

The Pathophysiology of Oral Biofilms and its relation to Initial Gum Disease and Caries

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Abstract

This study appraises diverse theoretical concepts into a coherent hypothetical concept of the pathophysiology of oral biofilms. The microbiota, their characteristics and properties are systematically analyzed and mechanisms of maturation clarified. Not all biofilms are the same as its stagnation and progress leads to development of gum disease and/or tooth decay. By deconstructing and assessing the various stages and types of oral biofilms, greater insight is attained in terms of assessing methods of prevention, its formation and control. Besides the local effect of biofilms on the teeth, bone and soft oral structures, some pathological effects and mechanisms derived from oral biofilms on systemic disease are high-lighted and articulated into a comprehensible hypothetical rationale.

Keywords: Bacteria; Biofilms; Decay; Gingiva; Gingivitis; Ecosystems; Endotoxin, Periodontitis; Plaque; Toxins;

Abbreviations

ECP=Extra-cellular Polysaccharide; PE=Pilot Ecosystem; CE= Climax Ecosystem; Ca=Calcium; ET=Endotoxin; TNF=Tumor Necrosis Factor; TLR=Toll Like Receptor; GIT= Gastro Intestinal Tract;

Introduction

Oral bacteria are the causes of the two most prevalent, globally ubiquitous diseases which affect Mankind, namely tooth decay and gum disease [1, 2].

Oral biofilms, commonly called “*plaque*”, this name is a descriptor term derived from ‘plaques’ of micro-organism colonies’ growth on nutrient microbial medium, as bacterial cultures in laboratories. Clinically it may be referred to “*materia alba*”, which can be easily demonstrated by scraping off the soft surface covering from tooth surfaces, or using disclosing dyes [3].

One mg of oral biofilm contain 10-6 bacteria include over 750 species. Severely infected mouths contain between 3 to 5 grams of microbial biomass. The vast majority of well-cared-for mouths will have 0.25G of *sessile biofilm*, and an equal amount of *planktonic* micro-organisms. Sessile biofilms are flat adherent bacterial colonies sticking to the tooth surface, especially in areas less prone to physical disturbance from eating food. These areas tend to be on the dental cervical margins and interdental. Planktonic micro-organisms are those which float about in

liquid medium, as in oral saliva, and may be individual, small clumps or in detached colonies. Oral plaque is not uniform, nor consistent in make-up, and different biofilms have different properties and characteristics [4].

Aim

This study appraises diverse theoretical concepts and articulates them into a coherent hypothesis of the pathophysiology of oral biofilms. Various types are discussed and grouped into stages of oral biofilms. Methods of clinical assessment are put forward, and methods are suggested on how to prevent formation, control its pathogenicity, and eliminate resulting morbidity. Besides the local effect of biofilms (as decay on the teeth and on gums as gingivitis), some systemic pathological effects derived from oral biofilms are discussed.

Pathophysiology of Oral Biofilms

Pilot Ecosystems

Pilot Ecosystems (PE), left undisturbed over a period of time, change into to Climax Ecosystems (CE). The initial PE’s are generally Gram positive, predominantly aerobic, non-motile, Extra-Cellular Polysaccharide (ECP) producing biofilms. PE produces mainly exotoxins, and an array of enzymes. The Gram-Positive bacteria are mainly *Staphylococci* and *Streptococci* with *Actinomyces* species. Among their cellular enzymes they produce, are an array of enzyme transferases which promote transforming glucose, fructose and galactose molecules into sticky gel-like polymers as Extra-Cellular Polysaccharide (ECP) [5]. The complexity of oral biofilm microflora has rendered it almost difficult to develop a successful vaccine to prevent decay and gum disease [6].

