BIOFILM AND ITS THERAPEUTIC APPROACHES

Suchetha A¹, Spandana Andavarapu², Sapna N³, Divya Bhat⁴, Apoorva SM⁵, Chitra Jayachandran⁶

¹ Head of the Department, Department of periodontology, DAPM RV dental college, Bengaluru
²³ Post graduate trainee, Department of periodontology, DAPM RV dental college, Bengaluru
³ Reader, Department of periodontology, DAPM RV dental college, Bengaluru
⁴ Lecturer, Department of periodontology, DAPM RV dental college, Bengaluru

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ABSTRACT

The microbial communities adhered to a substratum and encased within an extracellular polymeric substance (EPS) produced by the microbial cells themselves is defined as Biofilm. Treatment of these biofilm is usually supportive. Although dental plaque cannot be eradicated, it can be controlled with oral hygiene measures that include a daily regimen of brushing, flossing and rinsing with an antimicrobial mouth rinse, professional oral prophylaxis and many newer therapeutic modalities like replacement therapy, immunization, photodynamic therapy and laser. No matter what, biofilm control is fundamental to the maintenance of oral health and to the prevention of gingivitis and periodontitis. This can result in the prevention and development of associated sequel, including gingivitis, periodontitis and possibly the impact of periodontal diseases on specific systemic disorders.

Introduction

The microbial communities adhered to a substratum and encased within an extracellular polymeric substance (EPS) produced by the microbial cells themselves is defined as Biofilm. In simple terms biofilm is community of microorganisms attached to the surface. One of the important paradigm shifts that have taken place in the last decade has been the recognition and acceptance that dental plaque as a biofilm.¹ Treatment of these biofilm is usually supportive. Although dental plaque cannot be eradicated, it can be controlled with oral hygiene measures that include a daily regimen of brushing, flossing and rinsing with an antimicrobial mouth rinse, professional oral prophylaxis and many newer therapeutic modalities like replacement therapy, immunization, photodynamic therapy and laser.² No matter what, biofilm control is fundamental to the maintenance of oral health and to the prevention of gingivitis and periodontitis.³

DEFINITIONS

BIOFILM:
Costerton et al in 1995 defined biofilm as “a structured community of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface”.⁴

Keywords:
Extracellular polymeric substance, Biofilm, Replacement therapy, Immunization photodynamic therapy.
Percival et al in 2000 defined biofilm as “microbial cells immobilised in a matrix of extracellular polymers acting as an independent functioning ecosystem, homeostatically regulated”.

Marsh P D 2005 defined biofilm as “Orientated aggregations of microorganisms attached to each other or to a surface and enclosed in extracellular polymeric substance (EPS) produced by themselves”.

**FORMATION OF A BIOFILM**

Biofilm formation is a complex process that follows several distinct phases, beginning with adsorption on to the tooth surface of a conditioning film derived from bacterial and host molecules, which forms immediately after the tooth eruption or tooth cleaning. This adsorption is followed by passive transport of bacteria mediated by weak long-range forces of attraction. Covalent and hydrogen bonds create forces with strong and short range that result in irreversible attachment.

The primary colonizers form a biofilm by autoaggregation (attraction between same species) and co-aggregation (attraction between different species). Co-aggregation results in a functional organization of plaque bacteria and formation of different morphologic structures such as rosettes and corncobs. The microenvironment now changes from aerobic to anaerobic condition. The attached bacteria multiply and secrete an extracellular matrix, which results in a mature mixed-population biofilm.

After a day, the term biofilm is fully merited because organization takes place within it. Transmission occurs from other sites, leading to incorporation of new members into the biofilm and the formation of a climax community. The thickness of the plaque increases with time i.e from 20 to 30 µm after three days.

**CLASSIFICATION OF BIOFILM**

On basis of its location
1. Supra gingival - Present coronal to the gingival margin.
2. Sub gingival - Present apical to the gingival margin.

On basis of pathogenicity
1. Cariogenic - Generally acidogenic and gram-positive.
2. Periopathogenic - Mostly basophilic and gram-negative.

