Atomic force microscopy study of salivary pellicles formed on enamel and glass in vivo

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Abstract This study was performed to evaluate the use of atomic force microscopy (AFM) in examining the surface of the adsorbed layer of salivary proteins (salivary pellicle) formed in vivo on dental enamel and glass surfaces. Enamel and glass test pieces were attached to the buccal surfaces of the upper first molar teeth in two adults using removable intraoral splints. The splints were carried intraorally over periods ranging from 10 min to 1 h. Using the contact mode of AFM, pellicle structures could be recognised on intraorally exposed specimens compared to nonexposed enamel and glass surfaces. The surface of the adsorbed salivary pellicle was characterised by a dense globular appearance. The diameter of the globule-like protein aggregates adsorbed onto enamel and glass varied between 80 and 200 nm and 80 and 150 nm, respectively. The structure of the adsorbed protein layer was clearly visible on glass surfaces, even though minor differences in the protein layer between glass and enamel specimens were observed. This study indicates that AFM is a powerful tool for high-resolution examination of the salivary pellicle surface structure in its native (hydrated) state. AFM avoids artefacts due to fixing, dehydration and sputter-coating which occur with scanning electron microscopic analyses.

Key words Atomic force microscopy · Salivary pellicle · Protein adsorption · Enamel · Glass surface

Introduction

Water-soluble biopolymers have a great impact on numerous areas of applied medical science, such as bioengineering, implant research, biocompatibility and bioadhesion processes. Cellular adherence, bacteria adhesion and adsorption of protein molecules at solid–liquid interfaces are largely influenced by the structure and physicochemical properties of the different surfaces involved. In particular, the formation of protein layers on artificial and biological surfaces is essential for biocompatibility and for the protection of functional interfaces in almost any living system. Within the oral cavity all natural and artificial solid surfaces exposed to saliva become coated with a layer of proteins and related biopolymers, described as an acquired salivary pellicle [1–3]. Pellicle formation is the result of salivary protein adsorption at the tooth–saliva interface [1–5]. A number of salivary components have been identified to adsorb onto the enamel surface, for example, phosphoproteins, acidic proline-rich proteins, salivary α-amylase, immunoglobulins, mucins, lipids and glycolipids [1, 4–8]. The pellicle layer plays a critical role in determining the biocompatibility of any solid materials placed in contact with saliva. Ultrastructural studies of the pellicle layer on different solid surfaces could contribute to a better understanding of the pellicle formation and mechanisms involved. Many reports deal with the ultrastructural appearance of the in vivo formed pellicle on enamel surfaces using scanning electron microscopy (SEM) or transmission electron microscopy (TEM) [9–11]. This study was performed to evaluate the use of atomic force
microscopy (AFM) in examining the surface of the salivary pellicle formed in vivo on dental enamel and glass surfaces.

Materials and methods

Enamel specimens with a surface area of about $2 \times 2$ mm were cut from labial surfaces of freshly extracted bovine incisors. The surfaces of the enamel specimens were subjected to uniform wet-grinding and polishing with abrasive paper using grit sizes down to 4,000. The specimens were disinfected using 70% alcohol. Glass specimens with a surface area of about $2 \times 2$ mm were cut from commercially available glass cover slices. Intraoral exposure of specimens took place by use of removable acrylic appliances (minisplints, for details see Refs. [9, 12]). Specimens were fixed to the splints at the buccal aspect of the maxillary first molars (Fig. 1). The splints with fixed specimens were exposed to the oral environment in two subjects and carried over periods of 10, 30 and 60 min. During intraoral exposure, the consumption of food and beverages as well as any measure of oral hygiene were not allowed. Intraoral exposure of the enamel and glass specimens was repeated twice by the subjects. After intraoral exposure the specimens were removed from the acrylic splints, rinsed in distilled water and immediately mounted in the AFM sample holder. Measurements were performed using an Autoprobe CP including a 5-µm scanner (Thermo Microscopes, Sunnyvale, Calif.) operated in contact mode. Imaging was carried out using triangularly shaped silicon nitride cantilevers containing pyramidal tips (Microlever ML06F, Thermo Microscopes, Sunnyvale, Calif.). Images were taken in constant-force mode with typical scan rates and forces of 10 Hz and 17.5 nN, respectively. For reference purposes, enamel and glass specimens not exposed in the oral cavity were investigated by AFM under exactly the same conditions. Analysis of the AFM images were performed using the Park Scientific Instruments software package supplied with the AFM instrument.

Fig. 1 Schematic drawing of the upper jaw showing the acrylic appliance (hatched areas) with test pieces fixed at the left buccal site of the maxillary first molar teeth.

Fig. 2a-d Characteristic surface pattern of polished enamel specimen. a Reference surface, not exposed to the oral environment. The individual enamel crystallites measuring approximately 30-90 nm. The enamel crystallites of samples exposed for b 10 min, c 30 min and d 60 min towards the oral environment are masked owing to salivary protein adsorption (pellicle formation).