

COMBINATIONAL THERAPY OF NANOPARTICLES WITH ANTIBIOTICS& ANTIMICROBIAL PEPTIDES TO INHIBIT BIOFILM FORMATION

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Abstract

Biofilms are intricate communities of bacteria grouped together in a matrix that works against the effects of antibiotics and against the immune system of its host. Some of the most severe diseases occur due to the formation of resistant microbial biofilms. Most of the conventional antibiotics have become redundant due to the emergence of antimicrobial resistance that has rendered their inability to be used as a sole drug to be used as a therapeutic module. Recent advancement in the benevolent approach of nanomedicines has led to the minimization of overuse of antibiotics. One possible approach in treating these chronic diseases that come as an excellent platform for repurposing conventional drugs is to combine established antibacterials like antibiotics with nanoparticles. This technique has been successful in the removal of notorious biofilm associated with a number of pathogenic diseases. The latest developments in the use of nanoparticles used in conjugation with antibiotics and in certain cases with antimicrobial peptides for the possible eradication of microbial biofilms. This review thus focuses on the recent advancements in these synergistic combinational drug

therapies that can be used as a broadspectrum antimicrobial and as an effective strategy to meet antimicrobial resistance.

Keywords: *Antibiotics, antimicrobial peptides, biofilms, drug-resistance, nanoparticles.*

1.INTRODUCTION

Over the last few decades, rising bacterial infections, particularly those caused by antibiotic resistant bacteria, have emerged as a serious threat to global public health. Within the last century, these antibiotic-resistant bacteria have begun manifesting both intrinsic resistance to antimicrobial agents and acquired resistance from other strains through mutation and gene transfer. A number of bacterial strains termed “superbugs” have emerged due to these mutations and selection for years of antibiotic therapy, becoming complex challenges to manage. As older antibiotics used to treat these bacteria become less effective, infections caused by these resistant strains are becoming life threatening. In order to solve this issue, the use of nanoparticles that can be used as delivery agents such as liposomes, polymersomes, or solid lipid NPs as well as effective antimicrobials on their own such as metal and metal oxide NPs, has increased in hopes of improving existing antibiotics. Most of the modern antibiotics target bacteria using a single mechanism. On the other hand, metal and metal oxide NPs which possess intrinsic antibacterial activity are suggested to treat bacterial infections by employing several mechanisms simultaneously. While recent evidence suggests that the generation of reactive oxygen species (ROS) is a common contributor to cell killing downstream of drug-target interactions, antibiotics, particularly bactericidal drugs, typically cause cell death by inhibiting DNA replication (quinolones), RNA synthesis (rifamycins), cell wall synthesis (β -lactams), or protein synthesis (macrolides). However, through ion release, physical interactions, and ROS formation, metal and metal oxide nanoparticles can concurrently target intracellular targets as well as cell walls. This is why, the bacteria find it considerably more difficult in resisting nanoparticle antibacterial treatment (11).

When traditional antibiotic therapy is used, it is particularly challenging because many internal bacteria are dormant or latent. These bacteria are capable of undergoing periods of quiescence where they are alive, but non-culturable, which makes them able to survive for extended periods of time. Therefore, how susceptible they are to antibacterial drugs is affected due to drug targets being downregulated and cellular permeability changing drastically. Microorganisms in damaged tissues are protected by multiple biological structures that surround the infection foci. Under pathological conditions, these adhere to the tissues and show their protective qualities by secreting glycocalyx, which causes enhanced protection and increased resistance to antibacterial drugs(7).

The increase in antibiotic resistance is just as alarming as the infections caused by implanted devices. Biofilm forming microbes usually cause infections related to devices. It is estimated that 80% of hospital-acquired infections involve biofilms. A phenomenon called quorum sensing happens when the population of microbes on the surface of the host is high because there is sufficient cell-to-cell communication to trigger the expression of genes that lead to biofilm formation (11). Antibiotic resistance of biofilms in medical implants and catheters is one example of how the failure of traditional antibiotic therapy has resulted in worsening conditions worldwide. Because biofilms dynamically alter their Extracellular Polymeric Substance(EPS), they are resistant to a variety of antibiotics (6). Perhaps these chronic infections can be treated with nanoparticles-infused antibiofilm to combat the microbial biofilm(1).

