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miRNA-BASED THERAPEUTICS FOR CHRONIC BIOFILM-ASSOCIATED INFECTIONS

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Abstract

In the fight against persistent and resistant biofilm-associated infections, which present major difficulties in clinical settings because of their exceptional resistance to antibiotics and immune responses, microRNA (miRNA)-based therapeutics represent a promising new frontier. Complex microbial communities called biofilms, which are encased in extracellular matrices that the body produces on its own, are linked to chronic diseases like non-healing wounds, implant infections, and lung infections caused by cystic fibrosis. By targeting important bacterial genes involved in matrix synthesis, quorum sensing, and resistance mechanisms, certain miRNAs, such as human-derived miR-let-7b-5p, can impair biofilm formation and increase bacterial sensitivity to drugs, according to recent research. In order to diminish biofilm building and impede virulence pathways, miRNAs attach to bacterial mRNAs and either limit translation or promote RNA degradation. Additionally, miRNAs can influence host-bacterial interactions and disrupt bacterial stress responses by acting across kingdom boundaries. Clinical translation is hampered by issues such immune activation, off-target effects, miRNA stability, and effective administration, despite their therapeutic potential. These problems are intended to be addressed by developments in extracellular vesicles, nanocarriers, and biomaterial-based delivery systems. To eliminate biofilms and reduce antibiotic resistance, miRNA therapy can be used in conjunction with currently available antimicrobials. In addition to advancements in delivery systems for stable and site-specific administration, future initiatives will concentrate on creating tailored, focused strategies that use bioinformatics and AI for exact intervention. All things considered, miRNA therapies have revolutionary potential for precision medicine in the treatment of persistent infections linked to biofilms; nevertheless, more interdisciplinary research is necessary to ensure their safety and practical relevance.

Keywords: MicroRNA therapy; Biofilm disruption; Antibiotic resistance; Quorum sensing inhibition; Nanoparticle delivery; Chronic infection treatment.

1. Introduction

MicroRNAs (miRNAs) have emerged as a transformative class of endogenous regulators with significant therapeutic potential against chronic biofilm-associated infections, which represent a critical challenge in modern medicine due to their extraordinary resistance to conventional antibiotics and host immune defenses[1]. Complex microbial communities known as biofilms form when bacteria embed themselves in an extracellular matrix that they produce on their own[2]. This creates an environment that promotes persistent infections and offers physical protection, particularly in cases of implant-associated infections, cystic fibrosis lung infections, and chronic wounds. There is an urgent need for novel treatments since drug-resistant communities created by biofilm-forming bacteria, such as *Pseudomonas aeruginosa*, greatly contribute to the failure of current antimicrobial therapy[3].

Recent findings demonstrate that miRNAs are strong molecular agents that can break up biofilms and make bacteria more sensitive to antibiotics. Human airway epithelial cells secrete a particularly interesting miRNA called miR-let-7b-5p 4, which has shown promise in reducing the production of biofilms by targeting important bacterial proteins necessary for the integrity and proper operation of biofilms. Furthermore, by blocking bacterial resistance mechanisms including efflux pumps and beta-lactamase enzymes, this miRNA increases the effectiveness of beta-lactam medicines. MiRNAs are unique treatment possibilities that address the complex nature of biofilm resilience because of their dual action, which combines direct biofilm suppression with antibiotic sensitization[5].

Antisense suppression of bacterial gene expression is a major biological mechanism behind miRNA-based biofilm breakup. miRNAs can interfere with vital bacterial processes involved in biofilm formation, quorum sensing, stress responses, and metabolism by binding to complementary regions in bacterial

mRNAs and blocking translation start or encouraging RNA breakdown[6]. It is interesting to note that miRNA-mediated regulation can transcend kingdom boundaries. For example, eukaryotic miRNAs can alter the expression of bacterial genes, creating previously unheard-of opportunities for antimicrobial therapy by utilizing the host's own molecular arsenal[7].

Despite its potential, developing miRNA-based therapies is challenging due to issues such miRNA stability in biological environments, accurate delivery to infection sites, and minimizing immunological reactions and off-target effects. New delivery methods, including as extracellular vesicle engineering, nanoparticle encapsulation, and creative biomaterial carriers, are being actively pursued in an effort to overcome these challenges and enhance treatment outcomes[8]. Furthermore, the combination of miRNA treatments with already available antimicrobial medications offers a promising synergistic approach to dissolving formed biofilms and reducing antibiotic resistance.

miRNA therapies have the potency to improve the treatment of chronic infections with biofilm formation in clinical settings. These illnesses are notoriously hard to cure and cause a large amount of morbidity and medical expenses[9]. There is potential for more efficient, focused treatments that go beyond conventional antibiotics thanks to the capacity to modify bacterial communities and resistance features at the molecular level. To further improve precision medicine techniques, research is also looking into creating customized miRNA treatments based on unique infection profiles. A translational tool called Rocket-miR was created to advance mRNA research. It predicts cross-species miRNA-mRNA interactions by utilizing an existing bioinformatic tool[10].