PE Mechanisms of Attachment of Gram Positive Bacteria, to the Tooth Surface

The ECP through a variety of microbial mechanisms, allow attachment, growth and proliferation of Gram positive initially, and subsequent Gram negative bacteria on the teeth. To start the surface of the tooth is covered by the enamel pellicle, or has exposed enamel hydroxyapatite crystals which are negatively charged. ECP of the PE microbes accumulates as a glycocalyx surface covering the pioneer organisms, is also negatively charged. These electronic charges are called London van der Waal forces. The like charges (--&--) repel each other, but become linked through positively (++&++) charged proteins, called lectins, which interpose

between the glycocalyx (sometimes referred to as the fuzzy coat) and the tooth surface, to form a strong attachment bond. Other mechanisms assist in this attachment including divalent ions, like Mg⁺⁺ and Ca⁺⁺ which bind the tooth surface and glycocalyxes of the bacteria, as well as lipoteichoic

acid (an exotoxin from Gram-Positive bacteria), proline- and aniline-rich salivary proteins, and statherin (also a divalent charged protein) (Figure 1) [7-9].

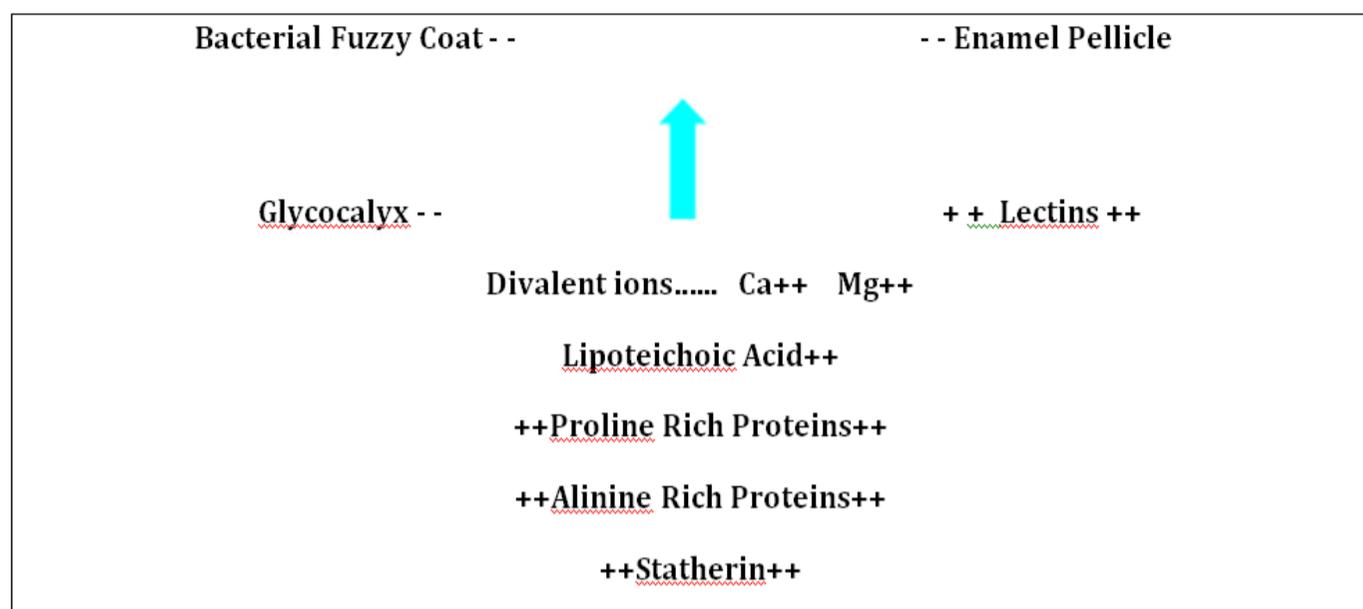


Figure 1: PE mechanisms of bonding attachment of Gram Positive bacteria, to the tooth surface. The bacterial fuzzy coat and enamel pellicle are negatively charged (- -); the arrow indicates where the positively (+ +) charged molecules bond to the negatively charges, and so allow attachment of microbes to tooth surfaces.

