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Influence of centrifugation treatment on the lubricating properties of human whole saliva

Y.F. Zhang[a,b], J. Zheng[a,*], L. Zheng[a], Z.R. Zhou[a]

[a]*Tribology Research Institute, Key Laboratory of Advanced Technologies of Materials, Ministry of Education of China, Southwest Jiaotong University, Chengdu 610031, P. R. China*

[b]*Key Laboratory of Testing Technology for Manufacturing Process, Ministry of Education of China, Southwest University of Science and Technology, Mianyang 621010, P. R. China*

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Abstract

An important function of human saliva is to serve as oral lubricant during mastication process and then effectively reduce tooth wear. Thus, centrifuged human whole saliva has been used as a substitute for human whole saliva for many in vitro studies on dental tribology. However, the difference in lubricating properties between human whole saliva and centrifuged saliva remains unclear. The objective of this study was to investigate the influence of centrifugation on the lubricating properties of human whole saliva. In this paper, the lubrication of both human whole saliva and centrifuged saliva on human tooth enamel were comparatively studied in vitro using a nano-scratch tester. The structure, composition, and mechanical properties of salivary pellicle were characterized. Result showed that food debris and high molecular weight proteins in human whole saliva were removed by centrifugation. However, the low molecular weight proteins were still in saliva. Under the lubrication of human whole saliva, the salivary pellicle formed on the enamel surface was uneven, and its mechanical properties were inhomogeneous. But a smooth and homogeneous salivary pellicle was obtained upon the enamel surface under lubrication of centrifuged saliva. Moreover, there were no significant deference in friction coefficient and wear volume of tooth enamel between human whole saliva and centrifuged saliva lubricating conditions. In summary, centrifuged saliva exhibited similar lubrication to human whole saliva. Centrifugation treatment does not impair the lubricating properties of human saliva. On the contrary centrifugation can help minimize the effect of cell and food debris.

Keywords: Human whole saliva; Centrifugation; Tooth enamel; Lubrication

1. Introduction

Saliva is secreted by the major (parotid glands, submandibular and sublingual glands) and minor salivary glands. Human whole saliva (HWS) is a complex mixture of fluids from salivary glands and gingival cervicular fluid [1], which has many functions in the oral cavity, such as digesting food, maintaining oral hygiene, preventing dental caries and lubrication [2]. The saliva lubrication property of is particularly crucial to swallow food bolus and to protect oral surfaces from abrasion and wear [3]. Due to the altered salivary glands function, a large number of people suffer from impaired salivary functions, displaying symptoms such as “dry mouth” (also called as xerostomia) which could result in excessive tooth wear [4]. It is the common treatment to use artificial saliva as an oral lubricant [5,6]. It is necessary to understand the lubricating mechanism of human saliva so as to develop new artificial saliva with similar lubricating performance.

The lubrication of saliva mainly depends on salivary pellicle [7,8], which is a biofilm that forms on the enamel surface by selective binding of proteins from saliva [1,9]. Salivary pellicle is composed of an initial layer and an outer layer [10], and this structure would vary with oral environment [11,12]. The compositions of diets and beverages could change the oral

*Corresponding author. Fax: +86 28 87603142.
E-mail address: jzheng168@home.swjtu.edu.cn (J. Zheng).
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environment, and then affect the structure of salivary pellicle. It was observed that decrease in the ionic strength below physiological conditions affected the structure and boundary lubrication of salivary pellicle [12]. Dickinson et al. found that the morphology and mechanical properties of salivary pellicle was dramatically changed when the pellicle was exposed to tannins [11]. Thus some methods were proposed to minimize the effect of diets and beverages on the HWS lubrication [8,13,14]. For example, saliva samples were collected following proper collection procedures. However, some food debris still exists in the collected saliva. To remove the food and cell debris, the collected HWS was always centrifuged [8]. The HWS subjected to centrifuged treatment is termed centrifuged saliva (CS). In previous studies, both CS and HWS were used as a lubricant between sliding surfaces, and the results showed significant differences [8,15]. Berg et al. observed that the friction coefficient between two sliding surfaces was 0.03 under CS lubrication using a atomic force microscopy [8]. However, Vardhanabhuti found that the friction coefficient between two surfaces was about 0.1 under HWS lubrication using a Mini Traction [15]. Of course, the friction coefficient closely depends on rubbing pair and lubrication conditions et al. However, that study does not consider the effect of centrifugation on saliva lubrication. As a result, the lubricating mechanism of HWS and CS has been unclear so far. Hence, this study is to explore the lubricating mechanism of saliva and determine whether the CS could be used as a substitute for HWS in vitro studies.

