Nanotechnology in Biofilm control: Emerging formulations and therapeutic potential

By

Amatun Noor

19346031

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School of Pharmacy

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.

I have acknowledged all main sources of help.

Student's full name and signature

Amatun Noor
19346031

Approval

The thesis titled by Nanotechnology in Biofilm Control: Emerging Formulations and Therapeutic Potential submitted by Amatun Noor (19346031) of Spring, 2025 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy on (Date-of-Defense).

Examining Committee:

Supervisor:
(Member)

_____

Faria Nasrin
lecturer
School of Pharmacy
BRAC University

Program Coordinator:
(Member)

_____

Namara Mariam Chowdhury
Senior lecturer
School of Pharmacy
BRAC University

Departmental Head:
(Chair)

_____

A F M Yusuf Haider , PhD
Acting Dean
School of Pharmacy
BRAC University

Ethics Statement

This study doesn’t involve any human and animal trial

Abstract

Nanotechnology offers novel methods for eliminating biofilms through the use of nanoparticles and nanostructures capable of infiltrating biofilm matrix, delivering antimicrobial chemicals with precision, and disrupting biofilm development. Nanoparticles, including silver (AgNPs), zinc oxide (ZnO NPs), and mesoporous silica, have demonstrated efficacy against bacterial and fungal biofilms. Certain nanoparticles, such as ZnO are acknowledged as safe by the FDA owing to their minimal toxicity. Nanotechnological platforms enhance drug bioavailability, stability, and controlled release, while minimizing negative effects and augmenting tissue penetration. Advantages include targeted delivery, efficient penetration, reduced toxicity, and synergistic action when combined with antimicrobial peptides or antibiotics. Disadvantages of nanomaterials include potential toxicity, stability issues, and challenges in encapsulation and clinical translation due to energy requirements in specific activation techniques. Nanotechnology has progressed to create systems for the co-delivery of antimicrobial peptides and antibiotics with stimulus-responsive release mechanisms. Ultrasound-activated nanoplates exhibit dual antibacterial activity and facilitate oxygen generation, enhancing therapeutic outcomes. The green synthesis of nanomaterials utilizing plant extracts enhances safety and environmental sustainability. Nanocomposite materials and dendrimer-based devices improve biofilm targeting and penetration. Nanotechnology enables several therapeutic approaches, such as liposomes, dendrimers, and nanoemulsions to enhance antimicrobial delivery. Photothermal and photodynamic therapies can effectively eliminate biofilms, utilizing ultrasound-assisted activation for targeted bacterial eradication.

Dedication

I would like to dedicate my thesis to my parents and my sisters , my little brother for their immense support and motivation .

Acknowledgement

I am profoundly appreciative of the unique opportunity granted to me by the School of Pharmacy at Brac University in Bangladesh. I wish to express my profound gratitude to the Almighty for the support received throughout my life.

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Chapter 1

Introduction

1.1 Biofilm

A complex microbiome, biofilm is made up of various bacterial colonies or a single type of that adheres to the surface in a group (abiotic & biotic). Extracellular polymeric material (EPS) obstructs the colonies in biofilms. Cellulose, polysaccharides, and Edna proteins make up the majority of EPS. Biofilm is protected by EPS against adverse environmental conditions, antibiotic medication, food restriction, and other factors. In contrast to non-biofilm cells (planktonic cells), biofilm cells are resistant to immunological attacks and antibiotics. Also they are significant contributors to persistent infection, especially medical implants & are associated with hospital acquired infections which is a major health global issue. So it is urgently needed to develop materials or technologies to prevent biofilm formation. (Kumar et al., 2023)

There are biofilms everywhere in organic life. In humid or non-sterile aquatic conditions, biofilm can grow on almost any non-shedding surface. Even under the most unfavorable conditions, such as highly hot, extremely acidic, or excessively alkaline environments, biofilms can grow reversibly. Both fungal and bacterial biofilms are possible. A biofilm is made up of only one kind of microbial cell. Mineral crystals, corrosion particles, clay or split particles, and blood components are examples of noncellular materials. Biofilms are key parts of the food chain in rivers and streams. Biofilm is easily encountered in industrial areas such as the food sector. As biofilms shield the cells that make them up in different ways industrial pollution is challenging to cure. As colonies that arrange themselves biofilms have evolved with distinct cell morphologies carrying out complementary tasks . Bacterial cells related cooperative activity which is mediated by cell to cell

interaction & other factors allows for greater resilience to environmental stressors & antimicrobial agents in addictive to higher metabolic diversity & efficiency. (Kumar et al., 2023)

1.2 Biofilm composition and properties

Biofilms consist of many elements that allow bacterial populations to evade the human immune response and endure antibiotic treatments. This complicates the management of biofilm infections. The main part of biofilm are extracellular polymeric substances. From the table 1 which I have attached below . we can see the extracellular polymeric substances are made up of water , bacterial cell debris, secreted proteins and nucleic acids and these all work together to move nutrients and materials around the biofilm . This layer supports bacterial cells and helps them grow and metabolize by giving them important nutrients. Basically the biofilm structure has two basic parts , a area of microbial cells with few gaps and the area is tightly packed another parts is a water channel . The water channel helps the nutrients to get to the cells . There is a spatial arrangement of the microbial cells within the biofilm which determines its physical and physiological features. The presence of "persisters" in bacterial biofilms makes antibiotics and the immune system less effective. These are a small number of bacterial cells that can survive an antibiotic level that would normally kill most of the germs. The presence of these cells was detected during the examination of penicillin's impact on a population of Streptococci. Furthermore, the presence of persisters biofilm bacteria turn on certain stress genes that change things like cell density , pH , osmolarity , nutrition and temperature to create resistant microbial phenotypes . The comparison between biofilm water channels and circulatory systems shows that they both work in ways which are similar to how simple multicellular organisms work . Environmental condition like nutrient availability and hydrodynamics influences biofilm production and maintenance . The polymorphism properties of biofilm allow it to change its form based on the availability of

nutrients which is involved with different glucose concentrations. Microcolonies proliferate swiftly, and biofilm thickness increases with elevated glucose concentrations; conversely, a decrease in glucose concentration leads to reduced thickness. Studies have demonstrated that different hydrodynamic conditions (flow type) can alter the biofilm structure. For example, In laminar flow, bacterial microcolonies exhibit a spherical form, whereas in turbulent flow, these microcolonies stretch in the downstream direction.

Elements	Element composition
Microbial cells	2–5%
Polysaccharides	1–2%
DNA and RNA	less than 1-2%
Proteins	Less than 1–2%
Water	Up to 97%

Table 1 : Components of biofilm matrix . (Banerjee et al., 2020)

Nanomedicine for biofilm control

The emerging topic of nanomedicine encompasses a diverse array of nanotechnology applications related to health. For the development of innovative biofilm controlling agents , the production of antimicrobial nanoparticles can be called as the most viable approach. Nanoparticles and nanomaterials which is made of metals (like silver, zinc, iron, copper, titanium, gold, and magnesium), metal oxides, nanopolymers, metal-containing polymers, liposomes, and peptide nanomaterials these have properties which make them effective against bacteria and biofilms. Additionally, it has been observed that the application of nanomaterials alongside antibiotics to eliminate biofilms has enhanced antibiotic efficacy.(Sedighi et al., 2024)

