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Short communication

Mechanical characterization of in vitro-formed short-term salivary pellicle

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ABSTRACT

Human saliva consists of approximately 98% water and a variety of electrolytes and proteins. Those proteins can be selectively adsorbed onto the enamel surface. The cuticular material formed on the enamel surface is termed acquired salivary pellicle (ASP), which is critical for the health of oral mucosa and teeth. The ASP is composed of a inner layer and a outer layer. The lubricating properties of ASP are closely associated with the inner layer. The aim of this research is to characterize the structural and mechanical properties of the inner layer of ASP. In this paper, enamel specimens were immersed for 1 min in human saliva. The ASP formed in vitro within 1 min was studied using a nanoindenter. The results show that the thickness of the inner layer of ASP is approximately 18 nm. Moreover, the inner layer is a heterogeneous pellicle with a gradient in density. From the surface of the inner layer to the enamel surface, the density and mechanical properties gradually increase. The research results may be helpful to extend the understanding of mechanical properties of salivary pellicle and to the oral hygiene industry for diagnose oral diseases.

1. Introduction

Human whole saliva, which consists of approximately 98% water and a variety of electrolytes and proteins, is secreted from salivary glands (Humphrey and Williamson, 2001). Salivary proteins have low solubility, high viscosity, high elasticity, and strong adhesiveness (Humphrey and Williamson, 2001). Moreover, they can selectively adsorb on the human tooth enamel to form an extended and highly-hydrated proteinaceous pellicle. The term acquired salivary pellicle (ASP) is first suggested to describe the cuticular material formed on the enamel surface after eruption by Dawes et al. (1963). An important function of the ASP is to serve as a lubricant between hard (enamel) and soft (mucosal) tissues (Berg et al., 2003; Zhang et al., 2015), which is critical for the health of oral mucosa and teeth (Kaplan and Baum, 1993).

In last decade, some research work has been done to study the physical and mechanical properties of ASP (Dickinson and Mann, 2006; Hannig, 1999; Hanning et al., 2001). Hannig (1999) studied the formation process of salivary pellicle on enamel surfaces for periods of 1 min up to 24 h. The results indicate that the ASP formation is initiated by the adsorption of an electron-dense layer of salivary proteins. A further adsorption of salivary biopolymers lead to the formation of an outer loosely arranged pellicle layer with the thickness of the formed ASP ranging from 10 to 1300 nm (Hannig, 1999). In a research work conducted by Hanning et al. (2001), the enamel specimens were carried intraorally over periods ranging from 10 min to 1 h. The results show that the surface of the adsorbed salivary pellicle is characterized by a dense globular appearance, and the diameter of the globule-like protein aggregates varies between 80 and 200 nm (Hanning et al., 2001). Moreover, a mature pellicle (formation time = 2 h) was examined by an atomic force microscope. The results indicate that the ASP has a dense undulating morphology and is an unexpectedly stiff, viscoelastic solid (Dickinson and Mann, 2006). As expected, the ASP is composed of an inner layer and a loosely arranged outer layer. Moreover, the inner layer is formed within seconds and probably takes a couple of minutes to be completed (Hannig et al., 2004; Vacca Smith and Bowen, 2000). A recent study shows that the outer layer can be easily removed during shear force. As a result, the lubricating properties of ASP primarily depend on the inner layer (Zhang et al., 2015). However, up to now, the microstructure and mechanical properties of the inner layer has not been fully understood.

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The aim of this work was to study the physical and mechanical properties of the inner layer. In this study, enamel specimens were immersed for 1 min in human saliva. Thereafter, the enamel specimens, with and without pellicle, were compared and analyzed using an XP nanoindenter based on the continuous stiffness measurement technique (CSM). Also, the thickness, structure, and mechanical properties of the in vitro-formed short-term salivary pellicle were investigated by exploring various nanoindentation parameters.

2. Materials and methods

2.1. Sample preparation

The study was approved by the Human Research Ethics Committee of Southwest University of Science and Technology. Informed written consent was obtained from all volunteers for the voluntary participation in the study. Fourteen enamel specimens were prepared from freshly extracted human mandibular permanent molars without caries, which were obtained from individuals between 18 and 25 years of age. At first, human teeth were embedded into a steel mold with self-setting plastic, and then were ground and polished to obtain enamel specimens with the exposed occlusal surfaces. The roughness of the polished specimens was measured using a 3D surface profiler based on scanning white-light interferometry (MFT3000, Rtec, USA).