Changes in the Biofilm Internal Environment

Fresh flows of saliva which, relative to plaque, is alkaline at pH 6.8, precipitates salivary Ca⁺⁺ and this promotes the start and ongoing progress of calcifying PE as calculus. Consequently calculus is always infective as it incorporates viable bacteria. PE takes several hours (about 4 to 6 hours) to form, and evolves into a different and highly complex biofilm. Initially the biofilm is easily permeable to elemental exchanges and this is facilitated by molecular ionic interchanges in the matrix of the ECP. Consequently when acidic hydrogen ions are produced (by bacterial metabolism) or introduced (by dietary acidic intake) they gain rapid ingress, and the *biofilm acts as a dynamic, ionic exchange gradient for charged elements*. The acidic pH environment is variable, but when the pH drops to below pH6.5, Ca⁺⁺ starts to be leached out of the tooth and dissipates into the relatively alkaline saliva. The pH at 5.5 is referred to as the absolute “critical pH”, but to start ... at pH 6.5, and definitely at pH below 5.5,...mainly calcium and phosphorous, and other trace elements are leached out of the constituent hydroxy-apatite crystals of the tooth. This loss of calcium may be reversible, but if this decalcification progresses, it is deemed the earliest stage of decay. The PE changes with stagnation after 48 hours into an Intermediate biofilm (Table 1) [8].

Table 1: Microbes, characteristics and properties of *pilot, early and intermediate ecosystem* in oral biofilm

Main constituent bacterial species	Properties & Characteristics
<i>Streptococci</i>	Gram +ves
<i>Actinomyces</i>	Aerobic
<i>Staphylococci</i>	Non-motile organisms- 40
<i>Fusobacteria</i>	Motile organisms - 1
<i>Lactobacilli</i>	Produces exotoxins: lipoteichoic acid
<i>Leptothricia</i>	Acidogenic
<i>Bacterionema matruchotii</i>	Non-invasive

The Intermediate Biofilm

The acid milieu of the ECP in the PE, provides a rich nutrient and ideal environment for Gram Negative bacteria to take hold and thrive. The ECP provides a medium for Gram positives and Gram negatives to grow. *Facultative bacteria, anaerobic bacteria, filamentous bacteria and anaerobic fusiforms* inter mingle with *cocci* and *streptococci* to produce a complex of microbiota. After about ten days the PE changes to an intermediate ecosystem with a progressively increasing count of virulent microbes in the biofilm. Characteristic of this stage are bacteria which look like corn-cobs or rosettes (Figure 2) [8-12].

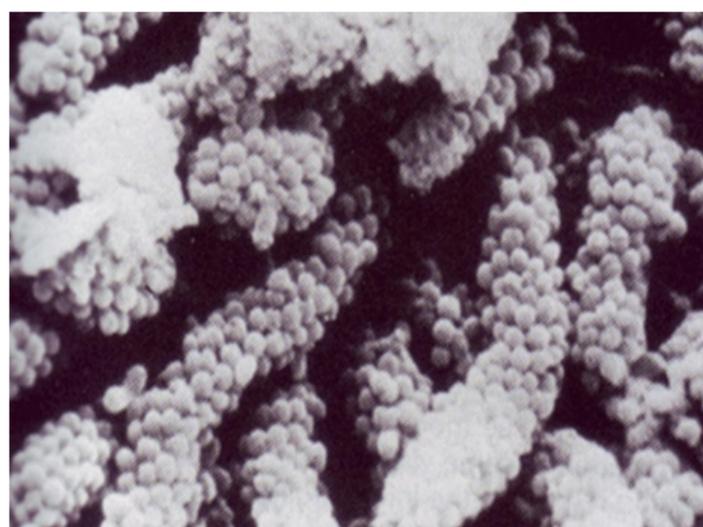


Figure 2: “Corn- cob” microbes from intermediate biofilms, *Bacterionema matruchotii* and *Streptococci*.

By 14 days the PE has morphed into a mature Climax Ecosystem (CE) with component bacteria into mainly gram negative, anaerobic and motile organisms. The major toxin of CE is endotoxin (Table 2).

