

Acquired Enamel Pellicle (AEP) - A Film with New Futuristic, Diagnostic and Therapeutic Perspectives.

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Review Article

Received: 19/08/2014
Revised : 13/09/2014
Accepted: 20/09/2014

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Keywords: Acquired Enamel Pellicle (AEP), Periodontal Disease.

ABSTRACT

The acquired enamel pellicle (AEP) is a thin acellular film that forms on tooth surfaces upon exposure to the oral environment. A wide range of techniques have been used to characterize the morphology and ultrastructure of the AEP. The knowledge of the 3D structure and composition of the AEP, along with an understanding of its functions, could lead to the development of new therapeutic options for the treatment and prevention of numerous oral diseases, such as periodontal disease.

INTRODUCTION

The initial stage of acquired enamel pellicle formation is triggered within seconds of exposure to whole saliva and is characterized by an increase in pellicle thickness within a few minutes that remains stable for about 30 minutes. The acquired enamel pellicle (AEP) is a thin acellular film that forms on tooth surfaces upon exposure to the oral environment. It consists predominantly of salivary proteins, but also includes non-salivary-derived proteins, carbohydrates, and lipids. Since it is the interface between teeth and the oral environment, the AEP plays a key role in the maintenance of oral health by regulating processes including lubrication, demineralization, and remineralization and shaping the composition of early microbial flora adhering to tooth surfaces.

Attempts to characterize the morphology and ultrastructure of the AEP used a wide range of techniques, including scanning electron microscopy, transmission electron microscopy ^[1], field emission in-lens scanning electron microscopy, cryo- electron microscopy, confocal laser scanning microscopy, and ellipsometry. More recent microscopy techniques include multiphoton modulation *microscopy*, optical coherence tomography, and optical coherence *microscopy*.



Origin of major constituents of the acquired enamel pellicle. (top, left) Proteins/peptides originating from salivary glands (major AEP constituents in protein/peptide abundance); (top, right) proteins/peptides from micro-organisms; (bottom, left) proteins/peptides originating from oral mucosa; and (bottom, right) proteins/peptides originating from gingival crevicular fluid.

Gingival inflammation

The Acquired Enamel Pellicle of individuals with gingival inflammation, in comparison with that of healthy individuals, presents

- A 2 to 10 times higher amount of total pellicle proteins
- Increased lactoferrin abundance, and
- Increase in plasma proteins on the incisal portion of teeth.

Gingival inflammation results in an increased flow of gingival crevicular fluid, altering the normal balance of local biopolymers, and thus changing the composition of the AEP [2].

Several potential biomarkers for periodontal disease have been identified within saliva and in gingival crevicular fluid from which many components of the AEP derive, so it is reasonable to expect that changes in protein concentration in these fluids may be reflected in the AEP composition.

With respect to the AEP in particular, more than 10% of the proteins identified have been associated with inflammatory responses and may serve as markers for oral inflammatory disease such as periodontal disease. Furthermore, elevated levels of transaminases have been found in the saliva of patients with periodontal disease. And this transaminase has been detected within the *in situ* pellicle. Present evidence supports AEP proteome analyses as a useful tool for assessing the diagnosis of oral inflammatory diseases and monitoring responses to therapeutic interventions [3].

When comparing the *in vitro* pellicles formed from unstimulated whole saliva in caries-free and caries-susceptible individuals higher levels of

Caries - Free Group

- A Proline Rich Protein1,
- Proline Rich Protein3 ,
- Lipocalcin,
- Cystatins-SN , S
- Histatin-1,
- Statherin

Caries - Susceptible Groups

- Increased concentrations of amylase,
- Immunoglobulin A,
- Lactoferrin.

Further research is necessary to elucidate the mechanism by which differences in AEP composition occur, it is hypothesized that there are differences in proteolytic capacities of whole saliva in caries-susceptible and caries-resistant individuals because of similar levels of proline rich protein1 (PRP1) levels within parotid and submandibular secretions of caries-susceptible and caries-resistant groups.