In this paper, the lubricating performance of HWS and CS saliva were explored using a nano-scratch tester. The composition of HWS and CS were investigated by gel-electrophoresis and a laser scanning confocal microscope (LSCM). Given that the boundary lubrication of saliva mainly depends on the salivary pellicle [8,14,16], the mechanical performances of salivary pellicle formed in HWS and CS were examined respectively using a nanoindenter. In order to further explore the wear mechanism of human tooth enamel under the HWS and CS lubrication, the wear volume and wear morphology of human tooth enamel were characterized.

2. Materials and methods

2.1. Sample preparation

Human teeth used in this study were prepared from freshly extracted human teeth without caries. The teeth samples were mandibular second permanent molars (M_2) of individuals aged between 20 and 22 years. Each tooth was cut into two parts under a water-cooling condition and then embedded self-setting plastic to obtain enamel samples. And then the samples were ground and polished to obtain a flat surface. The average roughness R_a of the polished enamel sample was controlled under 0.10 μm using a surface profilometer (TALYSURF6, England). The detailed preparation method of enamel samples was reported in our previous study [14].

Following proper collection procedures mentioned in the previous study [17], saliva samples were collected. The

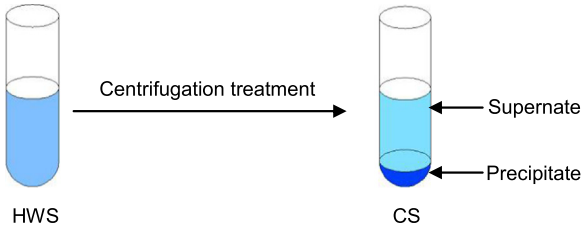

Fig.1. Schematic diagrams of HWS after centrifuged treatment.

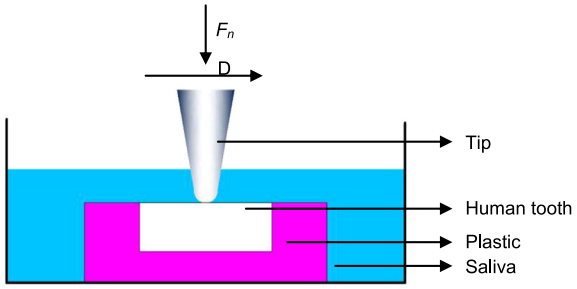

Fig.2. Schematic diagrams of testing method.

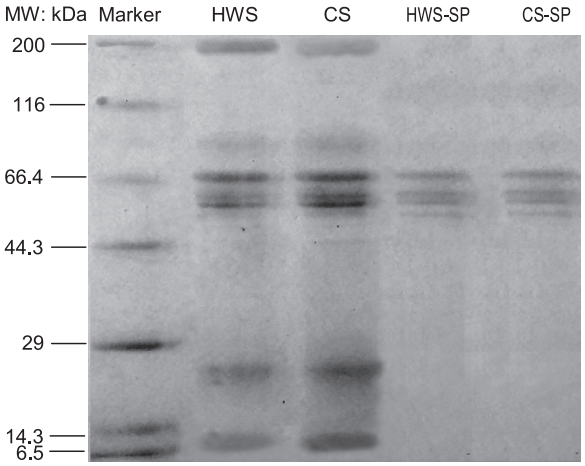

Fig.3. The protein-banding patterns of proteins presented in HWS, CS and salivary pellicle.