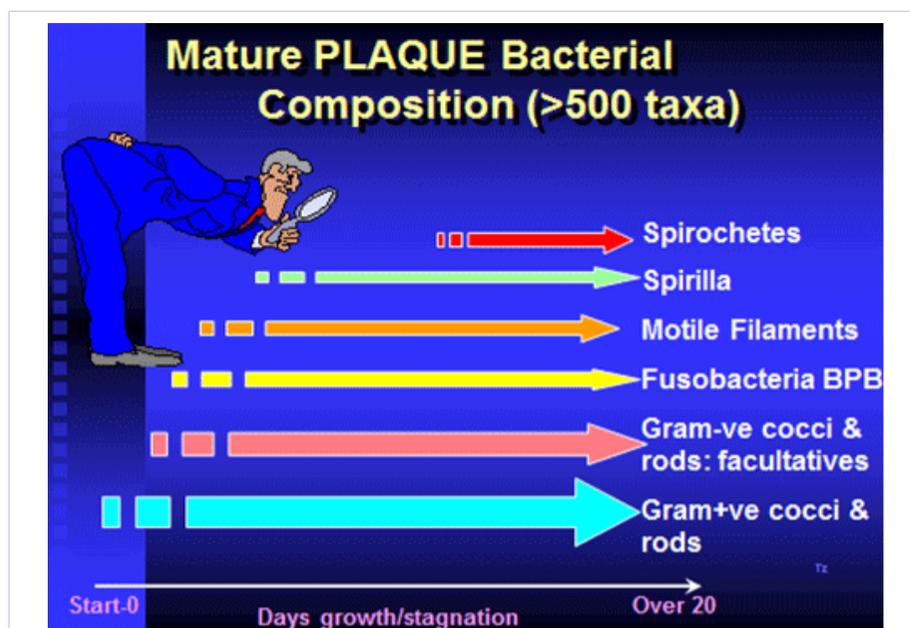


Figure 5: From Pilot Ecosystem to Climax: Progression of microbiota from Pilot ecosystems to Climax Ecosystems has Gram positive organisms attach first, then Gram negative bacteria use the gram positive products to attach and grow in the biofilm.

As undisturbed plaque stagnates, the conversion from initial biofilm, into an invasive ecosystem, progresses over weeks. The bacterial plaques grow into an adherent, sticky, interdependent biomass with transport channels, colonies of flat biologically glued sessile growths, as well as floating planktonic ecosystems. Each form of colony growth can successfully seed each other, and the surrounding ECP matrix may act as a barrier to deeper layers of organisms [10-11].

The internal structure of oral biofilm, produces multiple colonies of bacteria and grow nutrient channels which flow over and between the colonies. Metabolites are produced and exchanged as needed by the bacterial mix. Saliva provides and sustains moisture and nutrients which allow for continued growth. Floating planktonic clumps, seed off the sessile colonies and are transport to new sites. (Figure 6).

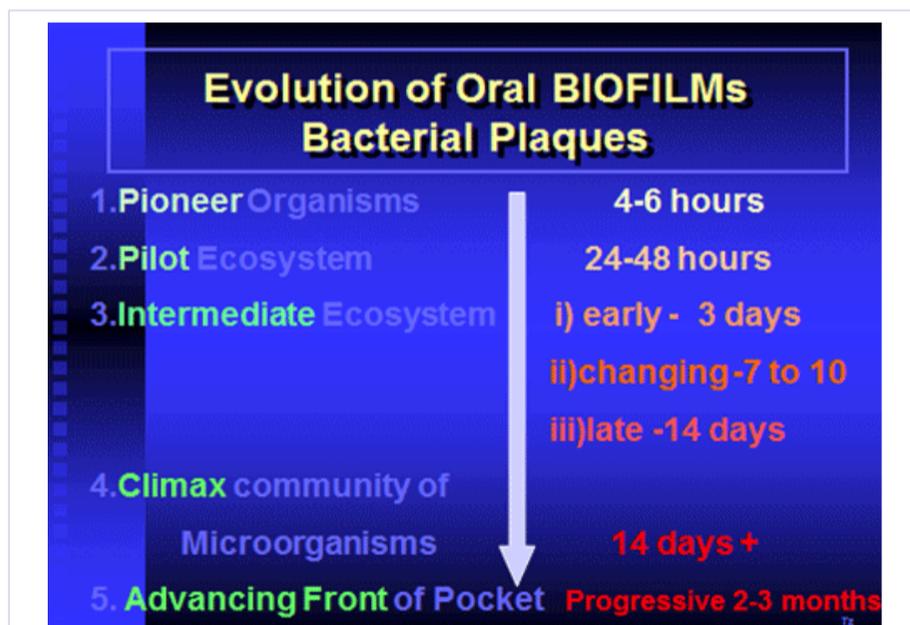


Figure 6: From Pilot Ecosystem to Climax: Time-line of maturation and transformation of oral biofilms.

