic acid (PAA, also known as carbomer) and 4) innovative mucoadhesive materials, such as thiolated polymers *e.g.* thiolated chitosan, thiolated PAA and thiolated xanthan gum. Among these materials, some are anionic polymers such as CMC, HA, PAA, pectin and gellan gum are rich in carboxylic moiety (-COOH) and function by virtue of hydrogen bonding with mucosal surfaces (Park and Robinson, 1987). Some materials are cationic polymers such as chitosan and cationic HEC which are hypothesized to undergo electrostatic interactions with residual anionic mucin in the mucus layer of the dry mouth patients, where hydrogen bonding and hydrophobic effects also happen, resulting in the enhanced mucoadhesive property (He *et al.*, 1998; Sogias *et al.*, 2008). Non-ionic polymers such as PEG and MC can also be used as adhesive agents. Although PEG lacks the functional groups *e.g.* carboxylic, hydroxyl or amine groups (Smart, 2005), it can interpenetrate into the mucus layer by diffusion and facilitate mucoadhesion (Serra *et al.*, 2006). As for thiolated polymers, they can form covalent disulfide bridges with the mucus layer *via* thiol–disulfide exchange reactions with mucus, thereby achieving strong mucoadhesion.

The bio-adhesion effectiveness of salivary substitutes containing proper adhesive and

moisturizing agent has been proven. For example, bio-adhesive properties of three saliva substitutes including Biotène® (HEC based), Oasis® (PEG and xanthan gum based) and Saliva Orthana® (mucin based) have been proven to be close to those of real human saliva tested by *ex vivo* indentation tests with pig tongues indicating adhesion force (Pailler-Mattei *et al.*, 2015). In the meantime, CMC, HEC or PEG-hydrogenated castor-based saliva substitutes are widely investigated. For example, in a study with 17 commonly applied saliva substitutes, only 3 items did not contain the aforementioned three mucoadhesive materials (Vinke *et al.*, 2020). Four of these tested 17 saliva substitutes including BioXtra gel (HEC based), Biotène gel (HEC based), Gum Hydral gel (xanthan gum, carrageenan and PEG-hydrogenated castor oil based) and Glandosane spray (CMC based) showed capability to increase the adsorption of saliva to these substitutes-coated surface of quartz crystals in QCM-D. The bio-adhesive properties of three saliva substitutes including Biotène® (HEC based), Oasis (PEG 60 hydrogenated based) and Saliva Orthana® (mucin based) were also reported to be similar to those of human saliva on pig tongues *ex-vivo*, except for the Aequasyal® (oxidised glycerol triesters based) (Pailler-Mattei *et al.*, 2015). However, in another study comparing the film-forming properties of CMC-based MAS 84 or porcine mucin-based Saliva Orthana®, CMC-based saliva substitute showed negligible adsorption on hydrophilic or hydrophobic silica surfaces tested by ellipsometry, while mucin-based Saliva Orthana® was adsorbed onto hydrophobic surfaces ($1.4\ \mathrm{mg\,m^{-2}}$) although not as effective as whole saliva ($2.8\ \mathrm{mg\,m^{-2}}$) (Christersson *et al.*, 2000).

As for moisturizing properties, contact angle measurements have been frequently used. For example, the contact angle of CMC-based and mucin-based saliva substitutes on human mucosa were comparable or even lower than that of human whole saliva on human mucosa layer, indicating good wetting properties of these saliva substitutes (Vissink *et al.*, 1986). Contact angle of saliva substitutes on buccal epithelial cell surface was also studied, proving a very high wettability of xylitol based mouth spray ($38.78 \pm 1.78°$) compared with $71.64 \pm 2.20°$ of unstimulated whole saliva (Spirk *et al.*, 2019). While contact angle of CMC based (Sialin-Sigma®) and macrogol based (Glandomed®) were $86.97 \pm 5.91°$ and $89.83 \pm 1.49°$ respectively, indicating poor wettability. These studies indicate the importance of standardised evaluation method for adsorption properties of saliva substitutes, such as standard surface, equipment and adsorption protocol.

Many clinical tests have also evaluated the effectiveness of aforementioned salivary

substitutes. Furness *et al.* (2011) assessed the risk of bias of 36 randomised controlled trials on topical interventions such as CMC, mucin, glycerol, xanthum gum, HEC citric acid and carbopol based salivary substitute gel or spray, in terms of random sequence generation, allocation concealment, blinding, incomplete outcome data, selective reporting and other potential sources of bias. However, no strong evidence was found for the effectiveness of any salivary substitutes due to the high risk of bias in most of the clinical trials. Therefore, further studies are needed for the design of promising salivary substitutes and controlled trials to guide clinical care.

## 3.3. Innovative technologies for salivary substitutes

In addition to these active agents added for different aspects of properties, some innovative technologies were also investigated for potential usage in salivary substitutes. For example, a self-assembly of mucin and lactoferrin has been shown by Xu *et al.* (2020), demonstrating promising wettability of hydrophobic surfaces, which was restored over 72 hours with similar adsorption compared to that of real human saliva. The study demonstrated that a synergistic lubrication by salivary components *i.e.* mucin and low molecular weight protein such as lactoferrin was key to mimic the lubricity (*i.e.* similar friction coefficients) of real human saliva (Xu *et al.*, 2020). The important role of low molecular weight proteins in saliva lubrication were also mentioned in other papers (Singh *et al.*, 2014; Yakubov *et al.*, 2015). This indicates future potential of such proteinaceous self-assembly as a novel technique to create salivary substitutes with better adsorption, lubrication and wettability properties. For instance, recently, in our laboratory, we fabricated microgel-reinforced hydrogel as a new, patented aqueous lubricant formulation (Hu *et al.*, 2020) that performs better than saliva in terms of lubrication performance. The synergistic effect between the components i.e. lactoferrin microgel and κ-carrageenan hydrogel was demonstrated to offer both boundary and viscous lubrication, respectivelu, resulting in significantly lower friction coefficient values in comparison to the sole components as well as real human saliva. The lubricant offers prospects in terms of acting as a salivary substitute in the future.

Table 2.3 summarises patents on salivary substitutes that have surfaced in the last 20 years focusing on textural property improvements.

For instance, polymers with gelling abilities might be converted into microgels thereby

potentially improving the hydration properties. Gellan gum-based microgel spray has been evaluated for prevention of oral dryness by *in vitro* study and clinical test (Table 2.3). Results showed that microgels were particularly effective for relieving dry mouth symptoms for patients with cancer (Ota *et al.,* 2012). In another instance, liposomes prepared by surrounding water with lipid bilayers have also demonstrated promise to act as effective salivary substitutes due to slower water release and prolonged moisture protection. For example, phosphatidylcholine-based (soya-PC) liposomes have shown to obtain higher water binding capacity than pectin (Adamczak *et al*., 2016). Polymer-coated liposomes showed even better properties with improved water binding capacity as compared to non-coated ones. High mucoadhesion and mucosal biocompatibility of polymer-coated liposomes were also demonstrated (Table 2.3). These findings indicate the great potential of liposomes and its derivatives in hydrating oral mucosa and relieving dry mouth symptoms.

**Table 2.3** Patents on inventions of salivary substitute formulations for dry mouth therapy filed in the last 20 years (Source of database: Espacenet).

| Patent number | Filing date | Assignee | Key technology in the invention | Property evaluation of the formulation (invention) | Reference |
|---|---|---|---|---|---|
| JP2005104966A | 2004-06-30 | Lion Corp | Microgel particle | (A) Average particle size measurement<br>(B) Viscosity measurement<br>(C) Evaluation of appearance, usage, dispersion stability and spray ability<br>(D) Clinical test (n= 20 healthy persons) for the evaluation of residual feeling in the oral cavity and cleaning feeling between teeth and gums | (Nakamoto and Ryoji, 2004) |
| US2005226822A1 | 2003-04-25 | Gaba International Ag | Mannoprotein and ovomucin | (A) Rheological behaviour measurement | (Garbers *et al.*, 2003) |
| US8540970B2 | 2008-02-22 | Biocosmetic SL | Olive oil, trimethylglycine and xylitol | (A) Clinical test (n=20 xerostomia patients) of unstimulated salivary flow rate at the beginning and after one week of application of composition<br>(B) Clinical test by xerostomia VAS questionnaire | (Rodriguez-Vilaboa, 2008) |

| | | | | | |
|---|---|---|---|---|---|
| KR101291413B1 | 2011-08-22 | Seoul National University Industry-Academic Cooperation Foundation | Yam mucilage extraction | (A) Viscosity measurement<br>(B) Lysozyme or peroxidase activity in solution | (Kho and Park 2011) |
| WO2012095774A1 | 2012-01-06 | Indian Institute of Technology, Bombay, India | Gellan gum linked with dipalmitoylphosphatidylcholine and palmitoyloleoyl phosphatidylethanolamine | (A) Fourier-transform infrared spectroscopy of composition<br>(B) Surface pressure<br>(C) Amphiphilic nature<br>(D) Viscosity measurement<br>(E) Viscoelasity measurement<br>(F) Atomic force microscopy of the formed films<br>(G) Height and roughness analysis<br>(H) Particle size analysis | (Banerjee and GuhaSarkar, 2012) |
| US2014093582A1 | 2013-09-24 | Golden Pearl Investment LLC | Serum composition | (A) Evaluation of the effect of serum extract on cell growth.<br>(B) Clinical test (n= 32 healthy female volunteers) to evaluate the effect of formulation (invention) on skin, focusing on satisfactory of maintenance, absorbance, moisturizing and so on.<br>(C) Animal test (n= 4 mice) to evaluate the effect of the formulation (invention) on burn injury. | (Qian, 2013) |
| US9334312B2 | 2013-10-04 | Rijksunviersiteit Groningen, Academisch Ziekenhuis Groningen | Recombinant cationic polypeptides | (A) Adsorption test on salivary conditioning films.<br>(B) Friction forces, repulsive force and glycosylation testa. | (Sharma *et al.*, 2013) |
| WO2018212771A1 | 2016-06-24 | Colgate-Palmolive Company | Combination of hemp seed oil and caprylyl glycol | (A) Friction measurement.<br>(B) *In vitro test* of moisture retention. | (Prencipe *et al.*, 2016) |