1.2 Biofilm formation

The process of biofilm production involves five phases 1) Bacteria on the surface move & stick together , a thin layer of exopolymeric material coats the connected bacteria as they begin to form biofilms under the right circumstances .2) The extracellular polymeric material (EPS) secreted by attached bacteria adheres to the surface ,causing bacterial aggregation & matrix formation .3) The formation of microcolonies & water channel architecture allows biofilms to fully mature & become more layered .4) Fully developed biofilms become three dimensional communities with their maximum cell density .5) Bacterial microcolonies are released from the major community by mature biofilms ,which seeds new places & disperses the infection .Antibiotics have a harder time penetrating such biofilms & eliminating the hidden gems.(Obi et al., 2018; Su et al., 2022)

1.3 Types of biofilm

There are two main forms of biofilms based on the types of bacteria they contain: Gram-positive and Gram-negative. Gram-negative bacteria have lipopolysaccharides (LPS), while gram-positive bacteria have teichoic acid (TA) in their cell walls. These parts give the outside of the bacteria charges, which may help them stick to surfaces and form biofilms. The length of LPS and how it differs across different strains of bacteria affect how well gram-negative bacteria stick to surfaces and form biofilms. Teichoic acid influences bacterial colonization in both Gram-positive and Gram-negative microorganisms, with lipopolysaccharide being a crucial factor. The teichoic acid in the cell wall of Staphylococcus aureus helps bacteria grow on both artificial surfaces, like heart valve prostheses and catheters, and natural surfaces, like tissues in the nose

.(Vani et al., 2023)

Chapter 2

2.1 Conventional Biofilm treatment

The ineffectiveness of conventional antibiotic therapies has led to worsening situations globally, including antibiotic resistance non biofilms linked to medical implants and catheters. Biofilms dynamically modify their extracellular polymeric components, making them resistant to various pharmaceuticals. Orally administered β-lactams, quinolones, aminoglycosides, and macrolide antibiotics illustrate instances of treatment failure. B-lactams are compromised by bacterial β-lactamases, resulting in their ineffectiveness and inability to infiltrate biofilms. Quinolones, aminoglycosides, and macrolides are extruded from bacterial cells by efflux pumps. Furthermore, quinolones have no enhanced efficacy in the anaerobic environment of the biofilm. Despite the difficulties in addressing biofilms with standard antibiotic treatment, they have shown susceptibility to particular kinds of medications when given correctly and at increased dosages. Diverse combination therapies, including high-dose topical treatments and antibiotic adjuncts, have been utilized to treat these conditions. Some medicines like Cephalosporins, aminoglycosides, monobactams, polymyxins, tetracyclines, and glycylglycines have demonstrated efficacy against biofilms via topical application at elevated dosages. To treat biofilm infections in the lungs, some treatment works well like inhaling tobramycin, aztreonam, ciprofloxacin, levofloxacin, and gentamicin. To stop biofilm formation when given directly to tubing or medical equipment, vancomycin, minocycline, linezolid, daptomycin, tigecycline, rifampicin, and cephalosporins are some antibiotics which is used .(Mirghani et al., 2022)

2.2 Photothermal and photodynamic therapy

For treating biofilm infection, photothermal therapy (PTT) and photodynamic therapy (PDT) have attracted considerable attention and they are acknowledged as effective treatment .Photothermal

therapy increases the ability of antibacterial chemicals to get into biofilms and it also slows the spread of antibiotic resistance . In the near infrared (NIR) causes irradiation and it diminishes biofilm by approximately 90% which demonstrates the therapeutic effectiveness of localized antibacterial intervention and hyperthermia. Nonetheless, the elevated irradiation doses and photosensitizer concentrations employed to eradicate biofilms by PTT and PDT may result in significant tissue damage and inflammation. The combination of therapy helps to enhance therapeutic efficacy and reduce the unwanted effects in the management of bacterial infections. Recent research increasingly emphasizes the implementation of new designs employing hydrogels , antimicrobial agents, plasmonic nanoplatforms, and analogous technologies. Moreover, synergistic strategies are sought as effective alternatives for treating bacterial infections and combating biofilm formation.(Sahli et al., 2022)

2.3 Ultrasound therapy by using nanoparticles

Ultrasound can get rid of biofilms in a number of ways, such as through antimicrobial sonodynamic treatment (aSDT) and microbubbles. In the aSDT method, ultrasound waves set off sonosensitizers, which then release deadly chemicals that kill bacteria cells. A scientist named Pourhajibagher et al. used ZnO and TiO2 because they are biocompatible, effective at killing germs, and good at photocatalysis. A FE-SEM study shows that ZnO/TiO2 NP samples have a strong effect on biofilm, which means they lower bacterial metabolic activity by 85.5%. The oscillation of microbubbles can change the shape of the P. aeruginosa biofilm in specific ways, so ultrasound activation is a new way to get around the biofilm matrix's physical barriers. Combining low-boiling point phase-change contrast agents (PCCAs) with ultrasound (US-PCCA) and medicines that target per sister cells was shown to be an effective way to get rid of biofilm. A study by the scientist named Bharatula et al. where he used high-intensity focused ultrasound (HIFU)

with different sound pressures to find out how bacteria in biofilms react to ultrasound treatment. With HIFU, a pressure amplitude of 4.5 MPa can separate live and dead cells from the surface, but it can't get rid of all of them. HIFU has physical effects, and it has also been shown to raise biomarkers that are linked to biofilm development (cyclic-di-GMP). More research into changes in acoustic pressure shows that colony-forming units (CFU) go down at higher temperatures, and at higher exposure levels, there are no more healthy CFU. A thorough study of how well ultrasonography works for treating chronic wounds found that there isn't enough clinical proof to support its usefulness. This means that more research needs to be done before any conclusions can be made. (Mukherjee et al., 2023)

2.4 Biofilm Physical Removal

The primary and most straightforward technique is the elimination of biofilms via physical removal. This step is crucial in biofilm infections linked to medical equipment, as the removal of the foreign body should precede antibiotic treatment whenever feasible. In alternative situations, including oral, wound, and joint biofilm-associated diseases, the application of irrigation with water jets and debridement prior to antimicrobial treatment is also prevalent. Nonetheless, the execution of this method is significantly constrained and heavily reliant on the accessibility of the infection site and the specifics of the patient situation. (Blanco-Cabra et al., 2024)

2.5 Biofilm Structural Damage

To overcome the limitations of physical biofilm removal, alternatives that affect the structural integrity of the biofilm have been proposed. These strategies focus on the extracellular matrix (ECM), leading to the suppression of biofilm production, destabilization, and disaggregation of biofilms ,sensitization of biofilm cells, and enhanced drug permeability. Numerous enzymes

targeting ECM components have been effectively evaluated, including enzymes generated from phages.