The key for the change of the ecosystems is the transformation occurs with stagnation. The plaque alters its character and properties when left undisturbed for periods over days, weeks and months. The biomass increases in volume, number and biochemical virulence, and invade the soft tissue, while simultaneously allowing a dynamic leaching

out of calcium and phosphorous from the dental hard tissues. Should the decalcified hard tissue fall prey to invading organisms, there is destruction, softening with liquefaction and finally cavitation. The early decalcification may be reversible, but once there is softening cavitation, frank tooth decay obtains and demands dentistry skills for arrest and repair.

Clinical Demonstration of Biofilm

Oral biofilms are usually camouflaged white and cannot easily be recognized when doing a clinical examination. This is why the term "materia alba" is used. However the biomass may be demonstrated using revealing stains and disclosing dyes, many of which are safely used in food colorings [15]. A relatively clean mouth, when using a disclosing solution, will not reflect major color stains on teeth. However a dentate mouth not brushed clean will show up the dye on the teeth. A neglected mouth, not sustained in health with oral hygiene care, will reflect progressive disease of the teeth and gums (Figure 7A, Figure 7B and Figure 7C) [15].



Figure 7A: This shows how a mouth cleaned of biofilm, shows nearly no staining of bacterial plaques after staining with a disclosing solution. Small stained, residual plaques may still be seen on the marginal cervical areas of the lower incisors and canines.

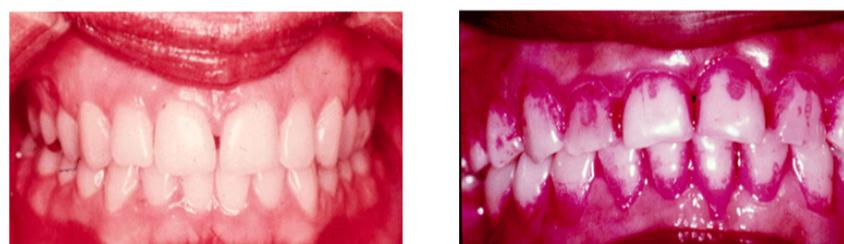


Figure 7B: mouth stained with erythrosine red dye reveals red stained biofilm on all the tooth surfaces [15].

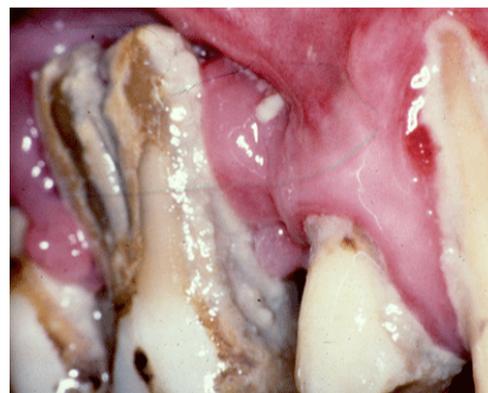


Figure 7C: A neglected mouth with ignored stagnating biofilm. The evolving biofilm progressively destroys soft tissue, and eventually demolishes all attachment to teeth with resultant tooth loss.

Discussion

Biofilm may harbor pathogenic bacteria which affect the health of the upper gastrointestinal system. For example gastric ulcers may be caused by *Helicobacter pylori* resident in the oral biofilm, with *H. pylori* elimination from oral biofilm moderating formation of aphthous ulcers, and Herpes simplex virus [16-18]. It is feasible that oral biofilm contributes in the formation of septic shock [19]. Understanding and deconstructing the entire pathophysiology of biofilm initiation, progress and evolution is important in controlling its formation, and maturation. This is because at different stages the biofilm behaves in different ways and induces different pathologies. Neutralizing charged surfaces of teeth, and blocking ionic precipitation of bacteria (*Streptococcus mutans*, *Staphylococcus albus* and others) by mouthwashes which act as an electronic sink, disrupts the linking charges, and successfully stops attachments of bacteria to the tooth. Chlorhexidine gluconate mouthwash is one example of how this is exploited. Cetyl-pyridinium chloride is another example. Some mouthwashes inhibit growth and colonization of bacteria. Listerine® and Scope® (constituted by mainly organic disinfectant oils) are examples of these. The entire cascaded evolution to a pathogenic CE is stopped by physical disruption, by way of brushing and flossing. Mouthwashes do help in reducing planktonic bacteria, but don't disrupt ECP in the depths of the stagnating areas, and don't replace the thoroughness of brushing and flossing. The Water Pik™ is minimally effective as even with a medicated lubricant, while it may remove some superficial planktonic bacteria, it does not remove all the sessile adherent subgingival microbial plaques, nor these from periodontal pocket's (PD > 3.5mm) radicular surface [20].