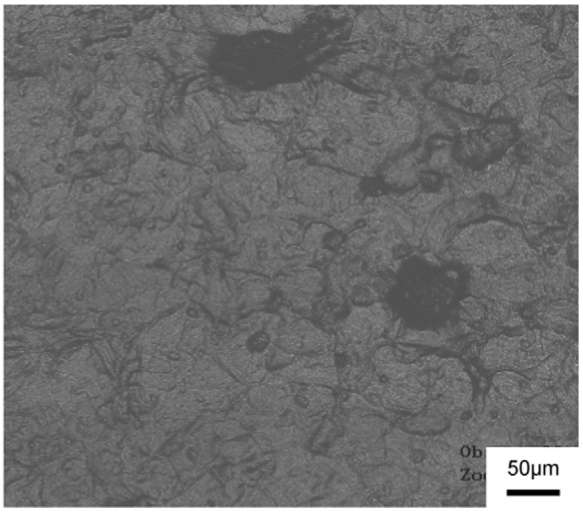

Fig.4. LSCM micrographs of the precipitates.

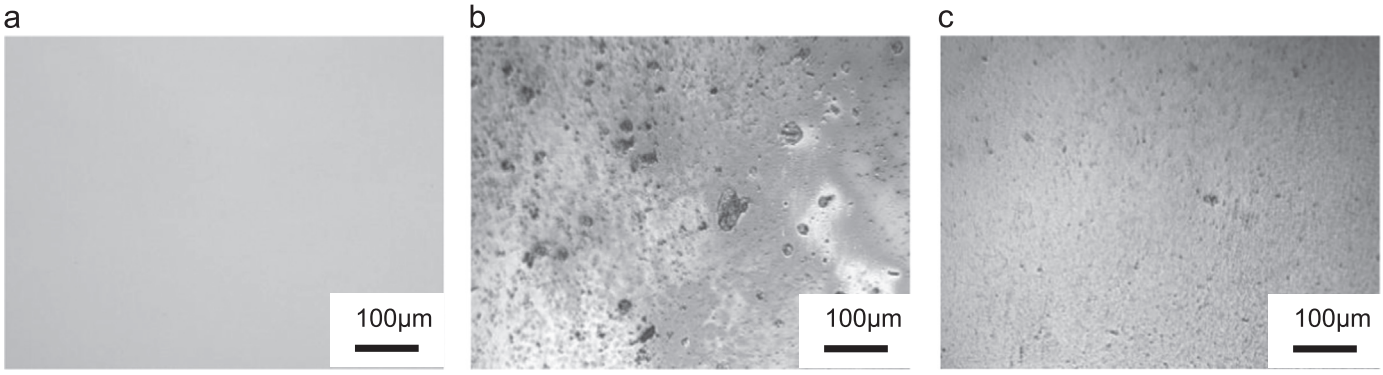

Fig.5. OM micrographs of salivary pellicle formed under different conditions, (a) Control; (b) HWS; (c) CS.

detailed collection methods of saliva were described in the literature [14]. The collected saliva was equally divided into two parts. One part was used to do experiments immediately in order to keep its natural performance. The other part was centrifuged for 30 min at 2000 rpm [8] and then the supernatant phase of the saliva was collected for tribological experiments, as shown in Fig. 1. Three samples were tested for each lubricant.

2.2. Composition of saliva

The HWS solution consists of supernate and precipitate after centrifuged treatment, as shown in Fig. 1. The protein molecular weight of the proteins presented in HWS and the supernatant phase of CS were measured by gel electrophoresis. The salivary pellicle formed in HWS and CS were collected by mean of combining mechanical (rubbing by toothbrush) and chemical actions, and then the protein molecular weight of the proteins presented in salivary pellicle were measured by gel electrophoresis too. The detailed testing method of the protein molecular weight was described in our previous study [14]. The precipitates were placed on a glass slide, and then observed by a LSCM.

