B7: Are there any specific agents that have been shown to be effective against biofilm in pre-clinical models?

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RESPONSE/RECOMMENDATION: Yes. There is pre-clinical evidence that antimicrobials, chemical disruptors, physical and energy-based disruptors, bacteriophages, enzymatic disruptors, and antibodies are effective modalities against biofilm.

LEVEL OF EVIDENCE: Moderate

DELEGATE VOTE: Agree: [% vote], Disagree: [%], Abstain: [%]

RATIONALE: Biofilm formation is an intrinsic defensive strategy that bacteria utilize to survive environmental stresses such as the host immune system and antibiotic therapy. There is strong evidence to support that biofilm formation poses numerous challenges in the management of periprosthetic joint infection (PJI) and contributes to the overall treatment failure of current standard therapy. The phenotypic and genotypic characteristics of biofilm are dependent on numerous factors, including the type of organism, the host environment, and the infected surface. Therefore, to improve treatment outcomes for PJI, novel modalities that target different components of the biofilm have been developed. To answer this question, we reviewed preclinical studies that tested therapeutic agents or interventions, against bacterial biofilm on a multitude of surfaces. We excluded studies that tested prophylactic approaches used to inhibit biofilm formation such as surface-altering treatments, and agents targeting planktonic bacteria. Therapeutic agents targeting biofilm were grouped into six main categories: 1) Antimicrobials, 2) Chemical disruptors, 3) Physical and energy-based disruptors, 4) Bacteriophages, 5) Enzymatic disruptors, and 6) Antibodies.

METHODS

A comprehensive literature search was conducted across four databases (i.e., Medline, Embase, Web of Science and SINAHL) designed to identify studies focusing on agents targeting biofilms in vitro or in vivo, which identified 1125 unique papers. From this screening process, 197 studies were selected for full-text review. Ultimately, 96 studies met the inclusion criteria for systematic review and were organized into 6 general classes of agents effective against biofilm in pre-clinical models.

RESULTS

1. Antimicrobials

Antimicrobials are agents that directly kill bacteria or other microorganisms. Antibiotics are the most common antimicrobial agent, followed by peptides, nanoparticles, and immunomodulators. We identified 33 relevant articles that tested antimicrobial agents in pre-clinical models. Among these studies, 25 addressed *S. aureus* and other coagulase-negative species. The majority focused on the MBEC (Minimum Biofilm Eradication Concentration), which is the lowest concentration of an antimicrobial agent required to completely eradicate a biofilm, and direct evaluation of biofilm integrity.[1] MBEC is often significantly higher than the Minimum Inhibitory Concentration (MIC), which applies to planktonic (free-floating) bacteria, and generally exceeds clinically achievable concentrations unless delivered locally with a carrier such as calcium phosphate. Common agents such as Vancomycin and Tobramycin penetrate biofilm at high doses (100-750 μg/mL) but may not be

effective against embedded bacteria.[2,3] Other drugs, such as Moxifloxacin showed greater efficacy against *S. aureus* than Vancomycin.[4,5] Rifampin was extensively tested and found to be more effective against biofilm-based infections when used in combination with other drugs such as linezolid, moxifloxacin, and doxycycline, but could inhibit Gentamycin.[4–8]

Some antimicrobials were found to have enhanced efficacy against biofilm. Rifabutin was shown to be superior in the reduction of biofilm at lower concentrations, compared to rifampin and rifapentine. Cefiderocol is a siderophore-conjugated antibiotic that demonstrated the ability to penetrate bacterial biofilms using bacterial iron transport systems.[6] Other antibiotics were found to be more effective against biofilm when used in combination, particularly when including Tedizolid.[9–14] Several plant-based extracts were significantly more effective than Chlorhexidine at removing biofilm from silicone surfaces with minimal soft tissue toxicity.[15] Several novel peptides designed to penetrate and destabilize biofilm matrix and disrupt bacterial membranes (eg: WLBU2, PLG0206, Camel Peptide, and others) show excellent eradication of biofilms and bacterial viability in vitro and are exceedingly promising agents.[16–19] For example, 8-hydroxyserrulat-14-en-19-oic acid (EN4) is a peptide with rapid biofilm reduction, achieving >3 log CFU reductions in *S. aureus* and *S. epidermidis* within five minutes.[18] Nanoparticles exhibiting strong antimicrobial activity such as Activated Zinc also show promise by disrupting cell membranes.[19] It should be noted that, like antibiotics, novel agents that have shown efficacy against one strain of bacterial biofilm may not be as effective against another strain, and further testing may be indicated.

