EARLY DENTAL PLAQUE FORMATION INVESTIGATED USING SURFACE PLASMON RESONANCE (SPR)

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The complex formation of the dental plaque is of vital importance in dentistry. Understanding this phenomenon could offer precious insight to clinical diagnosis and prognosis, increasing the predictability and success of dental treatments, and decreasing the incidence of dental affections. Surface plasmon resonance (SPR) offers a new perspective, that enables the direct observations on live interaction processes, using specific cells harvested from dental patients. The formation of the acquired protein pellicle, that coats all types of intraoral surfaces, could be observed with the help of SPR for the human oral micro-scale samples.

Keywords: dental plaque, *Streptococcus mutans*, *Escherichia coli*, hydroxyapatite, surface plasmon resonance

1. Introduction

Due to the increasing concern regarding oral health, a large array of investigations based on physical-chemical techniques exploring dental materials and processes have been developed in the last decade [1-3].

Clinical observations and evidence-based decisions are the modern golden standard in dentistry. Furthermore, the possibility to follow and understand *in vivo* interactions between oral proteins and cells opens new possibilities of refining and increasing the potency of oral drugs, further reducing the risk of dental affections.

The present paper is focused on investigating a major step in oral dental plaque formation, with the help of a Surface Plasmon Resonance (SPR) analysis. One of the main SPR applications is viewing live interaction processes between cells and specific receptors [4]. For this reason, we have investigated the primary interaction between the dental acquired protein layer and primary *Streptococcus*

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mutans (S. mutans) colonizer, by comparing its interaction pattern to *Escherichia coli* (*E.coli),* a theoretical periodontal passenger. Oral plaque has a very complex bio structure[5] with multipart layers. Its formation starts with the adherence of certain protein families to the tooth outer layer, made of hydroxyapatite. These proteins, mucins in their majority, form the acquired oral pellicle [6]. This structure has a dual role in the oral environment: it protects the dental hard surfaces from all types of harmful oral agents, but, at the same time, it offers a cellular scaffold, promoting microorganism adhesion. The first colonizers are the oral Streptococci. From this large family, *Streptococcus mutans* is one of the first pellicle colonizers and main microorganism involved in dental cavities formation [7]. It is a gram positive, facultatively anaerobic oral microorganism that metabolizes sucrose to lactic acid, decreasing oral pH levels. It adheres to the acquired oral pellicle, thus colonizing the dental structure [8]. By comparison, *E. coli* is a gram negative, facultatively anaerobic bacteria incriminated in severe cases of food poisoning [9], being a model bacteria for testing materials as well [10]. Despite the fact that its interaction to the acquired pellicle was investigated with various techniques [11, 12], there still is a dire need for more research in this domain.

2. Materials and method

The SPR system used was the Plasmonic® system (Wallenfels, Germany). It uses glass prisms deposited with 50μm of gold on the upper surface (Fig. 1). This prism is irradiated with a laser beam from the backside, determining the formation of an evanescent field (meaning a field which tends to vanish), formed by the electrons on the outer layers of the gold atoms. Within this field, of approx. 300nm thickness, bio-interactions can be conducted and observed (Fig. 2).

Fig. 1 Photographic image of glass prism with individual measuring channels and hydroxyapatite deposition

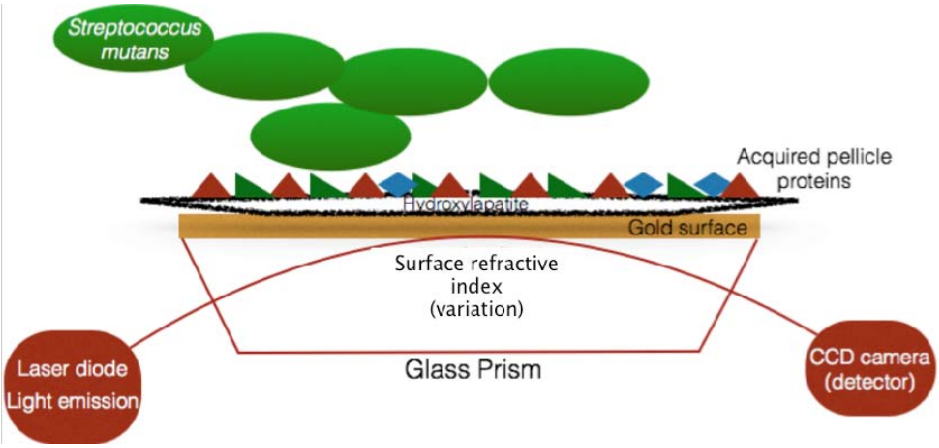

Fig. 2 The use of Surface Plasmon Resonance(SPR) technique to evidence the interaction of dental plaque with bacteria

In order to create a model of the intraoral environment, we have deposited a thin layer of hydroxyapatite on the gold prisms of the Plasmonic system, as described by Akira Monkawa et al. [13]. This layer was investigated using AFM to confirm surface aspect.