2.3. Structure and mechanical characterization

The polished enamel samples were immersed in the HWS and CS for 2 h respectively, and an acquired salivary pellicle would be formed on the enamel surface [18]. The morphology of the salivary pellicle was observed with an optical microscope (OM). The enamel surface without any adsorption treatments was referred to as “control”. In addition, the salivary pellicle formed in HWS was termed HWS salivary pellicle (HWS–SP), and the pellicle formed in CS was termed CS salivary pellicle (CS–SP). The mechanical properties of salivary pellicle were tested by continuous stiffness measurements (CSM) using a nanoindenter (G200, Agilent, USA). A triangular pyramid tip of Berkovich diamond with a radius of about 20 nm was used. The maximum indent depth was 1000 nm and the constant strain rate (0.05 1/s) was applied [19].

2.4. Friction and wear tests

To investigate the lubricating properties of HWS and CS, unidirectional nano-scratch tests at constant load mode were conducted on the surfaces of enamel samples, which were exposed to HWS and CS respectively, at room temperature using a nano-scratch tester (NST) (G200 , Agilent Technologies, USA). As shown in Fig. 2, the enamel surface and scratch tip were immersed in the HWS or CS, and the scratch testing was done on the surface of enamel sample. A conical diamond tip with a radius of 5 μm was used for all the tests. A normal load of 20 mN was used for each sample. The scratch length was 200 μm, and the scratch speed was 20 μm/min. The surface profile and the residual depth and width of scratch groove were measured by a surface profilometer (G200 , Agilent Technologies, USA). The wear morphologies of scratch grooves were investigated by a scanning electronic microscopy (QUANTA200, FEI Corp., England).

3. Results

3.1. protein molecular weight and sediments

The protein molecular weight of proteins presented in the HWS, the CS supernate and the salivary pellicle were measured by SDS-PAGE. As shown in Fig. 3, the protein-banding patterns of HWS have no obvious difference from those of CS supernate. The molecular weight ranged from 6.5 to 200 kDa. While for the proteins in the salivary pellicle, the molecular weight ranged from 44.3 to 66.4 kDa. The HWS–SP and CS–SP samples showed similar protein-banding patterns. LSCM results showed different shapes and sizes of particles on the glass slide. And many threadlike substances were observed (Fig. 4).

3.2. Morphology and mechanical behavior of salivary pellicle

The enamel sample without any adsorption treatment was referred to as “control”. The morphologies of control surface and covered surface by salivary pellicle were investigated with an optical microscope. As shown in Fig.5a, the control surface was relatively smooth and clean. An uneven salivary pellicle

