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A review on organic nanoparticles for treatment of bacterial biofilms

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Abstract

Biofilm is one of the significant problems that has to be resolved quickly for treating bacterial illnesses, that is important to the pathogenicity along with resistance of bacteria. A popular area of research now centres on examining effective ways to control bacterial biofilm. Organic nanoparticles (NPs) have demonstrated higher potential when compared to other metrics due to their special characteristics, in eradicating complications caused by bacterial biofilms. Additional advantages associated with NPs synthesis in biofilms include larger surface areas and increased biomass concentrations, which can result in more effective and expandable biosynthesis. This review began with an overview of biofilm formulation based on the publications that were searched. Second, the effectiveness of organic NPs in combating bacterial biofilms and potential anti-biofilm mechanisms (such as reduction of biofilm adhesion, improving permeability, increasing stability, and degradation of biofilms) were examined. Thirdly, the effects of NPs and biofilm characteristics on the effectiveness of organic NPs in combating biofilms was explored. Finally, challenges and prospects for organic NPs in the future against biofilm were in conclusion. Researchers can learn more from this review about the successes and limitations of NPs in the fight against biofilms, which will assist in facilitating the development of organic NPs that are more effective.

Keywords: Pathogenicity, Biosynthesis, Nanoparticles, Organic, Anti-biofilm

1. Introduction:

The term "biofilm" describes the bacterial adherence to their contact with the surface of either inanimate or live objects using their own extracellular viscous compounds that are secreted, including fibrin, lipoprotein, and polysaccharide matrix to develop numerous microbial aggregations when triggered by some external environmental factors. Greater than 90% of microbes, including *Staphylococcus aureus*, *Pseudomonas*

aeruginosa, *Candida sp*. develop in this way (Costerton, Stewart.,1999). It is a significant issue that needs to be resolved quickly for the treating the of microbial diseases because it is important to the pathogenicity along with the resistance of bacteria (Rasmusssn.,2000). Biofilm's three-dimensional structure acts as a natural barrier against antibacterial medications, decreasing its susceptibility to antibiotics and making treatment challenging. Antibacterial small molecules and surface-active compounds (such as some antimicrobial peptides (AMPs), surfactants, bromphenazine, etc.) typically have difficulty due to their hemolytic and acute toxicity, which limits the applications for them.

It has been determined through numerous investigations conducted in recent years that nanoparticles (NPs) with distinct chemical and physical characteristics, when exposed to physical damage, heat damage, oxidative stress, and other forms of damage, bactericidal activity and certain mechanisms can stop the growth of bacterial biofilm. This finding holds promise for the future control over diseases caused by bacteria, for enhancing their effectiveness of antibacterial medications, and for overcoming bacterial resistance brought on by biofilm. The majority of organic NPs exhibit improved biofilm dispersal and excellent biocompatibility, making them important subjects for future study on anti-biofilm therapies. (Duncan et al., 2015). In order to study the conceptual framework for more effective organic nanoparticles, this paper provided an overview of the development, hazards, and treatment issues related to biofilms as well as the advancement, organic nanoparticles' mechanism, challenges and possible future applications in combating bacterial biofilms are all examined. This review discusses the use of nanotechnology to prevent or eliminate biofilms, as well as the physicochemical interactions between EPS components of the biofilm matrix and NPs.

2. Formulation of Biofilm:

The dynamic process of biofilm formation is influenced to some extent by a number of parameters, including the external surroundings, variations in strains, and signal transmission. The development of biofilms generally occurs in five stages:

2.1 Adhesion-Bacteria improve the adherence of the cells to the carrier surface by using external organelles (like flagella, hyphae, fimbriae) and extracellular membrane proteins carrier that passes through the EPS released by the cells (Stewart et al., 2013). Once cell adheres to a solid support, division of cells, and signals from neighbouring bacteria change and increase its phenotype.

2.2 Proliferation- Following the previous step, cells that were attached to the surface of the carrier can form microcolonies. As the local environment is further perceived, secretion of proteins, lipids, nucleic acids, and polysaccharides are all present in the sticky extracellular matrix (EPS). During this process, microbial colonies clearly multiply, EPSs dramatically increase to cover the cell surface in a layer of hydrogel. (Flemming, Wingender, 2010).

2.3 Exodus- Time-lapse microscopy was only recently used to demonstrate that Nunc-mediated eDNA degradation took place prior to the formation of the biofilm and mediated exodus events. This stage of biofilm development is highly regulated (Moormeier et al.,2014). At this point, just a tiny percentage of the biofilm's cells secrete nuclease, which mediates the detachment of most of accumulated populations of biofilm.