Bacterial cells used for experiments were *S. mutans* and *E. coli*. *S. mutans* strain 5DC8 type containing serotypes c and d. They were grown on an infusion broth and incubated at 37^0C in a CO_2 environment. *E.coli* was grown on Luria-Bertani broth and incubated at 37^0C.

Natural saliva was gathered from dental patients and prepared by centrifugation at10.000 rot./10 min., and then filtered through 0.2µm filters to obtain a sterile solution which was placed into cold storage at 4^0C. All cells used for experiments were resuspended in filtered natural saliva 24h prior to experimental steps.

The SPR technique has a high degree of automatization, enabling a predefined program by which every experiment is conducted. The following steps should be performed:

1. Baseline acquisition with 20µL double distilled water
2. Removal of 15µL and addition of 15µL of natural filtered saliva
3. Waiting for 30min to observe acquired pellicle formation
4. Washing sequence to remove unbound material with 15µL distilled water (repeated three times)
5. Baseline assertion
6. Removal of 15µL and addition of 15µL of bacteria suspended in filtered natural saliva
7. Waiting for 2 hours

8. Final washing steps with distilled water (repeated three times)
9. Baseline assertion

3. Results and discussion

Fig. 3 shows the morphology and roughness aspect of dense hydroxyapatite layer on the glass-gold plasmonic SPR-chips. A relative uniform and dense layer may be observed by these 2D and 3D AFM images.

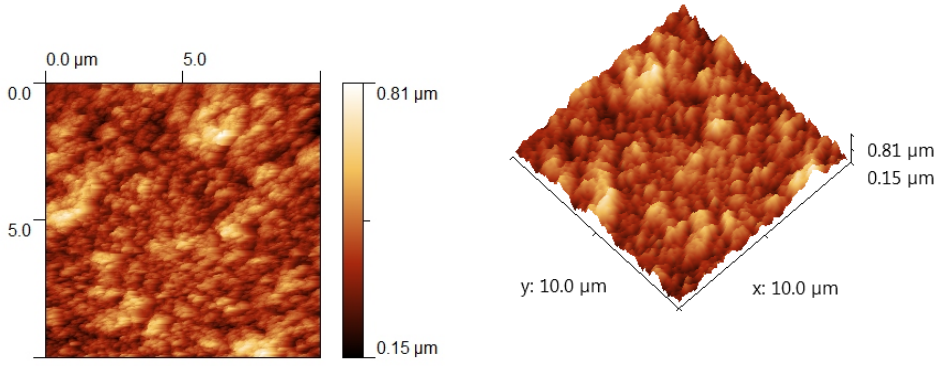

Fig. 3 AFM images confirming the deposition of a dense hydroxyapatite layer on the glass-gold plasmonic SPR-chips

3.1 Acquired pellicle formation and *S. mutans* interaction

Fig. 4 represents a complex SPR experiment. The first association curve represents a rapid binding of salivary proteins MG1 multimerizing protein and MG2 non-multimerizing protein to the hydroxyapatite fabricated surface. Furthermore, the binding and coating process has been almost instantaneous. The formation of the acquired dental pellicle is a complex process. It was shown that proline rich acidic proteins realize primary interaction [14]. They adhere to specific structures on hydroxyapatite. After this initial step, mucins families represented by MG 1 salivary mucin, with higher mass than 10^6 Da, and MG 2 salivary mucin, with lower values of $1.2\text{-}1.5 \times 10^5$ Da, form a dense layer [15]. MG2 is actively involved in peering and adhesive processes with *S. mutans* [16]. After 30 minutes of pellicle build-up, the washing cycles removed all un-bound solution proteins and removed sediment constituents from the adhered layer.

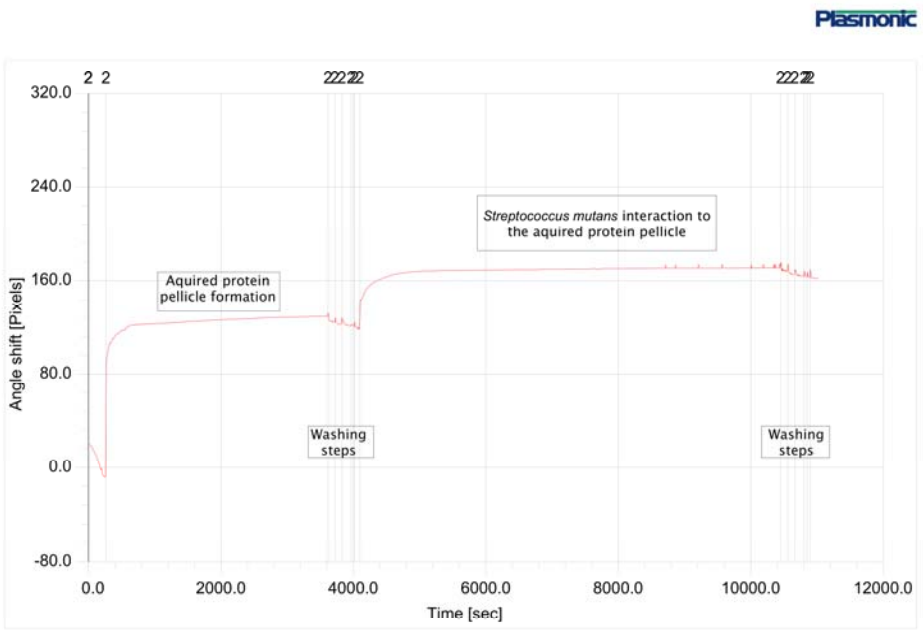

Fig. 4 Aquired pellicle formation and strong *S. mutans* interaction experiment showing initial salivary pellicle association curve, washing steps, secondary bacterial association curve and final washing cycle