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|---|---|---|---|---|---|
| WO2019102354A1 | 2018-11-20 | 3M Innovative Properties Company | Emulsion (oil in water): combination of plant based oils, an aqueous phase, surfactants and viscosity modifier. | (A) Viscosity measurement.<br>(B) Friction measurement.<br>(C) Stability (no phase separation) measurement.<br>(D) High temperature stability test.<br>(E) Freeze/ Thaw/ Centrifugation stability measurement.<br>(F) Spray-ability measurement.<br>(G) *In vitro* hydration retention measurement (Thermal gravimetric Analysis).<br>(H) Long term wash-off measurement (with artificial saliva)<br>(I) Biofilm disruption test<br>(J) Bovine tooth hardness measurement | (Wlaschin *et al.*, 2018) |
| CN109662981A | 2019-01-28 | UNIV Zhejiang Gongshang | Okra extraction | (A) Shear rheological property test<br>(B) Friction coefficient test<br>(C) Oral tensile rheological properties test<br>(D) Taste test (n=30 healthy participants) | *(Chen et al.*, 2019) |

Oil-based emulsions have also been investigated as potential saliva substitutes. The viscoelastic properties of lecithin-based emulsions were observed, with viscous behaviour at low frequency and increased elasticity at higher frequencies (Table 2.3). Clinical tests of lecithin-based emulsion showed superior retention compared with water and similar retention to that of methylcellulose solution. However, another clinical study of lecithin-based emulsion showed that no significant benefit of oily emulsion for relief of xerostomia (Table 2.3). These studies indicate larger well-designed clinical studies for product property assessment are needed to understand the future applications of these innovative technologies.

A variety of measurements were used to evaluate the properties of these patented formulations such as clinical trials, rheological tests, adsorption tests, wettability tests and tribological tests (Table 2.3). Among them, the most widely used evaluation is rheological tests. One major trend in these patents is the use of food-sourced components such as yam, okra and plant oil, since they are natural material easily accepted by human (Table 2.3).

For example, similar viscoelastic properties were found between yam solutions and human saliva (Kho and Park 2011). In summary, the saliva substitute development is a highly topical area of research and more efficient substitutes emulating the boundary lubrication properties of saliva appear to be a gap in the literature.

## 4. Conclusions

This review provides a comprehensive summary of various diagnostic tools for assessment of dry mouth conditions and examined the salivary substitutes providing textural properties emulating those of real human saliva for treatment of dry mouth condition. In terms of diagnosis, salivary flow rate test and questionnaire are commonly used in clinical setting with subjective questionnaires being the most common approach. However, to date, there has been little attention on assessing the alternation in biochemical composition and mechanical properties of saliva in dry mouth patients. Biochemical composition, rheological, adsorption and tribological properties are important feature of saliva contributing to its unique functions, which are widely studied by researchers. It is thus crucial to employ these mechanical measurements on saliva from dry mouth patients in order to rationally tailor the kind of saliva substitute needed for their relief. For instance, if the dry mouth patient has residual saliva which contains high levels of lubricating salivary proteins but lacking in the hydrodynamic properties, then a thickening agent might be an ideal solution. However, if the salivary quality of the dry mouth patient suffers from lack of adsorption and boundary lubrication properties that are measured using QCM-D and tribological analyses, respectively, more effective saliva substitute that can act as boundary lubricants should be approached. Such group-personalized design of saliva substitutes would likely provide optimum treatment outcome of xerostomia. Another important challenge is to find a correlation between objectively measured salivary properties (*e.g.* lubrication, adsorption, mucin content) and subjective assessment of dry mouth. The lack of correlations hinder clinical adoption of these techniques for routine evaluation of dry mouth conditions by dental practitioners.

For treatment, eight composition agents have been identified within the commercial saliva substitute products, while four of them were directly related to relief of oral dryness including lubricating, thickening, adhesive and moisturizing agents. Materials such as polysaccharides, mucin and cellulose-based derivatives were commonly discussed

materials in literature. In addition to these commonly used component agents, innovative development of saliva substitutes were summarised at the end of this review, indicating a trend of employing food-related materials such as yam, okra and colloidal technologies, such as self-assembly, emulsion, liposomes and microgels. In summary, further pre-clinical characterization of innovative technologies are needed and clear benefits of these technologies in terms of mucoadhesion, lubrication ad relief period over existing saliva substitutes need to be established before such materials can be used for clinical trials.

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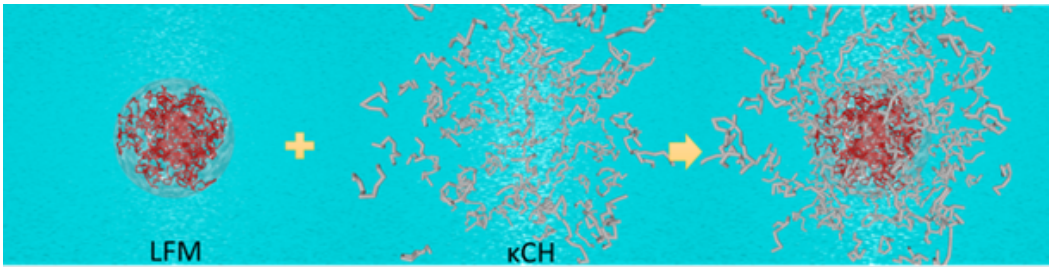
# Chapter 3

## Synergistic Microgel-Reinforced Hydrogels as High-Performance Lubricants [2]


### Abstract

The ability to create a super-lubricious aqueous lubricant is important for various biological and technological applications. Here, a nonlipid biolubricant with strikingly low friction coefficients is fabricated (patented) by reinforcing a fluid-like hydrogel composed of biopolymeric nanofibrils with proteinaceous microgels, which synergistically provide super-lubricity on elastomeric surfaces in comparison to any of the sole components. This two-component lubricant composed of positively-charged lactoferrin microgels and negatively-charged κ-carrageenan hydrogels, is capable of exceeding the high lubricating performance of real human saliva in tribo-tests using both smooth and textured surfaces, the latter mimicking human tongue's wettability, topography and compliance. The favorable electrostatic attraction between mutually oppositely-charged microgels and the hydrogel reinforces the mechanical properties of the hydrogel, allowing friction reduction by combining the benefits of both viscous and hydration lubrication. The superlubricity of these microgel-reinforced hydrogels offers a unique prospect for the fabrication of biocompatible aqueous lubricants for dry-mouth therapy and/or designing of nonobesogenic nutritional technologies.


[2] This chapter has been published as: J. Hu, E. Andablo-Reyes, S. Soltanahmadi and A. Sarkar (2020), Synergistic microgel-reinforced hydrogels as high-performance lubricants. *ACS Macro Letters* 1726–1731

# 1. Introduction

Water forms the basis of all biological lubrication systems such as tears, saliva and synovial fluids in humans (Lee *et al.*, 2008). Hydrogels and microgels are both composed of crosslinked water-swollen polymer networks, with the latter in the form of micron-sized particles. They have been extensively used to improve the rheological (Andablo-Reyes *et al*., 2019; Deshmukh *et al*., 2015) and mechanical properties of biomaterials and other technological applications (Hong *et al*., 2015; Lee and Mooney, 2001; Sun *et al.*, 2012). Recently, biopolymeric microgels have been reported as effective lubricants for elastomeric surfaces due to their capacity to trap water molecules providing hydration lubrication (Andablo-Reyes *et al*., 2019; Liu *et al*., 2014). Here, for the first time, we demonstrate that reinforcing a carbohydrate hydrogel with proteinaceous microgels can result in superlubricity on PDMS (polydimethyl siloxane), a commonly-used material for investigation of oral processes, and a novel 3D tongue-like biomimicking silicon surface. The two-component lubricant is composed of positively-charged lactoferrin microgels (LFMs) dispersed in negatively-charged κ-carrageenan hydrogels (κCH). While lactoferrin (80 kDa) is a globular protein, which is present in saliva (Hassoun and Sivamani, 2017). The synergistic effect between the components enhances the hydration lubrication, resulting in significantly lower friction coefficient values in comparison to the sole components. This patented lubricant formulation (Sarkar *et al*., 2020) is capable of exceeding the lubricity of real human saliva in orally-relevant tribo-contact conditions.

# 2. Methods

## 2.1. Sample preparation

**Materials**. Lactoferrin was purchased from Ingredia, France. HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) from ITW Reagents, UK was used for preparation of 10 mM HEPES buffer at pH 7.0, which is used as a solvent for all the experiments. All other chemicals including *κ*-carrageenan were purchased from Sigma-Aldrich (Dorset, UK).

**Preparation of lactoferrin microgels (LFM).** LFM were prepared using a top-down method (Sarkar *et al*., 2017). Briefly, 12.00 wt% lactoferrin was dispersed in HEPES buffer and then heated at 90 °C for 30 minutes to form thermally-crosslinked gel, which was mixed with HEPES buffer (3:1 w/w) before breaking down into macrogel particles using a hand blender (HB724, Kenwood, UK). The resulting macrogel particles (75.00 vol%) were transferred to a conditioning mixer (ARE-250, THINKY Corporation, Japan) for degassing before

homogenizing by passing twice through the Leeds Jet Homogenizer (University of Leeds, UK) at pressure of 300 ± 20 bars to produce LFM.

