Role of Nanoparticles as Antibiofilm Agents: A Comprehensive Review

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ABSTRACT
Biofilm forms with the colonization of different microorganisms resulting in a microbial community which is complex and diverse in nature. Biofilms are enclosed with different microbial colonies in a matrix known as extracellular polymeric substance. Microorganisms together in this complex structure communicate through cell signaling molecules. This process is known as quorum sensing which helps in maintaining the complexity and diversity. Biofilms are involved in causing various infections in human beings. Oral cavity and wounds are very common two sites where biofilm formation takes place. The complexity of a biofilm makes it tenacious and it’s tough to eradicate completely. There are several conventional methods such as physical, chemical, antibiotics, antiseptics and anti-biofilm agents for the eradication of dental/periodontal biofilms and biofilms associated oral wounds. Thus, various novel approaches can be proposed, one of them is Nanotechnology. Its extensive application in dentistry can be very helpful in curing various periodontal and dental diseases. Nanoparticles are one of new emerging potent system which can be designed and an improvement can be done to the available methods or some new method can also emerge.

Keywords: Biofilm, Oral biofilms, Periodontal diseases, Nanoparticles, dental plaque.

INTRODUCTION
Biofilm formation is a unique property of microbes. This property of microbes helps them to survive in harsh conditions and allow them to proliferate as a microbial community. Generally, biofilms have been depicted in several systems since Antony Van Leeuwenhoek examined the “animalcules” in the dental plaque on his own teeth in the seventeenth century, but the general theory of biofilm predominance was not promulgated until 1978 [1]. In human body, apart from normal microflora, microbe gets adhere to a surface and results in the formation of biofilm. The best salient description of a biofilm is given by Donlan and Costerton in 2002, who stated that a biofilm is “a microorganisms derived sessile community which are characterized by those cells that are irreversibly attached to a biotic/ abiotic substratum or interface or to each other, are embedded in a matrix of extracellular polymeric substances that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription” [2]. Such film like structures formed by the combination of different microbes is involved in etiopathogenesis of various diseases in animals and humans as well. According to IUPAC, “Aggregate of microorganisms in which cells that
are frequently embedded within a self-produced matrix of extracellular polymeric substances (EPSs) adhere to each other and/or to a surface" [3].

Microbial community adheres to biotic or abiotic surface and start to secrete some polymeric substances which result in a layer like structure. Later on, other microbes come and gets associated with this slime layer and a microbial community is formed. In a biofilm, microorganisms yield extracellular polymeric substances, such as exopolysaccharides (EPS), which procreate a high counteraction to host immune responses and limited penetration of antibiotic into biofilm [Figure 1] [4-6]. ‘Biofilm’ is a term given by Wilderer and Charaklis in 1989, depicted the comparatively ineffable microbial summation which is allied with a surface or some different substantial non-flicking material, conjecturally dispensed in a framed matrix or glycocalyx [7].

Biofilm implicated in etiology of different dental & periodontal diseases as the major cause of plaque formation in oral cavity. Some studies showed that biofilms are also responsible for causing severe periodontal inflammations, oral wound formation, acute to chronic. Presently, in a milestone study, James et al., used light and scanning electron microscopy to scrutinize the existence of microbial biofilms, manifestation of agglomerated colonies of microbes occupied by EPS, in wounds acute and chronic stages [8]. According to them, biofilm formation exhibit only in 80% of chronic wounds whereas in acute wounds only 6% of biofilm is incorporated.

Oral cavity is surrounded by various microbes present in the form of dental plaque and involved in the formation of biofilm. Though, it is not articulate whether chronic wounds with or without biofilms are impudence or sanative. Biofilms are mostly existing in chronic wounds than in acute ones [9-13].

**DENTAL AND PERIODONTAL MICROBIOLOGY**

Complete microflora located in the oral cavity is commonly referred as the oral microbiota, or oral microbiome, which can be defined as "whole microorganisms that are found on or in the human oral cavity and its proximate expansion". In early 1970’s, dental plaque was a challenging field among dental scientists. Scientists emphasized to find out the tenor rendering the multicity of oral microbial ecosystem such as pH, nutritional requisition etc. The importance of the plaque ecosystem was recognized by Loesche in 1976 and he proposed both nonspecific and specific plaque hypothesis individually for oral disease progression. Approximately, 1000 microbial species have been recognized in oral biofilms till now using different techniques [14].

Dental plaque is defined as, “a variable structural formation of highly organized intercellular matrix in which different microbes and their secreted products present forming a slime layer on the outer surface of the teeth enamel”. It is colourless layer at first but after tartar formation, it becomes yellow to brown in colour, covering teeth as well as the gum line. Progression of the dental plaque may lead to several oral problems like tooth decay, dental caries, gingivitis, etc.