Novel nanotechnology-based antimicrobials have recently been employed to target biofilm formations, antibiotic resistant species and planktonic bacteria. Antibacterial agents, along with these novel techniques, have become increasingly effective (1). Hydrogels, liposomes and even polymeric particles are micro- and nanocarriers that are being researched for the treatment of bacterial infections due to their ability to encapsulate and distribute therapeutic substances over an extended period of time. Polymeric biodegradable

nanoparticles(NPs), based on the biocompatible poly (lactic-co- glycolic acid) PLGA, are being researched in our lab as drug delivery vehicles. The great advantage of these NPs is that they can be produced with a variety of methods, most of which are readily scalable, making it possible to encapsulate many different compounds with a wide range of chemical and physical properties (3). The chronic nature of intracellular infections, even with advanced antibiotics in the market, are often not treated completely. Amid these challenges, emerging therapeutic approaches like loading antibiotics into liposomes, nanoparticles, and colloidal carriers have been utilized to improve contamination of infected cells. Hence, we will discuss and elaborate the issue concerning the limitations and pharmacological advantages of these particulate carriers for the targeted antibiotic therapy of intracellular bacterial infections (7).

Antimicrobial peptides, also known as cationic host defense peptides, are a very diverse class of tiny proteins with a range of amino acid counts. Numerous synthetic AMPs have been created in labs, but in addition to those that are naturally present in plants and animals, a vast array of AMPs are also made by bacteria and yeast. In addition to their anticancer and anti-inflammatory qualities and their capacity to function as immune modulators, AMPs have been shown to engage in a range of biological activities, such as antibacterial, antiviral, antifungal, and anti-mitogenic agents. AMPs are therefore a viable substitute for a large range of widely used medications. The majority of the research that is currently accessible also shows that AMPs have therapeutic effects in both in vitro and in vivo settings(16).

2. BIOFILM

Biofilms are collections of microorganisms (including bacteria, protozoa, algae, and fungi) that stick to surfaces and are encased in a very moist matrix of extracellular polymeric substances (EPS). It is estimated that around ninety percent of microorganisms in the world are structured in biofilms that are found in surface water, lakes, underwater bodies, and land- based ecosystems. They are also found in ecological niches that undergo extreme pH, chemicals like biocides and

antibiotics, as well as hydrodynamic stress, osmotic pressure, temperature, and pressure. Different forms of biofilms such as marine or subaerial biofilms are critically important for a variety of small-scale ecosystems(4). Bacteria that can develop biofilms have a higher tolerance against antibiotics and other antimicrobial treatments. Bacterial biofilms are thought to present the biggest problem, it is said to be from the increased degree of aminoglycoside resistance to macrophage and antimicrobial agents. Research has shown that the polysaccharide matrix within the biofilm, which anchor the whole group, is the main source of this type of bacteria. When used, the biofilm alters osmosis for the better. Furthermore, within the biofilm, oxygen levels and rate of nutrient supply differ, and so do the bacteria's metabolism and growth rate. Cell division consequently proceeds at substantially slower rates, resulting in persister cells that are extremely resistant to a range of antimicrobial treatments (2).

2.1. Biofilm Formation

- **Planktonic bacteria attachment:** -In aqueous circumstances, planktonic bacteria have the ability to adhere to surfaces. This includes transport mechanisms like motility and diffusion, environmental influences including organic conditioning films, and bacterial surface features like curli and fimbriae. Weak, non-specific interactions characterize initial attachment, which is reversible. Biofilms form when adhesion is stronger and more permanent.

- **EPS Production:-** Cyclic di-GMP, or c-di-GMP, is a signalling molecule found in bacteria that mostly controls the production of biofilms. A sequence of reactions occurs inside the bacterial cell when the bacteria detect changes in their surroundings, such as temperature, pH, or the availability of nutrients. In the end, this results in c-di-GMP production. High c-di-GMP levels encourage the production of biofilms. It facilitates bacterial adhesion to surfaces and promotes the synthesis of extracellular polymeric substances (EPS), a matrix that protects the bacteria by encasing them in a biofilm.