The second association curve is represented by the slow process of cell interaction with the surface. The complex cell adhesion process is initially represented by weak Van der Waals interactions that allow further interaction through specific receptors. The interaction of live bacteria onto the surface was accompanied by a new association curve, representing initial phases of cellular adhesion. After two hours of experiment time and three washing steps, the remnant signal registered was high, corresponding to important cell-to-surface interaction. The presence and colonization of the dental structure with *S.mutans* could clinically lead to a carious lesion. The importance of understanding the conditions leading to this adhesion curve is essential. The possibility to follow live cellular interaction is a great advantage, providing a further testing bench for different commercially available antibacterial substances and their interaction to the above discussed process.

3.2 Acquired pellicle formation and *E.coli* interaction

The SPR experiment represented an initial high association curve, corresponding to acquired pellicle formation, subsequent wash cycle to remove

unbound constituents, introduction of bacteria onto the surface, and final washing steps to remove all unbound constituents. The acquired pellicle construct has been formed in a similar way, further enforcing the experimental reproducibility offered by the high automatization of the Plasmonic SPR device. The first association curve corresponds to initial pellicle formation, and interaction with hydroxyapatite chip coating. Furthermore, the formed protein construct showed a high association curve, showing the high degree of salivary mucin interaction, even after the washing procedure. Fig. 5 shows the introduction of *E.coli* bacteria suspended in natural saliva, a step that has determined the absence of an association curve showing no surface interaction.

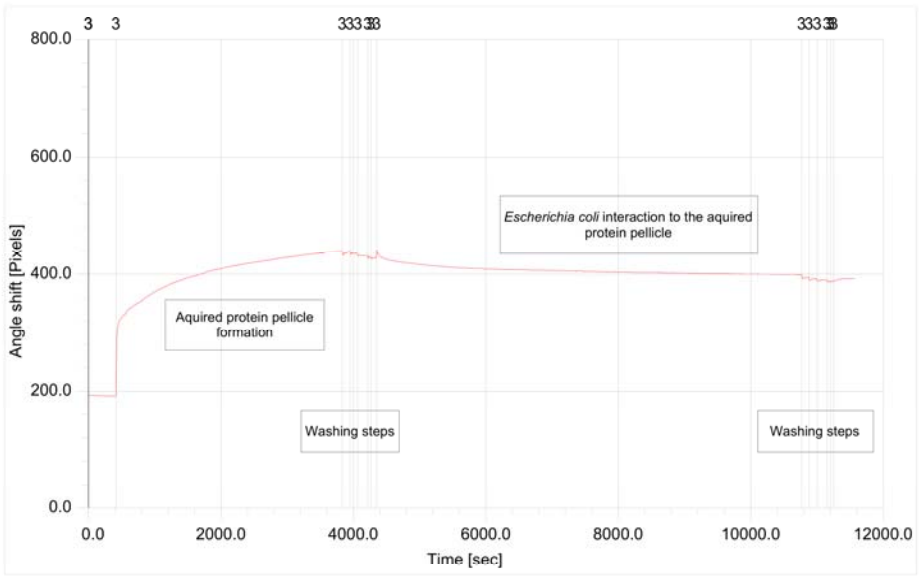

Fig. 5 Acquired pellicle formation and *E. coli* interaction experiment represented by initial salivary association curve, washing steps, bacteria interaction, and final washing

Even throughout the total two hour experimental time, there was no cellular interaction to be observed. This important result further supports the present theories that *E.coli* bacteria is only a passenger periodontal pathogen with no direct interaction to primary dental plaque construction [17]. Also, it has represented a negative experiment to the adhesion of *S. mutans,* showing that it had no specific interaction with acquired pellicle constituents.

4. Conclusion

SPR technique has proved a valuable technique in monitoring the interaction between the main bacteria incriminated in dental caries and acquired protein pellicle. Further research is required to take full advantage of such a complex testing platform. According to our experimental results, *S.mutans* bacteria have a strong interaction with dental plaque, because after two hours of experiment time and three washing steps, the signal remained still high, corresponding to important cell-to-surface interaction. On the contrary, *E.coli* has proved to be a passenger periodontal pathogen, showing no interaction to the protein pellicle. The fact that it lacks specific binding organelles decreases the chance of its presence in the early stages of plaque formation. Because the oral dental plaque is an intricate co-aggregation between different cells, its exact formation being still incompletely understood, we consider the SPR technique to be a valuable tool, offering a new perspective on complex oral processes.

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