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RESEARCH ARTICLE

STRATEGIES FOR BIOFILM INHIBITION.

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Abstract

Biofilms are groups of microorganisms in which cells stick to each other on a surface. A biofilm, is a polymeric mixture generally composed of extracellular DNA, proteins, and polysaccharides. Bacterial polysaccharides are a major component of the extracellular polymeric substance or matrix of biofilms, and mediate most of the cell-to-cell and cell-to-surface interactions required for biofilm formation and stabilization. Biofilm control is fundamental to oral health. The microorganisms involved are organize into complex biofilm communities with features that differ from those of planktonic cells. Control of oral biofilms is fundamental to the maintenance of oral health and to the prevention of gingivitis, and periodontitis. However, oral biofilms are not easily controlled by mechanical means and represent difficult targets for chemical control. This review deals with recent advances on novel strategies for biofilm dispersal and inhibition.

Introduction:-

Microbial cells (predominantly bacteria) in an extracellular polymer substratum are called *biofilms*. Complex physical, chemical, and biological processes govern bacterial attachment, biofilm formation, and persistence. The following fundamental processes comprise the development of a bacterial biofilm: (1) Substratum pre-conditioning by circumstantial or pre-meditated adsorption of fluid phase organic molecules; (2) bacterial cell transport to the surface; (3) cell desorption from the substratum; (4) permanent cell adhesion to the substratum; (5) bacterial metabolism (cell substrate conversion; cell growth and replication; extracellular exopolymer production; cell starvation, death, lysis); and (6) biofilm removal (cell and biofilm detachment; biofilm sloughing).

A diverse community of bacteria comprises tooth plaque biofilm. Early tooth colonizers (*e.g., Streptococcus*, *Hemophilus*, *Neisseria*, and *Veillonella*) adhere to enamel pellicle protein layers via specific and non-specific adhesion mechanisms.

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Biofilm Inhibition:-

Removal of cells from the biofilm colony is an essential stage of the biofilm life cycle because it enables biofilms to spread and colonize new surfaces. Strategies to plan against bacterial biofilm must be achieved by prevention of biofilm formation rather than dispersal of the formed biofilm. Strategies for prevention of biofilm formation include both "Chemical" and "Mechanical" methods.

Chemical methods:-

Chemical modifications are the main strategy for biofilm prevention.

Antimicrobial coatings:-

Antibiotics, biocides, and ion coatings are commonly used. They prevent biofilm formation by interfering with the attachment and expansion of immature biofilms.[1] These coatings are effective only for a short time period (about 1 week), after which leaching of the antimicrobial agent reduces the effectiveness of the coating.[1] The antimicrobial property of silver is known as an oligodynamic effect, a process in which metal ions interfere with the growth and function of bacteria. [2]

Polymer modifications:-

Antimicrobial agents can be immobilized on device surfaces using long, flexible polymeric chains. These chains are anchored to the device surface by covalent bonds, producing non-leaching, contact-killing surfaces. One *in vitro* study found that when N-alkylpyridinium bromide, an antimicrobial agent, was attached to a poly(4-vinyl-N-hexylpyridine), the polymer was capable of inactivating ≥ 99% of *S. epidermidis, E. coli,* and *P. aeruginosa* bacteria.[3] Dispersion forces between the polymer chains and the bacterial cells prevent bacteria from binding to the surface and initiating biofilm growth.

An in-vitro study, in the preparation of chitosan-based polymeric nitric oxides, showed sustained release of nitric oxide which provided potent antimicrobial and significantly reduced the level of viable bacteria living in preexisting biofilms and provided added or synergistic effects with a common antibiotic, amoxicillin, in killing pre-attached bacteria in biofilms.[4]

Mechanical methods:-

Hydrophobicity:-

Hydrophobicity also plays an important role in determining the ability of bacteria to form biofilms. Highly hydrophobic species of oral bacteria, including *Actinomyces viscosus, Actinomyces naeslundii, Streptococcus sanguis, Streptococcus mitis* and *Porphyromonas gingivalis,* have been shown to adhere to experimental salivary pellicles in significantly higher numbers than less hydrophobic or hydrophilic species, such as *Prevotella intermedia, Prevotella melaninogenica, Streptococcus mutans* and *Streptococcus salivarius.*[5]