- **MicrocolonyFormation:-**The critical stage in the development of biofilms is microcolony formation. Bacteria

multiply and create tiny, three-dimensional clusters known as microcolonies after first adhering to a surface. By promoting cell-to-cell contact and the synthesis of the extracellular matrix, which offers structural support and protection, these microcolonies form the basis for the mature biofilm structure.

- **Biofilm formation:** - Microcolony proliferation during biofilm maturation results in the formation of a larger, three-dimensional structure because of the creation of EPS. Antimicrobial resistance and biofilm protection are improved by this EPS. In contrast to planktonic bacteria, the mature biofilm exhibits unique and varied gene expression.

- **Dispersion:** - Dispersion is the last stage of biofilm formation. The life cycle of the bacterial biofilm is completed when some unattached bacteria attach to a new surface and create a new biofilm after the biofilm has matured(5).

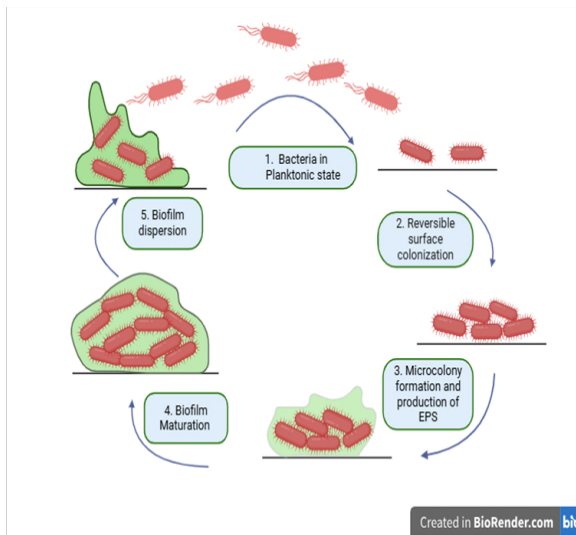


Fig. 1: LIFE CYCLE OF BIOFILM FORMATION

3. NANOTECHNOLOGY

Nanomedicine and other areas of nanotechnology have provided new possibilities in the diagnosis and therapy of a wide variety of diseases. Because of their size and surface

properties, nanoparticles can cross biological barriers (5). In addition, there is a great deal of research in various NPs for its potential antibacterial activity. For instance, metallic oxide NPs like CuO-NPs and ZnO NPs have been shown to be low in cytotoxicity while being highly effective as drug delivery systems. Numerous studies have shown that shape, surface charge, and many other factors can significantly affect the anti-microbial and anti-biofilm efficiency of a nanomaterial. It is worth mentioning that ZnO-NPs are also better in terms of solubility, bioavailability, and biocompatibility(2).

Some nanoparticulate **drug delivery carrier system's** function will be covered in detail in the sections that follow.

3.1. Solid lipid Nanoparticles

As nanoparticulate drug delivery devices, solid lipid nanoparticles were developed. Triglycerides, glycerides, fatty acids, steroids and waxes were the constituents of these nanoparticles.

The advantages of these nanocarriers are

- Control drug release
- Targeted delivery
- Improved drug stability
- Biocompatibility and
- Simple scale-up process (1).

According to the recent studies, the applications of this nanoparticles as a carrier of the compound with anti-biofilm activity are discussed here –

- Researchers assessed the in vitro anti-biofilm properties of SLNs loaded with rifampin against *Staphylococcus epidermidis*, which produces biofilms. They demonstrated that these nanoparticles could decrease biofilm biomass in a manner that was dependent on both time and concentration (1).
- In vitro anti-biofilm activity against *Staphylococcus aureus* biofilm was evaluated after the formulation of

clarithromycin-loaded solid lipid nanocarriers (CLA-SLNs). When compared to free drug, the results showed that CLA-SLNs had superior anti-biofilm activity in biofilm eradication at lower drug concentrations (1).