To sum up, miRNA-based therapies offer a fresh and creative approach to treating persistent illnesses linked to biofilms. This approach could transform the treatment of infectious diseases by utilizing the inherent regulatory ability of microRNAs to prevent biofilm formation and undermine mechanisms of antibiotic resistance. The translation of these encouraging results into safe, efficient treatments that tackle the urgent worldwide problem of persistent biofilm infections and antibiotic resistance requires ongoing multidisciplinary research combining molecular biology, bioengineering, and clinical sciences.

1. miRNAs, small non-coding RNAs, can regulate gene expression post-transcriptionally

A novel and promising strategy for battling bacterial biofilms is the use of microRNA (miRNA) therapies, especially when dealing with persistent and resistant infections. Complex bacterial colonies known as biofilms, which are entwined in an extracellular matrix that they produce on their own, are known to be resistant to antibiotics and to play a major role in chronic infections. These hardy bacterial communities pose serious problems for clinical therapy since they are linked to a variety of illnesses, such as those caused by medical implants, cystic fibrosis, and chronic wounds.

According to recent studies, certain miRNAssmall non-coding RNA moleculescan control the expression of bacterial genes related to resistance mechanisms, quorum sensing, and biofilm formation. For instance, let-7b-5p, a miRNA released by human airway epithelial cells, has been shown to specifically target proteins necessary for *Pseudomonas aeruginosa* biofilm formation. By reducing important biofilm-associated proteins like PpkA and ClpV1-3, this miRNA's distribution through extracellular vesicles efficiently inhibits the formation of biofilms[11]. In *V. harveyi*, the Qrr sRNAs repress the HAI-1 synthase LuxM12. This interdomain communication, in which host-derived miRNAs control the expression of bacterial genes, offers a new way to compromise bacterial defenses from within by utilizing the host's own molecular processes.

Through antisense processes, miRNAs can bind to corresponding bacterial mRNA sequences and prevent translation start or promote RNA degradation, thereby inhibiting bacterial gene translation and offering therapeutic potential. Crucial processes for the stability and persistence of biofilms are disrupted by this process, including the formation of exopolysaccharides, quorum sensing signaling, and stress response pathways. Targeting genes such as PpkA and ClpV1-3, for example, decreases the bacteria's capacity to create and sustain biofilms, increasing their vulnerability to traditional antibiotics[13].

In addition, miRNAs have the ability to increase antibiotic action in addition to their direct inhibitory effects. miRNAs can make bacteria more susceptible to antibiotics by inhibiting resistance mechanisms including the formation of beta-lactamases (like AmpC), which makes it possible to eradicate germs linked to biofilms more successfully[14]. Additionally, miRNAs can disrupt quorum sensing, the bacterial communication system that controls the maturation of biofilms, which hinders biofilm formation and encourages dispersal.

A variety of delivery methods, such as liposomes, extracellular vesicles, and tailored nanoparticles, are used in the use of miRNA therapies in order to improve stability, target specificity, and cellular absorption[15]. In order to get beyond obstacles including immune clearance, low tissue penetration, and nuclease degradation, these delivery methods are essential. If administered properly, miRNA therapies can be used as stand-alone treatments or in combination with antibiotics to eliminate biofilms that are otherwise challenging to cure.

Early research has shown that miRNA-based treatments can effectively lower biofilm biomass and restore antibiotic resistance in bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*. A new avenue for the treatment of persistent and drug-resistant infections is being opened by the possibility of customized miRNA therapeutics that are suited to certain bacterial strains and infection settings as research progresses[16].

Although still at the early stage, miRNA therapies have the potency to completely transform infection control methods by shifting the focus from conventional antibacterial drugs to precision molecular medicine. Translating this novel strategy from lab research into clinical practice will require overcoming present delivery and stability issues, which will ultimately result in more potent treatments for chronic illnesses linked to biofilms.

2. Mechanisms Targeted by miRNA Therapeutics

MicroRNA (miRNA) treatments are a novel way to fight bacterial biofilms because they target several important pathways that are necessary for the development, upkeep, and survival of bacterial populations. MiRNA therapeutics target four key mechanisms: downregulating bacterial stress response and resistance genes within biofilms, modifying bacterial metabolism to disrupt biofilm viability, interfering with quorum sensing signaling pathways, and inhibiting biofilm matrix synthesis by targeting the production of extracellular polymeric substances (EPS)[17].