Hydrophobic bond disrupting agents such as the Li+ cation and thiocyanate anion reduce the adherence of *Streptococcus sanguis* to saliva-coated hydroxyapatite by 81% and 94% respectively,(6) and a hydrophobic bond diluent, sulfolane (thiophene, tetrahydro- 1, dioxide), has been shown to inhibit the adhesion of *Streptococcus sanguis* to saliva-coated hydroxyapatite by >50%.[7]

Surface roughness:-

Rough, high-energy surfaces are more conducive to biofilm formation and maturation, while smooth surfaces are less susceptible to biofilm adhesion. The roughness of a surface can affect the hydrophobicity or hydrophilicity of the contacting substance.[8]

Surface charge:-

Modification of the surface charge has also proven to be an effective means of biofilm prevention. Both the bacteria and the surfaces of the mouth are charged. The repulsive force found at an interface constitutes the electrokinetic or zeta potential.[9] Zeta potentials of bacteria have been shown to increase with decreasing hydrophobicity, and it has been demonstrated that low surface charge and, high hydrophobicity favored bacterial adherence.[9]

Surface energy:-
The energy change at an interface between two substances was first described by Leslie,[10] that the liberation of heat when metal surfaces were wetted with a liquid. It has been demonstrated that this surface free energy influences dental plaque growth on various substrata *in vivo*, with high surface free energy promoting bacterial accumulation.[11] Majority of oral bacteria have high surface free energy values,[11] they bind most readily to substrata with high surface free energy, such as dental enamel.

Stannous and amine fluoride containing mouthrinse was shown to reduce the surface free energy of polished enamel and salivary pellicle-coated enamel, and in a 14-day clinical trial the mouthrinse significantly reduced plaque and gingivitis scores compared with placebo.[12]

Surface Modification:-
The strategy of surface modification involves altering the tooth surface or the salivary pellicle to impede bacterial colonization. Combination of an alkyl phosphate and a non-ionic surfactant alters the surface characteristics of the tooth, making it less attractive for microorganisms. Phosphate and phosphonate may be used to anchor water-soluble, protein repelling substances to the mineral surface.

Hirota K et al,[13] investigated the effect of mouthrinse incorporated with 2- methacryloyloxyl ethyl phosphorylcholine (MPC polymer) and revealed it as a potent inhibitor of bacterial adherence and biofilm development.

Alexandros et al,[14] has shown that application of silver nano-coating directly on dentine can successfully prevent the biofilm formation on dentine surfaces as well as inhibit bacterial growth in the surrounding media.

Strategies To Enhance Colonization Resistance:-

Replacement Therapy:-
The phenomenon by which one member of bacterial ecosystem can inhibit the growth of other member is called 'Bacterial Interference'. The possible use of antagonistic organisms to control the adhesion of pathogens and prevent infection is called **REPLACEMENT THERAPY.**

Replacement therapy has been suggested as a strategy to replace potential pathogenic micro-organisms with genetically modified organisms that are less virulent. There are two main approaches by which replacement therapy is being considered

1. Pre-emptive colonization
2. Competitive displacement

Pre-emptive Colonization:-
The ecological niches (functions) within plaque are filled by a harmless or potentially beneficial organism before the undesirable strain has had an opportunity to colonize or become established. The initial colonizer becomes integrated into the ecosystem and subsequently excludes the pathogen.[15]

Competitive Displacement:-
An alternative approach has been to derive a more competitive strain that would displace a pre-existing organism from plaque.[15] Competitive displacement is of potentially greater clinical value, since it is not dependent on treatment with the "effector" strain at or before colonization by the undesired organism.[16]

Guiding periodontal pocket recolonization:-
In 2007, Teughels et al.[17] conducted a study using a beagle dog model to test the hypothesis that the application of selected beneficial bacteria, as an adjunct to scaling and root planning, would inhibit the periodontopathogens recolonization of periodontal pockets. The beneficial bacteria selected for the guided pocket recolonization were *streptococcus sanguinis, streptococcus mitis*. They were choosen because they prevent colonization of hard tissues and epithelial cells from periodontal pathogens.