**Preparation of LFM-reinforced κ-carrageenan hydrogels (κCH).** 1.50 wt% κ-carrageenan powder was dispersed in the pre-heated (95 °C) HEPES buffer under constant stirring at 95 °C for 40 min to ensure complete dissolution and the formation of the hydrogel. Then the κCH was cooled to 37 °C and mixed with LFM at different ratios under stirring for 30 min to allow the formation of LFM-reinforced κCH. Note, all the nomenclatures of ratios used in the results are $\times 10^{-3}$ in wt/vol *i.e.* 0.0044 κCH/LFM (named as 4κCH/LFM), 0.008 κCH/LFM (named as 8κCH/LFM), 0.07 κCH/LFM (named as 70κCH/LFM), 0.120 κCH/LFM (named as 120κCH/LFM) and 0.239 κCH/LFM (named as 239κCH/LFM).

## 2.2. Human saliva collection

Whole human saliva was collected from healthy and young female subjects (n=15) at 10 am, subjects were refrained from at least eating and drinking for at least 2 h before saliva collection in accordance with the standard protocols of the University of Leeds Ethics Committee (MEEC 16-046). The collection of saliva required subjects with minimal oral movements and the saliva was collected at the same time of the days. After collection into pre-weighed tube, kept on ice, the human saliva was immediately diluted to 50% (v/v) in 10 mM HEPES buffer, and then centrifuged at 3,000 *g* for 3 min. The saliva samples were analyzed within 2 h of collection. The data is presented for one participant.

## 2.3. Particle size and ζ-potential

The hydrodynamic diameter of LFM was measured by means of dynamic light scattering using a Zetasizer Ultra (Malvern Instruments Ltd., Worcestershire, UK) at 25 ℃. Measurements were carried out using disposable cuvettes (ZEN0040) at the detection angle of 173.0°. The electrophoretic mobility of the samples was measured at 25 °C in folded electrophoretic cells (DTS1070, Malvern Instruments Ltd., Worcestershire, UK) and converted to ζ-potential using the Smoluchowski model. The size and ζ-potential results were reported as mean result of at least nine reported readings made on triplicate samples.

## 2.4. Transmission election microscopy

Electron microscopy images were acquired using a transmission electron microscope Tecnai G2 Spirit-T12 (ThermoFisher). Voltage of the electron gun was fixed at 120 kV and images

were captured using a Gantan CCD camera. In order to increase the electron contrast, the samples were negatively stained with two cycles of uranyl acetate while deposited in a carbon-coated TEM grid.

## 2.5. Quartz crystal microbalance with dissipation monitoring (QCM-D)

The real-time adsorption of real human saliva, microgel and the hydrogel samples was measured using a quartz crystal microbalance with dissipation monitoring (QCM-D, E4 system, Q-Sense, Biolin Scientific, Sweden) equipped with the hydrophobic polydimethylsiloxane (PDMS)-coated sensor.

For the preparation of PDMS-coated QCM-D sensors, 10 wt% PDMS in toluene solution was prepared and left under stirring conditions for 24 h. Then the solution was further diluted with toluene to 0.5 wt% which was again left under stirring conditions for 24 h. Silica-coated QCM-D sensors (QSX-303, Q-Sense) were immersed in RCA silicon wafer cleaning solution (5 parts of deionized water, 1 part of ammonia and 1 part of aqueous $H_2O_2$ (hydrogen peroxide, 30 %)) at 80 °C for 15 min to remove any organic material and insoluble particles, followed by three cycles of sonication in ultrapure water for 10 min each cycle before drying using liquid nitrogen gas. Finally, 100 µL of 0.5 wt% PDMS solution was placed on the substrate and was spin-coated at 5,000 rpm speed for 60 s.

The hydrophobic PDMS-coated sensors thus prepared were cleaned by 30 s immersion in toluene, followed by 30 s immersion in isopropanol, then 2 min immersion in ultrapure water, drying with nitrogen gas and letting the remaining solvent molecules to evaporate for 2 h. All samples except LFM-reinforced κCH (diluted to 0.01 wt% κCH and 0.02 wt% LFM) were diluted with HEPES buffer to 0.1 wt% and supplied into QCM-D chamber containing the PDMS-coated sensors with a flow rate of 100 µL $min^{-1}$ at 25 °C. The HEPES buffer was firstly injected for 20 minutes for a stable baseline, samples were then injected for a flow of more than 60 minutes until stable frequency and dissipation was achieved, followed by 20 minutes of rinsing with the buffer. The data were fitted using the Voigt model for viscoelastic solids (namely, “Smartfit Model”) by Dfind (Q-Sense, Biolin Scientific, Sweden) software to obtain the mass of hydrated samples. The QCM-D results were reported as mean result of at least three reported readings.

## 2.6. Rheology

Shear viscosity was measured using a Modular Compact Rheometer (MCR-302, Anton Paar, Austria) equipped with a cone-plate geometry (CP50-1, diameter 50 mm, angle 1°) at 37 °C. The steady state of the measured viscosity was achieved within a tolerance of 0.5% for a 10 s period. The frequency sweep and strain sweep were measured using a Kinexus ultra rheometer (Malvern Instruments Ltd. Worcestershire, UK) equipped with a cone-plate geometry (CP2/60 PL65). The strain sweep was measured firstly at 1 Hz and 37 °C to determine the linear viscoelastic region and then the frequency sweep was conducted at 1% strain and 37 °C. The rheological data were reported as mean result of at least nine reported readings made on triplicate samples.

## 2.7. Tribology

Tribological experiments of real human saliva, microgel and the hydrogel samples were firstly performed in a Mini Traction Machine (MTM2, PCS Instruments, London, UK), using silicone (PDMS, Sylgard, Down Corning) ball (Ø 19 mm)-on-disk (Ø 46 mm) with the surface roughness $R_a$ ~ 50 nm and Young's modulus of 2.8 MPa (Sarkar *et al*., 2017). Normal load ($W$) was fixed at 2.0 N, which is equivalent to a maximum Hertzian contact pressure about nearly 200.0 kPa (Sarkar *et al*., 2019). Sliding rolling ratio and temperature were fixed at 50.0% and 37 °C, respectively.

To emulate tongue-palate in dry mouth conditions, tribological experiments of real human saliva, microgel and the hydrogel samples were also performed using a Kinexus ultra rheometer (Malvern Instruments Ltd. Worcestershire, UK) equipped with a plate-on-plate geometry (diameter 50.0 mm) containing steel plate on soft textured elastomeric plate (Andablo-Reyes *et al*., submitted). The hydrophobic (without Span 80) and hydrophilic (with Span 80) soft elastomeric surfaces were created using Ecoflex™ 00-30, which has an order of magnitude lower Young's modulus as compared to PDMS and this 2.0 cm × 2.0 cm Ecoflex™ 00-30 surfaces (both hydrophobic and hydrophilic) were prepared by replica molding against a 3D-printed surface that contained random papillae distribution based on real human tongue masks[3], which were glued at the rim of the top plate. Experiments were performed in a controlled normal force of 1.0 N with torque recorded for the calculation of friction coefficient. The tribological data were reported as mean result of at least nine reported readings made on triplicate samples.

## 3. Results and discussion

All solutions are made in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES buffer) at pH 7.0. LFMs are fabricated by thermal gelation of lactoferrin protein solution followed by passing through a jet homogenizer as described in the Supporting Information, Experimental Section. Size and morphological characteristics of LFM are shown in Figure 3.1a. The size distribution of LFM, obtained using dynamic light scattering (DLS), is unimodal with an average hydrodynamic diameter ($d_H$) of 155.0 nm and a polydispersity index of 0.2. The transmission electron microscopy (TEM) image of LFM confirms well-dispersed spheres corroborating with the DLS data. κCH is prepared by dissolving κ-carrageenan in the buffer under heating and shearing conditions. In Figure 3.1b, a TEM image of κCH shows typical bundles of nanofibril-shaped κCnf (κ-carrageenan nanofibrils) (Hermansson and Jordansson, 1991). These κCnf form interconnected network with random distribution and orientation interacting via hydrogen bonds, resulting in gel-like structures with macroscale viscoelastic properties (Liu *et al*., 1991). LFM-reinforced κCH are fabricated by dispersing LFMs in κCH at the ratio of 0.07:1 wt/vol. Figure 3.1 $c_1$ clearly shows LFM particles dispersed in the interconnected network of κCnf-assisted hydrogel. Figure 3.1 $c_2$ is the magnified image of LFMs embedded in κCH indicating coverage of LFMs by κCnf.

The interaction between LFM particles and κCnf is expected to produce a synergistic effect towards the lubrication capacity of the LFM-reinforced κCH. Based on these TEM images, a conceptual representation of the system is presented in Figure 3.1d, where a spherical crosslinked proteinaceous network represents the microgel and grey-coloured rod-like flexible chains represent the nanofibrils. The visual image in Figure 3.1d visualize the stretchy behavior of the LFM+κCH which is similar to the "the extensional filament-like behaviour" observed for a stretched drop of real human saliva. In addition, they are highly viscous solutions that are dilutable with water or buffer.