The effectiveness of antimicrobials often depends on their ability to penetrate biofilms, highlighting the need for approaches that enhance biofilm disruption. For example, traditional antibiotics have shown increased effectiveness when used in combination with enzymatic disruptors such as Exebacase or physical disruption methods like ultrasound-assisted drug delivery.[20,21]

2. Chemical Disruptors

Chemical disruptors are agents that interfere with the structural integrity, signaling, or function of a biofilm without necessarily killing microorganisms. Of the 21 articles included, most involved in vitro microtiter biofilm models and biofilms on clinically relevant surfaces like metal, plastic, and ceramic. 2 studies incorporated in vivo models using titanium K-wires or polyester-thread implants.[22,23] The most common in vitro biofilms included *S. aureus*, *S. epidermidis*, and *P. aeruginosa*. Most chemical disruptors demonstrated significant reductions in biofilm biomass and bacterial CFUs achieving 2-4 log reductions, though complete eradication was rare. Activated zinc solutions, Bioactive glass, Polyhexanide (PHMB), and Chitosan Nanoparticles with Chloroquine & DNase I functionalization were some of the most effective at achieving MBEC reductions. Irrigation solutions such as XPerience and PHMB displayed superior antibiofilm activity compared to standard antiseptics like povidone-iodine and chlorhexidine, highlighting a shift toward specialized biofilm-targeting irrigation solutions.[24,25]

Several chemical agents were tested in isolation against biofilm. Metal-based agents, particularly activated zinc and bioactive glass, consistently outperformed conventional antiseptics, demonstrating significant biofilm reduction across multiple bacterial species. Activated zinc eradicated 100% of MRSA biofilm and reduced *P. aeruginosa* biofilm by 99.996%, while bioactive glass (BAG-S53P4) achieved up to 50% biomass reduction in methicillin-resistant *S. epidermidis*.[26,27] Plant-derived chemical biofilm disruptor, such as quercetin and allicin, showed promising results, reducing *P. aeruginosa* biofilm by up to 85%, though variability in efficacy suggests a need for further research.[28] Cell-free supernatants from Enterobacter strains demonstrated antibiofilm effects, inhibiting biofilm formation by >65% and disrupting mature biofilm by >85%.[29] Acetic acid, at clinically

acceptable concentrations (5%), showed a 96.1% reduction in MSSA biofilms within 20 minutes but exceeded safety limits at concentrations >10%.[30] Silver nanoparticles conjugated with DNA aptamers achieved a 63% reduction in biofilm biomass, enhancing penetration into the biofilm matrix.[31] Coraca-Huber et al., 2021 turned to Omega-3 fatty acids, and demonstrated eicosatetraenoic acid (EPA) had strong dose-dependent antibiofilm activity against *S. aureus* and *S. epidermidis*.[32] On the other hand, docosahexaenoic acid (DHA) was less consistent, which suggested that higher EPA concentrations (≥5 mg/L) may have clinical potential for managing infections. N-chlorotaurine (NCT) exhibited time- and concentration-dependent biofilm eradication against *S. aureus*, *S. epidermidis*, and *P. aeruginosa*, with a 1-log reduction in viable bacteria within 15 minutes.[33]

Synergistic therapies combining biofilm-chemical disrupting agents with antibiotics demonstrated enhanced efficacy, particularly with rifampin-based combinations for *P. acnes* biofilms, and antibiotic-anti-inflammatory synergies such as gentamicin with ketorolac.[34,35] Chitosan-based nanoparticles functionalized with DNase I and chloroquine provided a dual-action approach by disrupting extracellular DNA while exerting antimicrobial activity.[22] The combination of isobavachalcone with gentamicin and curcumin enhanced biofilm eradication and reduced inflammatory osteolysis in MRSA and MSSA biofilms.[23] Cis-2-decenoic acid, a quorum-sensing inhibitor, potentiated the efficacy of tetracycline, linezolid, and chlorhexidine, suggesting a role for quorum-sensing disruption in improving biofilm treatment outcomes.[36] Turner et al., 2022 demonstrated that while sodium salicylate reduced MBEC for rifampin twofold (from 1024 to 512 μg/mL), it had little to no effect on other antibiotics, making it a limited adjuvant therapy.[37]