Enzymes may be synthesized at high quantities in a laboratory environment, exhibit specificity and efficacy at relatively low concentrations, and encounter less resistance difficulties compared to antibiotics. Nonetheless, it is crucial to acknowledge that ECM composition significantly varies based on the strain, maturity, and environmental circumstances of the biofilm. A comparable constraint exists with phage-derived enzymes, as their specificity, while advantageous, necessitates prior knowledge of the infection's causal agent before therapy. To address this problem, it has been suggested to utilize a combination of enzymes targeting various ECM components, build phage cocktails, and incorporate conventional antibiotics, which may enhance therapeutic efficacy, particularly in polymicrobial biofilms. At present, the ECM-targeted enzymes dispersin B and pulmozyme have entered clinical use; nevertheless, the cytotoxicity of some other enzymes may hinder their clinical application. (Blanco-Cabra et al., 2024)

2.6. Antimicrobial Agents

An alternative method for biofilm therapy entails diminishing biofilm biomass by the induction of cellular apoptosis. The antibacterial efficacy of an agent does not inherently indicate its antibiofilm action. Various chemicals have shown effectiveness in eliminating biofilms by inducing cellular harm. Numerous compounds are inherently synthesized by flora and microbes. Furthermore, the mortality of biofilm cells resulting from stress-inducing therapy has been investigated. Antibiotics, nitroxide hybrids, and localized external stimuli (such as photodynamic treatment, voltage and electric current, and non-thermal plasma paired with water electrospray) have lethal effects within biofilms by generating reactive oxygen and nitrogen species. Various

alternatives have been evaluated in the pursuit of antibiofilm therapies, including the investigation of medication repurposing. This has resulted in the development of medications exhibiting substantial biofilm eradication efficacy, as observed with certain chemotherapy drugs, but the necessary dosages far beyond those sanctioned for oncological treatment. An alternative method involves the utilization of phages and predatory microorganisms. Their capacity to identify and lyse microorganisms influences biofilm elimination. The cocktail PYO, intended for the treatment of chronic wound biofilms, is presently accessible for commercial use; nevertheless, the possible detrimental effects of predatory bacteria on normal microbiota pose a limitation to its adoption. (Blanco-Cabra et al., 2024)

Chapter 3

Objective of study

The main objective of researching nanotechnology in biofilm elimination is to create novel and efficient methods to address biofilm-related infections, which are famously resistant to traditional therapies. This entails utilizing the distinctive characteristics of nanomaterials to infiltrate biofilms, administer antimicrobial drugs, and dismantle the biofilm matrix, hence enhancing therapeutic efficacy and diminishing dependence on conventional antibiotics. The aim of researching nanotechnology for biofilm elimination, specifically in formulating novel treatments, is to develop efficient, targeted therapeutic systems capable of surmounting the difficulties presented by biofilms—complex bacterial assemblages that exhibit significant resistance to standard antibiotics. Nanotechnology seeks to:

- Facilitate nanoparticles to infiltrate and break the biofilm matrix more effectively than conventional pharmaceuticals.
- Improve the administration and regulated release of antimicrobial drugs directly into biofilms. Enhance the solubility and stability of pharmaceuticals, particularly hydrophobic antibiotics - Offer multifunctional platforms that integrate antibacterial, anti-adhesion, and immune-modulatory properties.
- Mitigate toxicity and inflammation frequently induced by traditional therapies.
- Surmount bacterial resistance mechanisms through focused and prolonged intervention.

- Provide biocompatible, safe, and eco-friendly treatment alternatives. These programs deal with infections that are caused by biofilms that don't go away, especially in medical implants, chronic wounds, and other places where biofilms make treatment fail. Mesoporous silica which is a new formulation nanoparticles which helps to deliver antibacterial peptides at the same time and antibiotics as well as it is achieving over a 97% reduction in biofilm biomass and complete eradication of pathogenic cells without inducing inflammation in in vivo implant models. Polymeric nanoparticles augment antibiotic solubility and targeting, hence enhancing anti-biofilm efficacy compared to free medications. Moreover, the metal nanoparticles which is green-synthesized is used such as silver and zinc oxide, demonstrate substantial anti-biofilm effectiveness while maintaining positive safety profiles, highlighting the potential for clinically approved nanomaterials in biofilm treatment.

The investigation of nanotechnology-derived formulations for biofilm therapy seeks to create enhanced, targeted, and safer medicines capable of effectively eliminating biofilms, overcoming drug resistance, and minimizing side effects in comparison to the antibiotics which is conventional . (Campos et al., 2025; Mohanta et al., 2022; Sahli et al., 2022)

Chapter 4

Methodology

This review paper is done by using information from various review paper and journal article .Most of the journal article I choose from PubMed, Google Scholar , Royal Society of Chemistry ,frontiers, ACS Publication,Sciencedirect.com , ResearchGate , Theranostics etc . I have collected information from these review papers and used information in my review paper. To collect information, I have searched by using some keywords like biofilm eradication , conventional treatment , potential and drawbacks of treatment , nanotechnology ,nanoparticles , nanocarriers etc .

Chapter 5

5.1 Nanocarriers as a strategy to fight biofilm

Nanocarriers are tiny particles that are made to carry different substances. "Nanocarriers" denotes several forms of vehicles for drug delivery. Nanocarriers can demonstrate diverse behaviours to augment their efficacy, as outlined below. The following sections outline the biological relationships that enhance anti-biofilm capabilities. The integration of nanoparticles with current antibiotics may provide a highly promising approach for treating infectious diseases, especially in organisms with significant antimicrobial resistance. Various research teams have published findings investigating the use of nanoparticles alongside antibiotics to address multiple bacterial strains, as this strategy may reduce antimicrobial resistance and lessen the adverse effects linked to systemic antibiotic use. bacteria. Ag, Au, and ZnO nanoparticles linked to beta-lactams which have strong bactericidal effects against E. coli, S. aureus, A. baumannii, E. faecium, and P. aeruginosa by getting into the cell membrane and messing up with signal transduction pathways. So, it can be said that NPs works well against gram positive and gram negative bacteria which are resistant. Nanoparticles are made of Ag, Au, or Zn make certain drugs, like vancomycin, ceftazidime, clindamycin, ciprofloxacin, polymyxin B, and ampicillin, work better against bacteria which are very resistant to antibiotics. For example, NPs can assist antibiotics penetrate through the bacterial cell membrane, which kills the bacteria by breaking down their cells. As a result, AgNPs have been combined with antibiotics such vancomycin, erythromycin, streptomycin, and chloramphenicol, showing strong antibacterial activity and a large reduction in the growth of both Gram-positive and Gram-negative bacteria. The antibacterial efficacy of AgNPs depended on the specific antibiotic employed. (Ioannou et al., 2024)

Polymeric nanoparticles (NPs) are constructed from biodegradable polymers and exhibit strong structural integrity, enabling the stabilization of volatile pharmaceuticals and permitting their controlled release. Moreover, they are readily scalable, economically viable, and demonstrate commendable stability throughout storage. Poly(lactic-co-glycolic acid) (PLGA) is the predominant polymer employed for drug delivery owing to its biocompatibility and biodegradability. Numerous studies have demonstrated the efficacy of PLGA micro- and nanoparticles in delivering antibiotics to bacterial biofilms. Numerous polymers, such as chitosan, are extensively employed in the pharmaceutical and medical industries. Chitosan, derived from chitin, is biocompatible, biodegradable, and exhibits inherent antibacterial properties. Leveraging these characteristics, Tan et al. developed chitosan nanoparticles for the concurrent delivery of an antibacterial antibiotic (oxacillin) and a biofilm matrix-disrupting agent. (Ferraz, 2024)

5.2.1 Polymeric micelles

Polymeric micelles have attracted attention as a prospective strategy for addressing biofilms due to their unique properties, including the capacity to enhance the solubility of hydrophobic antimicrobial agents, hence augmenting their effectiveness against biofilms. Functionalizing polymeric micelles allows for direct delivery to biofilms, enhancing the concentration of antimicrobial drugs at the infection site while minimizing systemic side effects. Because they are small and can affect the surface properties, polymeric micelles can get through the thick extracellular matrix of biofilms better than other therapies. Polymeric micelles can be engineered to transport many medications, including antibiotics, enzymes, and anti-inflammatory drugs. This is an effective method for disrupting biofilms. It is hard to make and design polymeric micelles because we need to be very careful about the polymers' physicochemical qualities . Furthermore, they may lack stability within the body, potentially leading to premature release of the medication

prior to its arrival at the biofilm. Thorough testing of the polymers utilized in micelle formation for biocompatibility and potential toxicity is necessary. Producing numerous polymeric micelles and maintaining quality consistently is challenging, potentially restricting their application in medicine. (Ferraz, 2024)