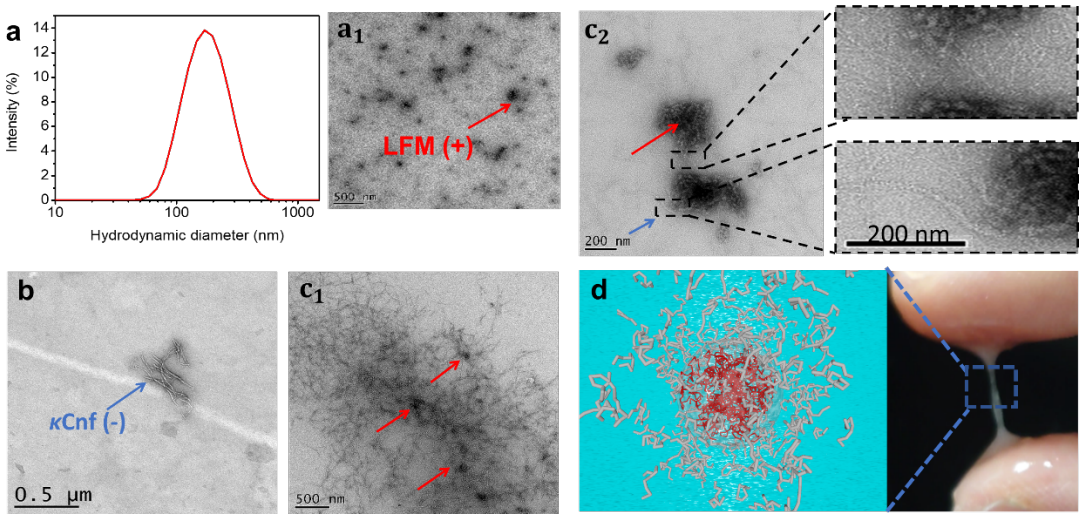



***Figure 3.1*** Mesoscopic structure of the lubricants. a) Particle size distribution of LFMs obtained by dynamic light scattering showing monomodal peak with hydrodynamic diameter ($d_H$) ~ 150 nm. $a_1$) TEM image (scale bar: 500 nm) of LFM. b) TEM image of κCH formed by an interconnected network of κ-carrageenan nanofibril (κCnf) *i.e.* mesoscale units of κCH. c) LFM-reinforced κCH (1.1 wt % κCH and 2.0 wt % LFM) showing how LFMs are finely woven by κCnf at the surface: $c_1$) lower magnification, $c_2$) higher magnification. Inset: zoom-in view of the LFM and κCH connection showing κCnf filaments at the surface (Scale bar: 200 nm); d) Schematic of LFM-reinforced κCH hydrogel and a visualized illustration of the stretchy behaviour of the LFM+κCH between thumb and forefinger which is similar to the "beads-on-a-string" phenomenon often observed for a drop of saliva.

The lubrication performance of the aforementioned lubricant compositions is first studied in rolling-sliding contacts on PDMS surfaces. Although the relevance of this conventional soft tribology test to study oral lubrication is of concern, PDMS as a model surface has been extensively used (Torres *et al.*, 2018) in oral-tribology studies. Therefore, the tests using PDMS surfaces, as a classic framework to understand the lubrication behavior offer cross-comparison of the obtained adsorption and friction results with literature.

In classical lubrication theories for hard-on-hard contacts, typically four lubrication regimes have been defined in tribology science, (Hutchings and Shipway, 2017), which have been exploited in soft tribology (Bongaerts *et al.*, 2007; de Vincente *et al.*, 2006). Although a distinct transition between two successive regimes is arduous, theoretical models and modified Stribeck curve (or friction vs a modified velocity parameter) have been proven useful in qualitative determination of regimes. (Supporting Information, Figure S.1) (Dowson, 1993). For ease of comparison in the tribo results, we use a product of lubricant viscosity and entrainment speed ($\eta U$), which is well-established in tribological studies of soft surfaces. We

also included the friction coefficient versus $\eta U$ for Newtonian fluids (as shown in Figure 3.2d) for comparison purposes. The $\eta U$ range in which the surfaces experience direct solid-solid contacts ($< \sim 5 \times 10^{-5}$Pa m) and the friction coefficient remains relatively constant is referred to as the "boundary regime" here-after. Upon increasing the entrainment speed, the friction coefficient reduces sharply where the lubricant film partially carries the load; that is "mixed regime".

The friction coefficient reduces as the contribution of the direct contact between surfaces reaches its minimum (*i.e.* elasto-hydrodynamic regime) and the onset of a full-film lubrication can be observed. Following transition to the full fluid-film lubrication, hydrodynamic forces bring about a lubrication regime where the contact-bodies are completely separated by a lubricant film and the lubricant viscosity renders an important role (*i.e.* hydrodynamic regime).

Figure 3.2a shows the friction curves for 2.0 wt% LFM, 1.1 wt% κCH and LFM-reinforced κCH (κCH: LFM at 0.07: 1 wt/vol, containing 1.1 wt% κCH and 2.0 wt% LFM) lubricant formulations in PDMS-PDMS tribo-contacts. Friction curves for LFM and κCH lubricants show nearly similar friction values across the speed range and lower values in comparison to those for buffer. The friction curves for LFM and κCH maintain such a decreasing trend in the friction coefficient values as speed increases regardless of the concentration changes, with relatively high friction coefficients at the relatively low entrainment speeds (*i.e.* $< 0.01\ ms^{-1}$) even at the highest concentration of original LFM and κCH (see Supporting Information, Figure S.2). On the other hand, LFM-reinforced κCH shows an unprecedented superlubricity behavior with friction coefficient values as low as $1 \times 10^{-2}$ throughout the whole entrainment speed range investigated here (*i.e.* $1.31 \times 10^{-2}$ and $0.49 \times 10^{-2}$ at $0.005\ ms^{-1}$ and $0.1\ ms^{-1}$, respectively). Interestingly, the LFM-reinforced κCH provides a superior lubricity as compared to the real human saliva, demonstrating its potential as an effective bio-lubricant.

The mixture of untreated i.e. non-microgelled LF protein and κCH (Supporting Information, Figure S.3) does not show superlubricity. This indicates the importance of using LF in microgel form in order to have allow high degree of interaction with κCH. To the best of our knowledge, such synergistic effect to yield super low friction coefficients has been particularly achieved through synthetic aqueous lubricants (Qu *et al*., 2015; Sun *et al*., 2019) and is rarely reported for natural biopolymeric aqueous lubricants.

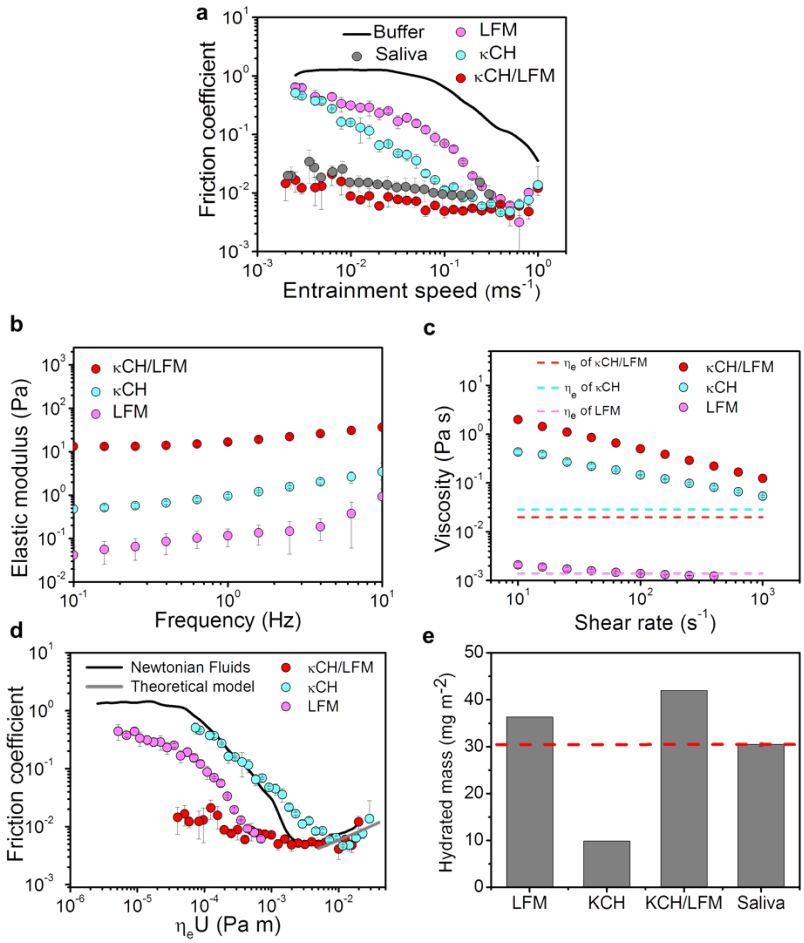



**Figure 3.2** Tribological and rheological performances of the lubricants. κCH/LFM in panels represents LFM-reinforced κCH. a) The friction results as a function of entrainment speed ($U$) obtained for lubricants using a MTM tribometer and PDMS specimens. The κCH/LFM delivered a lubrication performance exceeding that of real human saliva. b) Frequency dependence of the elastic modulus at a constant strain rate (1.0 %) for 1.1 wt% κCH, 2.0 wt% LFM and LFM-reinforced κCH containing 1.1 wt% κCH and 2.0 wt% LFM (see details in Supporting Information, Figure S4) c) Shear viscosity of LFM, κCH and LFM-reinforced κCH as a function of shear rate. $\eta_e$ represents the effective tribological viscosity (see Supoorting Information, Theory Section). d) The friction coefficient results as a function of $\eta_e U$. The gray solid line represents the estimated friction coefficient based on effective tribological viscosity (see Supporting Information, Theory Section). e) The hydrated mass of adsorbed LFM, κCH, real human saliva and LFM-reinforced κCH onto PDMS coated sensors obtained by QCM-D and measured using Voigt viscoelastic model applied to 3$^{rd}$-11$^{th}$ overtones (raw data of frequency and dissipation shifts of 5$^{th}$ overtone are available in Supporting Information, Figure S5). The red dotted line represents the adsorption level of saliva. Human saliva was collected from a healthy young female in the morning. The subject was refrained from eating and drinking for at least 2 h before saliva collection (Ethics number: MEEC 16-046, University of Leeds, UK) and the saliva was diluted with 10 mM HEPES at the ratio of 1:1 w/w, centrifuged, and the supernatant was used for the tribology measurements. Values are presented as the means ± SDs of nine readings on triplicate samples (n = 9 × 3) except for QCM-D data (n = 3 × 1).