2.1. Inhibition of Biofilm Matrix Synthesis Targeting EPS Production

One of the main components of biofilm resilience is the extracellular matrix, a complex mixture of proteins, lipids, polysaccharides, and extracellular DNA (eDNA) that shields bacteria from immunological reactions, environmental stressors, and antibiotics. Enzymes for the synthesis of polysaccharides and proteins are encoded by several genes that coordinate EPS synthesis, which is principally in charge of matrix structure and adhesion[18].

2.2. Interference with Quorum Sensing Signals

Bacterial quorum sensing (QS) is a communication mechanism that uses secreted signaling molecules known as autoinducers to coordinate gene expression in response to cell population density. Numerous biofilm-associated processes, such as the synthesis of EPS, motility, expression of virulence factors, and biofilm maturation, are regulated by QS. Therefore, QS disruption is a tactical area of action for biofilm management[20].

By suppressing the expression of important genes involved in autoinducer synthesis and reception, miRNA therapies can interfere with quorum sensing and impede communication networks that are necessary for the growth of biofilms. This interference slows the release of virulence factors, decreases the creation of extracellular matrix components, and lessens the coordination of biofilm formation.

Reduced biofilm biomass and enhanced antibiotic sensitivity are the outcomes of miRNAs or their analogs inhibiting the las, rhl, and pqs QS systems, which are key regulators of biofilm production and antibiotic tolerance in *P. aeruginosa*. Additionally, QS inhibition can enhance clearance by encouraging biofilm cell dispersal[21].

In addition to directly targeting genes, miRNAs help lessen QS-related resistance mechanisms. For instance, bacteria are shielded against antibiotics and reactive oxygen species via QS-regulated peroxidase production. miRNA therapies make biofilm bacteria more vulnerable to host defenses by suppressing these genes[22].

3.3.*Downregulation of Bacterial Stress Response and Resistance Genes*

In addition to offering physical protection, biofilms give bacteria more resilience by triggering stress response pathways and expressing resistance genes. When bacterial cells in biofilms are exposed to antimicrobials, oxidative stress, and nutritional constraint, adaptive reactions are triggered that increase the chances of survival[23].

Bacterial genes involved in these adaptive stress responses and antibiotic resistance can have their transcription and translation downregulated by miRNAs. To restore treatment efficacy, for example, miRNAs that target beta-lactamases, efflux pumps, and multidrug resistance-associated proteins hinder the bacteria's ability to neutralize or expel drugs[24].

miRNAs impair bacterial viability in biofilms by inhibiting molecular chaperones and stress response

regulators, which lowers the bacteria's ability to sustain protein folding and repair under stress. The persistence of antibiotic-tolerant persister cells, which are frequently linked to chronic infection recalcitrance, is reduced by this disruption[25].

3.4. Modulation of Bacterial Metabolism to Disrupt Biofilm Viability

The metabolic states of biofilm bacteria differ from those of planktonic cells; they frequently adopt anaerobic or slow-growth phenotypes that are tailored to the nutrient constraint and stress conditions found in biofilms. These metabolic changes support both virulence and antibiotic tolerance[26].

Enzymes and regulators that govern anaerobic respiration, central carbon metabolism, and energy production can be targeted by miRNAs to alter bacterial metabolism. By blocking important metabolic processes, bacterial colonies are weakened because ATP production, biosynthesis, and overall biofilm fitness are all reduced[27].

By changing matrix composition and influencing bacterial interactions, metabolic regulation also affects biofilm microenvironment factors including pH and redox potential. This results in heightened vulnerability and compromised biofilm maintenance[28].

3.5. Integration and Therapeutic Potential

The limitations of traditional treatments, which frequently target single pathways, are overcome by the diverse targeting capabilities of miRNAs, which enable the simultaneous disruption of biofilm construction, signaling, defense, and metabolism. Effective gene regulation is ensured by using tailored delivery vehicles, such as extracellular vesicles or nanoparticles, which improve miRNA stability and targeted distribution within biofilms[29].

To increase efficacy and safety, ongoing research includes refining miRNA sequences, delivery methods, and combination treatments. In summary, miRNA-based therapies target basic bacterial processes necessary for biofilm formation, survival, and resistance, offering a potent, all-encompassing strategy for treating chronic biofilm-associated illnesses. This multifaceted approach has enormous potential for improving antimicrobial precision medicine and resolving persistent infection issues in clinical settings[30].