However, several studies reported inconsistent outcomes, particularly with N-acetylcysteine, where its efficacy in weakening biofilm structures varied by strain and environmental conditions.[38,39] Berberine, a plant-derived bioactive alkaloid, showed mixed results against S. aureus biofilms, indicating its limitations as a stand-alone treatment, but potential as an adjunct to antibiotic therapy due to its MIC-lowering abilities against MRSA.[40–42]

While promising, combination therapies also introduce complexity in dosing strategies and potential cytotoxic effects, requiring further studies to optimize safety and effectiveness. Additionally, biofilm susceptibility varied significantly depending on bacterial strain and biofilm maturity, emphasizing the need for more standardized in vivo testing protocols before the commencement of clinical applications. Overall, chemical disruptors offer promising solutions for biofilm management, but their clinical applicability remains limited not just by variability in efficacy, but also the lack of standardized testing methodologies and validation in more robust in vivo models.

3. Physical Disruption & Energy-Based Agents

Physical disruptors are treatments that aim to physically alter or disrupt biofilms. They are most effective when used in conjunction with antimicrobial agents. A total of 23 studies met this criterion.

Photodynamic treatments were widely studied. In one study, Lasers combined with methylene blue showed promise against *S. aureus* Briggs, 2018) while two studies evaluated RLP068/Cl, a photosensitizing agent, and found it to reduce biomass and biofilm cell counts significantly in *S. aureus* and *P. aeruginosa*.[43,44] In another study, a single or fractionated dose of red-blue photodynamic therapy coupled to 5-ALA generated reactive oxygen species (ROS) disrupted 95% to 99% of biofilm in MRSA cultures.[45] A photothermic gel substance containing amino acids achieved 100% biofilm eradication.[46]

There were several mechanical approaches with good results. One study assessed pulsed lavage. While pulse lavage alone transiently reduced biomass, regrowth occurred within 24 hours. However, when followed with antibiotics, the regrowth did not occur, underlying the importance of combining mechanical treatment of the

biofilm with antimicrobial therapy.[47] Additionally, an atmospheric pressure non-thermal plasma jet resulted in a 99.99% reduction in biofilm CFUs, with the eventual complete eradication of *P. aeruginosa*.[48,49] Elevating substrate temperature was also shown to reduce biofilm and biomass.[50]

Several other physical disruptors were shown to be effective in disrupting the biofilm and thereby enhancing antimicrobial efficacy. An injectable hydrogel, "EMgel," activated by ultrasound, was also found to be effective in biofilm disruption.[51] Magnetic fields, extracorporeal shockwaves (both low and high energy), ultrasound, electrical stimulation, acoustic nanodroplets, and non-contact induction heating were also effective in biofilm disruption, particularly when combined with other agents, such as beta-defensins, SAAP-148, and antimicrobials.[21,50,52–63]

4. Bacteriophages (phages)

Phages are a class of viruses capable of specifically infecting and killing bacteria without infecting mammalian cells. They operate through mechanisms distinct from antibiotics, exhibiting strict host specificity and the ability to disrupt biofilms**.**[64] We identified 6 studies that primarily investigated phages. Four were in vitro models, one in vivo, and another used both models.

Over the past decade, there has been an increased interest to explore the therapeutic of phages , especially with the rising rate in antibiotic resistance, particularly in pathogens like *S. aureus*.[64] Phage therapy was highly efficacious across all studies, targeting mainly *S. aureus*, *P. aeruginosa*, and *E. coli* infections particularly when paired with antibiotics. The most commonly tested antibiotics in combination with phage were vancomycin, rifampin, ciprofloxacin, and gentamicin.