Figure 3.2b shows the linear response of the elastic modulus for the hydrogel, microgels and their mixture under shear in the frequency range of 0.1-10 Hz at a constant strain (see Supporting Information for determination of the linear regime, Figure S.4c). The elastic modulus of the LFM-reinforced κCH (13.26-36.63 Pa) is one order of magnitude greater than that of the sole components at equivalent concentrations (0.48-3.48 Pa for κCH, 0.04-0.93 Pa for LFM) throughout the whole frequency range measured here (Figure 3.2b). The SME for the LFM-reinforced κCH is greater than that of sole LFM or κCH at the higher concentrations (see Supporting Information, Figure S.4a-S.4c). This indicates that the microgels behave as active fillers and hence reinforce the hydrogel's nanofibrillar network. Such a synergistic effect raises a question towards the impact of viscous forces and surface adsorption on the lubrication performance of these hydrogels. The shear viscosity results for the same fluids are shown in Figure 3.2c. Both κCH- and LFM-reinforced κCH are shear thinning fluidsc. The LFM shows a relatively constant viscosity of 0.0016 Pa s which is at least two orders of magnitude lower than those of the other two fluids. The greater viscosity of LFM-reinforced κCH as compared to κCH implies the interactions between κCH and LFM (*i.e.* reinforcement and filling action, while retaining the fluidity).

The shear thinning behavior of LFM-reinforced κCH and κCH do not reach a plateau over the shear rate examined here. Therefore the effective tribological viscosity ($\eta_e$) is estimated using friction values in the elasto-hydrodynamic lubrication regime and de Vicente et al.'s (2005) theoretical model (see Supporting Information, Equation S1) (de Vicente *et al*., 2005). The obtained $\eta_e$ values are shown using dashed lines assuming the effective Newtonian behavior of fluids in the tribological limit. (Andablo-Reyes *et al*., 2019).

This brings us to evaluate the influence of viscous forces on the lubrication performance of the lubricant formulations by plotting the friction coefficient as a function of $\eta_e U$ (Figure 3.2d). This graph sheds light on the influence of hydrodynamic viscous forces of a lubricant when compared to the performance of aqueous based Newtonian fluid. Friction curve for κCH overlapped with Newtonian fluid almost in all regimes. This indicates that the viscous force is the main contribution to the tribological performance of κCH. Friction values for LFM are considerably lower in comparison to the values for a Newtonian fluid irrespective of the speeds. This indicates the impact of viscous and hydration action of LFM on its boundary lubrication. On the other hand, LFM-reinforced κCH shows a predominantly friction values which are

dramatically lower than the friction coefficient values of other tested lubricants. Since the viscous behaviour of LFM-reinforced κCH is similar to that of κCH (Figure 3.2c), it is reasonable to attribute its super-low friction behavior to the hydration force which is bestowed on LFM-reinforced κCH as a result of synergistic effects between LFM and κCH. As the entrainment speed increases, the friction curve of LFM-reinforced κCH overlaps with graph for Newtonian fluid within the elasto-hydrodynamic lubrication regime, indicating that the lubrication of LFM-reinforced κCH relies completely on the high shear rate viscosity of the solution in this regime.

The gray line in Figure 3.2d presents the theoretical prediction of the friction coefficient in the hydrodynamic regime (de Vicente *et al*., 2005) which shows a good agreement with the experimental results. The minimum film thickness ($h_m$) at the onset of the hydrodynamic regime (Supporting Information, Equation S2) for LFM-reinforced κCH, κCH and the Newtonian reference is estimated to be 1514.4, 2118.2 and 1762.5 nm, respectively (de Vicente *et al*., 2005). The film thicknesses which are greater than the diameter of microgels (an average size below 200 nm shown in Figure 3.1a) facilitate interposition of the microgels at the contact interface enabling fluids to act closely to a continuum.

As for the surface adsorption properties, the quartz crystal microbalance with dissipation monitoring (QCM-D) equipped with PDMS coated crystals is used and the associated results are shown in Figure 3.2e (also Supporting Information for frequency and dissipation shift data, Figure S.5). The adsorption data for LFM and LFM-reinforced κCH (hydrated mass of 36.0 and 40.0 $mg\ m^{-2}$, respectively) show that their adsorption behaviour on PDMS surfaces surpass the adsorption activity of greater values of adsorbed saliva (32.0 $mg\ m^{-2}$). Despite κCH having similar lubricating properties to LFM, absorption of the former is only about one third of the latter. Furthermore, κCH only slightly enhance the surface adsorption properties of LFM-reinforced κCH, despite their significantly different lubrication performance. Thus, unlike reported for simple carbohydrate solutions (Stokes *et al*., 2011; Zhang and Meng, 2015), fluids presented here do not show exclusive correlation between friction and surface adsorption. This lack of correlation can be attributed to the static nature of QCM-D measurements which differs from shear-dominated tribo-tests (Maid *et al*., 2014). Nevertheless, the high lubrication performance of LFM-reinforced κCH results from the

adsorption properties of LFM and the rheological properties of κCH, the latter helping the lubricant to remain at the contact interface even under tribological shear.

Further, we studied the lubrication performance of LFM-reinforced κCH compositions at different ratios to better understand the synergistic interactions between the lubricant components. For this purpose, the friction coefficient results as a function of the ratio of κCH to LFM (wt/vol) are presented in Figure 3.3a at 0.005 $ms^{-1}$ and 0.1 $ms^{-1}$. The full data for this investigation can be found in Supporting Information, Figure S.6. As shown in Figure Figure 3.3a, the friction coefficient at both speeds, increased around one order of magnitude upon decrease of the relative concentration ratio to below 0.01 wt/vol. This indicates that a sufficient amount of κCH is required to achieve the observed superlubricity. To further elucidate this observation, ζ-potential measurements are carried out on LFM-reinforced κCH compositions. ζ-potential results as a function of the κCH to LFM ratio are shown in Figure 3.3b. The ζ-potential for the sole LFM and κCH components is measured as +22.7 and -46.3 mV at pH 7.0, respectively. Oppositely charged components propel mutual electrostatic attraction resulting in a structure observed in TEM images (Figure 3.1).

The ζ-potential is sensitive to the κCH/LFM ratios, resulting in a decrease of electrophoretic mobility and consequently reduction in the net surface charge. As the κCH/LFM ratio increasees, thecoverage of LFM by κCnf is enhancened. As a result of this, the LFM-reinforced κCH undergoes a charge reversal process, at the shear plane, gradually from net positive towards net negative values. The coverage curve, calculated from ζ-potential results (Supporting Information, Equation S3) (Pallandre *et al*., 2007) reaches a plateau of around 90% at a ratio of 0.07:1 wt:vol. The friction coefficient curve plateaued out approximately at the same ratio (Figure 3.3a). These results are in agreement with TEM observations for LFM reinforced κCH at ratio of 0.07:1 wt:vol (Figure 3.1). These corroborate that an adequate level of coverage of LFM by the κCnf is required for LFM-reinforced κCH to deliver super-lubricity.

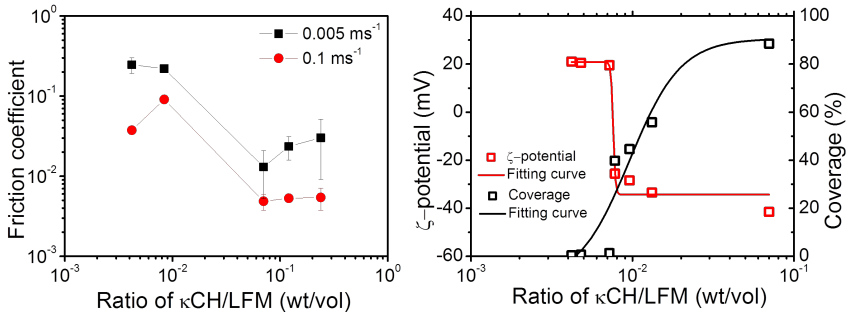



**Figure 3.3** Influence of concentration ratio of κCH to LFM on the tribological properties and ζ-potential characteristics of the LFM-reinforced κCH. a) The friction coefficient results obtained at relatively low (0.005 $ms^{-1}$) and high entrainment speeds (0.1 $ms^{-1}$). b) ζ-potential and surface coverage results, with the logistic fitting curve. Superlubricity (friction coefficient ~ $10^{-2}$) is achieved only at κCH/LFM ratios of greater than 0.07:1 wt/vol κCH/LFM. Values are presented as the mean ± SDs of nine readings on triplicate samples (n = 9 × 3).

Finally, the efficacy of the developed lubricant formulation on a tongue-emulated surface is assessed using the method developed by Andablo-Reyes et al. (Andablo-Reyes *et al.*, submitted). A set of experiments are performed under orally relevant conditions with respect to speed, pressure and surface properties. Briefly, a soft Ecoflex™ 00-30 was used to create silicone surfaces from a 3D printed mold, with Young's modulus of ~ 120 kPa, latter being considerably close to the modulus of the tongue as compared to PDMS. The mold contains papillae-shaped features with appropriate size and spatial distribution of fungiform and filiform papillae, mimicking those present on the real human tongue. Span 80 (0.5 wt%) was used to enhance the wettability of the Ecoflex™ 00-30 in some experiments. The water contact angle for the surfaces containing Span80 was measured to be 76.0° ± 2.0 (Andablo-Reyes *et al.*, submitted), resembling the wettability of tongue surfaces with some degree of adsorbed salvia on top. More details with reference to this testing approach can be found in a recent work by the authors. Figure 3.4 shows friction curves for all lubricants used in his study. Panels a and b present the results for soft and textured Ecoflex™ 00-30 (hydrophobic) and Ecoflex™ 00-30 + Span80 (hydrophilic) materials, respectively. LFM shows poor lubrication behavior, with a slight reduction in the friction coefficient when compared to the buffer. κCH provides relatively better lubricity as compared to buffer and LFM for both surfaces at speeds below $10^{-3}$ m/s. The lubrication performance of saliva is found to be dependent on the wettability behaviur of the surfaces, particularly at speeds below $10^{-3}$ m/s. For saliva, lower friction coefficients are observed on Ecoflex™ 00-30 +Span80 surfaces which surpasses the lubricity of both LFM and κCH.