**DENTAL AND PERIODONTAL MICROBIAL COMPLEXES**

In oral cavity the commonly found microbes, responsible for biofilm formation are Streptococcus spp., Lactobacillus spp., Actinomyces spp., Propionibacterium spp., Veillonella and other anaerobes which may vary as per the location in the mouth. Examples of such anaerobes include Fusobacterium spp., etc. [Figure 3] [15]. S. mutans and other anaerobes play important role in the initial colonization on tooth surface for the establishment of the early biofilm community [16]. In oral cavity, various microbial communities form complexes with each other and results in the formation of a tough layer which shows a characteristic color of plaque shown in Figure 4 [17].

**CLASSIFICATION OF DENTAL BIOFILMS:**

Dental and periodontal surfaces are peerlessly hard and non-flicking, unlike other parts of body. So, the dank and torrid environment of
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the oral cavity and the presence of rigid surface (teeth) create best condition for growth and development of dental plaque.

Biofilm present on the teeth and gum-line can be classified on two bases:

1. **On the basis of the location:** [18]
   a) **Supra-gingival biofilm:** It is the type of biofilm which forms on gum-sit. It is the initial stage of plaque which commonly forms after the brushing and generally found in between the teeth, tooth-pits, tooth-grooves and in gums. The deposited layer of plaque consists of aerobic bacteria. Prolong persistence of initial plaque allows growth of anaerobic bacteria and convert into complex matrix of biofilm [19].
   
   b) **Sub-gingival biofilm:** Sub-gingival biofilm proliferates upon the supra-gingival biofilm. This type of biofilm grows in downward direction up to the gum line (from top of the teeth to bottom). It constitutes anaerobic bacteria, which cause infections [20].

2. **On the basis of pathogenicity of microbes:**
   a) **Cariogenic biofilm:** Its formation takes place due to the deposition of acidogenic and gram-positive bacteria which particularly forms due to the regular long term uptake of cariogenic diet such as sugars.
   
   b) **Preiopathogenic biofilm:**
   
   Its formation takes place due to the basophilic and gram negative bacteria.

**FORMATION OF DENTAL BIOFILMS**

The complex process of oral biofilm formation begins with association and attachment of free microbes and sessile microbes present in the oral cavity on to the tooth surface. A film like layer started to form after the secretion of extra polymeric substances and some of host molecules.

The whole process of formation of dental biofilm is ensued by passive transport of bacteria mediated by weak long-range forces of attraction. Strong and short-range forces exerted by covalent and hydrogen bonds results in irreversible
The cell in the microbial community of oral cavity biofilms communicates with each other by process of Quorum sensing. It is a very important process of cell to cell signaling in dental biofilms as well as in other biofilms. This process is vastly carried out by different varieties of gram-positive and gram-negative bacterial species to co-measure with other species of microbes present in biofilm for various metabolic activities. This cell to cell signaling is the means of communication between all microbes present in the microbial community to regulate a wide range of behavior patterns among them. The various interfering compounds generated by the bacteria have an effect which can be either positive or negative on the expression of bacterial phenotypes generally regulated by quorum sensing (a process of communication between microbes in biofilm community). [22]

By the process of auto-aggregation (same species attraction) and co-aggregation (different species attraction) the cell to cell signaling ensures the coordinated behavior of microbes present in the biofilm community.
species attraction) early colonizers form a biofilm. Functional organization of dental plaque microbes results in co-aggregation, and conformation of structures such as ‘Corncobs’ and ‘Rosettes’. Now environment of oral cavity changes to facultative anaerobic from aerobic. Microbes which are already present started to multiply and secrete EPS, resulting in a paved mixed-population of microbes forming slime layer. [21] [23]

Fully organized mature complex slime layer formation takes place after one day, the term Biofilm is now suitable for it. Other new microbes get incorporated into the biofilm resulting in a complex summation. The plumpness of the dental plaque rises slowly and steadily day by day; it grows up to 20 to 30 μm in every three days. [21]

**PROBLEMS CAUSED BY PERIODONTAL MICROBES**

Oral problems/diseases have a significant economic impact on health care principles across the world, such as dental caries affects over 70-80% of the population in several countries, so there is an important impact of all dental problems on the quality of life, disease free life and implications for systemic health [24].

The dental biofilm is the basis of many oral problems; including endodontic infections, periodontal disease and dental caries. The most
common dental and periodontal problems among human community are tooth decay (cavities, dental caries) gingivitis, and periodontitis. Apart from this, evidences propounded that pathogenesis of systemic disease including rheumatoid arthritis, cardiovascular disease, respiratory disease, and other conditions are also caused by oral microbes [25-26].