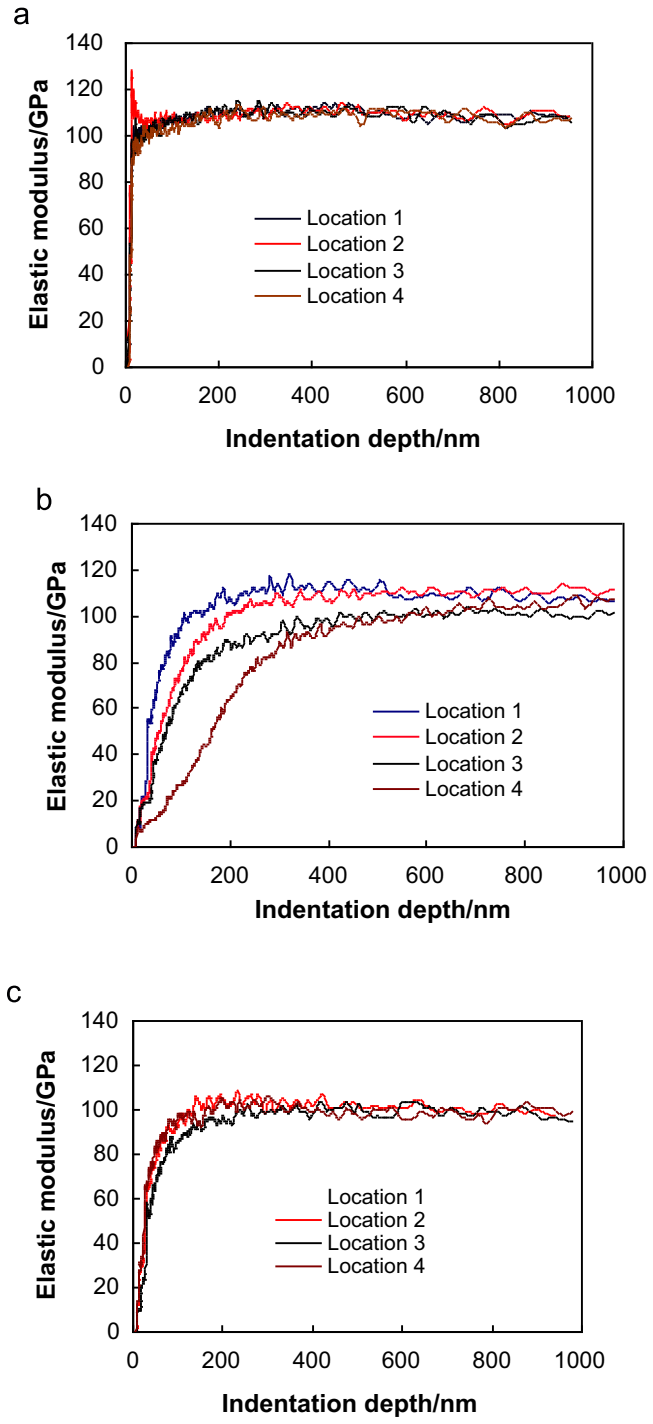

Fig.6. Elastic modules of the salivary pellicle as a function of indentation depth, (a) Control; (B) HWS–SP; (C) CS–SP.

was observed after the adsorption treatment with HWS, and some particles with various shapes and sizes distributed on the uneven pellicle (Fig.5b). However, the adsorption treatment with CS had no effect on salivary pellicle (Fig.5c).

The elastic modulus of enamel surfaces at different locations, were shown in Fig. 6, as a function of indentation depth. The elastic modulus of the control surface levelled off at 108 GPa when the indentation depth was more than 10 nm (Fig.6a). For the enamel surface covered with the HWS–SP, the maximum elastic modulus was different at different locations, ranged from 95 to 108 Gpa (Fig.6b). For the enamel surface covered with the CS–SP, the elastic modulus levelled off at 97.5 Gpa when the indentation depth was more than 200 nm (Fig.6c). It should be noted that the stable value of elastic modulus was lower than that the control.

3.3. Friction and wear behavior

The friction coefficient as function of displacement, at a normal load of 20 mN, was shown in Fig.7. And the mean of friction coefficient of HWS and CS was shown in Fig.8. Under both lubrication conditions, the friction coefficient of enamel surfaces was 0.13. No significant differences in friction coefficient were observed between HWS and CS lubricating conditions.

Typical nano-scratch profiles showed that there were significant differences in the morphologies of worn surface under different lubrication conditions, as shown in Fig.9. The scratch width of enamel surface under HWS and CS lubricating conditions was 12 μm. The scratch depth of the enamel surface was about 72 nm under CS lubricating conditions and was about 68 nm under HWS lubricating conditions. However, One-way analysis of variance (ANOVA) revealed that the differences were not statistically significant. The wear volumes of scratch on enamel surface under different lubricating conditions were similar (Fig.10).The typical micrographs of nano-scratch traces were shown in Fig. 10. It was seen that the worn surface morphologies of enamel surface were dominated by plastic deformation at 20 mN. There were no obvious differences in the worn enamel surface morphology in HWS and CS lubricating conditions (Fig. 11).

