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Antibacterial effect of enzymes on biofilms in orthopaedic implanted medical devices

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Abstract
Aim: This scoping review investigates the effects of supplemental enzymes on biofilm formation related to orthopedic implant infections, focusing on Staphylococcus epidermidis and Staphylococcus aureus, to enhance post-implantation recovery and reduce infection rates.
Materials and Methods: A systematic search was conducted across Web of Science, EMBASE, and Cochrane Central for studies published between 2012 and 2022. Using keywords related to enzymes, biofilms, and medical devices, we filtered for randomized controlled trials in English. A total of 918 articles were screened, resulting in 6 eligible studies after applying inclusion and exclusion criteria.
Results: The review highlighted various enzymatic strategies, particularly endolysins and polysaccharide depolymerases, which effectively disrupt biofilm integrity. One study demonstrated that a combination of endolysins significantly improved the efficacy of traditional antibiotics against S. aureus and S. epidermidis biofilms. These enzymes facilitate the degradation of protective biofilm matrices, thus enhancing susceptibility to treatment.
Conclusions: The findings indicate a promising trend in the use of supplemental enzymes to mitigate biofilm formation on orthopedic implants, suggesting potential improvements in patient outcomes. This review underscores the necessity for further research to refine enzymatic therapies and establish standardized treatment protocols to combat biofilm-related infections effectively.

Keywords: Enzymatic therapy, biofilm disruption, orthopaedic implants, *Staphylococcus aureus*, *Staphylococcus epidermidis*, endolysins, polysaccharide depolymerases

Introduction

Orthopedic implant related infections may cause serious surgical complications and can have a socioeconomic burden on the population. Around 5% of all elective and emergent orthopedic surgeries have been reported to have infections involving the orthopedic implant following the procedure [1]. *Staphylococcus epidermidis* and *S. aureus* are the most frequent causes of infections on indwelling medical devices, which involve biofilm formation [2]. *S. aureus* and *S. epidermidis* are estimated to cause about 40-50% of prosthetic heart valve infections, 50-70% of catheter biofilm infections, and 87% of bloodstream infections. Many biofilm eradication strategies have been put forward in an effort to enhance the post-implantation recovery time in patients with one being the use of supplemental enzymes. The purpose of this scoping review is to examine the effects of supplemental enzymes on medical indwelling devices. It has been determined that these supplemental enzymes aid in promoting the breakdown of biofilm to ensure proper recovery post implantation and prevent infection. The difficulties that staphylococci present to antibacterial therapy has brought awareness to the need of alteration of medical device surfaces and the molecular approaches to control biofilm formation [2].

Bacterial biofilm formation on implantable medical devices account for 80% of all medical device-related infections according to the National Institute of Health. Biofilms are formed from a complex accumulation of microorganisms which may include one or more species of communities [3]. Bacteria within the biofilms are protected from the surrounding environment due to a protective matrix formed that consists of lipids, proteins, and extracellular polysaccharides [4]. Supplemental enzymes have been found to target components of bacterial cell walls and the protective matrix formed. Endolysins are a form

of an enzyme that are derived from bacteriophages with hydrolase and lysing capability to cleave bonds within peptidoglycan, a major part of bacterial cell walls [5]. Implant related infections that involve multidrug resistance organisms have been known to have poorer patient outcomes. Many factors come into play while trying to overcome the specific implant infection such as the bacterial strain, species, age of the biofilm, and growth rate. The role of enzyme supplementation in the prevention of biofilm formation in indwelling orthopedic devices could prove beneficial in the future for better long-term patient outcomes.

Various enzyme mechanisms are currently being studied in the prevention of biofilm formation including the PNAG-degrading enzyme dispersin B. It was shown to be able to detach preformed biofilms produced by *S. epidermidis* and allow them to become sensitive to detergent killing however, it was found that dispersin B had no effect on the attachment of preformed *S. aureus* biofilms or their sensitivity to detergent killing [6]. The use of enzymes may prove to be an effective means of diminishing biofilms and a hopeful strategy to improve treatment of multidrug-resistant bacterial infections [7]. Biofilms prove to be advantageous to the bacteria by creating an anoxic and low nutrient environment. Incorporating ways to overcome biofilm formation is the main focus in improving post-implantation recovery time in medical indwelling devices.

Methods: A systematic search was conducted on Web of Science, EMBASE, and Cochran Central to identify relevant articles published within the last 10 years (2012-2022) pertaining to use of antibacterial effect of enzymes on biofilms in orthopedic medical device with a focus on randomized controlled trials.The search strategy utilized the following keywords and Medical Subject Headings (MeSH) terms: “enzyme”- proteases, metalloproteinase , “biofilm”- “staph aureus” and “staph epidermidis”, “medical device” - “indwelling” “internal device”. Filters were applied to restrict the search to articles published in the English language.

