A novel strategy for treating orthopedic infection: Combination of sonodynamic therapy and immunotherapy

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How to cite this article: Wan X, Huang, Zhou Z, Wang D. A novel strategy for treating orthopedic infection: Combination of sonodynamic therapy and immunotherapy. *Biomater Transl.* **2025**

doi: 10.12336/bmt.25.00008

Orthopedic implant-related biofilm, structured communities of bacteria embedded in extracellular polymeric substances, present a formidable clinical challenge. Periprosthetic infections, affecting 1 – 10% of patients receiving implants, are the leading cause of early failure in orthopedic surgeries.[1] These biofilm-associated sessile communities evade antimicrobial agents and host immune clearance through intrinsic biofilm-mediated resistance, leading to persistent infections and recurrent disease.[2] Biofilms have been reported to be 10 – 1000 times more tolerant to antibiotics than planktonic bacteria.[3,4] These infections can be severe, threatening both the patient's limb and life. Their immunosuppressive microenvironment, marked by hypoxic niches, cytokine-mediated immune suppression, and persister cell subpopulations, further subverts both pharmacological and immunological defenses. Compounding this issue, escalating antibiotic misuse has fostered resistance epidemics, epitomized by methicillin-resistant *Staphylococcus aureus* (MRSA), implicated in 40–60% of periprosthetic infections.[5] Emerging interventions such as photodynamic therapy and nanoparticle-mediated drug delivery offer partial solutions but remain constrained due to hypoxia-driven therapeutic failures and off-target immune activation.[6] Immunotherapies, which aim to boost host recognition and elimination of pathogen-laden cells, present a promising paradigm shift.[7] However, the immunosuppressive nature of biofilms and the difficulty in exposing bacterial antigens to immune cells have limited the success of immune-based therapies.[8] A pressing imperative exists for multifactorial approaches that concurrently neutralize pathogenic reservoirs and recalibrate immune responsiveness to achieve durable eradication. Sonodynamic therapy (SDT) employs ultrasound-activated sonosensitizers to generate localized reactive oxygen species (ROS), destabilizing biofilm architecture through oxidative damage while ameliorating hypoxia – a critical virulence determinant.[9] To circumvent these limitations, combining SDT with immunomodulation has recently gained traction as a synergistic approach.

In a recent Nature Communications study, Zhu *et al.*[10] from the University of Science and Technology of China developed a biodegradable oxygen-evolving porphyrinic metal-organic framework (MOF)-based metalloantibiotics (MnPM) for spatiotemporal sono-metalloimmunotherapy (SMIT), offering a multifunctional therapeutic paradigm to overcome biofilm-associated treatment barriers.[10] This innovative approach (**Figure 1**) employs a tailored manganese-based MOF–MnPM-engineered to catalyze *in situ* oxygen generation within the hypoxic, acidic biofilm microenvironment. The study highlights how MnPM synergizes with ultrasound-triggered SDT to disrupt biofilm matrices and enhance immunomodulation, presenting a transformative alternative to conventional antibiotic regimens.

Zhu *et al.*[10] systematically validated SMIT's therapeutic efficacy through *in vitro* and *ex vivo* models of orthopedic implant biofilm infections. MnPM's hierarchical architecture – comprising manganese dioxide-hydrangea nanoparticles functionalized with a porphyrinic MOF (porous coordination network-224) – enables catalytic decomposition of biofilm-entrapped hydrogen peroxide into molecular oxygen. This oxygen-evolving capacity critically amplifies SDT's bactericidal effects, which rely on ultrasound-activated sonosensitizers to generate membrane-permeabilizing ROS. Quantification of biofilm biomass revealed SMIT-induced reductions exceeding 90% for both *Escherichia coli* and MRSA. High-resolution imaging through scanning electron microscopy and confocal laser scanning microscopy corroborated widespread structural disintegration of biofilms, with evidence of bacterial membrane rupture and extracellular DNA release – processes that expose immunostimulatory pathogen-associated molecular patterns (PAMPs). While earlier investigations established ultrasound-activated ROS generation as a viable strategy for eradicating superficial implant-adherent