5.2.2 Dendrimers

Dendrimers are highly branched, consists of polymers which resembles tree and which is distinguished by an exact three-dimensional architecture. These synthetic polymers have a central core and numerous layers around it, which are called generations. Dendrimers have a unique structure that gives them better surface functioning and adaptability, which makes them useful for a wide range of medicinal uses, such as antibacterial therapies. Dendrimers have a core (the centre element from which branches emerge), branches (repeatedly branching structures that radiate from the core), and surface groups (functional groups on the outermost branches that can be altered to improve interactions with bacterial cells). Dendrimers can fight infections in several ways. These techniques can be put into two groups such as direct antibacterial activity and the delivery of antibacterial compounds. Dendrimers directly kill bacteria by breaking down cell membranes, which causes cell lysis. Cationic dendrimers can change the shape of bacterial cell membranes by binding to their negatively charged portions. Some dendrimers can also make the body create reactive oxygen species (ROS), which can kill cells by hurting their DNA, proteins, and lipids. Dendrimers can wrap around or connect with antibiotics, which makes them easier for the body to absorb, more stable, and more accessible. The dendrimer structure helps the antibiotic be released in a controlled way, which makes it stronger against bacteria. You can also modify the functional groups on the surface of the dendrimer to only target certain types of bacteria or biofilms. This focused technique sends antibacterial chemicals

exactly where they need to go, which means that the dose needed is smaller and there are less adverse effects. Poly(amidoamine) (PAMAM) is one of the dendrimers that scientists have looked at the most to see if they can kill bacteria. You can modify PAMAM dendrimers by adding other antibacterial chemicals or by making them antimicrobial on their own. Cationic dendrimers can interact with bacterial cell walls that have a negative charge because they have a positive charge. This destroys the membranes and kills the germs. Carbohydrate-functionalized dendrimers can bind to bacterial lectins, which helps bacteria adhere and take in the antibacterial agents more easily and makes their delivery more effective. Micelles, dendrimers, and polymeric nanoparticles are examples of polymeric nanocarriers that are used in drug delivery systems. They can be modified, are stable, and can release pharmaceuticals slowly. Polymers or the things that break them down normally work well with living organisms, but they could be dangerous if they don't break down completely. This could make you sick or hurt. The size, surface charge, and make-up of nanocarriers can influence how they interact with cells and tissues. This can make them less safe. It is crucial to properly construct these nanocarriers using materials that can be recycled and don't cause an immune response, and to thoroughly test their long-term impacts on living things

. (Ferraz, 2024)

5.2.3 Silica based nanocarrier in conventional treatment

Silica nanoparticles (SiNPs) are an effective technique to deliver pharmaceuticals since they are stable and biocompatible, which makes it easier to manage how the drugs are released. Their consistent pores provide them a huge surface area, which means they can contain a lot of medications and be functionalized with different groups for targeted delivery. These nanoparticles don't kill bacteria on their own, but they could be very helpful for getting

medicines straight to battle diseases that don't respond to many drugs. This is due to their capacity to include many medicines inside their structure. One of the main reasons SiNPs work against biofilms effectively is that they can get inside the biofilm and change its structure. Their small size lets them get into the biofilm matrix and reach the microorganisms inside. When SiNPs enter, they can be made to release antimicrobial compounds in a controlled way, which will keep attacking the biofilm for a long time. By following this technique it helps to enhance treatment effectiveness and diminishes the probability of resistance emergence. It is easy to change the surface of SiNPs to fit different medicines. This functionalization makes it easier for antibiotics, antifungals, or enzymes that break down the biofilm matrix to stick to it. The mesoporous structure of SiNPs has a large surface area that can hold a lot of these medications, making sure that they get to the biofilm effectively. Functional groups can also be added to the SiNP surface to selectively target certain parts of the biofilm or the microorganisms inside it. By adding the functional group the treatment gives more precise and diminish the side effects. Current research is continually expanding the potential use of SiNPs in biofilm cleanup. Studies have demonstrated the effectiveness of SiNPs in eradicating biofilms formed by various pathogens, including Staphylococcus aureus, Pseudomonas aeruginosa, and Candida species. Nanocarriers made of silica are highly valued for transporting drugs and for use in biology because they have a large surface area, pore size that can be changed, and are easy to functionalize. Even though they are usually thought to be biocompatible, concerns about toxicity come up because of things like particle size, surface properties, and dose. Smaller particles can penetrate cells more easily, but they may also cause more damage to cells, such as oxidative stress and inflammation. It is important to keep a close eye on the process by which silica turns into silicic acid in living things to avoid any possible injury. It is important to improve the design and surface modification of silica-based nanocarriers

and undertake rigorous in vivo tests to make sure they are safe and can be cleared from the body over time. (Ferraz, 2024)

5.2.4 Silver nanoparticles

The antibacterial effects of silver compounds have been recognized since antiquity. Nevertheless, the introduction of penicillin signified the beginning of the antibiotic age, resulting in the widespread disregard for silver. In light of increasing concerns regarding antibiotic resistance in bacterial infections and biofilms, the antibacterial characteristics of silver, especially in the form of silver nanoparticles, have re-emerged as a primary focus for researchers. In addition to fungi and viruses, silver nanoparticles work as a antiseptic which has broad spectrum that kills both Gram-negative and Gram-positive bacteria The diameter of silver nanoparticles is usually between 1 and 100 nm, and they have between 20 and 15,000 silver atoms. There are a few different ways to manufacture silver nanoparticles. The most frequent way to change Ag+ (like AgNO3) into Ag0 is to utilize a reducing agent like sodium borohydride. A stabilizer like poly(N-vinyl-2-pyrrolidone) is used to keep silver nanoparticles from adhering together. It is vital to choose reducing agents and stabilizers carefully so that they make particles more stable while lowering their reactivity and impact on the environment. One technique to manufacture silver nanoparticles that is better for the environment is to use living organisms, like bacteria. For example, you can put moist Bacillus licheniformis in a flask with AgNO3 solution and shake it at 37°C for 24 hours to create particles. Then the step which should be done is we should remove the cells , and washed off the silver nanoparticles . Using a biopolymer, like starch, as the stabilizing agent is another way to make silver nanoparticles that has less of an effect on the environment. A solution of silver nanoparticles in maize starch showed that it was stable in a colloidal way, with no particles sticking together and the duration was up to 90 days, as assessed using absorbance analysis. A reduction