Exclusion criteria were applied to filter out irrelevant studies. Inclusion criteria included past 10 years (2012-2022), articles (peer reviewed), English Language, article peer reviewed.

Studies were excluded if they were reviews (including meta-analyses, literature reviews, and scoping reviews). Following the initial search, duplicate articles were removed. The remaining articles underwent title and abstract screening based on the inclusion and exclusion criteria. Full-text articles were then assessed for eligibility.

Articles that did not meet the inclusion criteria were excluded. Specifically, articles were excluded if they were systematic reviews, case reports. A PRISMA diagram was utilized to showcase the article selection process [Figure 1].

The initial search yielded 918 articles. 35 duplicates were then extracted. EMBASE search found 716, Web of Science 196, and Cochran Central 6. Of these, articles were excluded based on publication type, text ineligibility and wrong focus of topic. This process resulted in a final selection of 6 articles meeting the inclusion criteria for further analysis.

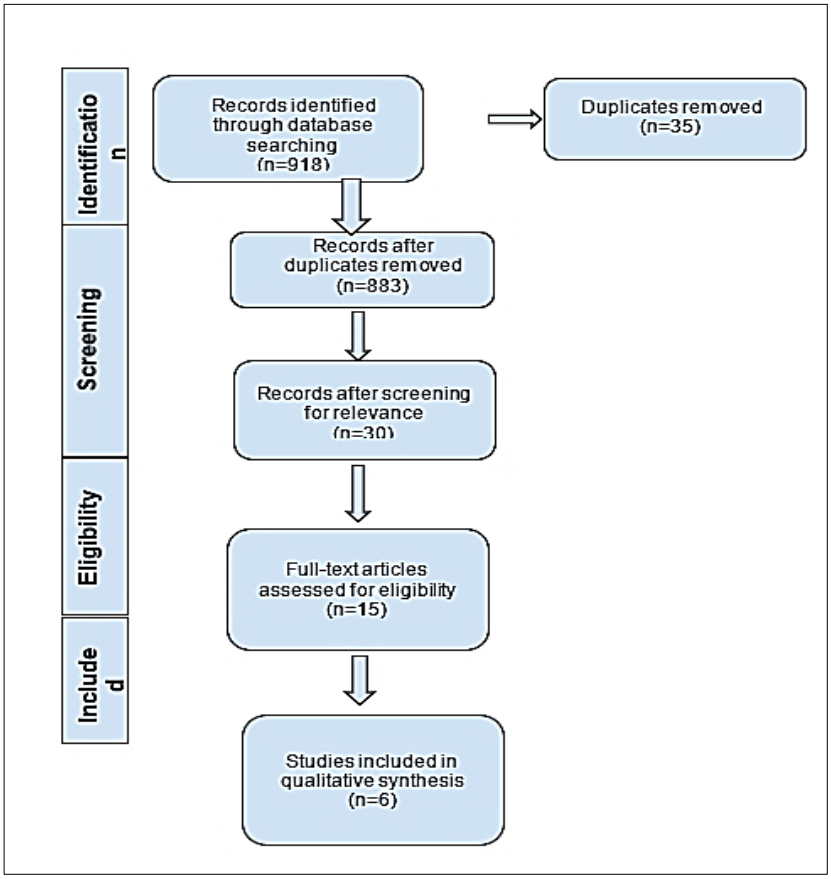

Fig 1: This diagram outlines the systematic search process conducted across Web of Science, EMBASE, and Cochrane Central, aimed at identifying relevant randomized controlled trials on the antibacterial effects of enzymes against biofilms in orthopedic medical devices. The search initially yielded 918 articles, from which 35 duplicates were removed. After applying inclusion and exclusion criteria, a total of 6 articles were selected for further analysis.

Results

Table 1: Summary of Enzymes Utilized in Selected Studies.

Author	Study	Bacteria	Enzymes/Supplemental Therapy	Methods for Quantification	Conclusion
Sumrall et al. (2021) [2]	Experimental trial	MRSA	Endolysins M23 and GH15	Scanning electron microscopy	Eradication of biofilm
Bottagisio et al. (2020) [7]	Comparative proteomic analysis	*Staphylococcus epidermidis*	N/A	Electrophoresis	External stimuli affect biofilm key genes
Lee et al. (2021) [8]	Experimental trial	*Staphylococcus aureus*	Curcumin	Scanning electron microscopy	Biofilm formation suppression
Zu et al. (2019) [9]	Experimental trial	*Staphylococcus epidermidis*	Cryptotanshinone	qRT-PCR	Down-regulation of biofilm key genes
Xia et al. (2021) [10]	Experimental trial	MRSA	Gallium	Scanning electron microscopy	Reduced-tolerance of biofilm
Asadullah et al. (2021) [11]	Experimental trial	*Staphylococcus aureus* and *E. Coli*	Tantalum oxide coating on polyimide	Scanning electron microscopy	Antibacterial and osteogenic

This table highlights the six articles reviewed, showcasing the primary enzymes employed in combating biofilm formation on orthopedic medical devices. Notable enzymes include endolysins and polysaccharide depolymerases, which demonstrated significant efficacy against biofilm-associated infections caused by Staphylococcus aureus and Staphylococcus epidermidis.