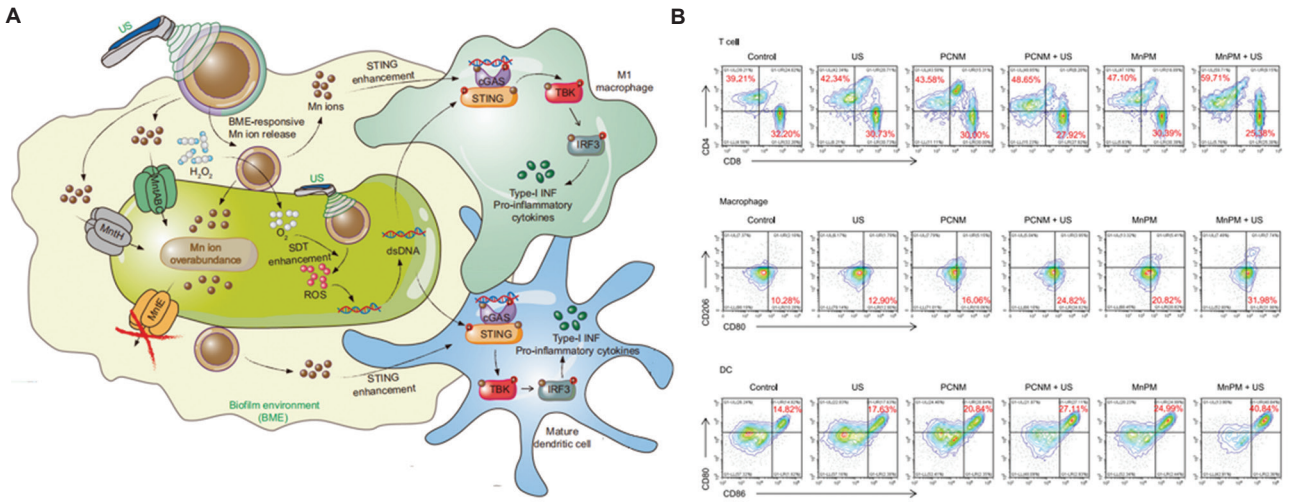

Figure 1. A spatiotemporal sono-metalloimmunotherapy strategy is proposed for biofilm ablation.[10] (A) Upon injection into the biofilm, MnPM acts as an oxygen self-supplying sonosensitizer, enhancing SDT against recalcitrant implant-related infections. Concurrently, manganese ions released sensitize the biofilm to SDT by disrupting intracellular homeostasis, further facilitating biofilm degradation. Bacterial fragments (e.g., double-stranded DNA and dsDNA), as bioactive agents released from the damaged biofilm, combine with manganese ions to initiate effective innate and adaptive antibiofilm immune responses by activating STING to suppress biofilm growth. (B) Representative flow cytometry plots of $CD4^+$ T cells gated on $CD3^+$ T cells, M1-phenotype macrophages ($CD80^+CD206^-$) gated on $CD11b^+F4/80^+$ cells, and mature DCs ($CD80^+CD86^+$) gated on $CD11c^+MHCII^+$ cells in spleens following MnPM treatment. With copyright permission: Image used with permission from Springer Nature, Copyright © 2024, Springer Nature.
Abbreviations: BME: Biofilm environment; DCs: Dendritic cells; MnPM: Porphyrinic metal-organic framework-based metalloantibiotics; PCN: Porous coordination network; PCNM: PCN+MnO2+neutrophil/macrophage membranes; STING: Stimulator of interferon genes; US; Ultrasound.

pathogens,[11] such monotherapeutic approaches fall short against bacteria entrenched in complex microenvironments, including intracellular niches. SMIT addresses this limitation through its dual-modality design, simultaneously disrupting biofilm recalcitrance and enhancing immune-mediated pathogen clearance.

