ORIGINAL RESEARCH PAPER VOLUME-6 | ISSUE-8 | AUGUST - 2017 | ISSN No 2277 - 8179 | IF : 4.176 | IC Value : 78.46

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

SURFACE DENTAL PELLICLE FORMATION INDUCED BY DIETARY SOURCES ENHANCED BY Streptococcus mutans- AN in vitro STUDY

Dental Science

Mani Sankari
Kumaravadivel

ABSTRACT

Dental pellicle formation on the human tooth surface is a physiological process which occurs every day. It is an organic biofilm and protects the enamel of the tooth from deminerlizaton. Food accumulation over the pellicle results in the formation of plaque. This on further calcification by the salivary phosphates leads to the formation of calculus. Hygienic oral practices eliminate the plaque accumulation, preventing calculus formation. Elevated microbial activity and lack of oral hygiene tend to increase the risk for dental caries.

KEYWORDS:

dental pellicle, dental caries, Streptococcus mutans, rice, wheat, multipurpose flour

INTRODUCTION:

Dental pellicle is defined as the acellular layer of adsorbed salivary proteins and other macromolecules on the dental enamel surface approximately 10 micrometers thick (1). Dental pellicle is assumed to consist mainly of' glycoproteins; studies of' experimental acquired pellicles have moreover demonstrated the presence of specific proteins such as immunoglobulins and lysozyme (2). Also, a salivary protein agglutinating bacteria has been reported to possess high affinity for powdered tooth enamel (3) and may be present in the pellicle. Agglutinating factors (3, 4) and specific antibodies (5, 6) are known to act on strains of Streptococcus, the numerically dominant genus in young plaque (7). Dental pellicle formation on the human tooth surface is a physiological process which occurs every day. It is an organic biofilm and protects the enamel of the tooth from deminerlizaton. Food accumulation over the pellicle results in the formation of plaque. This on further calcification by the salivary phosphates leads to the formation of calculus. Hygienic oral practices eliminate the plaque accumulation, preventing calculus formation. Elevated microbial activity and lack of oral hygiene tend to increase the risk for dental caries.

MATERIALS & METHODS:

Artificial saliva, containing the components listed in table 1 was prepared. The pH of artificial saliva was 6.4. Extracts from boiled rice, wheat and muti-purpose flour flour (multipurpose flour), were autoclaved. Glass slides, petri-dishes and glass rods were sterilised in the hot air oven. Glass slide was placed on the glass rod inside the petri-dish in a tilted manner and then extracts of each food sample, diluted with saliva was incorporated into separate petri-dish. 1ml of each food extract was diluted with 5ml of saliva. The petri-dishes were closed and left undisturbed for 6 hours and then observed for pellicle formation.

Pellicle formation was observed on glass slides after proper sterilization. Extracts from rice, wheat and muti-purpose flour were obtained and autoclaved. Glass slides were placed inside petri-dishes and the food extracts were added inside each petri-dish separately. After about 6 hours, the galss slides were taken out of the petri-dish and observed for pellicle formation.

The same process was repeated again with 12.5ml saliva and 2.5ml food sample in disposable containers. Colonies of *Streptococcus mutans* were then inoculated into the container under sterile conditions. The containers were placed on a tray, closed and another tray was placed over the tray and both were sealed by parafilm with a small opening for air circulation. The slides were left undisturbed for 72 hours. The slides were taken after 72 hours, wiped and Gram's staining was done. The slides were then focused under 100x of light microscope.

Table 1: Composition of artificial saliva

INGREDIENTS	CONCENTRATION/LITRE
Sodium bicarbonate	0.50g
Sodium phosphate, dibasic, dehydrate	0.85g
Calcium chloride	0.44g
Magnesium chloride	0.06g
Potassium chloride	1.40g
Sodium carboxyl & methyl cellulose	2.00g
Phosphoric acid to adjust pH 6.4, distilled water	QS

RESULTS:

In the first set of slides without bacteria, the amount of pellicle formation was highest on the slide with muti-purpose flour extract, followed by rice and wheat. The following images are photographs of slides containing pellicle films that were examined after 6 hours, before the inoculation of microorganism.