- Rifampin and cis-2-decenoic acid were recently added to SLN formulations, and anti-biofilm properties were evaluated against staphylococcal biofilm at both the creation and eradication stages. The formulation can be used as an effective nanoparticulate system with potential antibiofilm activities, as evidenced by the superior anti-biofilm activities that were observed (1).

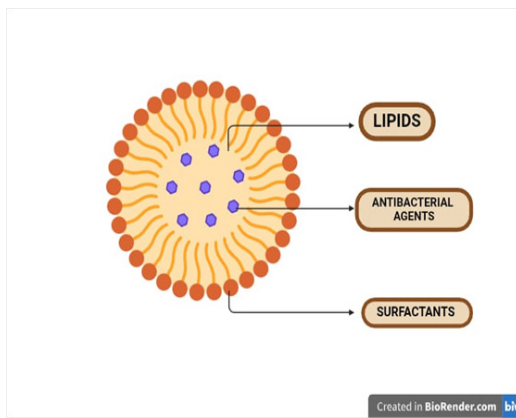


Fig. 02:SOLID- LIPID NANOPARTICLE

3.2. Polymeric Nanoparticle

Drug delivery systems widely incorporate the use of polymers. The polymeric carrier systems are primarily comprised of hydrophilic and hydrophobic segments. The hydrophobic segment of the molecule predominantly acts on a polymeric core which holds the drugs in place. While Elimination of the core is efficiently done by the hydrophilic segments and it is also responsible for the degradation of the core. These systems also present an opportunity to infiltrate hard to reach areas such as biofilm and super dense tissue (1).

Hydrogels

Hydrogels are cross-linked, hydrophilic copolymers or homo polymers which are highly biocompatible and form polymers of low or high degree of composition. These types of delivery systems are made up of natural or synthetic polymers in a three-dimensional network. These polymers can apply in the infusion of antibiotics owing to their unique net structure. For the controlled and sustained release of some antibiotics, they can take in and retain large volumes of water while exhibiting mass swelling characteristics (1).

Despite these benefits, the delivery of antibiotics via hydrogels has shown limited success; as a result, a few studies have concentrated on the delivery of antimicrobial hydrogels based on polymers -

a. In one investigation, the hydrogel/silicone composite made of cross-linked poly (2-hydroxyethyl methacrylate) embedded in a poly (dimethylsiloxane) silicone elastomer was sustained after ciprofloxacin was loaded into it. The antibiotic's release profile was noted. The danger of bacterial colonisation and subsequent biofilm formation on the device material was decreased by coating medical devices with hydrogel containing antibiotics (1).

b. Anderson et al. investigated polymeric hydrogels composed of crosslinked poly (2-hydroxyethyl methacrylate) (pHEMA), surface modified with octadecyl isocyanate, and loaded with norfloxacin. The results of this study showed that not only zero-order releasing profile of drugs be achieved from these hydrogels, also the antimicrobial activity of norfloxacin was improved to eradicate both planktonic and biofilm forms of *S. epidermidis* from medical implant surfaces (1).

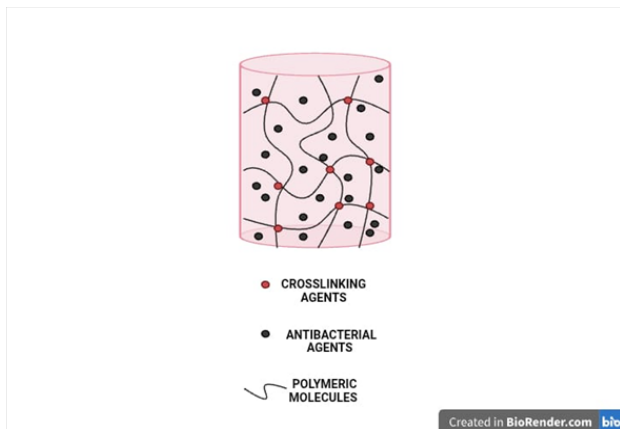


Fig. 03: HYDROGEL MATRIX

An overview of the various lipid-based carriers used to deliver antibacterial drugs to biofilm structures is given in this section of the article. These carrier systems were depicted, and Table 1 included some significant data(1).