Oral Diagnostics

Due to limitations on the amount of material that can be harvested the potential use of the Acquired enamel pellicle (AEP) in salivary diagnostics has been overlooked. However, with improvements to AEP collection techniques and the analytical tools available, it is now possible to determine the AEP composition of a single person or tooth. The AEP shows considerable inter-individual consistency, as demonstrated by studies of its amino acid and protein composition which makes the compilation of AEP "fingerprints" for healthy and disease states, such as caries or periodontal disease, a potentially valuable diagnostic tool [4].

Surface modification

The strategy of surface modification involves altering the tooth surface or the salivary pellicle to impede bacterial colonization. It is well-known that the salivary pellicle provides binding sites for the oral bacteria through a complex array of specific and non-specific binding mechanisms. Thus, the composition of the pellicle may modulate bacterial adhesion events by changing the surface characteristics by manipulating the protein film on the enamel, thereby reducing bacterial adhesion. Several routes of surface modification have been investigated. Functional groups like phosphate and phosphonate may be used to anchor water-soluble, protein-repelling substances to the mineral surface. It has been shown *in vitro* that the combination of an alkylphosphate and a non-ionic surfactant alters the surface characteristics of the tooth, making it less attractive for micro-organisms. Unfortunately, because of difficulties in securing persistent coating with the active component the clinical efficacy of such coating agents has been low. If these problems are resolved, a future approach to reduce colonization by coating the tooth surface with agents that interfere with two-component signal transduction in oral micro-organisms can be done.

Future Perspectives—Therapeutic Potential

Knowledge of the 3D structure and composition of the AEP, along with an understanding of its functions, could lead to the development of new therapeutic options for the treatment and prevention of numerous oral diseases, such as periodontal disease, dental caries, dental erosions.

A possible path for the modification of the AEP has been achieved *via* enzyme-containing toothpastes. Commercially available enzymatic toothpastes were shown to increase glucosidase and peroxidase activity in *in situ* pellicle. Whether these immobilized enzymes provide protection *in vivo* is unclear. Moreover, other vehicles assumed to deliver protective enzymes within the pellicle, such as mouthrinses, have had limited success, possibly because of pH or saturation issues ^[5].

Incorporation of protective non-enzymatic AEP proteins and/or peptides into mouthrinses should be considered. Histatins are subject to a rapid and extensive proteolytic degradation within the oral cavity, however, both intact and fragmented forms, exhibit antimicrobial, anti-fungal, and wound healing properties. Recently, the adsorption of histatin 1 onto hydroxyapatite provided resistance against its proteolytic degradation.

Other phosphoproteins with high affinity to hydroxyapatite, such as statherin and aPRPs, have also demonstrated a significant resistance against proteolytic degradation when adsorbed onto hydroxyapatite ^[6]. In contrast, histatin 2 (or His 18/32), a degradation product from histatin 1 identified in the AEP, has been demonstrated to enhance epithelial and fibroblast migration *in vitro*, suggesting its importance in early wound healing without exhibiting cytotoxic, pro-inflammatory, or pro-fibrotic responses. The role they play in the healing process of periodontal tissue requires further investigation.

It is reasonable to imagine, in the near future, the incorporation of synthetic proteins/phosphoproteins, with biological functions similar to those of histatin 2, into mouthrinses or toothpaste.

The first step in the initiation of periodontal disease is the colonization of tooth surfaces by microbial flora. One area of research focusing on the prevention of these diseases is *anti-adhesion therapy*. *Lectins* are a heterogeneous group of carbohydrate-binding proteins, several of which have been shown to be capable of binding to sugars in the pellicle with various intensities and patterns. Glucose/mannose-specific lectins were most effective in reducing several strains of *S. mutans* adhesion to hydroxyapatite *in vitro*. Another area of anti-adhesion therapy is *probiotic* bacterial strains, which are available in commercial products that can alter pellicle composition and have been shown to inhibit adhesion of *S. mutans* and *S. gordonii*. Dietary proteins (e.g., casein) can potentially amplify the biological function of specific or multiple AEP components.

