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Exposure to acids changes the proteomic of acquired dentine pellicle

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ARTICLE INFO

Article history:
Received 24 September 2014
Received in revised form
29 January 2015
Accepted 2 February 2015

Keywords:
Acquired pellicle
Dentine
Proteomics
Acidic exposure

ABSTRACT

Objectives: For the first time, this study characterized the proteome of the acquired pellicle formed on human dentine. The changes in this proteome after exposure to lactic or citric acid were also evaluated.

Methods: Volunteers ($n = 9$) wore a mandibular device containing 6 specimens of human root dentine. After the device remained in the volunteers' oral cavities for 10 min or 2 h to allow the formation of the acquired pellicle *in situ*, the specimens were immersed in citric acid (1%, pH 2.5) or lactic acid (0.1 M, pH 4.8) or deionized water for 20 s. In sequence, the pellicle was collected with an electrode filter paper soaked in 3% citric acid. This procedure was repeated for two additional days following a crossover protocol. After harvest, proteins were subjected to reverse phase liquid chromatography coupled to mass spectrometry (nLC–ESI-MS/MS). MS/MS data were processed and submitted to Proteome Discoverer software. Searches were done using SWISS-PROT and TrEMBL databases for human proteins.

Results: In total, 223 distinct proteins were identified in the dentine acquired pellicle in each of the different conditions. Exposure to citric acid dramatically reduced the number of identified proteins. This did not occur for lactic acid. Acid-resistant proteins, such as mucins, were identified after pellicle was exposed to lactic or citric acid.

Conclusions: These proteins could be related to protective effect of tooth homeostasis. Moreover, in the future, they could be candidates to the development of a supplemental therapy for the prevention and treatment of dental caries and dental erosion.

Clinical significance: This study indicates some acid-resistant proteins that could be used in dental products to prevent dental caries and erosion.

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http://dx.doi.org/10.1016/j.jdent.2015.02.001

1. Introduction

The acquired pellicle is an organic, bacteria-free film formed *in vivo* as a consequence of selective adsorption of salivary proteins and glycoproteins to the tooth surface.[1] It also contains, at lower concentrations, other macromolecules such as lipids.[2] The organic components of the pellicle confer to this integument important functions, such as lubrication and protection of the underlying tooth surface.[3,4]

The acquired pellicle has an important role in carious and erosive processes. Its selective permeability reduces the transport of ions into and out of the dental tissues. In addition, it promotes a mechanical barrier by reducing the contact between the tooth surface and acids and modulates bacterial colonization.[3,5] Several studies have evaluated the protective effect of acquired enamel pellicle against enamel erosive demineralization. It seems that pellicles formed at the short-term are able to confer significant protection, with no additional benefits of increased times of pellicle formation.[6–10] The formation of the acquired pellicle on the dentine surface exposed due to gingival recession or lost enamel is of great clinical relevance because, in general, dentine is more susceptible to erosive demineralization than enamel.[11] However, only few studies have evaluated the protective role of the acquired pellicle formed on dentine.[12–14] Due to the different composition of the dentine,[15] it can be assumed that the protein composition of the pellicle formed on dentine might be different from the enamel pellicle. This work hypothesis might have an impact on the protective properties of this acquired dentine pellicle (ADP) against acidic challenges. This study characterized, for the first time, the proteome profile of the ADP. The changes in this proteome profile after exposure to lactic or citric acid were also evaluated to mimic the harsh environment during the initial development of dental caries and dental erosion, respectively.

2. Materials and methods

2.1. Human subjects

The Ethics Committee of Bauru School of Dentistry, University of São Paulo approved the protocol of the present *in situ* study (Protocol 037/2011). Nine volunteers (18–35 year-old female) participated after signing and informed consent. The volunteers did not present any oral or systemic condition that could affect the salivary composition.

2.2. Preparation of the dentine specimens

Freshly extracted caries-free human third molars were selected after careful visual inspection in order to assure they did not present stains or cracks. The roots were scraped with a periodontal curette in order to remove the cement, which was confirmed by visualization of the dentinal tubules at the microscopic level. Teeth were stored in 2% thymol solution at 4 °C for 30 days. One hundred and sixty two dentine specimens (3 mm × 3 mm) were prepared from the buccal surface of the roots, using an ISOMET low speed saw cutting machine (Buehler Ltd., Lake Bluff, IL, USA) with two diamond disks (Extec Corp., Enfield, CT, USA) separated by a 3-mm thickness spacer. The surfaces of the specimens were protected with nail varnish, except for the surface that would be exposed in the oral cavity.

2.3. Custom-made silicon device for in situ assay

Custom-made silicon devices were obtained from the mandibular arches of the volunteers. Six recessed sites (3 mm × 3 mm) were carefully added to each silicon device. In each phase of the study, six dentine specimens were randomly assigned to each of the six sites and fixed with dental wax. After the specimens were fixed on the silicon device, they were subjected to prophylaxis procedure with coarse pumice containing no additives.

2.4. In situ ADP formation and harvest

ADP was allowed to form *in situ* on the dentine specimens in three different days in order to have enough material to be analysed. The same procedures were repeated during these 3 days. Pellicle material was always harvested between 9 and 11 am to avoid circadian effects on salivary composition.[16] Each day, acquired pellicle was allowed to form on the dentine specimens initially for a 10-min period. During this time, volunteers refrained from consuming food or beverages other than water. After 10-min ADP formation, three of six dentine specimens were taken out of the device and immersed in 1 mL of deionized water, or 1% citric acid (pH 2.5; Merck KGaA, Damstadt, Germany) or 0.1 M lactic acid (pH 4.8; Synth, Diadema, São Paulo, Brazil) for 20 s to simulate erosive ($pH < 4.5$)[17] or carious challenge ($4.5 < pH < 5.5$)[18]. This pellicle represents the initial stage of pellicle formation, where precursor proteins with high affinity for hydroxyapatite are found.[19]

The remaining three specimens on the silicon device were reinserted into the mouth and ADP was allowed to form over a 2-h period, in order that mature pellicle could be evaluated.[20] The three specimens were subjected to the same demineralization challenge as described above for 10-min ADP formation. For both periods, pellicle material was harvested after washed with deionized water and dried as previously described.[21] The harvested material was placed into a 2.0 mL polypropylene microcentrifuge tube and stored at −80 °C.

2.5. ADP harvest proteins extraction

Before starting the extraction of the ADP proteins, 27 collection strips corresponding to the same treatment and time of collection (9 volunteers × 3 days of collection) were pooled in one 1.5 mL polypropylene microcentrifuge tube, in order to have enough material to be analysed.

To extract ADP proteins from the collection strips, 400 μL of a solution containing 4 M urea, 10 mM DTT and 50 mM ammonium bicarbonate, pH 7.8, was added to each polypropylene microcentrifuge tube (that contained 27 collection strips) and incubated for 1 h at room temperature. After this, 300 μL of 50 mM ammonium bicarbonate (pH 7.8) was added and samples were sonicated for 2 min. The recovered solution