4. Discussion

It is generally accepted that human saliva plays a significant role in decreasing the teeth wear [14] and reducing the friction of tongue surface [20]. Due to the complexity of oral cavity environment, studying the saliva lubrication is a challenging task in vivo. Various experimental methods and instruments have been used to explore the lubricating properties of saliva in vitro [8,15]. In order to minimize the effect of cell and food debris, the collected saliva is always treated by centrifugation before testing. However, the effect of centrifugation treatment on the lubricating properties of saliva is still unclear. Previous studies demonstrated that the lubricating properties of saliva mainly depended on the salivary pellicle [14]. Thus it is very necessary to study the structure, composition, mechanical properties of the salivary pellicle.

4.1. Structure, composition, mechanical properties of salivary film

As shown in Fig.5, the structure of salivary pellicle formed in the HWS and CS were different. For the enamel surface

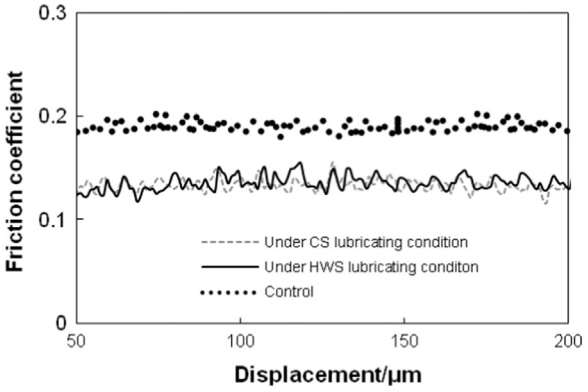

Fig.7. Variation of friction coefficient as function of displacement (F_n=20 mN).

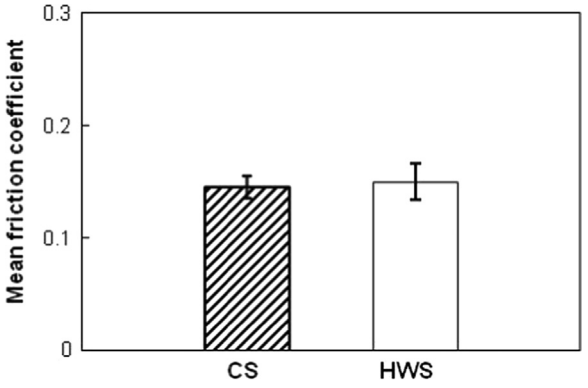

Fig.8. Mean of friction coefficient of HWS and CS.

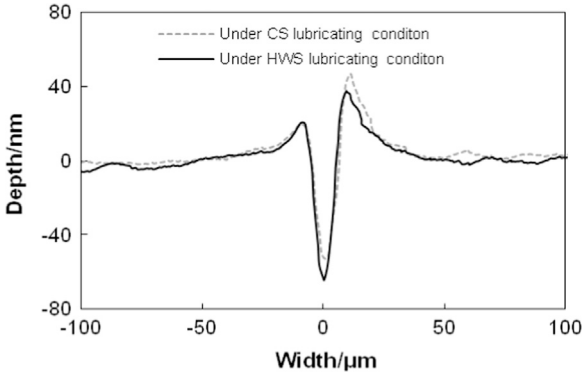

Fig.9. Profiles of scratches on the enamel surfaces under different lubricating conditions (F_n=20 mN).

treated with the HWS, an uneven salivary pellicle was observed, and some particles with various shapes and sizes stayed on this uneven pellicle (Fig.5b). However, for the enamel surface treated with the CS, a smooth and even salivary pellicle was observed (Fig.5c). The results indicated that some components in saliva were removed after centrifugation. In order to study the saliva composition change, the molecular weight of proteins in the HWS and the supernatant phase of CS were characterized using SDS-PAGE. Gel electrophoresis results showed that there were no significant difference of the protein patterns between the HWS and the supernatant