IMAGE 1: MUTI-PURPOSE FLOUR

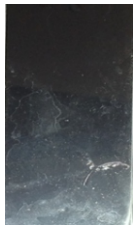


IMAGE 2: RICE

IMAGE 3: WHEAT

Images 1, 2, 3: pictures of slides that was obtained after 6 hours of remaining in the food extract and artificial saliva. The white outlines are the extents of pellicle formation of each food extract.

The amount of pellicle formation on each glass slide varied with the dietary source and with the organism after inoculation. The slide with rice extract sample had the maximum amount of attachment followed by wheat and then muti-purpose flour even after the inoculation of the organisms. Following are the pictures of microscopically examined, gram-stained slides.

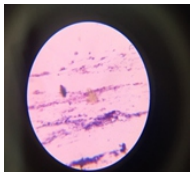


IMAGE 4: RICE

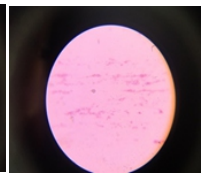


IMAGE 5: MUTI-PURPOSE FLOUR

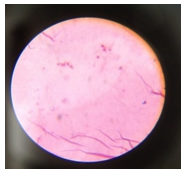


IMAGE 6: WHEAT

IMAGES 4, 5, 6: Microscopic pictures of Gram-stained slides that contained the food extracts along with ***Streptococcus mutans.*** *The bands and streaks represent the amount of pellicle that was formed along with the organism.*

DISCUSSION:

The surface of enamel is charged negatively in the normal oral pH range, and is due to the structure of hydroxyapatite, in which phosphate groups are arranged close to the surface of the enamel. Thus positively-charged ions like calcium are attracted to the enamel surface forming a hydration layer with unevenly distributed charges. As calcium is the

predominant ion in the hydration layer, the resulting net charge of the hydration layer is positive. Thus, the hydration layer will attract negatively charged macromolecules with acidic side chains and end groups of phosphate or sulfate. The amino acids like aspartate and glutamate, which are negatively charged at physiological pH, also have a high affinity for the positively charged hydration layer of the tooth surface.

The exact composition of the hydration layer is dependent on several factors including pH, ionic strength and the types of ions present in the saliva; usually the hydration layer contains mainly calcium and phosphate ions in the ratio 10:1. Other ions present include sodium, potassium and chloride. The adsorption of the first molecule layer on a clean tooth surface is immediate. The initial rate of formation of the dental pellicle is fast during the first hour, after which it decreases. Rate of formation of the pellicle varies in individuals due to differences in salivary flow and composition (1).

Gram staining method, the most important procedure in Microbiology, was developed by Danish physician Hans Christian Gram in 1884 (8). This differential staining procedure separates most bacteria into two groups on the basis of cell wall composition:

1. Gram positive bacteria (thick layer of peptidoglycan-90% of cell wall)- stains purple
2. Gram negative bacteria (thin layer of peptidoglycan-10% of cell wall and high lipid content) –stains red/pink

The differences in cell wall composition of Gram-positive and Gram-negative bacteria accounts for the Gram staining differences. Gram-positive cell wall contain thick layer of peptidoglycan with numerous teichoic acid cross linking which resists the decolorization(8).

IMAGE 7: Principle of gram staining

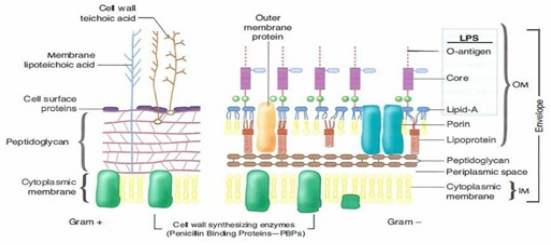
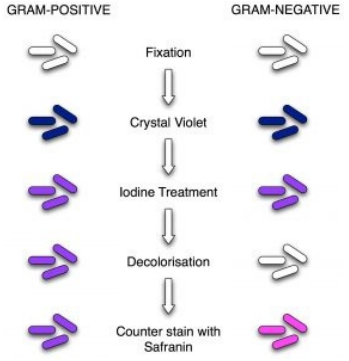


IMAGE 8: Gram- Staining protocol



Gram's-staining was done in this study since, *Streptococcus mutans* is a Gram-positive organism.

