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On the thickness and nanomechanical properties of salivary pellicle formed on tooth enamel

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ABSTRACT

Objective: To determine the thickness and nanomechanical properties of salivary pellicle formed on tooth enamel.
Methods: In vitro adsorption experiments were conducted by immersing enamel samples in centrifuged saliva for 1 min, and then the nanomechanical properties of the salivary pellicle/tooth enamel system were measured firstly using nanoindentation based on a continuous stiffness measurement technique. Finally, a model was proposed to obtain the thickness and the intrinsic nanohardness of this biofilm.
Results: The composite nanohardness of salivary pellicle/tooth enamel system varied with indentation depth. The model can describe the experimental date at both shallow and deep indentation depths very well. The fitted average thickness of salivary pellicle was about 17 nm, which was in good accord with the scanning probe microscopy experimental results. The intrinsic hardness of salivary pellicle and tooth enamel was about 0.52 Gpa and 4.88 Gpa respectively, which was consistent with previous studies.
Conclusions: It was convenient to extract intrinsic hardness and thickness of salivary pellicle from the indentation curve according to the model. Moreover, this model was applicable to plasticity-dominated behaviour of the soft film/hard substrate system.
Clinical significance: The research results may be helpful to extend the understanding of our lubricating and anti-caries behaviours of salivary pellicle and to the oral hygiene industry for diagnose oral diseases.

1. Introduction

Human whole saliva (HWS) is a complex mixture of fluids secreted by major and minor salivary glands [1,2]. HWS is composed of water (approximately 99%), a variety of electrolytes and proteins [3]. In the oral cavity, these proteins are known to adsorb selectively from saliva to form a highly hydrated proteinaceous layer on mucosa and teeth, which was termed salivary pellicle (SP) [4]. SP is critical for preserving and maintaining the health of oral mucosa and teeth [2]. For example, SP is a special lubricant to reduce the friction and wear between teeth and the surrounding tissue [5]. In addition, SP is thought to protect tooth enamel surface against acid attack [6]. It should be noted that these functions of SP mainly depend on the thickness and mechanical properties of this proteinaceous biofilm on the substrate [7,8]. Consequently, quantifying the thickness and mechanical properties of SP is of great importance to the oral hygiene industry for diagnose oral diseases [9].

Previous studies indicated that the thickness of salivary pellicle can be obtained using null ellipsometry, atomic force microscope (AFM), transmission electron microscope (TEM) et al. [10,11]. Null ellipsometry is based on analyzing changes in the polarization of light upon its reflection at an interface, so the measured surface needs to be planar, smooth and reflecting. Moreover, the biofilm on the surface need to be homogeneous. If the biofilm is inhomogeneous, the average thickness of the film will be obtained unreliable [12]. It is generally accepted that salivary pellicle is a inhomogeneous biofilm consisting of a thin and dense inner layer and a more thicker and diffuse outer layer [13]. Consequently, it is hardly to acquire the actual thickness of salivary pellicle using null ellipsometry. AFM is a powerful technique for high-resolution examination of the surface microstructure and morphology. AFM imaging is based on scanning a surface using a cantilever with a very sharp tip. To make sure that biofilm is without alterations

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caused during the probing, the sample topography can be acquired in non-contact atomic force microscopy mode. Therefore, it is should be note that AFM is an appropriate choice for investigating the topography rather than the thickness of biofilm [14]. TEM is a particularly useful method technique to examine salivary pellicle formation on substrate surfaces. Hannig obtained the ultrastructural and thickness of salivary pellicle by mean of TEM [15]. However, it was hard to obtain high quality data by TEM because the process of sample preparation was complicated and difficult. In addition, it was difficult to obtain the thickness and mechanical properties of SP at the same time using the aforementioned methods. Previous studies indicated that the thickness of salivary pellicle depended on experimental conditions and substrate properties, and varied with the topography of substrate [11,15,16]. Consequently, it is very necessary to acquire the thickness and mechanical properties of SP simultaneously using a reliable and convenient method.