The etiology of different dental and periodontal microbial diseases has been pervasively studied; however, the complexity of nature and diversity of composition in dental and periodontal biofilms makes it difficult to identify the main culprit involved to carry out the whole process. Early researches solely focus on identification of that pathogen which is responsible for oral problems such as dental caries, gingivitis, and chronic periodontitis. It is now generally accepted that these diseases result from the concerted actions of multispecies microbial communities [27].

There are several conventional culture methods available which have traditionally been used for the characterization of oral microbiota; however, most of the proportion remains unculurable, which leads to partial knowledge of wild type microbial communities involved in various oral problems. [28] Such microbial appurtenant and huddle of various pathogenic biofilms on surfaces of implant plays considerably important role in peri-implantitis pathogenesis [29-31].

**BIOFILMS ASSOCIATED WITH PERIODONTAL DISEASES AND WOUNDS**

For many years in periodontal infections, biofilms have been studied and accepted widely. Wounds can be extensively categorized according to the etiology condition as an acute or a chronic wound. Wounds in chronic condition generally have deferred healing pattern and repeatedly have some endogenous factors that conciliate the process of healing in which microbes associated with biofilms plays active role in wounds. In severe inflammation and wound/ ulcer formation inside the oral cavity, two anaerobic oral bacteria are the subject of extensive study 'Porphyromonas gingivalis' and 'Fusobacterium nucleatum'. These two bacteria are commonly found in vicinity of periodontal region attached with the epithelium of the gingiva [32-33].

In recent years, most of the researchers are focusing on wound associated biofilms. It has been observed in few studies, that the wound healing gets delayed due to the persistence of pathogenic biofilms, which may lead to the several chronic diseases. [34-36].

**CONVENTIONAL METHODS AVAILABLE UP TO DATE:** Conventional methods available for the removal of dental biofilms which causes various oral problems are brushing, flossing, gargling, interdental brushing, tongue scraping and sub gingival irrigation. [37-41]

Conventional methods have been traditionally used to remove harmful oral microbiota; however, a large proportion remains as it is in mouth, leading to the formation of plaque and helpful in generating new oral problems. Microbial biofilms are generally characterized as highly resistant towards antibiotic treatment and host immune responses. There are several drugs which are used as a conventional method to control plaque and other inflammatory infections. One of the widely used drugs are;

1. **Chlorhexidine** which is an antibiotic used to control dental plaque and periodontal diseases caused by microbial community. It increases the staining of tartar present on teeth. Brushing helps in removal of tartar build up and staining. This medication is available as mouth rinse and gelatin- filled chips that are placed in deep gum pockets which released slowly over seven days.

2. **Doxycycline** is used to help treat periodontal diseases. It prevents growth of microbes. It is placed in gum pockets next to teeth and it dissolves naturally over seven days. This drug is having some side effects such as permanent discoloration of teeth, slow bone growth and therefore not recommended during breastfeeding, etc.
3. **Fluoride** used to prevent tooth decay. It is available in many types of toothpaste. It is absorbed by teeth and strengthens teeth to resist acid and blocks the cavity-forming action of microbes and helps in tooth sensitivity reduction.

4. **Tetracycline** used commonly in various therapies or alone, to reduce or eliminate microbes temporarily which are associated with periodontal diseases, to suppress the destruction of tooth attachment.

Dental antibiotics used conventionally are easily available in market in the form of gels, thread like fibers, microsphere, mouth rinses, and medicated toothpastes.

In general, the antibiotic drugs have certain chemical properties which lead to various side-effects. Moreover, regular use of antimicrobial drugs may raise anti-drug resistant property in microbes. Generally, microbes secrete certain products for stability maintenance of biofilm such as EPSs & eDNA which is an important component of both fungal and bacterial biofilms and both of these are related to antimicrobial resistance. [42]

In case of biofilms present in wounds treatments, modalities present up to date are;

1. **Debridement**: This process can be autolytic, enzymatic, surgical, or mechanical (hydrotherapy). Effective controlling of biofilms in oral wounds can be done by this method. In the case of chronic wounds, it is suggested for removal and suppression of biofilm [43, 44]. There are various debridement techniques present such as plasma-mediated bipolar radiofrequency ablation technique (PBRA).

2. **Antibiotics and antiseptics**: Biofilm generally consist of subpopulation of survivor cell of antibiotic resistance. In local oral wound infection, it is suggested that antibiotics are effective only 25-30% [45]. 0.2% solution of povidone-iodine, chlorhexidine gluconate, benzalkonium chloride or alkyl diamino ethyl glycine hydrochloride shown effective and fast bactericidal activity in the case of periodontal diseases [46, 47].