The current caries paradigm holds that dental caries is caused by acidogenic bacteria that produce lactic acid as a result of the anaerobic fermentation of carbohydrates, coupled with their aciduric properties that allow their survival in a low pH milieu (9). Indeed, lactobacilli have been regarded as secondary invaders rather than the primary initiator of caries, a role traditionally reserved for the *Streptococcus mutans species* (10). Van Houte and coworkers recognized that the relatively low affinity of lactobacilli for teeth implied that mechanical retention may play an important role in their colonization of the tooth surface (11).

Streptococcus mutans, was chosen as the organism to be inoculated in the food extracts, due to its ability to initiate dental caries. Dental pellicle is one among the most important oral biofilms that plays a vital role in caries initiation and progression, which has again been re-emphasized through this in vitro study. Continuous formation of dental pellicle acts both as a saviour and a threat to oral health.

CONCLUSION:

The etiology of dental caries is more attuned to the polymicrobial nature of dental biofilm. Sucrose is the most important dietary contributor to dental caries in modern humans. *Streptococcus mutans* exemplifies the cariogenic properties of sucrose metabolism, acidogenicity via fermentation, and adherence and biofilm formation via synthesis of extracellular glucans. Surface pellicle formation was minimal in the absence of *Streptococcus mutans.* The rate of pellicle formation was accelerated after the inoculation of the organism. The oral cavity hosts over 700 different species of microorganisms. Diet influences the rate at which the dental pellicle gets calcified to form plaque and calculus, leading to the development of dental caries by hosting these organisms. Since rice and multipurpose flour contain higher percentages of carbohydrates, the amount of pellicle formation was more on those samples. Repeated sampling with a combination of other cariogenic microorganisms will add up to the credibility of this study.

REFERENCES:

1. Fejerskov, O., & Kidd, E. (2003). Dental Caries: The Disease and Its Clinical Management (1st ed.). Oxford: Blackwell Munksgaard Ltd.
2. Xrstavik, D., and F. W. Kraus. 1973. The acquired pellicle: immunofluorescent demonstration of specific proteins. J. Oral Pathol. 2:68-76.
3. Hay, D. I., R. J. Gibbons, and D. M. Spinell. 197 1. Characteristics of some high molecular weight constituents with bacterial aggregating activity f'rom whole saliva and dental plaque. Caries Res. 5:111-12,3.
4. Kashket. S., and C. G. Donaldson. 1972. Saliva-induced aggregation of oral streptococci. J. Bacteriol. 112:1127-1133.
5. Kraus, F. W., and J. Konno. 1963. Antibodies in saliva. Ann. N.Y. Acad. Sci. 106:311-329.
6. Sirisinha, S. 1970. Reactions of human salivary immunoglobulins with indigenous bacteria. Arch. Oral Biol. 15:551-554.
7. Carlsson, J. 1967. Presence of various types of nonhaemolytic streptococci in dental plaque and in other sites of the oral cavity. Odontol. Revy 18:55-74.
8. Tankeshwar Acharya in Bacteriology, Staining techniques in Microbiology
9. Oral Lactobacilli and Dental Caries-A Model for Niche Adaptation in Humans P.W. Caufield, C.N. Schön, P. Saraithong, Y. Li, and S. Argimón J Dent Res. 2015 Sep; 94(9 Suppl): 110S–118S. doi: 10.1177/0022034515576052
10. Bacterial specificity in the etiology of dental caries. van Houte J. Int Dent J. 1980 Dec; 30(4):305-26.
11. Ecology of lactobacilli in the oral cavity: a review of literature. Badet C, Thebaud NB Open Microbiol J. 2008; 2():38-48.