of 88% and 85% in Pseudomonas aeruginosa biofilm was observed after 48 and 24 hours of treatment, respectively, indicating significant suppression of biofilm formation using these starch-stabilized particles. Habash and colleagues evaluated the response of P. aeruginosa biofilms to treatments including citrate-capped silver nanoparticles of varying sizes (10 nm–100 nm), the antibiotic aztreonam, and the combination of silver nanoparticles with antibiotics. The significance of particle size was ascertained subsequent to the treatment of premade biofilms with silver nanoparticles of varying sizes and concentrations. Habash et al. showed that silver nanoparticles that were 10 nm and 20 nm in size worked better than 100 nm particles to reduce the biomass of biofilms and the number of living bacterial cells in the biofilm. There were no changes in the structure of the biofilm or the shape of the cells. Radzig and colleagues found that 8.3 nm silver nanoparticles did not work against Escherichia coli biofilms at doses that stopped planktonic bacteria; however, at higher concentrations of 100–150 μg/ml, the nanoparticles did get rid of most of the E. coli biofilm bacteria interactions. Jena and her team observed that chitosan-coated silver nanoparticles between 55 nm and 275 nm did not break up 24-hour-old biofilms of P. aeruginosa and Staphylococcus aureus. However, they did slow down the growth of P. aeruginosa biofilms by 65%. Palanisamy and others have found that chemically made silver nanoparticles (20 nm–30 nm) have a comparable effect on P. aeruginosa biofilm. Many researchers have looked into therapy plans that use both antibiotics and silver nanoparticles together. A study found that the antibiotic aztreonam, when given by itself, did not kill biofilms; instead, it helped them grow, increasing biomass by up to 250% after treatment ended. Cationic antimicrobial peptides (CAMPs) show a lot of promise for treating lung infections caused by P. aeruginosa that are resistant to many drugs in people with cystic fibrosis. There is an immediate necessity for inhalable formulations capable of delivering intact CAMP in conductive airways while protecting it from interactions with airway

mucus and bacterial biofilm, hence augmenting CAMP/bacteria interactions. The goal of this study was to make and build nano-embedded microparticles (NEMs) that would distribute CAMPs to the lungs for a long time.

To attain this goal, nanoparticles composed of poly(lactide-co-glycolide) (PLGA) encasing the model molecule colistin (Col) were synthesized using the emulsion/solvent diffusion technique. Adding poly(vinyl alcohol) (PVA) and chitosan (CS) to nanoparticles changed the way their surfaces worked, making it easier for them to move through synthetic cystic fibrosis mucus. To produce a long-lasting dosage form that is safe for NP inhalation, NPs were spray-dried with different carriers (lactose or mannitol) to make NEM. We chose the best NEM formulations based on how well the nanoparticles spread out in the carrier and how smoothly they flowed. We then checked how efficiently they aerosolized when given with a dry powder inhaler that worked when you breathed. Some Col-loaded NEMs were able to completely get rid of P. aeruginosa biofilm, which was better than free Col. Confocal imaging shows that nanoparticles can get inside bacterial biofilm and assist Col release inside of it. This is probably what prompted this action. Our results show that making PLGA nanoparticles in a way that works well is a promising way to use novel antimicrobials, including cationic antimicrobial peptides, to treat P. aeruginosa lung infections, especially in people with cystic fibrosis. The combination of tiny silver nanoparticles with aztreonam showed very encouraging outcomes. After being exposed to a mixture of 10 nm silver nanoparticles and aztreonam, it was found that the biomass dropped by more than 98%, the thickness dropped by around 50%, and the cell walls of the bacteria were damaged. Moreover, there was a notable rise in the generation of reactive oxygen species (ROS) when silver nanoparticles were given with an antibiotic, as opposed to when each drug was given on its own. The precise mechanism by which silver nanoparticles (NPs) exhibit antimicrobial properties

remains ambiguous; however, it is widely recognized that silver NPs, akin to other metal NPs, significantly induce bacterial cell damage through reactive oxygen species (ROS) such as hydroxyl radicals, superoxide anions, and hydrogen peroxide, which can compromise bacterial proteins and DNA. Silver nanoparticles emit silver ions that interact with thiol groups in proteins, blocking important respiratory enzymes. This leads to higher quantities of harmful reactive oxygen species that damage and kill cells. Reactive oxygen species produced on the surface of silver nanoparticles can engage with cell membrane lipids, resulting in the impairment of membrane functions. Silver nanoparticles are known to stick to and go inside the bacterial cell wall, which makes the cell wall more permeable and kills the cell. Using silver nanoparticles with regular antibiotics looks like it could work for treating biofilms. (Han et al., 2017)

5.2.5 Gold Nanoparticles

Unlike silver, gold is not acknowledged for having inherent antibacterial properties. Even so, the features of nanoscale gold make it possible to effectively functionalize particles, which is why researchers are looking into using gold nanoparticles to cure biofilms. Gold nanoparticles, similar to silver nanoparticles, are predominantly produced through the reduction of gold salts using various recognized techniques. We used an antimicrobial extract from Plumbago zeylanica to make silver nanoparticles (about 63 nm), gold nanoparticles (which formed into gold nanotriangles), and silver-gold bimetallic nanoparticles (about 93 nm) via bioreduction of silver nitrate (AgNO3) and chloroauric acid (HAuCl4). Gold nanoparticles inhibited Staphylococcus aureus biofilm formation by 97% and eradicated 24-hour-old Staphylococcus aureus biofilms by 95%, while also disrupting Acinetobacter baumannii biofilms by 40%. Silver and bimetallic nanoparticles were more effective than gold nanoparticles at stopping and disrupting all of the bacteria that were tested. Ramasamy et al. examined the anti-biofilm properties of gold-silver

bimetallic nanoparticles synthesized by the γ proteobacterium Shewanella oneidensis MR-1. Cultures of E. coli, P. aeruginosa, Enterococcus faecalis, and S. aureus were incubated with gold-silver bimetallic nanoparticles (~209 nm) or gold nanoparticles (~57 nm) for 24 hours and subsequently assessed with crystal violet to measure biofilm suppression. At a concentration of 250 μM, bimetallic gold-silver nanoparticles stopped all strains from growing. At particle concentrations as low as 10 mM, the production of E. coli biofilm was totally stopped. Methylene blue (MB) photosensitizers were attached to the surface of gold nanoparticles (about 26 nm after conjugation) to attack Candida albicans biofilms that were 24 hours old. A light source can activate photosensitizers, which then produce a lot of reactive oxygen species to kill infections The conjugation transpired through electrostatic interactions between negatively charged gold nanoparticles and positively charged methylene blue. After administering a 660 nm diode laser at 38.2 J/cm² for 40 seconds every 6 hours over a 24-hour duration, the proliferation of C. albicans biofilm was inhibited by 63.2% using 20 μg/ml methylene blue (MB) alone, and by 82.2% with MB-conjugated gold nanoparticles containing the same concentration of MB, as evidenced by crystal violet staining results. The 2,3-Bis(2-methoxy-4-nitro-5-sulfophenyl)-5-([phenylamino]carbonyl)-2H-tetrazolium hydroxide (XTT) biofilm reduction assay demonstrated that 81.9% and 95.4% of the biofilm bacteria were eradicated under identical treatment conditions. Furthermore, the architecture of biofilms treated with MB-conjugated gold nanoparticles exhibited significant deformation compared to untreated reference biofilms during SEM analysis. It was also shown that MB made bacterial cell membranes more permeable. .When the right conditions are met, photosensitizers like MB can make reactive oxygen species (ROS) that are very poisonous and kill germs. The incorporation of gold nanoparticles enhanced the anti-biofilm efficacy of the photosensitizer methylene blue. It is imperative to examine whether gold nanoparticles can provide

analogous outcomes with other photosensitizers, such as rose bengal and erythrosine. The chemically synthesized gold nanoparticles did not prevent or disrupt the biofilms of A. baumannii and E. coli. The chemically synthesized silver nanoparticles and bimetallic nanoparticles shown inadequate effectiveness in both biofilm inhibition and eradication. The authors determined that the anti-biofilm effectiveness of gold nanoparticles resulted from the inclusion of antibacterial plant extracts in their manufacture. Even though gold nanoparticles show promise, more research is needed to see how they can be used alone or with other treatments.

