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

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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

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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

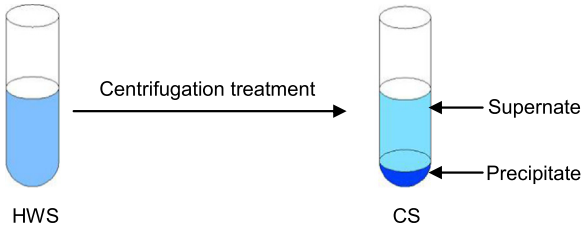

Fig.1. Schematic diagrams of HWS after centrifuged treatment.

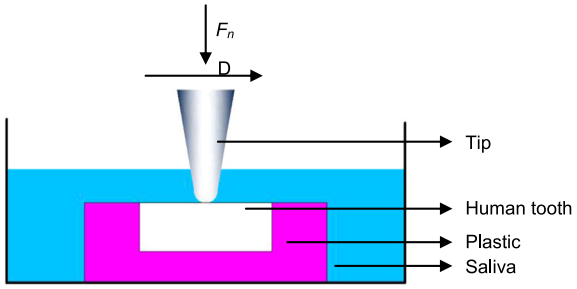

Fig.2. Schematic diagrams of testing method.

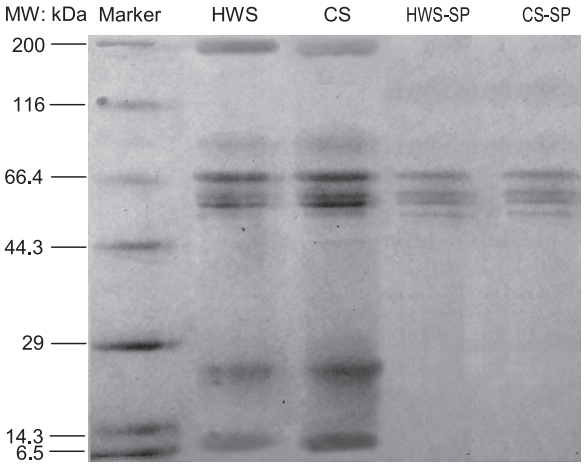

Fig.3. The protein-banding patterns of proteins presented in HWS, CS and salivary pellicle.

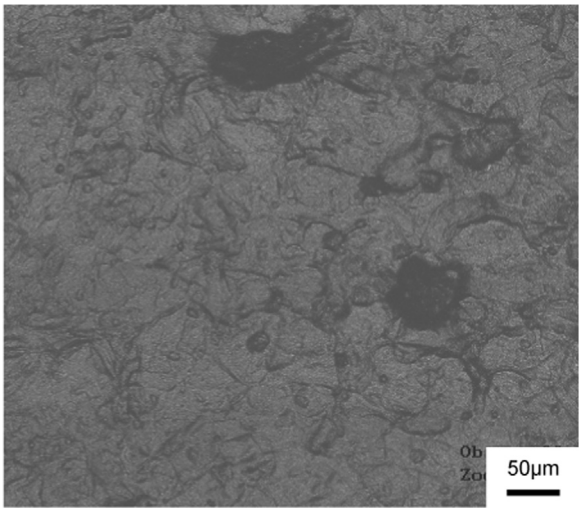

Fig.4. LSCM micrographs of the precipitates.