Results show that tribo-test conditions (contact pressure, contact geometry and sample topography and chemistry) used with tongue-mimicking surfaces and surface properties have a large impact on the performance of the fluids (compare Figure 3.4 to Figure 3.2). For example, all the friction measurements are higher with the new Ecoflex patterned surfaces than the smooth PDMS ones. Interestingly and in agreement with the MTM results (Figure 3.2a), LFM-reinforced κCH outperformed the other fluids within the whole speed range measured here, with friction coefficient values an order of magnitude lower with respect to the values for the buffer. These results prove the superlubricity of LFM-reinforced κCH for soft silicon surfaces that emulated the stiffness and topography of human tongue with the Ecoflex™ 00-30 representing extreme dry mouth conditions and the modified material (Ecoflex™ 00-30 + Span80) representing dry tongue with some residual saliva.

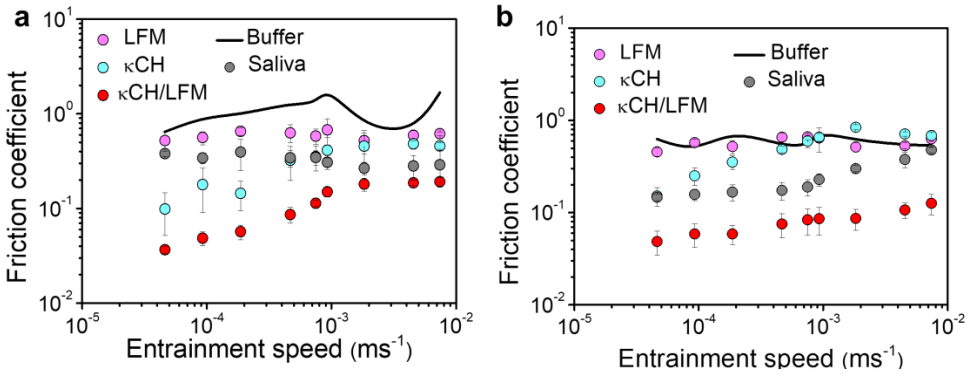



**Figure 3.4** Tribological performances of the lubricants obtained using tongue-mimicking tribological surfaces. LFM-reinforced κCH demonstrates lowest friction values as compared to buffer, saliva, κCH or LFM. Friction results obtained using soft textured a) Ecoflex™ 00-30 (hydrophobic) and b) Ecoflex™ 00-30 + Span80 (hydrophillic) surfaces. Human saliva was collected from a healthy young female at the morning of the testing day. The subject was refrained from eating and drinking for at least 2 h before saliva collection (Ethics number: MEEC 16-046, University of Leeds, UK). The saliva was diluted with 10 mM HEPES at the ratio of 1:1 w/w, centrifuged, and the supernatant was used for the tribology measurements. Values are presented as the means ± SDs of nine readings on triplicate samples (n = 9 × 3).

## 4. Conclusions

In summary, we have developed a new sophisticated non-lipidic bio-lubricant composed of submicron-sized lactoferrin microgels dispersed in κ-carrageenan hydrogel. The bio-lubricant possesses superlubricity characteristics which exceeds the lubricity of real human saliva in different oral mimicking conditions. The excellent lubrication performance of this microgel-reinforced hydrogel is attributed to the synergistic effects between LFM and κCH. The synergistic effects impart superlubricity to the lubricant which is facilitated through viscous

forces and surface adsorption (Xu *et al*., 2020). This new hydrogel has significant potential for applications in oral care products where lubrication without lipid content is desired. For instance, dry mouth syndrome or xerostomia (Porter *et al*., 2004) pose a limitation to the lubrication of oral surfaces in absence of natural saliva. In addition, intake of extra lipid for lubrication can be undesirable for these patients especially for the elderly population, where this condition is prevalent. Thus, the development of bio-inspired lubricants as alternative to saliva, such as our LFM-reinforced κCH, is a high priority. Additionally, the present formulation can be potentially used to replicate the lubricating properties of fat content in food products, providing the possibility of decreasing caloric content, without sacrificing sensory related attributes (Sarkar *et al*., 2019).

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# Chapter 4

## Concluding Remarks and Future Directions

### 1. Key findings

This research is driven by the goal to develop a novel bio-lubricant as a saliva substitute that provides effective lubrication similar to or exceeding that of real human saliva for the treatment of dry mouth conditions.

Firstly, a comprehensive literature review in **Chapter 2** has summarised various tools for dry mouth diagnosis and examined the properties of existed salivary substitutes. In terms of dry mouth assessment, questionnaire is the most common approach for subjective evaluation, with certain kinds of questions (*e.g.* dry mouth symptoms and eating behaviour related questions) followed by measuring the objective salivary flow rate change (Fox et al., 1987, Thomson et al., 1999, Pai et al., 2001, Suh et al., 2007). Although rare attention has been given on employing biochemical, rheological, adsorption and tribological tests of saliva for dry mouth diagnosis, researchers have widely used these tests to study the alteration in biochemical and mechanical changes of saliva in dry mouth patients. By correlating these measurements with subjective assessment of dry mouth, these tests can be useful as objective diagnosis tools for rational tailoring of the exact property of saliva substitutes for patients with different salivary changes.

In terms of the properties of existing saliva substitutes, most of commercial saliva substitute products contain dry mouth relief agents such as lubricating, thickening, adhesive and moisturizing agents mainly composed of mucin, modified celluloses, polysaccharide gum or polyethylene glycol (PEG). However, no strong evidence was found for the effectiveness of these saliva substitutes by clinical or laboratory studies. As for innovative development of saliva substitutes, various food-related materials such as yum, okra as well as colloidal technologies, such as self-assembly, emulsion, liposomes and microgels were found in saliva substitute patents in the last decade. Further characterization and identification of clear benefits of these materials and techniques are needed before they can be employed in clinical settings.

To solve the above-mentioned question of designing an effective saliva substitute, **Chapter 3** demonstrated the development of a novel water-based lubricant for dry mouth treatment with effective tribological properties by reinforcing a fluid-like hydrogel with proteinaceous microgels. This aqueous lubricant composed of positively-charged lactoferrin microgels dispersed in negatively-charged $\kappa$-carrageenan hydrogels demonstrates a synergistic interaction offering super-lubricity in the low speed lubrication regime in comparison to any of the pure components alone. In addition, it lubricates better than real human saliva in oral contact mimicking conditions (hard PDMS as well as soft textured Ecoflex 00-30) at certain component ratios. Such friction reduction is attributed to the combining of both viscous lubrication and surface-effects *i.e.* hydration lubrication supported by adsorption onto hydrophobic surfaces. The reinforcement of combined hydrogel is also evidenced by one-order of magnitude increase in elastic modulus as compared to separate components in oscillatory measurements. Therefore, this thesis has shown the development of a novel aqueous lubricant with superlubricity mediated by synergistic interactions, which offers a unique prospect towards the fabrication of biocompatible aqueous lubricants for dry mouth conditions.

## 2. Practical implications

The novel bio-lubricant with super lubricity developed in this research has been filed in a patent in May 2020 (GB Patent Application number 2007546.1). Such water-based lubricant composed of food-related materials can be applied in mainly three ways:

- Serving as saliva substitute as a symptomatic treatment for dry mouth patients, which provides relief for the dryness and roughness of the oral surfaces.

- Application or addition of the composition in the form of beverages or solid foods, such as chewing gum, candy and chocolate to facilitate mastication and deglutition of the food products for people with swallowing disorders which is also one of the common results of dry mouth.

- Application or addition of the composition in food to replicate the lubricating properties of fat content in food products, providing the possibility of decreasing caloric content, without sacrificing sensory related attributes.

## 3. Recommendations for future work

Four key areas of future work including methodology, material, commercial application and clinical trial viewpoint can be explored as next steps.

**Methodology viewpoint.** This thesis fully studied the macro-scale mechanical properties of this newly-developed bio-lubricant in terms of tribological, rheological and structural properties. Nevertheless, to fully understand the mechanism of superlubricity, one requires focusing on the adsorption and molecular boundary lubricant film properties of the lubricant. Although, QCM-D is used to understand hydrated mass, it is important to employ surface plasmon resonance (SPR) to study the dry mass of the lubricant and to identify any correlation with the boundary lubrication as well as transition between mixed and elastohydridynamic regime (Stokes et al., 2011). In addition, atomic force microscopy (AFM) in the force spectroscopy mode with a colloidal probe can be used to understand the influence of contact area on frictional properties at the nanoscale (Liamas et al., 2020).

**Material viewpoint.** This thesis proved the effectiveness of reinforcing negatively-charged *κ*-carrageenan hydrogels with positively-charged lactoferrin microgels in producing the super-lubricity behaviour in the low speed lubrication regime. It is worthwhile to explore if similar synergistic effect happens in the combination of other oppositely-charged microgels and hydrogels. The proteinaceous or non-proteinaceous microgels can be selected from the group consisting of: lysozyme, gelatine, milk protein, bovine serum albumin, whey protein, caseinate, egg protein, albumin, gluten, gelatine Type B, pea protein, rice protein, legumin, corn protein, peanut protein, chitosan and chitin. The hydrogels might be selected from the group consisting of: *ι*-carrageenan, *λ*-carrageenan, agar, agarose, alginate, pectin, dextran sulphate, cellulose, xantham gum, gellan gum, and any negatively-charged polysaccharide. Also role of ionic strength need to be evaluated. Also, in real system, Ca2+ may influence the behavior of LFM reinforced κCH, therefore should be taken into account.