2.4 Maturation- The layer rich in nutrients promotes the quick growth of microorganisms by utilizing the biofilm environment. A mature biofilm has complex diffusion channels that transfer other elements, such as oxygen and nutrition that are necessary for bacterial growth as well as waste materials and dead cells. On the carrier's surface, a fully developed membrane with a three-dimensional structure appears as attached colonies grow and EPS standardises sequentially.

2.5 Dispersal- A part of the matrix is degraded during this process, and the biofilm is actively dispersed, leading to recolonization afterward. Particular enzymes play a role in breakdown and reformation of the biofilm. (Flemming et al., 2007)

3. Organic Nanoparticles to Eradicate Biofilm:

3.1 Reduction of adhesion property- Bacterial infection and biofilm formation are inevitable consequences once the bacterial adhesion turns into an irreversible bond and starts to generate free energy on its surface.

Therefore, one of the main goals of treating biofilms is to decrease their adhesion. The adhesion-reducing properties of organic NPs can be engineered primarily based on the two factors: the biofilm environment and the characteristics of NPs. Since NPs have distinct physical characteristics, their hydrophilicity can change the surface roughness that makes up the biofilm attachments, causing them for challenging to adhere and inhibiting the growth of the biofilm. Research has revealed that the first stage of biofilm development is hampered by the extent of surface which differs because of their smoothness, and the amount of biofilm that forms is decreased. Significantly greater hydrophilicity in a naturally occurring polysaccharide, chitin, is employed to prevent biofilms from adhering. Coaxial electrospinning of nanocrystals could virtually eliminate adhesion of biofilm and consequently demonstrated a strong antimicrobial activity directed against *Escherichia coli* (Jalvo et al.,2017). By altering the surface morphology of hydrophobic organic nanomaterials and employing repulsion, biofilm adhesion can be decreased. For example, polystyrene, polyethylene, and superhydrophobic polycarbonate all effectively decrease the adhesion of *E. coli*.

3.2 Stronger Penetration- Organic nanoparticles can play a role as carrier because of their small size and surface charge, which increases the permeability of their packaged antibacterial drugs through bacterial membranes, thus increasing the antibacterial effectiveness. The biofilm's negatively charged matrix of polymers and its negatively charged surface make it more likely for positively charged NPs to disrupt the bacterial membrane's integrity. After entering biofilm, nanoparticles release an antimicrobial agent and exhibit an improved antibacterial effect. The chitosan nanoparticles' positive charges are combined with the biofilm's adverse charges for increasing the permeability of curcumin across biofilm enabling it to permeate the biofilm (Ma et al.,2020). The increased penetration efficiency throughout biofilms is also made by positively charged pH-sensitive NPs under pathological circumstances along with negatively charged particles under physiological pH, a viable option to treat biofilms. It is clear that increasing the permeability of nanoparticles is a key strategy and a game-changing development for drugs carrying nanoparticles in their anti-biofilm properties. Because DNA degradation by DNase type- I, is an important step in the formation of biofilms, nanoparticles triggered by DNase I cannot just disrupt biofilm formation but also inhibit their formation. *Pseudomonas aeruginosa* biofilms are reduced, but the established biofilm is also reduced(Baelo et al.,2015).

3.3 Enhanced Stability- Organic nanoparticles are used for improving antibiotic penetration, can also improve antibiotic stability in the body through encapsulation. It might not be possible to guarantee that all antibiotics along with other small-molecule anti-biofilm drugs reach the infected area of the biofilm and kill it due to their inadequate stability in the body. As a result, the application of NPs that are comparatively stable in order to increase the antibacterial agents' stability, increase efficiency, decrease waste and resist biofilm. As an example, some phytochemicals, are promising agents for combating multi-drug resistant bacteria (MDR) because they are inexpensive, biocompatible, and may have anti-biofilm characteristics. Furthermore, multi-layer modification of the surfaces of organic nanoparticles can improve composite stability. Using the unique layer-by-layer (LbL) method, stable polyelectrolyte-modified NPs have been produced. (Ivanova et al., 2018). The nanosize of this layer-by-layer conjugate with the abundance of accessible amino groups on NPs outer layer enhanced their stability. Without affecting the viability of human cells, an association between the bacterial phospholipid bi-layer and NP, ruptures the membrane and prevents *Staphylococcus aureus* and *E. coli* from forming biofilms (94% and 40%, correspondingly). Organic NPs can be employed for increasing biological stability of enzymes. Polymer nanoparticles, for example, can be used for encapsulation of PDH (pyruvate dehydrogenase) and successfully improve its stabilizing property. It takes in pyruvate (which facilitates the growth of *Pseudomonas aeruginosa* biofilm), thus disrupt *Pseudomonas aeruginosa* biofilm formation (Han et al.,2019).