Nanoindentation testing is a technique that determines mechanical properties of a material in the micron or submicron scale. It has been widely used to study biomaterial such as hair, enamel and wood cell walls [17–19]. Moreover, nanoindentation is a useful tool for characterizing mechanical properties of film/substrate system. For example, Dickinson et at [9] used nanoindentation to examine the mechanical properties of SP formed on dental enamel. The results showed that the nanohardness of unstained salivary pellicle ranged from 0.5 to 1.5 Gpa. However, the obtained data was somewhat equivocal because of the unnegligible substrate effect and indentation size effect. The measured data included the combined contributions from both tooth enamel and SP. The nanohardness was the composite nanohardness of film/substrate system rather than the intrinsic nanohardness of SP. Previous studies indicated that in order to obtain the mechanical properties of film, a commonly rule was to limit indentation depth to less than 10% of film thickness [20]. However, the 10% rule did not applicable to SP/tooth enamel system in the current technology because the thickness of SP just ranged from 10 to 200 nm [9,11,15,16]. Consequently, it was necessary to propose a model to describe the mechanical response of SP/tooth enamel system in the process of indentation, and then to extract the intrinsic nanohardness and the thickness of SP from experimental data. However, up to now, almost no research work has been done to propose a model to accurate extract the intrinsic nanohardness and the thickness of SP from the indentation experimental data.

In this paper, the nanomechanical properties of SP were investigated using nanoindentation based on a continuous stiffness measurement technique (CSM). The aim of this paper is to propose a model to extract the thickness and the intrinsic nanohardness of SP form the CSM data simultaneously. The results indicated that the model can describe the experimental date at both shallow and deep indentation depths very well and the fitted intrinsic thickness values of the biofilm was in good accord with the scanning probe microscopy experimental results. The finding of this study will extend the understanding of the nanomechanical properties of SP, and then give valuable insights into the clinical treatments for tooth wear and dental erosion.

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 detailed preparation method of enamel samples was reported in our previous study [8].

Saliva samples were collected following proper collection procedures to help obtain high quality data [21]. According to saliva collection and handling advice [21], unstimulated HWS was collected in glass tubes in the morning on the day of the experiment. In order to avoid the influence of contaminating substances introduced from foods or beverages to saliva, the donor was required to rinse mouth with water 10 min before saliva collection [8]. Furthermore, in order to avoid the influence of contaminating substances introduced from foods or oral cavity to saliva, the collected saliva was centrifuged for 30 min at 2000g and then the supernatant phase of the saliva was collected for adsorption experiments [5]. In vitro adsorption experiments were conducted by immersing the enamel samples in the centrifuged saliva for 1 min at room temperature, and then the enamel samples covered SP were used to do CSM experiments immediately without any treatments.

In order to validate the fitted thickness of SP, an experiment was designed to measure the actual thickness of SP. For this experiment a part of the polished enamel sample was exposed to saliva for 1 min, and then two different areas appeared on the surface of enamel sample. One part was the bare surface without saliva-adsorption treatment, while the other part was the saliva-adsorbed surface. The thickness of the SP was obtained by observing the junction of the two parts using a scanning probe microscopy (SPM) in Nanomechanical Test System (TI 900, Hysitron Corp., USA).

2.2. Nanoindentation test

The polished enamel samples were immersed in the saliva for 1 min at ambient temperature, and an salivary pellicle would be formed on the enamel surface [6]. The morphology of the salivary pellicle was observed with an optical microscope (OM). The mechanical properties of SP formed on tooth enamel were tested by continuous stiffness measurements (CSM) using a nanoindenter (G200, Agilent, USA). A Berkovich indenter with a radius of about 20 nm was used. The maximum indentation depth was 1000 nm and the constant strain rate (0.05 1/s) was applied. The enamel surface without any adsorption treatments was referred to as “original enamel surface”. The mechanical properties of original enamel surface were tested under the same conditions.

2.3. The theoretical model

To obtain the intrinsic hardness of a thin film, the work of indentation (the energy required to produce an defined indentation depth) based hardness model was proposed to analyze the variation of composite hardness with the relative indentation depth [22,23]. According to the model, Korsunsky et al. derived an expression for the composite hardness of coating/substrate system given by [22]:

$$H_c = H_s + \frac{H_F - H_S}{1 + \kappa\beta^2} \quad (1)$$

Where H_c is the composite hardness of coating/substrate system, H_s is the intrinsic hardness of substrate, H_F is the apparent hardness of film,β is termed the relative indentation depth (RID), denotes the indentation depth normalized with respect to the coating thickness. In the plastically-deforming films, κ is a constant and only be weakly dependent on the thickness of coating.

In order to accurate fit the experimental data, an improved model more general than Eq. (1) have been constructed by Tuck