3. **Anti-biofilm agents**: A component which is present in human tears, mucus, and milk known as Lactoferrin. It is considered that this component clogs biofilm formation by comming microbial motility and reduction in surface attachment. Lactoferrin synergistically with Xylitol impairs biofilm matrix development. Lactoferrin with xylitol in methylcellulose gel works as a good anti-biofilm agent. [45]. There are some polysaccharides designed by the researchers which shows the anti-biofilm property by inhibiting EPS formation. *P. aeruginosa* secretes PsI and PeI which show anti-biofilm property [48, 49].

**NANOPARTICLES AND NANOMATERIAL A NEW APPROACH IN THERAPEUTICS**

The ‘nano’ is a Greek word, which means small in size which is used as the prefix for the billionth from the range 9 to 10. Nanoparticles are the particles which have two or more dimensions in the size range as 1 to 100 NM (ASTM International) [50]. It is well known with the ongoing researches nanoparticles have unprecedented physical and chemical properties in comparison to their solid bulk materials as their sublime surface area and unique electronic properties so it has been utilized in many aspects of science such as photochemical, electrochemistry, and biomedicine [51]. Nanoparticles have great application podium that can be exploit for various therapeutic aspects and different functions. Such particles can be easily synthesized from various inorganic and organic metals and compounds, but for simultaneous therapy and diagnosis, the inorganic materials are very important because they can be modifying easily, high loading capacity for drug and stability [52]. Such particles can also be utilizing in drug delivery systems and for the different drug determination in pharmaceuticals as well [53].

**Types of Nanoparticles:**

There are various types of nanoparticles differing in size, shape, compositions, and functions. Each type of NP’s can potentially be concoct using various techniques, such as both nanoprecipitation and lithography for polymeric nanoparticles.

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There are three basis of classification:  
1) Origin, [54] 2). Dimension, [55-57] and 3). Structural organization [58-63].

**Synthesis of nanoparticles:**
To synthesize nanoparticles, there are two approaches: 1) Top down approach and 2) Bottom up approach. In the process of bottom up approach, the particles get arranged as smaller components into more complex assemblies. Chemical or physical forces are used for the operating at nanoscale for the assembling of basic units. Whereas, in the top down approach bulk material is converted into smaller sized nanostructure.

There are three methods of synthesis, physical, chemical and biological;

1) **Physical method:** It’s been carried out via two different methods such as Mechanical method and Vaporization

2) **Chemical method:** It is the most promising method of nanoparticle synthesis. It’s been carried out via colloids and colloids in solution and Sol gel method

3) **Biological method:** It is also known as green synthesis. It is done by three types, by the use of microorganisms such as fungi/yeast, plant extracts or enzymes and
templates like DNA, membranes, viruses and diatoms.

**ADVANCE CONTROL OF ORAL BIOFILMS**

Dental and periodontal biofilms are intensely pliable microbial assemblies that are challenging to eradicate. Due to lacunae in existing methods of biofilm eradication and development of antimicrobial resistance in conventional drugs, there is necessity to explore the development of new anti-biofilm agents. Polysaccharides and eDNA, which are complex biofilm matrix, seem to be a good target for future therapeutic strategies [66].

Use of Nanoparticles is a suggestive approach in developing various methods to eradicate or control oral pathogenic microbes. Neoteric microbiological studies have umbilici on developing novel antimicrobial agents with higher efficacy while being non-invasive and non-toxic and not causing drug resistance such as nanoparticles and new drug delivery systems which will be helpful in enhancement of medical therapeutics [67, 68]. Nanotechnology potentially benefited in the field of biomedical, it has become widely conceded for the treatment of various microbial diseases with a new generation of hopeful strategies [69]. Metals are widely used in the synthesis of nanoparticles due to the antibacterial properties commonly used metals as antimicrobials are silver, gold, zinc, copper, and titanium. For centuries, these metals have been known and been avail in modern medicine for infection control. Due to the broad-spectrum antibacterial activities Silver containing (Ag-containing) nanomaterials is of extensive interest [70-76]. Some of the metals showing antibacterial

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![Diagram of top-down and bottom-up approaches to generate nanoparticles.](image)
properties which can be used as promising and effective agent to design nanoparticles which can increase the efficacy of conventional methods to combat with different infectious pathogens causing diseases in human body. A list has been compiled showing the metal nanoparticles having potential anti antimicrobial properties (Table 1).

**CONCLUSION**

Improper tooth care leads to the deposition of biofilm which is not easy to eradicate using conventional methods. Nanotechnology is a new approach in medical therapeutic field of dentistry as well as wound healing. It can be very helpful in providing sustainable methods to increase accuracy, attributes and fast treatment procedure which will be economically cheap and easily available. The current review article enlightens the literal perspectives of nanotechnology in dentistry to cure problems caused by pathogenic oral microorganisms. In near future, this technology may become the core of therapeutics in dental and medical science.

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