The additive or synergistic reduction of bacterial adhesion by these combination therapies needs to be investigated. Moreover, toxicity studies of modifiers, such as lectins and casein, and their potential impact on pellicle function are required.

Protein Precipitants

Formaldehyde and glutaraldehydes precipitate salivary proteins in dentinal tubules, can be used to manage dentinal hypersensitivity.

Lactic acid bacteria are known to have a wide range of effects on the immune system. They may have general immune-enhancing effects, which include augmentation of phagocytic function, i.e., neutrophils, monocytes, macrophages, and natural killer cells. Specific immune responses, both humoral and cellular, can also be enhanced by lactobacilli. Perhaps some of the modulation of the inflammatory response may be more related to regulating or modulating the immune system.

The most commonly used probiotic bacterial strains belong to the genera *Lactobacillus* and *Bifidobacteriu*. Both lactobacilli and bifidobacteria can be found in breast milk, suggesting early exposure of the oral cavity to these bacteria. Bifidobacterial species isolated from oral samples include *B. bifidum*, *B. dentium*, and *B. Longum*.

Various clinical conditions

Halitosis results from the action of anaerobic bacteria that degrade salivary and food proteins to generate amino acids, which are in turn transformed into volatile sulphur compounds.

The capacity of lactobacilli to inhibit the growth of periodontopathogens, including *P. gingivalis*, *Prevotella intermedia* and *A. actinomycetem comitans* have been studied. Together, these observations suggest that lactobacilli residing in the oral cavity could play a role in the oral ecological balance. The beneficial effect of *L.reuteri* against gingivitis was assessed. Probiotic strains included in periodontal dressings at optimal concentrations of 10⁸ CFU ml were shown to diminish periodontal pathogens.

Replacement therapy

A possible future approach is to use *genetically modified micro-organisms* to deliver tailored molecules that could interfere with adaptive pathways such as two-component signal transduction or quorum-sensing systems. However, one cannot exclude the possibility that a genetically modified replacement strain might later undergo transformation in oral biofilms and then become an opportunistic pathogenic strain.

Immunization

Immunization against oral diseases—particularly periodontal disease—has been a central research topic in recent decades. The aim is to inhibit adhesion or reduce the virulence of putative microbial etiologic agents targets. Micro-organisms could, for instance, be cleared from the oral cavity by antibodies prior to colonization. Efforts have been made to immunize both actively and passively. In active immunization, an antigen which will elicit a protective immune response is administered. In passive immunization, the antibody itself is administered. Animal studies using either active or passive immunization approaches have been successful. There are also data to show that passive immunization in humans impedes recolonization of selected target micro-organisms in periodontal disease [7].

However, no vaccination scheme in humans is yet clinically applicable. The question that still needs to be resolved is whether the systemic or the mucosal immune system needs to be stimulated. A vaccine against periodontal disease should probably involve both systemic and mucosal immunity. Periodontal disease being ecologically driven multi-microbial in origin immunization approaches face a major problem as they are generally directed against single bacterial species epitopes.. Furthermore, since micro-organisms have the ability to form biofilms and to adapt and undergo transformation that may lead to altered antigenicity, it is questionable whether immunization will provide lasting protection.

Conclusion

Our knowledge of its composition has undergone a rapid expansion due to the application of novel techniques. The balance between AEP composition and structure modulates the complex nature and biological function of this unique interface between the teeth and the oral environment. Moreover, the comprehension of the AEP can enhance our understanding of oral health and can lead to innovative therapeutic approaches regarding the diagnosis, prevention, and treatment of specific oral pathologies

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