.(Han et al., 2017)

5.2.6 Iron Nanoparticles

The multifunctional applications of magnetic nanoparticles in the biological field have been the subject of extensive research. They have the ability to improve magnetic resonance imaging (MRI), targeted medication administration, and hyperthermia-based therapy. Moreover, magnetic nanoparticles are considered biocompatible and economically viable. Magnetic nanoparticles can be produced by coprecipitation or thermal decomposition techniques. Adding a base to a water solution with Fe3+ and Fe2+ salts at room temperature or higher and without oxygen is how the co-precipitation method works. This makes a black magnetite precipitate. Co-precipitation is an excellent way to make a lot of magnetic nanoparticles that stay in a stable colloidal state. It is difficult to obtain nanoparticles of uniform size by the co-precipitation method, as size distribution is influenced by various parameters, including temperature, pH, the salts used, and their concentrations. Durmus et al. and Taylor et al. utilized superparamagnetic iron oxide nanoparticles (SPIONs) to address methicillin-resistant Staphylococcus aureus (MRSA). Before being chelated with Fe3+ from FeCl3 or Zn2+ from ZnCl2, the SPIONs were covered with dimercaptosuccinic acid (DMSA). DMSA is a chelator that makes SPION more soluble by binding to metal ions to

form complexes. Twenty-four-hour-old S. aureus biofilms were subjected to in vitro treatment for 24 hours with either iron- or zinc-conjugated SPION or DMSA-coated SPION. The OD and crystal violet stains demonstrated that zinc-conjugated SPION inhibited biofilm development at a concentration of 0.01 mg/ml, the lowest concentration evaluated. The concentration of 1 mg/ml of all SPION stopped biofilm growth. On the other hand, using just ZnCl2 helped the biofilm grow more faster. The authors found SPIONs in biofilm bacterial cells that had been treated for two hours and then aged for 48 hours, as shown by TEM examination. This suggests that SPIONs brought zinc and iron ions into the cells, which messed up their chemical balance and killed the bacteria in the biofilm. Fructose was used with the metal-conjugated SPION that Durmus et al. talked about to treat 24-hour-old S. aureus biofilms for 24 hours. Fructose greatly improved the ability of SPION to fight biofilms, regardless of whether it was conjugated with metal ions. Adding fructose made SPION build up more in the biofilms, which caused more ROS to be generated. Unconjugated SPION and 1 mM or 4 mM iron-conjugated SPION attained a mortality rate of up to 99.99999% in the presence of 1 mM fructose. All SPIONs, irrespective of conjugation, exhibited superior effectiveness compared to vancomycin. Fluorescence microscopy images of cells undergoing live/dead labelling confirmed this conclusion. Also, the biomass went down by around 50% after treatment with iron-conjugated SPION and fructose, which is the same amount as what vancomycin did. When combined with 100 mM fructose, even 0.1 mg/ml SPION had the same effect on biofilm as 1 mg/ml SPION. This shows that fructose makes SPION's anti-biofilm action stronger. This therefore diminishes the likelihood of systemic toxicity of SPION, as a reduced dosage is necessary for therapeutic efficacy. Promising results were seen in Gram-negative biofilms, like P. aeruginosa and E. coli, showing that they were quite effective at killing biofilms. A scientist named Salunke et al. after research showed that metal nanoparticles that were reduced

by a plant extract high in fructose worked better than those that were made in a lab. Magnetic nanoparticles can penetrate the full depth of biofilms when directed by an external magnetic field, gathering at the base and eradicating bacterial cells situated deep within.

Subbiahdoss et al. functionalized superparamagnetic iron oxide nanoparticles (SPIONs) with carboxyethylsilanetriol (CES) to address 24-hour-old gentamicin-resistant Staphylococcus epidermidis biofilms. The CES graft did not alter the dimensions of SPIONs but reduced their zeta potential and it reduced the zeta potential from +43 mV to -15 mV. A magnet facilitated the aggregation of CES-SPIONs at the center of biofilms. Live/dead stained biofilms showed dead bacteria at the base, and gentamicin administration did not affect the anti-biofilm efficacy of SPIONs. Utilization of magnetically focused CES-SPIONs resulted in the eradication of 38% of mature biofilm bacterial cells. In the absence of the magnetic field, only 9% were lethally affected. The lack of a significant difference in anti-biofilm efficacy between unmodified SPIONs and CES-SPIONs indicates that the production of reactive oxygen species is prompted by the small size of the nanoparticles , may be more pivotal in anti-biofilm efficacy than the electrostatic interactions between SPIONs and the cell walls of biofilm bacteria. Hyperthermia caused by magnetic nanoparticles also improves anti-biofilm treatments. When planktonic S. aureus was cooked in tryptic soy broth (TSB) for 20 minutes at 60°C, the number of bacteria dropped by as much as 99.9%. For magnetic hyperthermia to work, the temperature had to be decreased to 43°C, which killed 99.9% of S. aureus biofilms. In biofilms of P. aeruginosa, hyperthermia induced by magnetic nanoparticles caused the dispersion of the biofilms upon achieving a temperature increase of 5°C or more. Hyperthermia demonstrated no detrimental effects on the microorganisms but enhanced the the efficacy of gentamicin treatments . This result was expected, as planktonic bacteria demonstrate markedly higher susceptibility to conventional

antibiotics than biofilm bacteria; hence, heat enhanced the susceptibility of dispersed bacteria to antibiotic treatment .Iron oxide particles demonstrate considerable potential as targeted delivery agents, magnetically directed particles, or agents for inducing hyperthermia. Further investigation into the effects of size, chemical composition, and other formulation characteristics is necessary. In the case of Fe2O3 iron oxide nanoparticles, 2 nm particles did not inhibit biofilm formation; instead, they enhanced its production. Borcherding et al. posited that these small Fe2O3 nanoparticles could have provided an iron source for the development of biofilm bacteria in this study. Moreover, Fe2O3 nanoparticles were found to inhibit the activity of antimicrobial peptides and deactivate ciprofloxacin. Additional investigation into the effects of iron oxide nanoparticles is crucial to understand their effectiveness in biofilm treatment . (Han et al., 2017)

5.2.7 Copper Nanoparticles

Copper nanoparticles has advantageous features for therapeutic applications owing to their antibacterial and antifungal efficacy, and they are more cost-effective than other metals like silver and gold. Copper nanoparticles have been utilized as coating agents on biomedical devices to impede pathogen proliferation. Copper nanoparticles function as industrial fungicides, facilitate water filtration, and serve as antifouling agents. A series of experiments has been undertaken regarding the influence of copper nanoparticles on biofilm formation, emphasizing cellular quantities and culturing capacities . Copper nanoparticles can inhibit biofilm formation and limit pathogen growth, suggesting their potential use in hospitals and healthcare environments to reduce pathogen transmission on equipment and prevent nosocomial infections in patients. A range of methods for synthesizing copper nanoparticles is documented in the literature, including polyol synthesis, thermal plasma, inert-gas condensation, one-pot synthesis, and the solvothermal