Table 01: The lipid-based drug delivery systems to biofilm.

Type of carrier	Encapsulated agent(s)	Active against	Advantages
1. SLN	Clarithromycin	<i>Staphylococcus aureus</i>	• High potential for eliminating biofilms • cellular biocompatibility and characteristics of reduced acute toxicity
2.SLN andNLC	Levofloxacin and DNase type I	<i>Pseudomonas aeruginosa</i>	• Reduced biofilm formation

3. Liposomes	Vancomycin	Staphylococcus aureus	• Fusogenic liposome-encapsulated vancomycin exhibits improved biofilm penetration.
4. Hydrogels	Norfloxacin	S. epidermidis	Targeted drug delivery • Sustained release of the antibiotic • Improved tissue compatibility • The potential for sustained therapeutic effects • Controlled drug release

SLN :Solid Lipid Nanoparticle

NLC : Nanostructured Lipid Carrier

4. Silver Nanoparticle:

The silver cationic nanoparticles pose a promise in battling bacterial infection linked with burns and wounds. Silver nanoparticles have shown promise for kitchen as an antibacterial adjuvant for treatment of several infectious diseases due to the synergy that they have with other antibiotics such as ampicillin and vancomycin. The results obtained by the joint efforts against some Gram positive and negative bacteria including *Streptococcus pneumoniae*, *P. aeruginosa*, *S. aureus*, and *Shigella flexneri*, demonstrate Ag-NP's anti biofilm and antimicrobial activity. (1).

Ag-NPs have been shown in numerous surveys to be useful in reducing infections linked to biofilms. The synergistic effect of Ag-NPs and curcumin NPs (Cur-NPs) against *S. aureus* and *P. aeruginosa*, which are Gram-positive and Gram-negative bacteria, was examined by Loo C-Y et al. A phenolic plant extract called curcumin has anti-biofilm qualities by

weakening the quorum sensing systems of bacteria. When Ag-NPs and 100 µg/ml Cur-NPs are combined, 50% of bacterial biofilms may be disrupted(<https://doi.org/10.1016/j.jddst.2020.101880>).

But from several studies Ag-NPs has been showing several disadvantages that are listed below:

- Cytotoxicity that might restrict usage in therapeutics
- Silver nanoparticles cause the central nervous system to become inflamed by releasing pro-inflammatory cytokines and producing reactive oxygen species (ROS) and nitric oxide.
- Additionally, the cytotoxic effects of silver nanoparticles on the circulatory, respiratory, osteoclast and osteoblast systems may limit their usage in biomedical applications(2).

But according to recent research by Panacek et al., following repeated exposure, Gram-negative bacteria such *Escherichia coli* 013, *Pseudomonas aeruginosa* CCM 3955 and *E. coli* CCM 3954 become resistant to silver nanoparticles (11).

5. Zinc Oxide Nanoparticle:

A broad range of NPs are being evaluated for their antibacterial properties. For example, metallic oxides NPs like copper oxide (CuO-NPs) and zinc oxide (ZnONPs) are unique because they have low cytotoxicity and enhanced drug delivery properties. It has been shown that a lot of parameters, for instance shape and surface charge, can affect a nanomaterial's antibacterial and anti-biofilm properties (2).

ZnO-NPs in particular possess a wide range of benefits including soluble, bioavailable, and biocompatible. Thus, ZnO-NPs can reside within numerous biological systems, mimic the function of biomolecules, and maintain the equilibrium of cells. Because ZnO-NPs are less expensive and is of lower toxicity than other nanomaterials, they are more likely to be utilized for the treatment of diabetes, inflammation, cancer, and infection. ZnO-NPs are differed from other nanosized materials by their catalytic effectiveness

combined with superior adsorption capacity and chemical stability (2).