3.4 Degradation of Biofilm- Some nanomaterials have the ability to either strengthen the QS inhibitors' inhibitory action or directly regulate the QS mechanism, eliminating mature biofilms. It has been found that QS significantly affects biofilms, and this effect is typically mediated by a limited class of chemicals called autoinducers (AI). Organic nanoparticles can be used to boost their anti-QS effect. As a result, this inexpensive process and the creation of biomaterials that are not toxic has considerable potential for the prevention of biofilm formation. Aside from Quorum Sensing, interacting with proteins is a helpful strategy to prevent and eradicate biofilms since particular proteins in biofilms are crucial to their development (Kłodzi´nska et al., 2019). Interaction of organic NPs with biofilm shown in figure 1.

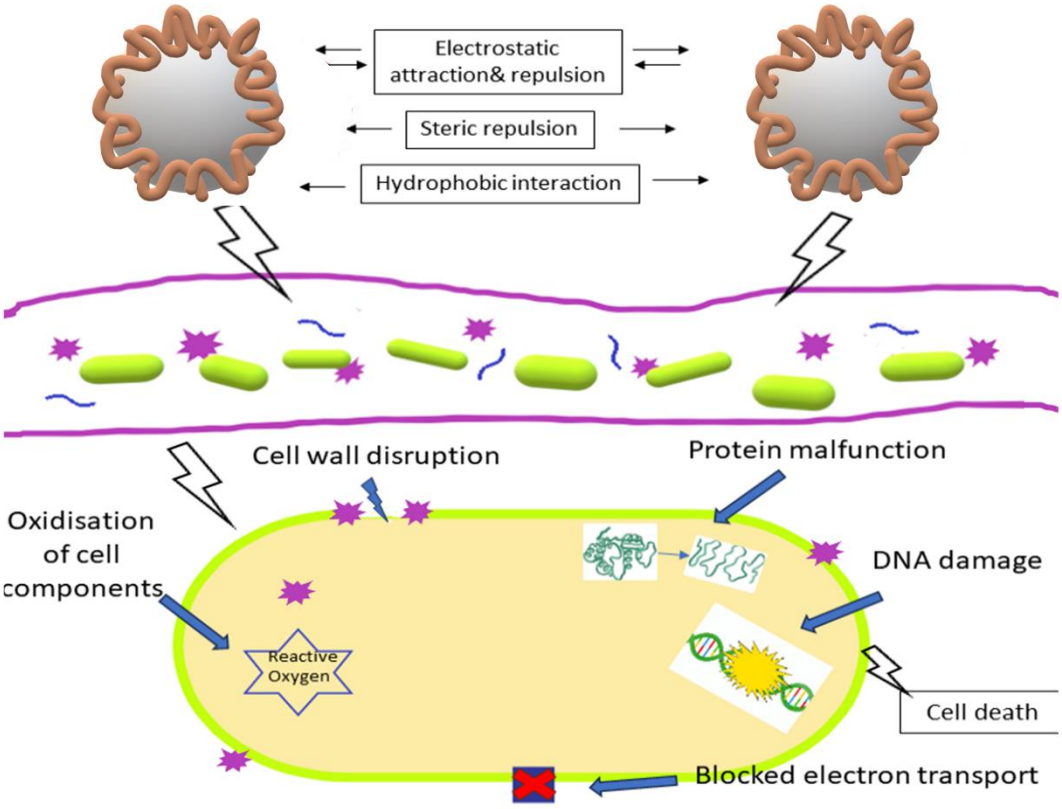

Figure 1: Organic NPs and biofilm interactions

4. Factors influencing organic nanoparticles as anti-biofilm:

4.1 Physical and chemical properties: Organic nanoparticles' activity against biofilms has been shown in studies to be intricately linked to their chemical and physical characteristics, including particle size, surface charge, surface features, and particular alterations.

Surface charge: Controlling the charge of organic NP's surface allows them to maintain their anti-biofilm property. NPs with positive charge, can adhere to the biofilm surface layer that is negatively charged using the electrostatic action. Organic NPs which are positively charged are capable to improve antimicrobial agent invasion (Mu et al., 2014).

Particle size: Smaller nanoparticles can more specifically target specific bacteria (Natan et al., 2015). The size of the particle influences nanoparticle delivery inside the body. In between a specific range, more deeply the nanoparticles invade the body, the tinier they are. and the greater their permeable property.

Surface characteristics: The substance is more suitable to bacterial adherence and formation of biofilm the rougher its surface**.** An excessive rise in surface roughness decreased adhesion rather than promoting biofilm formation. However, as the surface roughness increases, air in the space between the NP and the bacteria increases, reducing the contact area and, as a result, the biofilm's adhesion (Mi et al., 2018). Several studies have demonstrated that higher hydrophobicity is capable of lowering biofilm adhesion. This is due to higher hydrophobicity may decrease contact surface, and the introduction of air layer reduces protein adsorption, which further lowers biofilm adhesion.