**COMPOSITION**

a) Micro-organisms associated with dental plaque (key pathogens)
1. *Aggregatibacter actinomycetemcomitans*
2. *Porphyromonas gingivalis*
3. *Prevotella intermedia*
4. *Tannerella forsythus*
5. *Fusobacterium nucleatum*
6. *Peptostreptococcus micros*
7. *Campylobacter rectus*

b) Organic constituents
1. Polysaccharides
2. Proteins
3. Glycoprotein b
4. Lipids

c) Inorganic constituents
Calcium

**DENTAL PLAQUE IDENTIFICATION**

Identification of the supra- gingival dental plaque is difficult for both dentist and patient, because of the color similarity between the tooth surface and dental plaque. Plaque identification may be done either by
1. Screening the plaque directly from the tooth surface.
2. Changing its color with a disclosing solution.
Using the ability of natural teeth to fluoresce under blue light.\(^{14}\) 

Disclosing dyes work by changing the color of dental plaque so that it contrasts with the white tooth surface. Dental plaque has the ability to retain a large number of dye substances which can be used for disclosing purposes.\(^{15}\) The particles are bound to the surface by electrostatic interaction (proteins) and hydrogen bonds (polysaccharides). Over the years, different staining agents have been used (table 1).\(^{16}\)

### SIGNIFICANCE OF BIOFILMS

Epidemiologic evidence indicates that biofilms are a source of several infectious diseases; although the exact mechanisms by which biofilm-associated bacteria induce disease are poorly understood.\(^{8}\) The pathogenicity of the biofilm in the oral cavity is increased by two biofilm characteristics:

1. Increased resistance to antibiotics.
2. Phagocytosis by host inflammatory cells.

Current intervention strategies are designed to prevent initial colonization by mechanical removal, minimizing microbial cell attachment to the oral tissues and increasing penetration of the biofilm matrix by antimicrobials. In the future, treatments may inhibit the genes involved in cell attachment and biofilm formation.\(^{7}\)

### Table 1

<table>
<thead>
<tr>
<th>Disclosure Dye</th>
<th>Staining Agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodine</td>
<td>Brilliant blue</td>
</tr>
<tr>
<td>Fuchsin</td>
<td>Crystal violet</td>
</tr>
<tr>
<td>Erythrosine</td>
<td>Fluorescein</td>
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<tr>
<td>Merbromin</td>
<td>Gentian violet</td>
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<tr>
<td>Methylene blue</td>
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### Table 2

<table>
<thead>
<tr>
<th>DEVICE</th>
<th>TYPES</th>
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<tbody>
<tr>
<td>Tooth brushes</td>
<td>Manual tooth brush</td>
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<tr>
<td>Electrical tooth brush</td>
<td></td>
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<tr>
<td>Interdental cleaning aids</td>
<td>Dental floss</td>
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<tr>
<td>Wooden tips</td>
<td></td>
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<tr>
<td>Perio-aid</td>
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<tr>
<td>Interdental brushes</td>
<td></td>
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<tr>
<td>Gum massagers</td>
<td>Rubber tip stimulator</td>
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<tr>
<td>Gum stimulator</td>
<td></td>
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<tr>
<td>Oral irrigation devices</td>
<td>Supra gingival</td>
</tr>
<tr>
<td>Sub gingival</td>
<td></td>
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<tr>
<td>Tongue cleaners</td>
<td>Stainless steel tongue cleaner</td>
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<tr>
<td>Copper made tongue cleaner</td>
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### MANAGEMENT PROTOCOL

1) Plaque control
   a) Mechanical
   b) Chemical
   c) Professional oral prophylaxis

2) Replacement therapy
   a) Probiotics
   b) Prebiotics

3) Immunization

4) New treatment approaches like:
   a) Laser
   b) Photodynamic therapy

### REMOVAL OF PERIODONTAL BIOFILMS/PLAQUE CONTROL:

Plaque control is a key element of the practice of dentistry. It is the most important predictive factor in determining the overall prognosis of the treatment therapy. It is an effective way of treating to prevent
BIOFILM AND ITS THERAPEUTIC APPROACHES 3(2);2017

Table 3

<table>
<thead>
<tr>
<th>Bisbiguanides</th>
<th>Chlorhexidine, Alexidine</th>
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</thead>
<tbody>
<tr>
<td>Non-ionic polyphenolic compound</td>
<td>Triclosan</td>
</tr>
<tr>
<td>Herbal extracts</td>
<td>sanguinarine</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>Delmopinol, Thymol</td>
</tr>
<tr>
<td>Metallic salts</td>
<td>Zinc, Tin, Copper</td>
</tr>
<tr>
<td>Quarternary ammonium compounds</td>
<td>Benzalkonium chloride, Cetylpyridinium chloride</td>
</tr>
<tr>
<td>Amino alcohols</td>
<td>Octapenol, Decapenol</td>
</tr>
<tr>
<td>Bispyridines</td>
<td>Octenidine</td>
</tr>
<tr>
<td>Other surfactants</td>
<td>Sodium lauryl sulphate</td>
</tr>
</tbody>
</table>

gingivitis, periodontitis, and perhaps any microbial aetiology disease as related to oral health.17

There are two modes of plaque control:
1. Mechanical plaque control
2. Chemical plaque control

Mechanical plaque control
Mechanical plaque control is the removal and prevention of microbial plaque accumulations on the teeth and adjacent gingival surface by the use of tooth brush and other mechanical oral hygiene aids without the use of chemicals. The removal of microbial plaque leads to cessation of gingival inflammation.19 The removal of plaque also decreases the rate of formation of calculus. Thus eliminating the plaque is the key to prevent the occurrence of periodontal disease or halting the progression of the disease.