Discussion

The systematic review has explored the potential of enzyme supplementation to prevent the formation of biofilms in internal orthopedic medical devices, focusing on the prevalent pathogens Staphylococcus epidermidis and Staphylococcus aureus. The reviewed literature underscores the prevalence of S. aureus and S. epidermidis in biofilm-related infections, accounting for a substantial percentage of infections in prosthetic heart valves, catheters, and the bloodstream. The intrinsic tolerance of these bacteria to standard antibacterial therapy emphasizes the need for alternative approaches to prevent and manage infections associated with medical devices.

S. Aureus and S. Epidermidis biofilm formation on mechanical biomedical devices has presented a noteworthy challenge to medical professionals worldwide. This challenge can be primarily attributed to the microenvironment surrounding the microbes in biofilms. To be specific, S. Aureus and S. Epidermidis microorganisms synthesize a protective layer known as a glycocalyx [8], which consists of a variety of extracellular polysaccharides that classic antibiotics have trouble breaking. Consequently, it is imperative that the medical community continues to invest substantial resources into the exploration of alternative means for eliminating biofilms. Sumrall ET et al., a 2021 experimental trial, did just that. They inserted a plasmid into *E. Coli* to synthesize chimeric endolysins, M23 and GH15. Endolysins are bacteriophage-derived enzymes that degrade peptidoglycan [9]. Bacteriophages use these enzymes to break down bacterial cell walls while transporting genetic material from one microorganism to the next. Thus, they have recently gained traction as alternatives to the standard of care treatments for Methicillin-Resistant S. Aureus infections: Vancomycin and Gentamicin [9]. Their study returned a variety of promising results; the combination of both M23 and GH15 demonstrated a vastly superior ability to kill both free bacteria and bacteria protected by biofilm as compared to antibiotics alone. What is more, the group supplemented the endolysin combination with a polysaccharide depolymerase, DA7, which exerts its effect on poly-B-1, 6-N-acetyl glucosamine (PNAG), a major constituent of the biofilm extracellular matrix [9]. The DA7, M23, GH15 regimen showed great efficacy in reducing CFU counts.

To take it a step further, this enzymatic regimen was supplemented with gentamicin, demonstrating even superior efficacy to the DA7, M23, GH15 combination alone [9]. The group hypothesized that the addition of the DA7 polysaccharide depolymerase weakened the glycocalyx, allowing M23, GH15, and gentamicin easier access to cell wall peptidoglycan. This trial clearly demonstrates that endolysins and polysaccharide depolymerases have a seat at the table when considering innovation in biofilm treatment. While the results above are promising, more scenarios must be explored. Intuitively, the previously-discussed mechanism of biofilm destruction is most effective against gram-positive infections, such as staphylococcus and streptococcus species. Covering such organisms is a top priority, as they account for over 80% of all infections associated with orthopedic devices. However, one must also consider *E. Coli* biofilms and their implications in catheter-associated UTIs. The cell wall composition of gram-negative biofilm colonies warrants tweaking to the regimen elucidated by Sumrall ET, et. al.

In an effort to break up a gram-negative biofilm, Bruno B et al. proposed that endolysin therapy be supplemented with Ethylenediaminetetraacetic acid (EDTA). This is because EDTA destabilizes lipopolysaccharides of the gram-negative outer membrane, granting endolysins access to peptidoglycan [10, 11]. It is clear that the enzymatic therapy used to destroy a biofilm's dominant microbe must be gram dependent. Therapeutic standardization, however, should be explored with respect to breaking the biofilm's extracellular matrix. Ruhal R et al. highlighted that there are a variety of polymeric substances common to both gram positive and gram-negative biofilms. Regardless of gram stain, microbes secrete exopolysaccharides that allow for intercellular adhesion [12]. As such, the development of an enzymatic therapy that incorporates depolymerases to weaken the biofilm's extracellular matrix should be standard practice.

The remainder of the therapy can be geared towards destruction of the case-specific microbe hiding within the biofilm. As demonstrated by Sumrall ET et al., endolysins alongside gentamicin are extremely effective against S. Aureus and S. Epidermidis biofilms. EDTA should be added in the context of gram-negative catheter-associated biofilms [10, 11]. The conclusions that can be drawn from these studies should significantly propel forward the medical community's ability to combat such devastating, resilient infections.