In this study, unstimulated human whole saliva (HWS) specimens were collected from 3 donors with no dental or oral health problems in a convenient, non-invasive manner by passive drool into a small vial (Zhang et al., 2013). To reduce the effect of contaminants from food or beverages on saliva, the specimens were collected in the morning before breakfast, and the donors were required to rinse their mouth with water 10 min before saliva collection (Zhang et al., 2015). Thereafter, the collected saliva specimens were centrifuged for 30 min at 2000*g*, and the supernatant phase of the saliva specimens (centrifuged saliva) was collected for adsorption experiments (Berg et al., 2003). In vitro adsorption experiments were conducted by immersion of the tooth enamel specimens in the centrifuged saliva for 1 min at room temperature. In this paper, the tooth enamel specimens covered with ASP are termed as “TESP”, while the enamel surfaces with no adsorption treatments are referred to as “Control”.

2.2. Nanoindentation tests

Nanoindentation testing was carried out with XP nanoindenter (G200, keysight, USA) by using the continuous stiffness measurement technique (CSM). Fourteen enamel specimens were used in this paper. Firstly, for each specimen, four indents were made on the control surface. Secondly, the control surfaces were conducted by immersion in the saliva for 1 min. And then the enamel specimens covered with ASP (TESP) were used to perform nanoindentation experiments. Consequently, fifty-six indents were made on the control and TESP surface respectively. For indentation, the Berkovich diamond tip with a radius of about 20 nm was employed with the maximum indentation depth of 1000 nm. The thermal drift of the instrument was maintained below 0.05 nm/s during the indentation. All the CSM tests were performed at a constant strain rate (0.05/s) and the chosen frequency of 45 Hz. The applied harmonic displacement was maintained at about 2 nm. Both the diamond tip and the nanoindenter were calibrated using fused silica before measurements.

3. Results

3.1. Harmonic displacement

Harmonic displacement is the amplitude of the harmonic force (Li and Bhushan, 2002), which reaches a stable value when the indentation tip makes a complete contact with the specimen surface (Yang et al., 2009). As shown in Fig. 1, the harmonic displacement of a typical control surface started to level off at approximately 28 nm while the corresponding value for the TESP surface was approximately 46 nm. The indentation depth is termed D_C and D_T respectively. The difference in the indentation depth is associated with the thickness of the ASP (Yang et al., 2009). As shown in Table 1, the average thickness of the ASP was calculated as approximately 18 nm.

3.2. Phase angle

Phase angle is defined as the phase difference between the force and displacement signals (Oliver and Pharr, 2004). The slope of the phase angle curve can be influenced by the chain length and ordering structure of the adsorbed molecules (Chang and Liao, 2008). In order to elucidate the structure of the ASP, five regions were specified to analyze the phase angle of the TESP surface. As shown in Fig. 2, a decrease in the phase angle in the region 1 is caused by the sensitivity of the XP indenter and tip’s radius of curvature. The slope of the curve in the region 2 is greater than that in the region 3, which implies that the density of the salivary protein layer in the region 2 is lower than that in the region 3. Also, the slope of the curve in the region 4 is lower than that in the region 3, which suggests that the density of the salivary protein layer in the region 3 is lower than that in the region 4. In the region 5, the slope of the curve is close to zero signifying that the indentation tip was completely pressed onto the enamel surface. Based on the above analysis, one could infer that the inner layer is a heterogeneous pellicle with a gradient in density. From the surface of the inner layer to the enamel surface, the density gradually increases.

3.3. Hardness and elastic modulus

The hardness and elastic modulus versus indentation depth graph is shown in Fig. 3. It can be found that the hardness and elas-

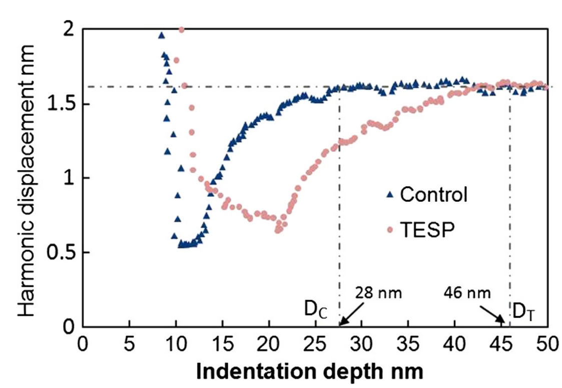

Fig. 1. Typical Harmonic displacement-indentation depth curve. The harmonic displacement of the control surface starts to level off at approximately 28 nm while the corresponding value for the TESP surface is approximately 46 nm. The average thickness of the ASP was calculated as approximately 18 nm, using control surface as the reference.