approach. Nevertheless, chemical reduction often functions as an essential technique for the synthesis of copper nanoparticles. Chemical reduction is a straightforward and cost-effective technique for synthesizing metal nanoparticles, utilizing a reducing agent to convert metal salts and a capping agent to limit nanoparticle size to a specified dimension. The reactivity of copper with air often leads to the formation of a surface oxide layer on copper nanoparticles during synthesis. Nonetheless, various techniques employ an anoxic environment to prevent the formation of this layer. Similar to other metallic nanoparticles. The many synthesis processes available offer facilities the flexibility to manufacture copper nanoparticles, potentially enabling large-scale manufacturing. An environmentally sustainable approach for synthesizing copper nanoparticles through chemical reduction was developed. The researchers examined the impact of copper salts and reducing agents on the dimensions and morphology of copper nanoparticles, and subsequently assessed the effect of these nanoparticles on foodborne pathogens, specifically the Gram-positive bacterium Listeria monocytogenes and the Gram-negative bacterium E. coli. Employing two reducing agents, sodium hydroxide (NaOH) and ascorbic acid (AA), with three copper salts, they evaluated the effectiveness of particles synthesized with each reducing agent against two bacterial strains using an agar well diffusion method to measure zones of inhibition. L. monocytogenes (10.5 mm–12.5 mm) shown heightened susceptibility to copper nanoparticles compared to E. coli (11.7 mm–18.5 mm), as seen by a broader zone of growth inhibition. Furthermore, nanoparticles produced with sodium hydroxide shown enhanced effectiveness against both L. monocytogenes and E. coli relative to those synthesized with ascorbic acid. Lewis Oscar et al. evaluated the escalation of antibiotic resistance in P. aeruginosa in older samples and elucidated the role of copper nanoparticles in inhibiting biofilm formation during the early developmental stages. The methods utilized included two control strains of P. aeruginosa and 17

additional clinical isolates cultivated on Luria-Bertani (LB) agar at 37°C for 24 hours. This study demonstrated a maximum biofilm inhibition of 94% and emphasized the utilization of copper nanoparticles as a more economical alternative to silver nanoparticles in preventing biofilm formation. Recent discoveries minutes demonstrate that copper nanoparticles are more efficacious in preventing biofilm formation than in eliminating existing biofilms. (Han et al., 2017)

5.2.8 Liposomes

Biocompatible amphiphilic phospholipids readily form spherical vesicles made of a single lipid bilayer. Liposomes are categorized into multilamellar and unilamellar varieties according to the number of lipid bilayers, and they are capable of encapsulating both hydrophilic and hydrophobic compounds. Methods for synthesizing liposomal nanoparticles with high encapsulation effectiveness include the Bangham process, detergent-depletion technique, ether/ethanol injection, reverse phase evaporation, and emulsion methods. The bilayer membrane structure provides liposomes with remarkable permeability, enhancing their cellular uptake. Hydrophilic antimicrobial agents can be safeguarded against deactivation elements in vivo through encapsulation within the extensive aqueous core of liposomal nanoparticles with high drug loading capacity The efficacy of the liposomal formulation of the antibiotic amikacin (Arikace®), was evaluated by the scientist Meers at el, and the formulation is a nebulized solution used for cystic fibrosis treatment ,in vivo against Pseudomonas aeruginosa biofilms. Similar amounts of free tobramycin and liposomal amikacin (~300 nm) was inhaled by uninfected rats and the durations was for 60 or 80 minutes . Around 65% of the amikacin remained in liposomal form after deposition in the lungs and the amounting was 782 μg, compared to 645 μg of free tobramycin . The fast elimination of the unbound aminoglycoside during respiration is probably the reason. The given tobramycin was effective for under two hours before it was predominantly

cleared from the lungs. Liposomal amikacin exhibited an extended drug release duration, persisting for up to 175 hours following an initial burst release triggered by the inhalation of liposomal amikacin transitioning to its free form. When incubated with diluted P. aeruginosa sputum from a cystic fibrosis patient, the prolonged release of amikacin persisted for nearly 48 hours suggesting that the lytic components of biofilms may enhance the release of amikacin. After the amalgamation of liposomal amikacin with Di-rhamnolipid (RHL) and mono-rhamnolipid, and a 24-hour incubation period, almost all of the amikacin was liberated. This suggests that amikacin is more easily released in areas affected by biofilm contamination. Rats infected with P. aeruginosa for four days were administered equal dosages of free amikacin and liposomal amikacin three times weekly during the two time week period. The cohort group received free amikacin exhibited no significant alteration in colony-forming units (CFUs), while liposomal amikacin resulted in a 40% reduction in CFUs. Three days post-treatment, approximately 1300 μg of amikacin was detected, indicating prolonged drug release. In cystic fibrosis patients, the viscous mucus enveloping biofilms necessitates the effective delivery of sufficient antimicrobial agents through the mucus to the biofilm-infected regions, which is contingent upon size. In a mixture which includes 1 μm polystyrene particles, 62% of fluorescent liposomes penetrated a sputum layer within 24 hours, along with 60-hour-old pure P. aeruginosa biofilms. In comparison, only around 9% of the 1 μm polystyrene particles were found at the distal end of the sputum layer. However, some 200 μm polystyrene particles were found to have penetrated the sputum. A separate study also found that penetration depended on size. Cationic, smaller-sized (128 nm–141 nm), unilamellar particles penetrated P. aeruginosa and S. aureus biofilms at a significantly higher rate and achieved greater penetration depth compared to larger, multilamellar particles. After 24 hours of exposure, the small, simple, cationic liposomes cut the growth of P. aeruginosa biofilms

by 75% in terms of relative viability. They also cut the growth of S. aureus biofilms by 43%. It is believed that unilamellar cationic liposome particles might upset the electrostatic balance of biofilm colonies, which makes anti-biofilm treatments work better. The synthesis of neutral and anionic liposomal tobramycin involved dissolving a combination of cholesterol and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), along with a mixture of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and 1,2-dipalmitoyl-sn-glycero-3-phosphoglycerol sodium salt (DPPG) in chloroform. In this trial, however, liposomal tobramycin did not show any improvement in effectiveness over free tobramycin. Liposomal antibiotics have been investigated as an alternative to conventional free antibiotics, which provide only limited amounts at the site of biofilm infections and are rapidly eliminated from the lungs. However, the therapeutic use of liposomal formulations is constrained by the suboptimal physicochemical characteristics of liposomes. Meer et al. discovered that 35% of liposomal amikacin failed to enter the lungs in vivo. The adverse consequences of antibiotic leakage from liposomes may be detrimental. Even though Meer et al. revealed promising results, there is still a debate over whether liposomal medicines can get through the complicated biofilm biomass in sick people. (Han et al., 2017)

5.2.9 Chitosan Nanoparticles

Chitosan, a natural polymer, is efficacious in biofilm remediation owing to its biocompatibility, biodegradability, and extensive antibacterial characteristics. The nanoparticles, possessing a positive surface charge, are better adapted for targeting sick regions. The ionic gelation procedure is a common method for synthesizing chitosan nanoparticles. In chitosan acetic acid solution, amine groups quickly engage with polyanions such sodium tripolyphosphate (TTP), which causes chitosan nanoparticles to form through cross-linking. Chávez de Paz et al. suggested the fabrication of chitosan nanoparticles for biofilms at neutral pH. The anti-biofilm effect was evaluated by

administering low molecular weight chitosan nanoparticles to 24-hour-old S. mutans biofilms. After treatment, more than 95% of the bacterial cells in the biofilm were damaged, but the structure of the biofilm did not alter significantly.