Innovative treatments outside the realm of enzyme therapy are currently under exploration, as well. Bottagisio et al. (2021) investigated the proteomic changes in bone tissue around prostheses during periprosthetic joint infections (PJI). They noted significant alterations in immune response, energy metabolism, and osteoblast and osteoclast activity in the surrounding tissue. These insights could guide new strategies for managing infections [13]. Furthermore, Lee et al. (2021) reported on functionalized hydroxyapatite (HA) bone substitutes with curcumin, which effectively inhibits bacterial biofilm formation and is safe for clinical use, offering potential for improved post-surgical implant outcomes [14].

The biofilm is a complex structure that like any bacterial community thrives on oxygen, nutrients and the ability to fight off pathogens. Biofilms are employed as a defense mechanism for bacteria, and Zur et al. (2019) explored the genes that must be upregulated to form such a community. Knowledge of these genes makes treatment options more clear; we must learn to downregulate them. They concluded that cryptotanshinone, a medicinal herb, can effectively downregulate biofilm-related genes, demonstrating potential as an alternative biofilm therapy. What is more, cryptotanshinone is less toxic than traditional antibiotic therapies, such as Vancomycin [15]. Meanwhile, Xia et al. (2021) proposed gallium-based treatments, which disrupt iron-dependent biofilm formation, making mature biofilms more susceptible to treatment [16]. Asadullah et al. (2020) also identified tantalum oxide coatings on polyimide as having promising osteogenic and antibacterial properties, which could enhance implant material performance [17].

Methicillin-resistant S. aureus (MRSA) is one of the most successful strains and biofilm-forming pathogens responsible for life-threatening infections [18,19]. Lee et al. developed a bioactive bone substitute with enhanced anti-biofilm activity by incorporating curcumin onto hydroxyapatite (HA) [14]. The synthesis of HA composite effectively inhibited bacterial cell attachment and biofilm formation. The study suggests that curcumin-loaded HA could serve as an alternative antimicrobial agent to control infections in post-surgical implants. These researchers highlight the need for further studies and new insights into the potential effect of supplemental therapies to prevent and eliminate biofilms from medical devices.

The use of enzymes, as suggested by Watters et al., in enzymatic degradation of S. aureus biofilms supplemented with human plasma provides further evidence of the potential effectiveness of enzymatic approaches [7]. The results from the proteomic analyses on a S. epidermidis clinical isolate, however, add a layer of complexity to our understanding of the use of enzymatic therapy in the dissipation of biofilms. The study noted an upregulation of glycolytic enzymes and elongation factors that may explain the initial adhesion process of biofilms on biomechanical equipment. Thus, enzymatic therapy may demonstrate varying success at different stages of biofilm development. We surmise that they may be most effective in the early stages. It is clear that enzymatic supplementation to traditional antibiotics improves biofilm combat. The next step in exploration of this topic is to deduce exactly when in the biofilm formation process is enzyme supplementation most useful.

Some limitations of this study include sample size, date range of studies conducted, and types of medical devices. First, the sample size restricts the robustness of the conclusions, as a larger sample could provide more comprehensive insights into the efficacy of enzymatic treatments across different scenarios. Additionally, the study focuses on a limited range of medical devices, which may not represent the diversity of orthopedic devices used in clinical practice, potentially affecting the applicability of the results. Furthermore, the review of studies within a narrow 10-year timeframe may overlook long-term trends in both enzyme technology and biofilm management. Future directions should address these limitations by incorporating a broader array of devices, extending the study period to capture long-term effects, and increasing sample sizes to enhance the reliability and applicability of the findings across various contexts.

Conclusion

The use of orthopedic implants and indwelling medical devices has allowed for promising outcomes in the field of medicine. Aiming for beneficial long-term outcomes in this patient population has always remained a goal. The proper process to prevent implant related infections should start with proper preoperative care, the use of proper surgical techniques, and proper postoperative care. In some situations, biofilms may form on medical devices due to many factors involved. In order to prove the full effectiveness of the orthopedic implant or medical device used, preventing implant related infections should be a major focus. Our scoping review showed that there was an increasing trend in the number of publications reporting enzyme supplementation in preventing biofilm formation in medical indwelling devices. Overall, many different enzyme supplemental strategies have been put forward to overcome biofilm formation including bacteria such as Staphylococcus epidermidis and S. aureus. The response from these strategies has been seen as beneficial in the biofilm degradation process. The results of this scoping review could guide subsequent systematic reviews regarding this topic. In summary, the use of supplemental enzymes plays a significant role in eradicating the biofilm formation that may result in the implanted orthopedic medical device.

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