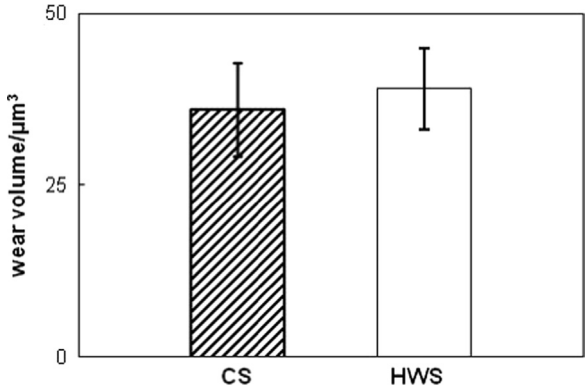

Fig.10. Wear volume of scratch on enamel surface under different lubricating conditions.

phase of CS. However, the LSCM result showed the centrifugation precipitates consisted of particles and threadlike substances. It was the food debris, cell debris and high molecular weight proteins (molecular weight more than 200 kDa) that precipitated after centrifugation. But the proteins with molecular weight ranged from 6.5 to 200 kDa would not precipitate during centrifugation. According to the gel electrophoresis results, only proteins with molecular weight ranged from 44.3 to 66.4 kDa adsorbed on the enamel surface. Thus the formation of the salivary pellicle resulted from highly selective adsorption. Since there were no significant differences of the protein patterns of salivary pellicle formed in HWS and CS. it can be concluded that the salivary proteins selectively adsorbed on the enamel surface could not be influenced by centrifuged treatment.

Given that the lubricating property of saliva mainly depends on boundary film, the mechanical properties of salivary pellicle were measured. Because salivary pellicle is viscoelastic [11,21] and is a multilayer biofilm, the measured data from the conventional nanoindentation method are not reliable. A technique, continuous stiffness measurement (CSM), has been used to measure the properties of polymeric materials, graded materials and multilayers [22]. Based on the thickness and special structure of salivary pellicle, CSM was used to measure its mechanical properties. Human tooth enamel is mainly composed of hydroxyapatite crystals [23], which has relatively stable mechanical properties [24]. Therefore, for the bare enamel surface, no significant differences were found among the elastic modulus-versus-indentation depth curves obtained at different locations of the enamel surface. For the curves, the rapidly increasing of elastic modulus ahead of 10 nm, might be caused by the sensitivity of indentation tip with respect to the ambient condition [19]. For the enamel surface covered with the HWS salivary pellicle, the elastic modulus -versus-indentation depth curves were different at different locations. After treated by the HWS, the structure of salivary pellicle would be influenced by the food and cell debris, as shown in Fig.5b. The salivary proteins could not be adsorbed evenly on the enamel surface. As a result, the elastic modulus varied with the pellicle structure. While the enamel surface treated in the CS, salivary proteins would be evenly adsorbed onto the enamel surface