**Commercial application viewpoint.** This thesis has developed a water-based lubricant composed of food-related materials with super-lubricity. To convert this lubricant into a relevant commercial application such as saliva substitutes or food products, different delivery vehicles and additives should be taken into future consideration. As for delivery vehicles, the lubricant can be transformed into gel or spray as saliva substitutes or added into food products

as texture modifier. Meanwhile, different additives such as sweetener, cool sensation agent, surfactant, colorant and preservatives can be added to the formulation for customer acceptance and shelf life extension.

**Clinical trial viewpoint.** This thesis did not conduct any clinical trial to test the acceptance and effectiveness of this novel bio-lubricant in real dry mouth patients or healthy population. Therefore, further studies of well-designed human trials with proper subjective questionnaires and quantitative evaluation of oral lubrication with VAS scores are needed in future to see the translation of this technology in a commercial setting.

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# Appendices

# Supplementary Information of Chapter 3

### THEORY

According to the de Vicente et al. (de Vicente *et al.*, 2005), the friction coefficient for an elastic ball on disc contact is

$$\mu = 1.46\overline{U}^{0.65}\overline{W}^{-0.70} + SRR(3.8\overline{U}^{0.71}\overline{W}^{-0.76} + 0.96\overline{U}^{0.36}\overline{W}^{-0.11}) \quad \text{(S1)}$$

where, $\overline{U} = \frac{U\eta_e}{E^*R'}$ and $\overline{W} = \frac{W}{E^*R'^2}$, $R'$ is the ball radius and $E^*$ is the Young's modulus, W is the applied load, U is the entrainment speed, SRR is the sliding/rolling ratio and $\eta_e$ is the effective tribological viscosity as the only free parameter. Only, the second term in the equation S1 (*i.e.* $SRR(3.8\overline{U}^{0.71}\overline{W}^{-0.76} + 0.96\overline{U}^{0.36}\overline{W}^{-0.11})$) was used to fit the friction data and obtain the effective viscosity in the tribo-contact.

The minimum thickness is calculated as (Vicente *et al.*, 2005):

$$h_m = 2.8R'\overline{U}^{0.65}\overline{W}^{-0.21} \quad \text{(S2)}$$

The coverage% of LFM by κCH is calculated using the following empirical equation by modeling the ζ-potential vs polysaccharide concentration curves (Pallandre et al., 2007):

$$Coverage\% = \frac{c}{c_{sat}} = -\frac{1}{3}ln\left(\frac{\zeta_c - \zeta_{sat}}{\zeta_0 - \zeta_{sat}}\right) \quad \text{(S3)}$$

where, $\zeta_{sat}$ is the ζ-potential when the LFM are completely saturated by κCH; $\zeta_0$ is the ζ-potential of LFM in the absence of κCH; and $\zeta_c$ is the ζ-potential of the LFM-reinforced κCH at κCH concentration c. $c_{sat}$ is the minimum amount of κCH required to completely cover the surface of LFM.

## SUPPLIMENTARY FIGURES

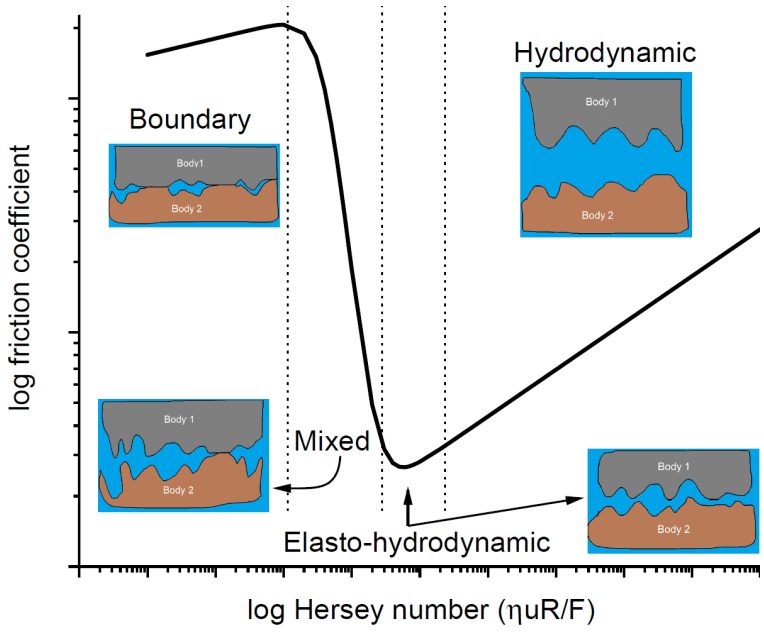


**Figure S.1** The friction coefficient versus a modified velocity parameter (Sommerfeld number, a product of the lubricant viscosity in the contact, lubricant entrainment speed, normal contact load, and contact geometry). Typical Stribeck curve divided into four lubrication regimes (Chong and De la Cruz, 2014). The measured friction coefficient depends on the adsorbed moieties and the lubricant film thickness between the two contacting surfaces. In the boundary regime, the moving surfaces are almost in full contact and a significant proportion of the contact load is supported by the contact-bodies, therefore the friction coefficient is mainly dependent on the characteristics of the contacting surfaces and adsorbed molecules of the lubricants. In this regime, hydrodynamic forces are believed to be negligible and the capacity of the lubricant to decrease the friction coefficient relies on rapid adsorbance of lubricant-derived surface-anchored layers which are few molecules to nanometer in thickness. In the mixed regime, the film thickness is comparable to the height of the surface asperities resulting in the contact load being borne by the lubricant at the interface as well as the surface asperities of the tribopairs. The friction coefficient reduces as the contribution of the direct solid-contact between surfaces reaches its minimum where the elasto-hydrodynamic regime takes place. In the hydrodynamic regime, the surfaces are completely separated by the lubricant film and the friction force is a function of hydrodynamic forces.

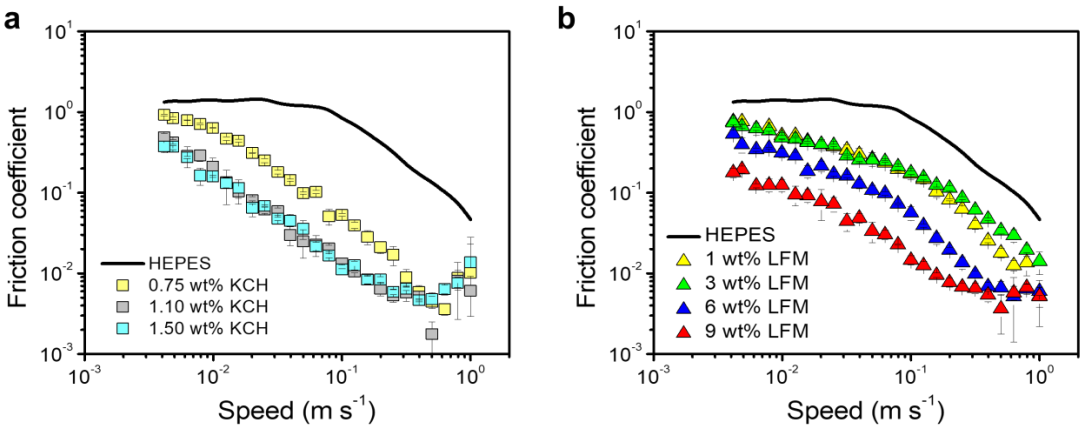


**Figure S.2** Tribological performances of κCH and LFM at different concentrations using PDMS-PDMS contacts in a MTM tribometer. a) Friction coefficient obtained for κCH as function of entrainment speeds. Friction curves for κCH at different concentrations have similar shape, showing steep decrease of friction coefficient with increasing speed. The friction coefficients are similar irrespective of concentration increasing at above 1.10 wt%. b) Friction coefficient obtained for LFM as function of entrainment speeds. Friction curves for LFM start to shift to lower values at concentration above 3 wt%, but maintains relatively high friction coefficient at low entrainment speed even at the highest concentration (9 wt%). In summary, both κCH and LFM show lubrication enhancement versus the buffer but both the systems demonstrate relatively high friction coefficients irrespective of the concentration increase. Values are presented as the means ± SDs of nine readings on triplicate samples (n = 9 × 3).

