ICM 2025 Question B14: Is there a role for the use of proteolytic enzymes to treat orthopedic infections?

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RESPONSE/RECOMMENDATION: No. While enzymatic therapies have demonstrated effects against implant related biofilms in pre-clinical models, their safety and efficacy needs to be demonstrated in the clinical scenario. Thus, clinical trials are necessary to clarify the translational potential of proteolytic enzymes to treat orthopedic infections.

LEVEL OF EVIDENCE: Weak

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

RATIONALE: Databases Searched: PubMed, Medline Embase from inception till Nov 2024
Using the following terms ((("Prosthesis-Related Infections"[Mesh] OR "orthopaedic infection*" OR "orthopedic infection*" OR "joint infection*"[tw] OR "infected joint*"[tw] OR PJI[tw] OR ((endoprosthe*[tw] OR prosthes*[tw] OR prosthet*[tw] OR periprosthe*[tw]) AND joint*[tw] AND infect*[tw])) OR (("Orthopedic Procedures"[Mesh] OR "Orthopedics"[Mesh] OR "Joint Prosthesis"[Mesh] OR "Arthroplasty"[Mesh] OR orthopaedic*[tw] OR orthopedic*[tw] OR arthroplast*[tw] OR "artificial joint*"[tw] OR "prosthetic joint*"[tw] OR "joint implant*"[tw] OR "spine surger*"[tw] OR "spinal surger*"[tw] OR "spine implant*"[tw] OR "spinal implant*"[tw]) AND ("Bacterial Infections"[Mesh] OR "Infections"[Mesh] OR "Infection Control"[Mesh] OR "Surgical Wound Infection"[Mesh] OR infect*[tw] OR SSI[tw]))) AND (Proteolytic enzyme* OR "Peptide Hydrolases"[Mesh] OR Protease* OR "Viral Proteases"[Mesh] OR proteolysis OR enzymatic debridement OR Bromelain OR Papain OR Trypsin OR Chymotrypsin)) AND ((1990:3000/12/12[pdat]) AND (english[LA])). Search Results yielded 694 publications in English language. Two of the coauthors went through title and abstract and scored inclusion or exclusion, discrepant results were adjudicated by a third person. Thirty-one articles were reviewed, of which 26 articles was finally deemed to be suitable, one author drafted the original draft and all the contributors commented and agreed final draft. Due to the heterogeneity of the studies, a narrative review was performed and summarized below.

Biofilm related musculoskeletal infections (MSKIs), including prosthetic joint infections (PJI), remain a major health burden in orthopedics and are associated with the inability of the host immune systems to effectively clear infections, and with phenotypic antibiotic resistance. These charactaristics are linked to bacterial multicellularity, and an evolving theory posits that dispersal of bacterial aggregates partially restores susceptibility. At the core of these concepts lies the identification of enzymes that specifically interfere with intercellular adhesive properties. As an example, the pathogenesis of foreign-body-associated infections by coagulase negative staphylococci (CoNS) and particularly of *S. epidermidis* is related to their ability to grow as an adherent biofilm[1; 2]. Poly-N-acetylglucosamine, referred to as polysaccharide intercellular adhesin (PIA), plays a key role in biofilm formation and protection of *Staphylococcus epidermidis* from innate host defenses. PIA was originally defined by its biological properties as an intercellular polysaccharide adhesin. The icaADBC locus, which encodes the biosynthesis of PIA biosynthesis, is widespread in clinical staphylococcal isolates. However, a significant percentage of ica-negative strains has been identified by various researchers in *S. epidermidis* populations isolated from foreign body infections[3]. Staphylococci and other pathogens can form biofilms using PIA-independent mechanisms. Polysaccharides and protein binding between cells and cells-surface can be detected. Also, extracellular DNA (eDNA) supports biofilm formation[4]. Besides the use of antibiotic substances to

manage biofilm infections, the enzymatic detachment of biofilms may support treatment of biofilm associated infections in Staphylococci spp with the understanding that heterogeneity and the gap in knowledge for other organisms are major limitations of target specific approaches!

Leite (2020) as well as Lacey (2024) and colleagues investigated the role of caspases on the bacterial clearance of bone infections. Caspases are a family of proteases that play a key role in the immune response, regulating cell death and inflammation. There role in the treatment of implant related biofilm infections is less understood. Caspase-1 is an important component of the innate immune response involved in the production of inflammatory cytokines through proteolytic cleavage. The results of Leite and colleagues demonstrate in an *in vitro* model that caspase 1 is essential to the generation of inflammatory cytokines and control of bacterial replication in infected cells[5]. Similar findings were reported by Lacey and colleagues while investigating caspase-1 and caspase-11 in a mouse model of *Brucella* joint infections, where both caspases induce pyroptosis, which limited *Brucella* infection in macrophages in vitro[6].