Strategies For Biofilm Dispersal:-

Strategies for more effective biofilm dissolution treatments become fundamental. Conventional antibiotics work by either preventing bacterial cell division (bacteriostatic) or killing the cell (bactericidal).

Bacterial Antibiofilm Polysaccharides :-

Polysaccharides, as sugar polymers, have the capacity to act as lectin inhibitors. Lectins are proteins that specifically recognize and bind sugars without modifying the molecules. In bacteria, the primary function of lectins is to facilitate attachment or adherence of bacteria to host cells. These proteins play an important role in biofilm formation, and are essential for bacterial colonization and infection. Lectins are mainly located on the surface of bacteria cells where they can access and bind to the glycan substrates present on the surface of host cell. By competing for the sugar binding domain of lectins, polysaccharides can inhibit lectin-dependent adhesion of pathogens and biofilm formation.

Polysaccharides mediate cell-to-surface and cell-to-cell interactions that are critical for biofilm formation and stabilization. Antibiofilm properties of polysaccharides are believed to lie on their ability to: a) alter the physical characteristics of bacterial cells or abiotic surfaces. b) act as signaling molecules that impact the gene expression patterns of susceptible bacteria. c) competitively inhibit multivalent carbohydrate–protein interactions, thereby interfering with adhesion.

Many studies are reported about the ability of some bacterial polysaccharides to inhibit biofilm formation by several bacteria, including *E. coli strains*, *P. aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus* and *Enterococcus*.[18] Most of these antibiofilm agents are able to inhibit the biofilm formation of a broad range of bacteria, suggesting that they may play an essential role in microbial competition and niche exclusion.

Extracellular polysaccharides:-

Recent evidence indicates that some bacterial exopolysaccharides inhibit or estabilize biofilm formation by other species. It forms slime layers or capsules surrounding bacteria that confer protection against host defense factors, such as antibodies and phagocytic white blood cells, and against antibiotics. Exopolysaccharide, principally dextrans, is important for the cohesion of bacteria forming dental plaque.

Delmopinol, a low-molecular-weight amino alcohol, displays significant substantivity and antiplaque and antigingivitis properties in short and long term studies.[19,20] It appears to target dextrans in the extracellular matrix by blocking synthesis,[21] reducing viscosity[22] and also by selectively inhibiting dextrans-producing streptococci.[23]

Aggregation and Coaggregation:-

The interbacterial aggregation include protein-carbohydrate interactions such as the 38-kDa surface protein of *S. sanguis* with adhesin properties capable of mediating coaggregation with *A. naeslundii*.[24] Such interactions have been demonstrated using lactose to block the binding of proteins with carbohydrate receptor groups.[25]

Protein-protein interactions are possible, such as between *p. gingivalis* and *S. sanguis*, as demonstrated by the inhibition of coaggregation using saliva and protease treatments.

Anti-biofilm Enzymes:-

N-acetyl-D-glucosamine-1-phosphate acetyl transferase is an essential peptidoglycan and lipopolysaccharide precursor in Gram-positive and Gram-negative pathogens, respectively, is among the enzymes targeted for matrix disruption.[26] Treatment with such enzymes prevented *Staphylococcus* and *Enterococcus* biofilm formation and disperse preformed biofilms *in vitro*. For example, Dispersin-B is a glycoside hydrolase that cleaves β 1–6 N-acetylglucosamine polymers in the bacterial peptidoglycan layer. Dispersin-B treatment has been shown to be effective against *S. aureus* and *S. epidermidis* biofilms and bacteria.[27]