Recently claims that evidence for flossing is absent and consequently omitted by leading health authorities as a recommendation for oral hygiene practice [21]. This notion is refuted as spurious, deduced from global observations with thousands of dentists examining millions of dentate mouths, claiming it is self-evident that interdental gum disease and interproximal tooth decay does not form with daily flossing, and flossing does indeed disrupt early stagnant interproximal biofilm formation [22].

The regular daily brushing and flossing interdentally, ECP build-up is disrupted and decalcification stops. Consequently with brushing there should be no decalcification and/or erosion, and no interproximal caries or gum disease. Subsequent cavitation by bacteria of decalcified tooth often leads to decay (or caries), but decalcification without cavitation manifests as erosion. Controlling the frequency, amount, timing and type of acidulated drink consumed, and the method of tasting and swallowing moderates the formation of erosion and attrition. Prevention of erosion is achieved mainly by avoiding swishing before swallowing.

The harmful effect of biofilm locally is to cause gingivitis, and later the CE destroys gingival attachments to teeth. Neglected uninterrupted gingivitis often, but not always, leads to periodontitis, especially in individuals with systemic predisposing co-factors like diabetes mellitus [23].

Conclusion

Florid periodontitis presents the equivalent of a gram negative infected wound which could measure 30cms long. This hypothetical figure is derived from summing the total length of the advancing front of

infected periodontal pockets surrounding the teeth. Severe periodontitis acts as an ongoing source of toxins, enzymes antigens and toxins which are continuously being drained into the circulation system.

Endotoxin (ET) is an intrinsic part of most Gram-negative bacteria cell walls. Dead bacteria in blood will release reactive ET. There may be a negative blood culture for live bacteremia, but ET producing bacteria release residual ET which remains highly potent. Bacteremia and endotoxemia are not the same, and may not always be present together. ET is inactivated at 250°C or more when heated at least one hour, and even though ET is heat labile, ET is not inactivated by conventional heat sterilizers (which rarely exceed 120°C,) and boiling water. ET from gum disease may contribute to critical threshold levels of endotoxemia which predisposes to systemic disease [13].

Most ET is produced from Gastrointestinal (GIT) bacteria but rendered inactive by bile salts. Changes in GIT function which alter intestinal permeability allows for endotoxemia with resultant symptoms. Severe Gram-negative cellulitis of any organ produces local and/or systemic effects of ET. The resultant systemic endotoxemia targets multiple organs and often renders them dysfunctional. The immune-compromised are at risk, with children and seniors being most frequently affected. An endotoxin concentration as low as 10⁻⁹ g/ml causes the hypothalamus to provoke pyrexia. Non-bonded ET in the blood binds with protein which attaches to a CD 14 receptor on macrophages, monocytes and circulating polymorphonuclear cells. Extremely low concentrations of ET [like 10⁻¹²/ml or 10 pico-grams/ml] sets off a complex cascade of intra-cellular mechanisms, starting with Toll-like Receptor Protein 4 [TLR-4], to release Interleukin-1 [IL-1] and Tumor Necrosis Factor-α [TNF-α] cytokines. Invading microbes are controlled through innate immunity which is enhanced by the TLR-receptor cascade [14].

Concluding Hypothesis

Not all biofilms are the same, as its stagnation and progress leads to development of gum disease and/or tooth decay. This hypothetical deconstruction and assessment of the various stages and types of oral biofilms provides greater insight for assessing and devising methods of CE prevention, formation and control. Besides the local effect of biofilms on the teeth, bone and soft oral structures, some pathological effects and mechanisms derived from oral biofilms on systemic disease are discussed and articulated into a comprehensible hypothetical rationale.