Various devices are used for mechanical plaque control measures (table 2).

Chemical plaque control
As many clinical studies did not show 100% plaque removal with mechanical therapy, using chemical plaque control measures came into existence.18 There appears to be a consensus that antiplaque agents cannot replace conventional mechanical plaque removal methods but should be used as adjuncts to mechanical cleaning.19 The rationale for the use of antiplaque agents as adjuncts to mechanical cleaning methods is based on two premises.
1. Firstly, plaque is the major etiological factor in gingivitis.19
2. Secondly the prevalence of gingivitis.20

Chemical plaque control requires a vehicle and an anti-plaque agent.21 Vehicles required for the delivery of these chemical anti-plaque agents include:22
1. Toothpaste
2. Mouthrinses
3. Spray
4. Irrigators
5. Chewing gum
6. Varnishes

Various chemical antiplaque agents are used (table 3). The most tested and effective antiplaque agent well known today is chlorhexidine, which has been used for more than two decades. The mode of action of chlorhexidine against periodontopathogenic bacteria is well understood and is concentration dependent. It can be either bacteriostatic or bactericidal depending on the dose.22 23 It is used as an adjunct to oral hygiene, professional prophylaxis and also as a post periodontal surgery or root planing. By understanding the properties
and limitations of the chlorhexidine, the efficacy can be maximized and the side effects can be minimized. Hence, chlorhexidine remains as the gold standard of chemical antiplaque agents and also a positive control to compare with other agents.23

**Professional oral prophylaxis**

Professional oral prophylaxis is purely a therapeutic measure. It is used to remove dental plaque and other irritants from the oral cavity. Prophylaxis is one way to ensure a clean bill of oral health, at least for the next six months.17 Oral prophylaxis is essential not only for maintaining your teeth, but is also used to treat the early stages of gum disease. If left untreated eventually leads to periodontal disease which may cause tooth loss.

1. Calculus-associated biofilm can effectively be removed by scaling and root planing.
2. Tissue-associated biofilms can be effectively removed by gingival curettage.8

**REPLACEMENT THERAPY:**

To replace potential pathogenic micro-organisms with genetically modified organisms that are less virulent. This can be done in two ways.

**Probiotics:**

Probiotics can be defined as “Live microorganisms that, when administered in adequate amounts, confer a health benefit to the host”.24 Earlier these probiotics were used to control gastro-intestinal diseases and prevent dental caries. Later, oral administration of probiotics has also been explored in the treatment of periodontal disease by fighting against plaque formation. Probiotics have many positive influences in both direct and indirect interactions. They combat against plaque formation and on its complex ecosystem by compromising and intervening with bacterial attachments. Through its direct interactions, these probiotics compete with oral microorganisms of substances available and produce chemicals to inhibit oral harmful bacteria that damage oral hygiene.27 This process involves the substrate metabolism. On the other hand, the indirect interactions of probiotics are effective in the process of removing harmful bacteria and stabilizing normal conditions.

The International Study Group on New Antimicrobial Strategies (ISGNAS) developed a concept for the detailed definition of probiotics in three categories:25

1. **Medical probiotics (drugs):** A medical probiotic is a microbial preparation which contains live and/or dead microorganisms including their components and products determined to be employed as a drug for therapeutic purposes

2. **Pharmaceutical probiotics (food supplements):** A pharmaceutical probiotic is a microbial preparation designed for production of food supplements.

3. **Alimentary probiotics (food):** An alimentary probiotic is a microbial preparation designed for use in food fermentation or food production.

The main fields of probiotic activity in general medicine include; gastrointestinal infections, urogenital infections, cancer therapy, cardiovascular diseases, diabetes, organ transplant patients etc.26 The probiotics can also be used to treat oral infections like; oropharyngeal infections, streptococcal pharyngotonsillitis, oral candidiasis, bacterial and fungal infections, caries management, gingivitis, periodontitis and halitosis.