Evaluation of the impact of ZnO-NPs and antibiotics together on the formation of *S. aureus* biofilms. The test tube approach was used to examine the synergistic effect of ZnONPs and tested antibiotics on biofilm inhibition against *S. aureus* ATCC 6538. The findings indicate that ZnO-NPs by themselves could reduce the production of biofilm by 34–37%. However, a 65–85% inhibition of biofilm formation was seen when ZnO-NPs and different antibiotics were combined (2).

Characterization of ZnO-NPs-induced suppression of *S. aureus* biofilm formation under a microscope: ZnO-NPs were further studied, with emphasis on their effect on *S. aureus* biofilm, through SEM and light microscopy. *S. aureus* biofilm was grown on polystyrene discs with 0.78 mM ZnO-NPs (64 µg/mL), gentamicin (2 µg/mL), and control, and was compared to the untreated cells. The structure of *S. aureus* biofilm was illustrated using a photograph captured on the microscope at different magnifications. Untreated cells displayed in a dense fashion at higher magnification (>5000x) showed evidence of a thick biofilm. After the treatment of either gentamicin or ZnO-NPs there was a significant decrease in biofilm formation which resulted in minimal biofilm mass on the polystyrene discs. Microscopic examination of the infected surfaces revealed the presence of a scattered single layered biofilm of bacteria of 1–2 cell thickness with clumped cells in multilayered structures. Higher magnification revealed the cruder form of untreated *S. aureus* (>10000x) which showed monolayer thick dense clumps. Whereas after treatment, both organisms appeared to have turned into single cell structures resulting in a smooth uniform cell surface. The combination treatment of gentamicin and ZnO-NPs managed to halt bacterial biofilm development leading to the significant disruption of biofilm production and formation resulting in a minimal biofilm mass.

6. Gold Nanoparticle

The range of surface plasmon resonance (SPR) exhibited by Ag, Au, Pt, Zn, and Cu green synthesized metal nanoparticles

is unique, and versatile, with Au-NPs demonstrating a distinct anti-biofilm activity against clinically relevant multi-resistant enterococcal strains. Augmented antibacterial, antimalarial, anti-viral (anti-HIV), anti-tumour, and anti-biofilm effects have been accredited to Au-NPs. Au-NPs, synthesized with the use of MPPE, were tested by Vijayakumar S. et al. for the anti-biofilm activity against Gram-positive *Enterococcus faecalis*, a multiple antibiotic resistant (MARS) bacterium (1).

It was also reported by Giri K et al. that the surface charge of Au-NPs is responsible for the antibacterial activity against *S. aureus* and *P. aeruginosa*, the major pathogens in ventilator associated pneumonia (VAP) and also important in the destruction of biofilms. The growing importance of Au-NPs as anti-biofilm agents can be attributed to the ease of surface functionalization (1).

Table 02 lists the antibacterial characteristics, potential antibacterial mechanisms, and key variables influencing the antibacterial activity of other metal and metal oxide nanoparticles, including gold nanoparticles, iron oxide nanoparticles, and zinc oxide nanoparticles. These NPs have also been thought to be effective antimicrobials (11).

Table 02 : Nanoparticles and nanomaterials used for antibacterial applications.

Nanoparticle	Mechanism of Action	Antibacterial properties	Active against
1.Ag-NPs	- o ROS production - o silver ion release - o membrane integrity disruption	Antibacterial activity against Gram-negative bacteria is superior to that against Gram-positive bacteria.	- o <i>S. aureus</i> - o <i>p.aeruginosa</i>

2. ZnO-NPs	- o ROS generation - o Zinc ion release	Good antibacterial activity against both Gram-positive and Gram-negative bacteria	- o <i>S. aureus</i> - o <i>p. aeruginosa</i>
3. GoldNPs	- o ROS generation - o disruption of membrane integrity - o suppression of rRNA binding to the ribosomes	High stability, nontoxicity, simple surface modification, photothermal therapy, and effective antibacterial qualities	o <i>Enterococcus faecalis</i>

7. Antimicrobial Peptides:

Small molecular weight proteins have a wide range of antimicrobial properties against bacteria, viruses, and fungi known as antimicrobial peptides (AMPs). The molecule can be soluble in aqueous conditions and penetrate lipid-rich membranes because these evolutionarily conserved peptides are often positively charged and possess both hydrophobic and hydrophilic sides (12).