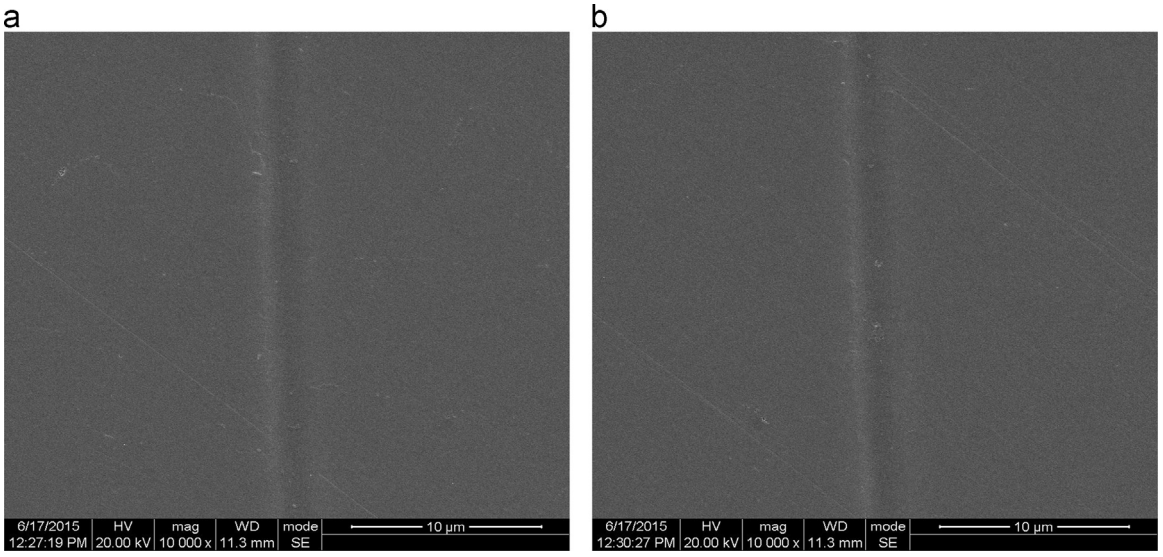

Fig.11. SEM micrographs of the scratches on the enamel surfaces under different lubricating conditions (a) HWS; (b) CS.

because the food and cell debris were removed by centrifugation (Fig.5c). As a result, no significant differences were observed for the elastic modulus-versus-indentation depth curves at different locations.

4.2. *Lubricating properties of HWS and CS salivary pellicle*

The proteins in saliva would selectively adsorb on the enamel surface, and then a biofilm termed salivary pellicle is formed. Salivary pellicle is a bilayer membrane consisting of dense initial layer and looser outer layer [10]. Initial layer is formed rapidly and the outer layer is formed relative slowly [14]. The mechanical test results of salivary pellicle indicated that elastic modulus-versus-indentation depth curves have significant difference between HWS and CS salivary pellicle. For the enamel surface immersing in HWS, food debris and high molecular weight proteins would random deposit on the outer layer of salivary pellicle. As a result, the thickness of salivary pellicle is not homogenized. The elastic modulus of salivary pellicle varies with the thickness of salivary pellicle [25]. For the salivary pellicle formed in CS, the thickness and mechanical properties of salivary pellicle is homogenized due to the food debris and high molecular weight proteins are removed. The food debris and high molecular weight proteins in HWS would deposit on the outer layer of salivary pellicle, however, the initial layer of HWS salivary pellicle would not be affected. As a result, the structure and performance of initial layer of HWS–SP and CS–SP were similar. Previous study demonstrated that the adhesion strength between outer layer and initial layer was lower than that between initial layer and enamel surface, and the outer layer would be removed easy under shear force [26]. Consequently, the lubricating property of salivary pellicle mainly depends on the initial layer. Consequently, no significant differences in friction coefficient and wear behavior were observed between HWS and CS lubricating conditions. Centrifuged saliva exhibited similar lubrication to human whole saliva. Centrifugation treatment does not impair the lubricating properties of human saliva. On the contrary centrifugation can help minimize the effect of cell and food debris.

5. Conclusions

Influence of centrifugation treatment on the lubricating properties of human whole saliva was studied in this paper. Based on the given testing conditions, the conclusions could be summarized as follows:

1. Food debris, cell debris and high molecular weight proteins were removed after centrifugation treatment. The low molecular weight proteins would not precipitate during centrifugation.
2. The salivary pellicle formed in the CS on the enamel surface was even and homogeneous, and have superior mechanical properties to the salivary pellicle formed in the HWS.
3. Food debris, cell debris and high molecular weight proteins would deposit on the outer layer of salivary pellicle. However, the initial layer of salivary pellicle would not be affected. Centrifuged saliva exhibited similar lubrication to human whole saliva. Centrifugation treatment does not impair the lubricating properties of human saliva. On the contrary centrifugation can help minimize the effect of cell and food debris.

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