4. Strategies and Delivery

4.1. Synthetic miRNA Mimics and Antagonists

Chemically modified compounds known as synthetic miRNA mimics are intended to enhance or restore the activity of natural miRNAs that inhibit the formation of biofilms. These molecules bind to bacterial mRNAs encoding important proteins involved in biofilm production, quorum sensing, and resistance mechanisms by imitating natural miRNAs. This prevents the translation of these proteins or causes mRNA destruction. For instance, miR-210 gene promoter can suppress the expression of this miRNA33 which are critical for the formation and functionality of biofilms. Bacterial adhesion and persistence are decreased, and biofilm architecture is effectively weakened by such targeted inhibition.

Conversely, miRNA antagonists, sometimes referred to as anti-miRNAs, are made to stop the action of miRNAs that can unintentionally increase pathogenicity or biofilm resilience. By attaching themselves to mature miRNAs, these antagonists stop them from interacting with target mRNAs. Bacterial gene networks that normally aid in biofilm survival under stress can be altered by this selective repression. Phosphorodiamidate morpholino oligomers (PMO), peptide nucleic acids (PNA), and locked nucleic acids (LNA) are a few examples of chemical modifications that improve the stability and specificity of these artificial molecules, enabling them to withstand in biological settings and effectively inhibit[34].

4.2. Nanoparticle and Liposome-Based Delivery Systems

Effective delivery of miRNA-based therapies to bacterial biofilms, which are naturally impervious to penetration because of their thick extracellular polymeric substance (EPS) matrix, is one of the main obstacles. In response, delivery systems based on nanotechnology have been created that shield miRNAs from deterioration and enable tailored transport over biofilm barriers[35].

Synthetic miRNAs are encapsulated in nanoparticles, such as lipid-based nanocarriers, polymeric nanoparticles, and inorganic nanosystems, to improve their stability and regulate their release kinetics. For instance, cationic liposomes and negatively charged miRNAs can combine to generate electrostatic complexes that facilitate fusing with bacterial membranes or biofilm EPS, which in turn promotes absorption. In a similar manner, miRNA mimics have been conjugated using DNA nanostructures such as DNA tetrahedrons, which enhance cellular uptake and shield oligonucleotides from nucleases[36].

To enable targeted accumulation within biofilms or release triggered by pH, enzymes, or redox conditions common in infection locations, these delivery vehicles can be functionalized with biofilm-targeting ligands or environmental stimuli-responsive moieties. Such accurate targeting optimizes local treatment efficacy while minimizing systemic off-target consequences. When miRNA-loaded nanoparticles enter biofilms, they effectively knock

down genes that support biofilm formation and compromise the integrity of the biofilm[37].

4.3. Combination with Antibiotics to Overcome Tolerance

In order to overcome the infamous antibiotic tolerance of bacteria associated with biofilms, miRNA therapies may work in concert with conventional antibiotics. Through physical defence and modified metabolic states, biofilms lessen antibiotic susceptibility; however, these adaptive defences can be undone by miRNAs that target resistance genes, quorum sensing, and biofilm matrix formation[38].

For instance, by stopping enzymatic breakdown and drug extrusion, miRNAs that target beta-lactamase genes and efflux pumps restore susceptibility to beta-lactam antibiotics. Antibiotics can efficiently reach bactericidal concentrations within biofilms thanks to this re-sensitization. According to studies, biofilm biomass is considerably decreased[39]. Growth inhibition and higher tolerance to ofloxacin and cefotaxime but not tobramycin were caused by overexpression of ygiU. Type V, a novel TA module type, was recently linked to persistence in E. coli. In this instance, the antitoxin GhoS prevents the manufacture of GhoT by selectively cleaving its mRNA, masking its toxicity[40].

5. Applications and Challenges

Chronic biofilm-associated illnesses, which are particularly difficult to treat because of their tenacity and resistance to traditional antimicrobial medications, have significant promise for treatment with miRNA therapies. The use of miRNA-based treatments covers a wide range of clinical situations, including implant-associated infections, lung infections associated with cystic fibrosis, infections linked to medical equipment, and chronic wounds[42]. Although these applications have potential, achieving their full therapeutic potential is fraught with difficulties. For miRNA therapies to be successfully implemented in clinical settings, accurate delivery systems and optimal dosage schedules must be developed[43].