Two papers investigated single phage activity, one using biomimetic apatite powder targeting *S. aureus* biofilms. Totten et al., 2024, and the other exploring single phage therapy on isolates from PJI cases.[65] They reported up to 100% of biofilm eradication, indicating that phage therapy may be a viable therapeutic option for biofilm-associated PJI infections.[65,66]

Four papers investigated phage synergy for enhanced results. Taken in combination, these papers suggest that combined phage therapy is superior to monotherapy. They also suggest that phage-derived-lysin, combined with vancomycin, is moderately effective against biofilm and that phages combined with multiple antibiotics were more effective than single antibiotic regimens. While promising, it is worth noting that the overall efficacy of phage treatment against biofilm is not on par with that observed with other agent classes reviewed.[60,67–69]

5. Enzymatic Disruptors

Enzymatic disruptors are a novel class of agents that target and break down the biofilm matrix. There were 5 articles from this search that matched this definition.

Enzymes in this category can be plant-based, such as Bromelain which is derived from pineapple stems, or phages (Enzybiotics). Bromelain powder combined with mechanical scrubbing can achieve 91% biofilm dissolution.[70] Prior studies have found bromelain to be useful in surgical trauma, thrombophlebitis, debridement of wounds, and enhanced absorption of drugs such as antibiotics.

Four papers looked at several enzybiotics finding in some cases an important dose-response curve and in others important synergy with antibiotics.[20,71–74] Exebacase (CF-301) is of particular interest in the literature due to its ability to effectively lyse *S. aureus* biofilms by breaking down peptidoglycan within bacterial cell walls.[20]

DNase is another enzyme that targets specific components of the biofilm matrix, facilitating its breakdown and increasing susceptibility to antimicrobials.[22]

6) Antibodies:

Passive immunization with antibodies has been used clinically to treat various infectious diseases including SARS-CoV-2.[75] This approach has also been investigated as treatment to eradicate established biofilm.[76] A notable example is the pre-clinical efficacy of a native human monoclonal antibody, TRL1068, against the DNABII family: integration host factor (IHF) and histone-like (HU) proteins, which was recently evaluated in a phase 1 clinical trial.[77,78] Antibodies conjugated with photoactivatable compounds and drugs have also been demonstrated to have preclinical efficacy against biofilm.[79,80]

CONCLUSION

PJIs present a significant clinical challenge, primarily due to the resilience of biofilms and the limited effectiveness of antibiotics in these complex infections (Staats, Li, Sullivan, & Stoodley, 2021). Although several agents show preclinical efficacy against biofilms, a multimodal antibiofilm therapeutic strategy is required to enhance efficacy. Our review shows that out of the six main categories of antibiofilm agents, the antimicrobial group was the most pre-clinically studied. Traditional antibiotics have poor efficacy against biofilm when used alone. However, their efficacy in clearing biofilm is enhanced when combined with other antibiofilm agents, such as peptides, nanoparticles, enzymes, phages, or energy-based disruptors. Due to the heterogeneity in the mechanisms of action of the various antibiofilm agents being studied, as well as the various pathogen being treated, it is not possible to determine which group of agents are the most efficacious to be translated into clinical studies. In order to address this knowledge gap, further research is necessary to identify how each of these groups of agents can be tailored to maximize its therapeutic efficacy in the clinical setting.

Supplementary Legends

Table 1: Classification and Examples of Agents active against Biofilm.

Table1

Category	Key Mechanism of Action	Types of agents	Examples
Antimicrobials	Direct bacterial or microorganism killing	Antibiotics, Peptides, Nanoparticles, antimicrobials, immunomodulators	gentamycin, citric acid, silver, SLS
Chemical Disruptors	Chemical interference with biofilm	Quorum sensing inhibitors, Chelators, Chemical Agents, Biofilm Disruptors, antiseptics and disinfectants	Chlorhexidine, Povidone-iodine, Hydrogen peroxide, PHMB,Bleach, Alcohol (at high concentrations), Quaternary ammonium compounds
Bacteriophages	Viral lysis of bacteria	Phage therapy	CF-301,
Enzymatic Disruptors	Biofilm matrix degradation	DNase, Dispersin B	N-Acetylsysteine, Cis-2-Decenoic acid, Chitosan Nanoparticles
Physical Disruption & Energy-Based	Mechanical or energy-based biofilm disruption	Ultrasound, Shock waves, Photo Dynamic Therapy, ROS	Titanium Oxide Nanorod Arrays, Phase-Change Nanodroplets, Acoustically Responsive Hydrogel Microsph
Antibodies	Targeted neutralization of biofilm-associated proteins or bacterial components	Monoclonal antibodies, engineered antibodies, immunotherapies	TRL1068, Aurograb, Anti-Psl

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