Functional modification via biomolecules: The pH sensitive NPs may be intended to encourage drug accumulation in target tissues by manipulating pH fluctuations. At the moment, an increasing number of studies are giving insight in an alteration of NPs by biological molecules. According to research, double or multiple biological molecules modifications may considerably improve the anti-biofilm effect. As an example, the combination of photodynamic treatment and matrix metalloproteinase (MMP)-sensitive supramolecular NPs (S-NPs) enhances antibacterial activity against biofilm-related bacterial keratitis (Han et al., 2019).

4.2 Biofilm Characteristics: The characteristics of the biofilm, besides to the nanomaterial, influence its formation. (Fulaz. et al., 2019). Bacterial biofilm heterogeneity is influenced by a variety of factors, including types of bacterial species, variations in strains, and serotypes. Studies have been clarified that different bacteria's biofilm might respond differently to the exact same nanoparticles. It has also been shown that when temperature rises, it increases the quantity of *Staphylococcus aureus* biofilm generated as a result. However, the amount of biofilm generated decreases when it reaches 37°C, possibly as a result of increased bacterial metabolism. (Kim et al., 2020). In conclusion, biofilm heterogeneity has made it more difficult to eradicate harmful microorganisms using nanoparticles and has also led to unpredictability and inconsistency in safety evaluations, especially when it comes to the risk diminution efficiency assessment of sterilisation procedure. Some examples of organic NPs used as anti-biofilm agent tabulated in table 1.

Table 1: Some examples of organic NPs used as anti-biofilm agent

Sl. No.	Organic Nanoparticles	Targeted Bacteria	Performance	Reference
1	Chitosan NPs	*Staphylococcus Aureus, Candida albicans*	Compared with unrestricted curcumin, bactericidal and biofilm activities against bacterial biofilms was superior.	Ma et al., 2020
2	Polythioene nanoparticles	Multidrugresistant *Klebsiella pneumoniae*	Higher anti-MDR Klebsiella pneumonia activity and a lower minimum inhibitory concentration (MIC) in contrast to drugs	Lou et al., 2018
3	Cellulose nanocomposites	*Staphylococcus Aureus E. coli*	Mainly lowering the Cordier in pigments' production which is QS-related	Demircan et al., 2017
4	Ag-Nps	*S. aureus, K. pneumoniae, P. aeruginosa, E. coli, S. flexneri, and S. mutans*	ROS generation, anti-bacterial drug carrier	Prateeksha et al., 2019
5	Hydrogels	*Acinetobacter baumannii, MRSA, S. aureus, and P. aeruginosa*	Biofilm disruption, healing of wounds	Yeo et al., 2018
6	MMP-S NPs	*P. aeruginosa*	After an 8-minute effect, MMP-S NPs' antibacterial rate increased to 99.997%.	Han et al., 2020
7	Polyhydroxybutyrate (PHB) nanoparticles	*Staphylococcus aureus*	Polyhydroxybutyrate NPs, loaded with melanin, successfully inhibited the development of Staphylococcus aureus biofilm.	Kiran et al., 2017
8	Polymer nanoparticle carrier (NPC)	*Streptococcus mutans*	Farnesol/pH responsive nanoparticles reduce colony forming units (CFU) by about 2-4 logs	Sims et al., 2019
9	SPIONs	*S. aureus, S. mutans, M. tuberculosis, H. pylori, and P. aeruginosa*	Cell lysis, oxidative stress, and preventing colonisation	Hasanzadeh et al., 2015
10	Nanogels with hyaluronic acid (OSAHA)	*P. aeruginosa*	lowering interactions between mucins and pre-formed biofilms while retaining azithromycin's low eukaryotic cytotoxicity	Kłodzi´nska et al., 2019

5. Challenges and Future Perspectives:

Though organic nanoparticles exhibit strong effectiveness against the biofilm; research on the biofilm's ability to suppress both quiescent and persistent microorganisms is yet scarce; In this moment, primary treatment for persistent cells is targeted administration of unbound substances. Although biofilms are the most common type of microorganism in nature, their resistance poses a significant challenge that conventional antimicrobial agents have generally failed to meet. The matrix of the self-produced biofilm creates a chemically as well as physically intricate barrier, that protects the bacteria in part. Antibacterial therapy, environmental challenges and immunological responses all result in embedded cells.

Some multidisciplinary approach to dealing with the various challenges about organic NPs as anti-biofilm agents are- a) Future research should concentrate on total breakdown of the biofilm by simultaneously targeting the cells and the EPS matrix, increasing therapeutic impact and reducing toxicity and the emergence of resistance. b) This also critical for emphasising the necessity for organised in vivo research to evaluate these novel technologies' effectiveness involving organic NPs in biological context. c) Another important aspect to look into is the way NPs become altered in biological environments such as the blood, and how these modifications influence their function.

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