Enzymatic disruption of biofilm by attacking both PIA and the extracellular DNA (eDNA has been investigated. Arciola and colleagues (2009), showed that both dispersin B, an enzyme active against PNAG, and DNase I are able to inhibit biofilm formation, detach preformed biofilms and sensitize bacteria to be killed by other antimicrobials[7]. Drawbacks related to enzyme-based anti-biofilm therapy is the possible spread of cells from the biofilm, which may increase the risk of bloodstream infections and seed infections to distant sites. Enzyme-based therapies could be used in combination with antimicrobial agents, or their benefit may be limited to prevention rather than treatment[8]. Chaignon and colleagues (2007), also tested dispersin B, comparing to periodate, Pectinex Ultra SP, proteinase K, trypsin and pancreatin to treat preformed *Staphylococcal* biofilms *in vitro*. They found that the enzymatic detachment of staphylococcal biofilms depends on the nature of their constituents and varies between the clinical isolates tested. In their conclusion, they suggest that a treatment with dispersin B followed by a protease (proteinase K or trypsin) might be able to eradicate biofilms of a variety of staphylococcal strains on inert surfaces[9].

Serratiopeptidase (SPEP), a proteolytic enzyme produced by the enterobacteria Serratia E15 is also a candidate for the enzymatic treatment of PJIs[10]. It is produced in the intestines of silkworms to break down cocoon walls[11]. Mecikoglu and colleagues tested this enzyme in animal models for *S. epidermidis* PJI. One of the groups tested received SPEP enzyme injections into the infected knee joint in addition to antibiotic therapy for four weeks while the other was treated only with antibiotics. Bone samples from mice that were treated with SPEP + antibiotics were more likely to be nagtive than those from mice treated with antibiotics alone.This suggests that the anti-biofilm property of the enzyme may enhance antibiotic efficacy in the treatment of Staphylococcal infections[12]. Further, Selan and her team evaluated the ability of SPEP to control *S. aureus* invasion of osteoblastic MG-63 cells and the secretion of the pro-inflammatory chemokine MCP-1. She showed that SPEP impairs invasion of staphylococci into osteoblasts, without affecting the viability and proliferation of bone cells, and reduces their production of MCP-1. Selan and colleagues recognize SPEP as a potential tool against *S. aureus* bone infection and destruction[10; 13].

Urokinase is an enzyme produced by the kidneys and excreted in the urine. Used as a thrombolytic drug to treat blood clots, it may assist in the degradation of both fibrin and the biofilm produced by *S. epidermidis*, thus improving penetration of antibiotics and local host defense mechanisms. It has been studied in an *in vivo* model of vascular graft infection and shown in combination with antibiotics to reduce numbers of viable bacteria[14]. Alternatively, it can be used as a coating substance for preventing implants of being colonized by biofilms[15].

Phage-derived enzymes with antibacterial properties have been isolated and tested against biofilms.

One of them, LysECD7, showed outstanding broad range activity against the planktonic forms[16]. Fursov et al tested the activity of LysECD7 as anti-biofilm agent in animals implanted with biofilm-inoculated intra-abdominal cages. As result, they showed that LysECD7 possesses the ability to act against emerging and formed biofilms both in vitro and in vivo, comparable with the activity of amikacin but with less inflammatory infiltration[1]. Still related to phage-derived enzymes, Kuiper and colleagues evaluated XZ.700, a chimeric endolysin built combining parts of *S. aureus* bacteriophage endolysin ply2638[17] and lysostaphin[18]. In their study, XZ.700 reduced MRSA biofilms, especially under flow condition, without toxicity for surrounding bone cells[19]. Other studies testing lysostaphin against biofilm-infection showed promising results. Walenka and colleagues shows the synergistic effect of subBIC lysostaphin+oxacillin in diminishing MSSA and MRSA biofilms[20]. Coating implants using lysostaphin could be a promising tool in the therapeutic strategies for avoiding PJI[21]. In further studies, BMP-2–loaded lysostaphin-delivering hydrogel therapy effectively eliminated *S. aureus* infection while simultaneously regenerating functional bone resulting in defect healing in animal model[22; 23]. The chimeric lytic enzymes M23LST(L)_SH3b2638A(M23) and CHAPGH15_SH3bALE1 (GH15), as well as the DA7 polysaccharide depolymerase, used as a combination regimen, showed effectiveness at eradicating established *S. aureus* infection[24]. Exebacase (CF-301), a phage-derived lysin with anti-staphylococcal activity, is the most advanced lysin tested in clinical trials and is currently in phase III for treatment of *S. aureus* bacteremia/right-sided endocarditis (ClinicalTrials.gov Identifier: NCT04160468)[25]. Suche et al tested exebacase against bacteria isolated from PJI and concluded that it enhanced the activityof rifampicin, vancomycin, and daptomycin[26].

In summary, enzymatic disruption of biofilms, or the use of enzymes as coating substances to avoid infection of implants is a very attractive idea. The data are heterogenous and limited to pre-clinical studies. Different substances have been tested with positive results. However, their use present certain drawbacks. For example, the release of planktonic bacterial cells from a disrupted biofilm could cause infection in other sites, or even bloodstream infection. In this case, the parallel administration of systemic or even local antibiotics is highly indicated. Further, because enzymes cause the lysis of the bacterial cell, they can promote the release of intracellular toxins that could be harmful to the host tissues. Further studies considering these risks with the aim to establish the use of enzymes as a toll agaist PJIs are welcome. Human studies and clinical trails are required to establish whether enzymatic disruption of biofilms improves outcomes over the standard of care for PJI.

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