5.1. Therapeutic Potential in Chronic Biofilm-Associated Infections

Biofilm-related chronic infections commonly arise on medical equipment such cardiac implants, prosthetic joints, and catheters. Because biofilms create protected niches that hide bacteria from medications and the human immune system, these infections are challenging to remove. miRNA therapies offer a novel approach to upsetting these protective communities by focusing on resistance mechanisms, quorum sensing pathways, and genes that generate biofilms[44]. For example, it has been demonstrated that miRNAs, miRNA 708, miRNA 23a are used to prevent the formation of biofilms by downregulating proteins necessary for biofilm construction in pathogens such as *Pseudomonas aeruginosa*, a crucial bacterium in lung infections caused by cystic fibrosis (CF)[45]. Polymicrobial infections create long-lasting biofilms in CF lungs, which worsen lung damage and hyperinflammation. By simultaneously controlling inflammation and infection, miRNA therapies that target biofilm and resistance pathways can enhance the course of disease.

Another context in which miRNA-induced disruption of biofilm can promote healing is chronic wounds. In chronic wounds, biofilms increase antibiotic resistance, encourage inflammation, and postpone re-epithelialization. miRNA mimics that target genes involved in bacterial adhesion and matrix formation can be applied topically or systemically to decrease the load of biofilms, which will aid in wound closure and lessen the severity of infections[46].

Morbidity is greatly increased by biofilm-associated infections in orthopedic and dental implants, which necessitate lengthy antibiotic courses and frequently implant removal. It has been shown that miRNA-based strategies and antimicrobial nanoparticles work well together to lessen bacterial colonization and encourage tissue regeneration. The integration of infection control and healing promotion is demonstrated by multifunctional scaffolds that include bone regeneration factors and miRNA inhibitors that target pathogenic biofilms[47].

5.2. Challenges in miRNA Therapeutics for Biofilms

The use of miRNA therapies for biofilm infections is fraught with difficulties. Due to nuclease degradation, miRNAs are unstable in the body and have trouble penetrating thick biofilm matrices, which limits their usefulness. Effective, targeted delivery to infection locations is challenging, with risks of immunological responses and off-target consequences, while preserving host cells and beneficial bacteria. For delivery systems to address the varied and ever-changing bacterial populations in biofilms, controlled and sustained release must be possible. Additionally, customized methods taking patient immune state and biofilm variability into account are needed to optimize dosage in order to balance toxicity and efficacy[48].

5.3. Advances in Delivery Platforms and Dosing Regimens

Researchers are creating novel delivery technologies such lipid nanoparticles, polymeric systems, extracellular vesicles, and bioengineered scaffolds to overcome these obstacles. These carriers facilitate targeted delivery through surface ligands that recognize bacterial or tissue markers, improve biofilm penetration by taking advantage of charge interactions and biofilm-responsive release processes, and shield miRNAs from destruction[49]. Delivery via extracellular vesicles improves absorption and reduces immunogenicity by simulating normal cellular communication. Enhancing penetration and bacterial binding is achieved by functionalizing nanoparticles with biofilm-disrupting agents such as enzymes or targeting moieties. Controlled release technologies that minimize frequent administration and offer continuous miRNA availability are essential for optimizing dose schedules. Co-delivery platforms that combine miRNA administration with antibiotics or anti-inflammatory drugs take advantage of synergistic effects to get better results. Dosing and delivery options for certain biofilm-associated infections are guided by preclinical models that incorporate biomimetic infection settings. Clinical translation pipelines are facilitated by regulatory advancements that acknowledge oligonucleotide medicines[50].

6. Future Directions

The integration of AI and customized medicine is the main focus of future developments in miRNA therapies for biofilm infections. AI helps create precise compounds that maximize miRNA targeting and reduce off-target effects, improving the effectiveness of treatment[51]. By examining bacterial strains, gene expression, and immune responses, personalized therapeutics customize miRNA interventions to patient-specific infection profiles, increasing results, particularly in intricate polymicrobial biofilms. Treatments that disrupt biofilms while promoting immunological balance will be developed with the aid of increased study on miRNA interactions with human immunity and polymicrobial biofilms. These developments hold promise for resolving present issues and enabling chronic biofilm precision medicine[52].

7. Conclusion

miRNA therapies that target biofilm-associated infections have enormous therapeutic potential in a variety of clinical settings, including for implants, chronic wounds, cystic fibrosis, and device-associated infections. However, there are still major obstacles to overcome in the areas of immunological safety, stability, targeted distribution, and dosage. By directly dissolving biofilms, restoring antibiotic sensitivity, and boosting tissue repair, miRNA-based techniques hold the potential to transform the treatment of chronic infections through novel delivery platforms, biomimetic testing, and combination medicines. For miRNA therapies to have a revolutionary effect on the treatment of chronic biofilm infections, further interdisciplinary research and clinical development are required.

Declaration of competing interest

The authors declare no competing interest.

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