There is great potential for using AMPs as novel antibiofilm agents, and this is a field of study that is being investigated more and more. The microbial, animal, and plant kingdoms all include AMPs, which are important elements of innate immunity. Most AMPs are amphipathic, cationic, small molecules (12–50 amino acids) with a mechanism of action that disrupts bacterial membranes. AMPs have a broad spectrum of activity, a lower propensity to induce resistance, and a high ability to target metabolically inactive cells because of the low specificity of their molecular target. Numerous investigations have shown that AMPs can disrupt different phases of biofilm formation by stopping bacterial cells from adhering to surfaces in the first place, focusing on planktonic cells before they

integrate into the biofilm structure, or eliminating established biofilms by separating and/or eliminating bacteria that are embedded in the biofilm (13).

An international summary of the most promising AMP-based combinations against biofilms is given in this article, along with potential explanations for the synergistic effect. The following combinatorial strategies are given special attention: (i) traditional antibiotics; (ii) compounds that can break down the extracellular matrix of biofilms or prevent their synthesis; (iii) compounds that inhibit QS and/or other signalling pathways involved in biofilm formation; and (iv) additional AMPs and/or peptide-based molecules. Examples of combinations where AMPs are used to combat bacterial biofilms in combination with other substances (13). By disrupting their essential biological functions, including DNA and protein synthesis, AMPs can efficiently eradicate MDR bacteria. Compared to antibiotics, their modes of action are less likely to cause bacteria to develop drug resistance. Additionally, it has been observed that AMPs can destroy bacterial biofilms once they have formed and prevent the production of the signals (ppGpp) needed for biofilm development and spread. To increase the antibacterial arsenal, a variety of synthetic AMPs with strong antimicrobial efficacy against MDR bacteria and biofilms have been created based on an understanding of the structure-activity connections of AMPs (15).

7.1.2 Chitosan

N-acetyl-D-glucosamine and D glucosamine are two monosaccharides connected by glucosidic β -1-4 bonds to form chitosan, a linear high molecular weight molecule that is produced by the alkaline deacetylation of chitin. In addition to its bactericidal effect, its biodegradability via hydrophilic enzymes is one of its most significant biological characteristics.

Chitosan has antibacterial properties because of:

- Presence of amino group in its composition
- disrupt the bacterial membrane's regular processes and cause intracellular components to seep out

- as well as the breakdown of nutrient delivery to the cell.

Chitosan's antibacterial action is only effective at pH values below 6, hence it needs to be artificially altered in order to be studied in physiologically neutral environments.

- Derivatives of chitosan have demonstrated notable bactericidal action when combined with other substances. For instance, certain bacteria belonging to the ESKAPE group can be effectively combatted by chitosan-based biocomposites containing multi-walled carbon nanotubes (MWCNT). Additionally, when chitosan and chlorhexidine are used in endodontic sealants, the antibacterial action of chitosan against *Enterococcus faecalis* is greatly enhanced.
- Furthermore, two efficient combinatorial approaches against resistant bacteria have been investigated; one showed that nisin, teapolyphenols (TP), and chitosan work best together to inhibit both Gram-positive and Gram-negative bacteria, while the other showed how chitosan hydrolysates work in concert with divalent metal ion-EDTA compounds. Lastly, it was shown that, when combined with the zinc-EDTA chelate, active molecular chitosan (AMC) exhibited superior inhibitory efficacy against *Penicillium italicum* (14).

7.1.3 Alginate

Mannuronic and guluronic acids combine to form alginate, a naturally occurring polymer. It is non-toxic to human cells and possesses exceptional biocompatibility and biodegradability qualities. By treating them with aqueous alkaline solutions, brown algae (Phaeophyceae) are the primary source of this biopolymer. The alginate salt precipitate is obtained by filtering the extract and combining it with calcium chloride. The water-soluble sodium alginate (Na-Alg or SA) is then produced by purifying the alginate salt with diluted hydrochloric acid (HCL), which transforms it into alginic acid.