References

1. Haffajee ADD, Bogren A, Hasturk H, et.al. Oral Biofilms Differs Geographically. J Periodontol. 2004;31:996-1002.
2. NIDCR. Oral Biofilms Differs Geographically. J Clin Perio; 2004: www.nidcr.nih.gov/ News And Reports/media
3. Roberts FA, Darveau RP. Beneficial bacteria of the periodontium. Perio.2000. 2002;30:40-50. 10.1034/j.1600-0757.2002.03004.x
4. Lamont RJ, Yilmaz O. In or out: the invasiveness of oral bacteria. Periodontol.2000 .2002;30:61-69. 10.1034/j.1600-0757.2002.03006.x
5. Overman PR. Biofilm: A new view of Plaque. J Contemp Dent Pract. 2000;1(3):18-29.
6. Smith DJ. Dental caries vaccines: prospects and concerns. Crit Rev Oral Biol Med. 2002;13(4):335-349.

7. Lindhe J, Karring T, Lang NP. Clinical Periodontology & Implant Dentistry. Dental Plaque and calculus Ch-3. 2003;81-102; Blackwell Munksgaard. Microbiology of Periodontal disease Ch-4. 2003:106-139.
8. Kolenbrander PE. Oral microbial communities; Biofilms, interactions and genetic systems. Ann Rev Microbiol. 2000;54:413-437.
9. Biofilm/Adhesion. Archives of Oral Biology. 2013;58(10).
10. Socransky SS, Haffajee AD. Microbial mechanisms in the pathogenesis of destructive Periodontal diseases. A critical assessment. J Periodontal Res. 1991;26:195-201.
11. Loesche WJ. Bacterial mediators in Periodontal diseases. Clin Infect Dis. 1993;16(4):S203-210.
12. Kornman KS. The role of supra-gingival plaque in the prevention and treatment of periodontal diseases. Review. J Periodontal Res. 1986;5-22. 10.1111/j.1600-0765.1986.tb01511.x
13. Rietschel ET, Brade H. Bacterial Endotoxins. Sci Am. 1992;267(2):54-61.
14. Gaffin SJ. Control of septic shock. SA Jnl Hospital Medicine. 1982;8(1):4. [For detailed description of Endotoxins structure & function].
15. Arnim SS. The use of disclosing solutions for measuring tooth cleanliness. Journal of Periodontology. 1963;34(3):227-245. DOI 10.1902/jop.1963.34.3.227
16. Dye BA, Kruszon-Moran D, McQuillan G. The Relationship Between Periodontal Disease Attributes and *Helicobacter pylori* Infection Among Adults in the United States. Am J Public Health 2002;92(11):1809-1815.
17. Abanidou-Fermaki E, Giannoulis L, Markopoulso A, Fotiades S, Aggouridaki X, Farmakis K, et al. Outcome following treatment for *Helicobacter pylori* in patients with recurrent aphthous ulcers. Oral Dis. 2005;11(1):22-26. 10.1111/j.1601-0825.2004.01053.x
18. Baccaglini L, Schoenbach VJ, Poole C, et al. Association between Herpes simplex virus -1 and *Helicobacter pylori*. Oral surg, oral Med, oral Pathol Radiol endod. 2006;101(1):63-69.
19. Touyz LZ. Periodontitis contributes to initiation, progress and aggravation of septic shock; a feasible hypothesis. Medical Hypotheses. 2013;81(4):650-652. doi: 10.1016/j.mehy.2013.06.033
20. Goyal RC, Lyle DM, Qaqish JG, Schuller R. Comparison of Water Flosser and Interdental Brush on Reduction of Gingival Bleeding and Plaque: A Randomized Controlled Pilot Study. J Clin Dent. 2016;27(2):61-65.
21. Donn J. No evidence flossing improves oral health. National Post and Montreal Gazette. 2016: <https://www.pressreader.com/canada/montreal-gazette/20160803/281874412783867>
22. Touyz LZ. Don't give up on flossing just yet. Flossing advice approved by practicing dentists. Letters to the Editor. The Montreal Gazette. 2016:A10.
23. Chapple IL, Iain LC, Genco R. Diabetes and periodontal diseases: consensus report of the Joint EFP/AAP Workshop on Periodontitis and Systemic Diseases. J Periodontol. 2013;84(4):S106-12. doi: 10.1902/jop.2013.1340011.