**Prebiotics**

Prebiotics are non-digestible dietary supplements. Their function is to enhance the growth and activity of
beneficial organisms and simultaneously suppress the growth and activity of potentially deleterious bacteria. Prebiotics have been proved to complement probiotics in the treatment of oral diseases. In this way prebiotics modify the balance of the intestinal micro-flora. The characteristic feature of prebiotic ingestion is mainly to change microbial population density. Some of the usually known prebiotics are lactose, inulin, fructo-oligosaccharides, galacto-oligosaccharides and xylo-oligosaccharides. Naturally found prebiotics include certain fruits like bananas, asparagus, garlic, tomato and onion.

**IMMUNIZATION:**

Immunization against periodontal disease has been a central research topic in recent decades. The aim is to inhibit adhesion or to reduce the virulence of putative microbial etiologic agents. Several virulence factors of various periopathogens are used as vaccine targets. These vaccines can be of three types which include:

1. Vaccines prepared from pure cultures of streptococci, and other oral microorganisms.
2. Autogenous vaccines
3. Stock vaccines

These vaccines can be administered systemically or locally in the periodontal tissues. Even though all the above three types of vaccines are producing effective results, autogenous vaccines are having a greater efficacy against plaque control therapy. These autogenous vaccines were prepared from the dental plaque of patients with destructive periodontal diseases. Plaque samples were removed from the diseased site, "sterilized" either by heat or by immersion in iodine or formalin solutions, and then re-injected into the same patient, either in the local periodontal lesion or systemically.

The vaccine should be investigated first in animal models followed by nonhuman primates, before being studied in human beings. Various bacterial strains being investigated and used for periodontal vaccine preparation include: *Porphyromonas gingivalis, Tannerella forsythia, Prevotella intermedia, Bacteriodes macacae, Aggregatibacter actinomycetemcomitans* and *Campylobacter rectus.*

There are many limitations in periodontal vaccination, which may include:

- High risk of reactivity with the human counterparts
- Contamination with unwanted proteins, toxins or live viruses in hypersensitive individuals.
- If killed vaccines are not completely killed they may cause serious problems in immune-compromised patients.

However, vaccination may be an important adjunctive therapy to mechanical debridement in humans to prevent colonization of periodontopathic microorganisms. When present in subgingival plaque as an undisturbed biofilm, specific antibodies restrict the progression of disease by blocking penetration into gingival tissue and neutralizing key virulence factors associated with acquisition of essential nutrients.

Hence, the concept of periodontal vaccine will come into reality in near future if research is carried out in right manner in right direction.

**NEW TREATMENT APPROACHES**

**LASER**

A laser is defined as a photo-thermal device that produces a monochromatic, coherent, and collimated light with a specific wavelength. It acts right on cellular structures, destroy cell walls, altering DNA, modifying metabolic processes, and ungluing the polysaccharide
structure of the biofilm. There are many in vivo and in vitro studies stating the bactericidal effect of lasers in reduction of subgingival periodontopathic bacteria. Hence, laser light can be used as a new modality of anti-infective approach to treat biofilm/plaque-induced periodontal diseases. The laser light eliminates periodontal pathogens through its antibacterial property and promotes the return of the periodontium to a state of health through its anti-inflammatory effect.

**Photodynamic Therapy**

As the chemical antimicrobial agents are difficult to maintain at a therapeutic concentration in the oral cavity and can be rendered ineffective by resistance development in the target organisms, there is a need to develop alternative antimicrobial approaches. So another approach has been developed for killing bacteria in the biofilm namely photoactivated disinfection (PAD) or photodynamic therapy (PDT). Photosensitizers like, methylene blue, toluidine O blue, tolonium chloride are applied to the biofilm and are transformed by laser light into a reactive state which forms free radicals, oxidizing cellular constituents, causing cell death. There are many advantages of this approach, which include:

1. The bacteria can be eradicated in very short periods of time (seconds or minutes).
2. Resistance development in the target bacteria is unlikely.
3. Avoids damage to adjacent host tissues and disruption of the normal microflora.

Hence, this approach may be a useful alternative to antibiotics and antiseptics in eliminating periodontopathogenic bacteria from disease lesions.

**CONCLUSION**

Plaque thus represents a true biofilm involved in a wide range of physical, metabolic and molecular interactions. Dental plaque biofilm cannot be eliminated. However, the pathogenic nature of the dental plaque biofilm can be reduced by reducing the total microbial load and maintaining a normal flora with appropriate oral hygiene methods that include daily brushing, flossing and rinsing with antimicrobial mouth rinses. This can result in the prevention and development of associated sequel, including gingivitis, periodontitis and possibly the impact of periodontal diseases on specific systemic disorders.

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