Chelating Agents:-

Metal cations, such as calcium, magnesium, and iron have been implicated in maintaining matrix integrity. Chelating agents have been shown to destabilize biofilm architecture besides interfering with bacterial membrane stability. For example, sodium citrate inhibited biofilm formation by several *Staphylococci* species *in vitro*.[28]

Antimicrobial Peptides:-
Lytic peptides are antimicrobial peptides assessed for their inhibitory effects on biofilm formation. Lytic peptides bind the lipopolysaccharide moieties of the bacterial cell membrane, disrupting membrane stability.[29] Studies in *Staphylococcus aureus* have shown that a lytic peptide prevented *in vitro* biofilm formation and was also capable of diffusing into the deep layer of preformed biofilm, killing 99.9% of biofilm bacteria. This peptide retained activity under highly acidic environments and in the presence of excess of metals, conditions that mimic the *S. aureus* biofilm environment.[29]

In 2007 Novak et al,[30] evaluated the effectiveness of a novel synthetic anti microbial lentivirus lytic peptide against *Streptococcus gordonii* an early colonizer, *Fusobacterium* a bridging colonizer and *Porphyromonas gingivalis* a late colonizer associated with multiple forms of periodontal disease.

Anti-adhesion Agents:-
Attachment constitutes the first step in biofilm formation, thus studies have focused on preventing bacterial adherence. Efforts have been made to inhibit assembly of different types of pili, through the use of pilicides, which are compounds rationally designed to interfere with export of the corresponding pilin subunits. Pilicides were shown to inhibit biofilm formation *in vitro* by 50%, at concentrations as low as 3 μM.[31]

Antagonistic Strains To Reduce Adhesion:-
Van Hoogmoed et al.[32] evaluated 6 antagonistic bacterial strains for their capacity to reduce the adhesion of periodontopathogens and block their attachment to a surface. Their results indicated that *Actinomyces naeslundii, Haemophyllus parainfluenza, and Streptococcus mitis* provided the strongest blocking of adhesion by *Porphyromonas gingivalis.*

Immunization:-
The immunization inhibit adhesion or reduce the virulence of putative microbial etiologic agents. Passive immunization in humans impedes recolonization of selected target microorganisms in periodontal disease.[33] Immunization approaches are directed against single bacterial species epitopes, but periodontal disease are ecologically driven multi-microbial diseases.[34]

Signal Transduction Interference:-
- ❖ Two Component Systems
- ❖ Quorum Sensing Inhibition

Two-component signal transduction systems and histidine kinases represent potential prophylactic targets.[35] Histidine kinases and response regulators of the two component systems exhibit both conserved and variable domains and can be used for the development of inhibitors with species.

Quorum-sensing inhibitors:-
The biofilm formation can be disrupted by alarming the quorum sensing mechanism utilized by the various species of bacteria that together form the plaque biofilm. The inhibition of quorum sensing is commonly referred to as quorum quenching.
It can be accomplished in several ways, including:[36]
- ❖ Enzymatic degradation of signaling molecules
- ❖ Blocking signal generation
- ❖ Blocking signal reception.

They are grouped into two categories according to their structures and functions.[37]
a. The structural mimics of quorum-sensing signals, such as the halogenated furanones and the synthetic AIPs that are similar to the AHL and AIP signals, respectively.
b. The small chemicals is the enzyme inhibitors.

Strategies Based On Nanobiomaterials:-

Nano elements (NEs) for Biofilm Inhibition:-

Even if bacteria adhere, there are promising antibacterial approaches to prevent further growth and biofilm initiation through NE-based assemblies that target bacteria or biofilm components. The bioactivity can be "programmed" through a) physicochemical mechanisms (i.e., cell membrane rupture, charge interaction, blockage of membrane channels) b) biochemical mechanisms (i.e., enzyme inhibition, prevention of DNA replication) c) toward the biofilm matrix by disrupting exopolysaccharide (EPS) synthesis.[39]

Surface attachment in situ can be attained with the use of mouthwashes and dentifrices containing NEs. The efficiency of this approach was explored in a recent clinical study by using formulations with silver nanoparticles.[40]

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