- Zinc oxide-cellulose sodium alginate nanocomposite fibres (ZnO-SACNF), which have demonstrated strong antibacterial activity against *E. coli*, are an example of how

sodium alginate's (SA) antimicrobial qualities have recently been investigated in combination with other substances. At the same time, *S. aureus* and *E. coli* were instantly eliminated by copper alginate hydrogels in the form of microspheres.

- Moreover, the best antibacterial efficacy against *Listeria monocytogenes* was seen in sodium alginate film containing 5% hydroxyapatite nanoparticles (HA NPs).
- The green production of sodium alginate (SA)-silver nanoparticles (AgNPs), in which SA served as a stabilizer for AgNPs, was reported in another study. Because of their increased membrane permeability and breakdown of the bacterial wall, these NPs caused *S. aureus* and *E. coli* to undergo cell death.
- Zinc alginate films with and without 1% graphene oxide (GO) have also been created using sodium alginate, and they exhibit strong antibacterial action against *S. aureus*. Unfortunately, adding 1% w/w of GO had an impact on a number of mechanical properties. Similarly, films made of crosslinked alginate, hydroxypropyl methylcellulose, and ϵ -polylysine with minimal plasticizer content demonstrated a 99.9% reduction in bacteria in strains of *S. aureus* and *E. coli*. Because ϵ -PL competes for alginate chains, making the films more fragile, it should be used sparingly. Last but not least, a brand-new antimicrobial nanocomposite was created using corona-treated polypropylene (CPP) and copper oxide nanoparticles and alginate. This nanocomposite shown outstanding antibacterial activity against *Candida albicans*, *S. aureus*, and *E. coli* (14).

8. Conclusion and future directives:

The inability of the antibiotic targeting approach to effectively clear infected cells may be due to the development phase of the bacteria, and the inability of non-dividing bacteria to effectively respond to the traditional antibiotics. When bacteria attach themselves into the glycocalyx matrix, their resistance to antibacterial treatment increases, as earlier studies have shown. New strategies for the use of antibacterial medicines when the target bacteria are stubbornly inaccessible or in inactive states are essential. In addition, chronic carrier or

persistent infection models of *Salmonella* spp. can be of great assistance. The advance in the antibacterial treatment will require a mix of both genetic and biochemical skills. Perhaps a more reasonable option for enhancing the antibacterial treatment is the combination of different forms of carriers(7). One example is Linhaliq, a liposomal formulation of ciprofloxacin aimed at *P. aeruginosa* infections in cystic fibrosis patients, which is currently pending regulatory approval (3).

The way devices get infected with biofilm is becoming a more serious problem, caused somewhat by the overuse of antibiotics. This will lead to the future design of more efficient systems being based on the comprehension of how the nanoparticles and nanostructured surfaces interact with the bacteria and which technologies will be used to them for material science and biology (3). Although antibiotics have proven to be an effective weapon against pathogenic bacteria, it is crucial to develop new therapies for pathogens that have devised means to evade their mechanisms. New materials like polymers, nanoparticles, and AMPs provide distinct benefits compared to traditional antibiotics in the fight against different infectious diseases. It was noted, however, that certain therapies can have undesirable toxic effects on human cell lines. Only a small number of combinatorial treatments have made it to the clinic. Challenges regarding the clinical use of combination strategies include cytotoxicity effects, production costs, bioavailability, and efficacy. To address these challenges, a number of strategies have been developed, including chemical modifications, innovative delivery systems, and advanced synthesis technologies like electrospinning and three-dimensional printing(14).

This review article discusses some research studies where all the nanotechnology came into practice for drug delivery systems, especially for biofilms. It is expected that it will be used for the treatment of microbial biofilms as well. The NPs' ability to deliver the conventional antibiotics to the infection sites and increase the anti-biofilm properties makes them promising for biofilm elimination (1).

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