Chen and colleagues encapsulated the photosensitizer ER into chitosan nanoparticles to augment photodynamic treatment. These nanoparticles were utilized in vitro to combat biofilms of S. mutans, P. aeruginosa, and C. albicans. After 12 hours, ER-loaded chitosan nanoparticles completely eliminated Gram-positive S. mutans biofilms. Similarly, RB-loaded chitosan nanoparticles enhanced cell membrane permeability, facilitating the release of intracellular contents. Encapsulated RB exhibited superior cellular uptake compared to free RB. Encapsulated RB completely eradicated biofilm when photodynamic treatment was administered in parts. Two applications of 20 J/cm² demonstrated more efficacy than a single application of 60 J/cm². Chitosan nanoparticles inhibit the release of singlet oxygen and then it helps to extend the bactericidal effect. Chitosan nanoparticles coated with linoleic acid were employed to immobilize the enzyme β-N-acetyl-glucosaminidase(DspB), which possesses the ability to suppress and dislodge biofilms by degrading poly-β(1,6)-N-acetyl-glucosamine (PNAG). The encapsulated enzyme retained 33.4% of its activity at 37°C, while the unencapsulated enzyme demonstrated a decline in activity after 7 hours. The immobilized enzyme demonstrated increased stability under temperature fluctuations. Chitosan nanoparticles exhibited a negligible anti-biofilm effect during treatment; yet, prolonged administration may lead to biofilm elimination. Some good properties of chitosan nanoparticles like chitosan's biocompatibility, degradability, and antibacterial properties make it an ideal candidate for antibiotic delivery via biofilms. However, its hydrophobic characteristics promote aggregation, which might be mitigated by integrating poly(ethylene glycol) into the nanoparticles, potentially influencing the future of this drug delivery method. (Han et al., 2017)

Chapter 6

Conclusion

The utilization of nanotechnology in biofilm elimination offers a potential and novel strategy to address the difficulties presented by tenacious microbial communities. The application of nanoparticles and nanoscale materials enables the efficient disruption and elimination of biofilms, a challenge that conventional approaches frequently inadequately address. This nascent discipline integrates advancements in nanomaterials with microbiology to precisely target biofilms at the molecular level, hence augmenting antibacterial effectiveness and mitigating resistance. There are many conventional treatment are used in nanotechnology like photothermal and photodynamic therapy ,by using nanocarriers , drug delivery etc. Some new development like laser irradiation , magnetic disturbance ,nanoparticles mediated strategies etc are used in biofilm inhibition /eradication . Overall nanoparticle is used in biofilm eradication as well as in medical devices ,to treat wound etc.

Chapter 7

Reference

Banerjee, D., Shivapriya, P. M., Gautam, P. K., Misra, K., Sahoo, A. K., & Samanta, S. K. (2020). A Review on Basic Biology of Bacterial Biofilm Infections and Their Treatments by Nanotechnology-Based Approaches. In *Proceedings of the National Academy of Sciences India Section B - Biological Sciences* (Vol. 90, Issue 2, pp. 243–259). Springer. https://doi.org/10.1007/s40011-018-01065-7

Blanco-Cabra, N., Alcàcer-Almansa, J., Admella, J., Arévalo-Jaimes, B. V., & Torrents, E. (2024). Nanomedicine against biofilm infections: A roadmap of challenges and limitations. In *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology* (Vol. 16, Issue 1). John Wiley and Sons Inc. https://doi.org/10.1002/wnan.1944

Campos, J. V., de Pontes, J. T. C., Canales, C. S. C., Roque-Borda, C. A., & Pavan, F. R. (2025). Advancing Nanotechnology: Targeting Biofilm-Forming Bacteria with Antimicrobial Peptides. In *BME Frontiers* (Vol. 6). American Association for the Advancement of Science. https://doi.org/10.34133/bmef.0104

Ferraz, M. P. (2024). Advanced Nanotechnological Approaches for Biofilm Prevention and Control. In *Applied Sciences (Switzerland)* (Vol. 14, Issue 18). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/app14188137

Han, C., Romero, N., Fischer, S., Dookran, J., Berger, A., & Doiron, A. L. (2017). Recent developments in the use of nanoparticles for treatment of biofilms. In *Nanotechnology*

Reviews (Vol. 6, Issue 5, pp. 383–404). Walter de Gruyter GmbH. https://doi.org/10.1515/ntrev-2016-0054

Ioannou, P., Baliou, S., & Samonis, G. (2024). Nanotechnology in the Diagnosis and Treatment of Antibiotic-Resistant Infections. In *Antibiotics* (Vol. 13, Issue 2). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/antibiotics13020121

Kumar, L., Bisen, M., Harjai, K., Chhibber, S., Azizov, S., Lalhlenmawia, H., & Kumar, D. (2023). Advances in Nanotechnology for Biofilm Inhibition. In *ACS Omega* (Vol. 8, Issue 24, pp. 21391–21409). American Chemical Society. https://doi.org/10.1021/acsomega.3c02239

Mirghani, R., Saba, T., Khaliq, H., Mitchell, J., Do, L., Chambi, L., Diaz, K., Kennedy, T., Alkassab, K., Huynh, T., Elmi, M., Martinez, J., Sawan, S., & Rijal, G. (2022). Biofilms: Formation, drug resistance and alternatives to conventional approaches. In *AIMS Microbiology* (Vol. 8, Issue 3, pp. 240–278). AIMS Press. https://doi.org/10.3934/microbiol.2022019

Mohanta, Y. K., Chakrabartty, I., Mishra, A. K., Chopra, H., Mahanta, S., Avula, S. K., Patowary, K., Ahmed, R., Mishra, B., Mohanta, T. K., Saravanan, M., & Sharma, N. (2022). Nanotechnology in combating biofilm: A smart and promising therapeutic strategy. In *Frontiers in Microbiology* (Vol. 13). Frontiers Media SA. https://doi.org/10.3389/fmicb.2022.1028086

Mukherjee, A., Bose, S., Shaoo, A., & Das, S. K. (2023). Nanotechnology based therapeutic approaches: an advanced strategy to target the biofilm of ESKAPE pathogens. In *Materials Advances* (Vol. 4, Issue 12, pp. 2544–2572). Royal Society of Chemistry. https://doi.org/10.1039/d2ma00846g

Obi, C. N., Nkemnaso, C., & Chibueze, I. K. (2018). Advances in Industrial Biofilm Control with Nanotechnology-A Review. *International Journal of Chemical and Biomolecular Science*, *4*(4), 41–59. http://www.aiscience.org/journal/ijcbshttp://creativecommons.org/licenses/by/4.0/

Sahli, C., Moya, S. E., Lomas, J. S., Gravier-Pelletier, C., Briandet, R., & Hémadi, M. (2022). Recent advances in nanotechnology for eradicating bacterial biofilm. In *Theranostics* (Vol. 12, Issue 5, pp. 2383–2405). Ivyspring International Publisher. https://doi.org/10.7150/thno.67296

Sedighi, O., Bednarke, B., Sherriff, H., & Doiron, A. L. (2024). Nanoparticle-Based Strategies for Managing Biofilm Infections in Wounds: A Comprehensive Review. In *ACS Omega* (Vol. 9, Issue 26, pp. 27853–27871). American Chemical Society. https://doi.org/10.1021/acsomega.4c02343

Su, Y., Yrastorza, J. T., Matis, M., Cusick, J., Zhao, S., Wang, G., & Xie, J. (2022). Biofilms: Formation, Research Models, Potential Targets, and Methods for Prevention and Treatment. In *Advanced Science* (Vol. 9, Issue 29). John Wiley and Sons Inc. https://doi.org/10.1002/advs.202203291

Vani, S., Vadakkan, K., & Mani, B. (2023). A narrative review on bacterial biofilm: its formation, clinical aspects and inhibition strategies. *Future Journal of Pharmaceutical Sciences*, *9*(1). https://doi.org/10.1186/s43094-023-00499-9