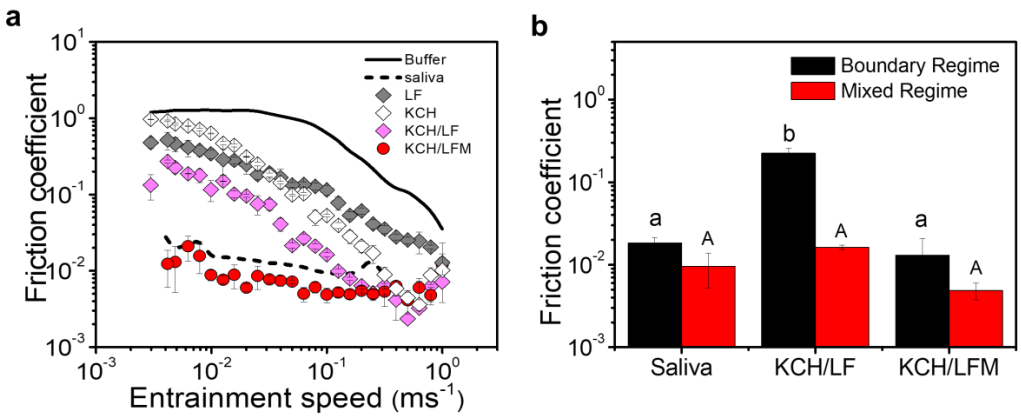


**Figure S.3** Comparison of tribological performances of κCH/LFM with non-microgelled κCH/LF using PDMS-PDMS contacts in a MTM tribometer. a) LFM-reinforced κCH obtained similar friction curves as those obtained using real human saliva, with lower friction coefficients compared with buffer. While the combination of non-microgelled lactoferrin solution (LF) with κCH at similar ratio of LFM-reinforced κCH does not show significant synergistic effect, with similar friction curves as compared to lactoferrin solution and κCH alone. b) Friction coefficient obtained for aforementioned real human saliva saliva, κCH/LFM and κCH/LF at 0.005 $ms^{-1}$ (boundary reime) and 0.1 $ms^{-1}$ (mixed regime). Significant differences are analysed by one-way ANOVA, followed by Turkey's test. Different letters on the top of each bar are significantly different. At 0.005 $ms^{-1}$, κCH/LF shows around one magnitude higher ($p < 0.05$) value of friction coefficient as compared to real human saliva and LFM-reinforced κCH, which shows no significant difference with each other ($p > 0.05$). In summary, no synergistic effect is observed for non-microgelled κCH/LF, indicating the importance of reinforcing the hydrogels with microgels. Human saliva was collected from a healthy young female in the morning, subject was refrained from eating and drinking for at least 2 h before saliva collection (Ethics number: MEEC 16-046, University of Leeds, UK) and was diluted with 10 mM HEPES at the ratio of 1:1 w/w, centrifuged, and the supernatant was used for the tribology measurements. Values are presented as the means ± SDs of nine readings on triplicate samples (n = 9 × 3).

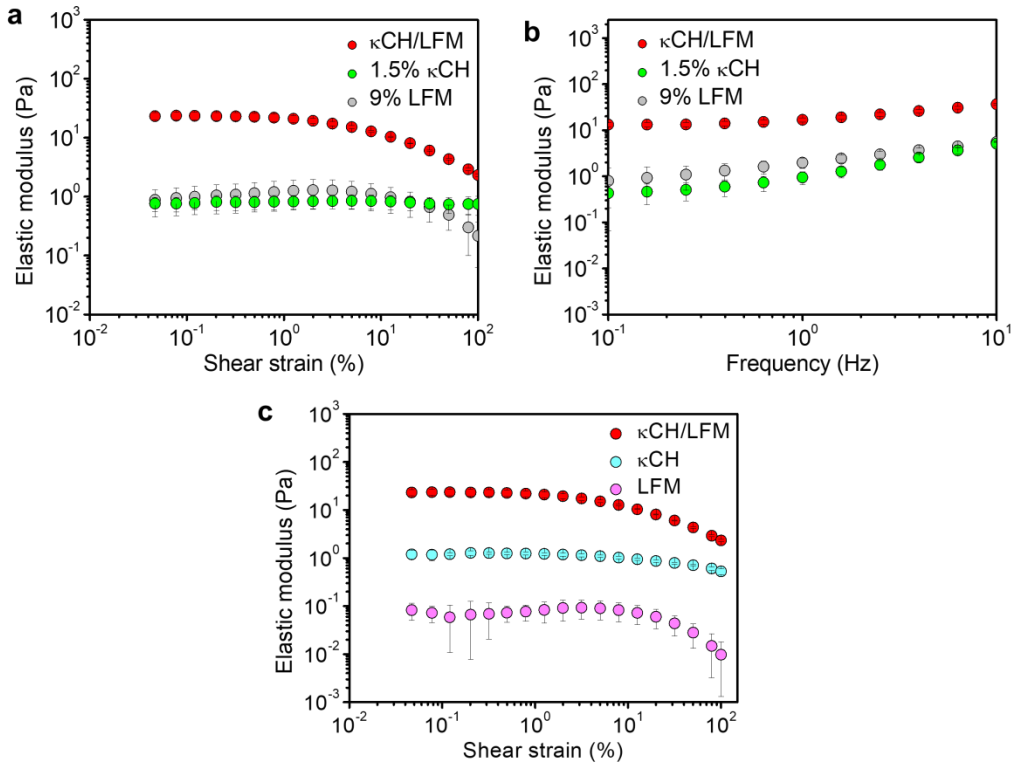


**Figure S.4** Rheological characterization of microgel-reinforced hydrogels. Strain and frequency sweep of LFM-reinforced κCH as well as LFM and κCH. a) Strain dependence of the elastic modulus at a constant frequency (1 Hz) for 1.5 wt% κCH, 9.0 wt% LFM and LFM-reinforced κCH containing 1.1 wt% κCH and 2.0 wt% LFM. It is clear that all three fluids are in the linear viscoelastic range before the shear strain of 1.0% b) Frequency dependence of the elastic modulus at a constant strain (1.0 %) for 1.5 wt% κCH, 9.0 wt% LFM and LFM-reinforced κCH containing 1.1 wt% κCH and 2.0 wt% LFM. It is clear that the LFM-reinforced κCH hydrogels have even higher modulus than the highest concentration of LFM or κCH when used individually. c) Strain dependence of the elastic modulus at a constant frequency (1 Hz) for 1.1 wt% κCH, 2.0 wt% LFM and LFM-reinforced κCH containing 1.1 wt% κCH and 2.0 wt% LFM. The linear viscoelastic range where elastic modulus is independent of the applied strain is determined below 1.0 %. Values are presented as the means ± SDs of nine readings on triplicate samples (n = 9 × 3).

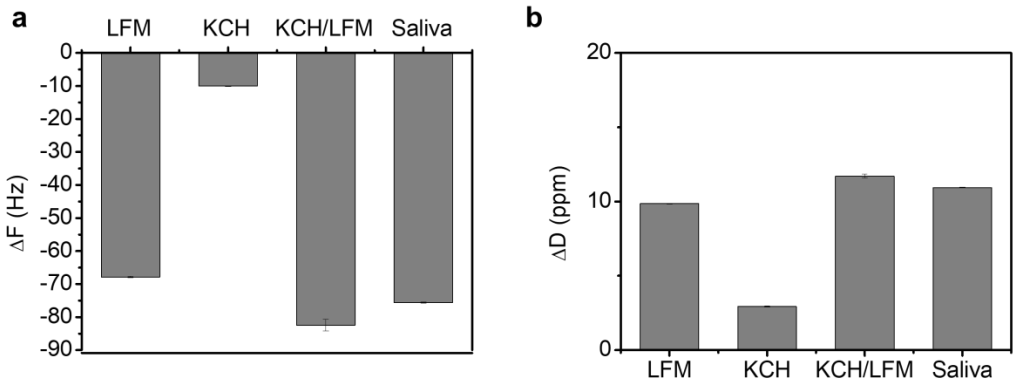


**Figure S.5** Quartz crystal microbalance with disspation monitoring (QCM-D) data of of microgel-reinforced hydrogels. a) Frequency and b) dissipation shifts of LFM-reinforced κCH, saliva as well as LFM and κCH. Both frequency and dissipation shift of LFM-reinforced κCH is significantly higher than all of the rest samples. Values are presented as the means ± SDs of nine readings on triplicate samples (n = 3 × 1).

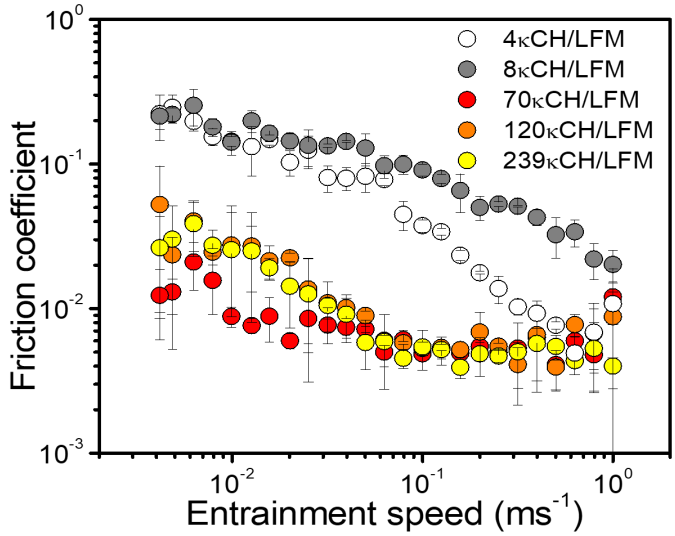


**Figure S.6** Tribological performance of κCH/LFM at different ratios using PDMS-PDMS contacts in a MTM tribometer. Friction coefficient obtained for LFM-reinforced κCH at different ratios as function of entrainment speeds. Friction curves for LFM-reinforced κCH above 0.07:1 wt/vol are in similar shape with relatively low friction coefficients through whole entrainment speeds. An obvious shift of friction curves to higher levels is observed for LFM-reinforced κCH at ratio below 0.07:1 wt/vol, with around one order of magnitude higher friction coefficient at the entrainment speed before 0.1 $ms^{-1}$ as compared to those have higher relative concentrations of κCH (0.120:1, 0.239:1 wt/vol). This indicates the importance of sufficient κCH to generate superlubricity at low entrainment speeds. As for 4κCH/LFM, 8κCH/LFM, they obtain similar friction coefficient at low sppeds, while 4κCH/LFM has lower friction coeffient as speeds are increasing.This indicates not enough κCH might has an inverse effects on LFM lubrication. Values are presented as the means ± SDs of nine readings on triplicate samples (n = 9 × 3). Note, all the nomenclature in ratios are × $10^{-3}$ in wt/vol *i.e.* 4κCH/LFM is 0.0044: 1 wt/vol, 8κCH/LFM is 0.008: 1 wt/vol, 70κCH/LFM is 0.07: 1 wt/vol, 120κCH/LFM is 0.120: 1 wt/vol, 239κCH/LFM is 0.239: 1 wt/vol.

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