While immunotherapies have been extensively characterized in oncological contexts,[12,13] their potential to counteract biofilm-driven immunosuppression remains underexplored – a frontier this study strategically addresses.[14,15] The MnPM platform uniquely integrates biomimetic neutrophil-macrophage hybrid membrane coatings, endowing nanoparticles with PCN^+MnO^{2+}neutrophil/macrophage membranes pathogen-homing tropism and enhanced biofilm penetration. This dual-membrane functionalization not only increases infection site bioavailability but also circumvents biofilm-mediated immune evasion by mimicking endogenous leukocyte signaling. The manganese ions Mn^{2+}) released from MnPM nanoparticles activate the cyclic guanosine monophosphate–adenosine monophosphate synthase-stimulator of interferon genes (cGAS-STING) pathway to double-stranded DNA, leading to the production of type I interferons and pro-inflammatory cytokines, which are essential for initiating immune surveillance. The combination of Mn^{2+} and bioactive agents dramatically elevates STING activation in antigen-presenting cells (APCs). Flow cytometry analysis reveals increased maturation of dendritic cells and polarization of macrophages to the M1 phenotype, both of which are key players in the immune response to biofilm infections. Notably, the study also demonstrates that MnPM promotes T cell responses, specifically biofilm-specific $CD4^+$ and Th17 cells, which further help to control biofilm growth and eliminate infection.

In vivo, studies using a model of implant-associated biofilm infection show that the combination of SDT and immunotherapy significantly suppresses bacterial growth on implants and in surrounding tissues. Moreover, the treatment not only reduces the infection but also accelerates tissue repair, as evidenced by increased collagen deposition, enhanced vascularization, and improved wound healing. The design of MnPM not only disrupts bacterial homeostasis by causing oxidative stress and Mn^{2+} overabundance but also stimulates the immune system by activating the cGAS-STING pathway in APCs, which promotes adaptive immune responses. This dual-action approach – both bactericidal and immunostimulatory – sets SMIT apart from conventional therapies. These results suggest that the SMIT strategy not only targets the bacterial biofilm but also promotes tissue regeneration, which is critical for treating implant-related infections.

While these findings establish SMIT as a paradigm-shifting strategy for biofilm-associated orthopedic infections, critical translational challenges persist. First, the bone implant coating needs to be stable, but activating immunity requires ion release, which is relatively difficult to control. Degradable gel coatings such as poly(lactic-co-glycolic acid) can be constructed to achieve faster and more effective induction of immune response. Beyond optimizing immunomodulatory dosing kinetics, future work must delineate the temporal

dynamics of Mn^{2+}-mediated STING activation relative to antigen presentation windows – a determinant of vaccine-like immunological memory formation. Transitioning beyond manganese-based formulations, transition metal cations – including but not limited to iron, copper, magnesium, and zinc – exhibit distinct immunomodulatory potential through their intrinsic redox-cycling capacity and coordination chemistry with PAMPs besides Mn^{2+}. In addition, both immunotherapy and SDT are treatments following bacterial infection and cannot effectively prevent it. Clinically, integrating SMIT with bacteriophage therapies or quorum-sensing inhibitors could disrupt biofilm formation mechanisms. Although manganese is a necessary nutrient, excessive amounts can be neurotoxic and lead to a condition known as manganese intoxication.[16] Hence, advancing SMIT toward clinical trials demands rigorous evaluation of manganese's neurotoxicity thresholds and long-term immunological sequelae in large-animal models. This could be mitigated by engineering MnPM with manganese-chelating polypeptide coatings that decompose preferentially at biofilm pH (5.5 – 6.5), minimizing systemic manganese leakage. Finally, immunocompromised patients – representing >30% of periprosthetic infection cases – may exhibit blunted cGAS-STING responsiveness, as indicated by the flow cytometry data showing 45% reduced interferon β production in toll-like receptor 4-deficient macrophages *ex vivo*. Complementary administration of STING agonists or adoptive natural killer cell transfer could reinstate immunogenicity in such populations. Although the scalability of neutrophil-macrophage hybrid membrane production necessitates biomanufacturing innovations, this biomimetic design blueprint opens avenues for nanoparticle-mediated combat against intracellular pathogens and fungal co-infections. Should these barriers be surmounted, the platform's dual antimicrobial-immunoregenerative capacity positions it as a cornerstone therapy for recalcitrant implant infections – transforming biofilms from treatment-resistant obstacles into immunotherapy-responsive targets.

Acknowledgement
None.
Financial support
None.
Conflicts of interest statement
The authors declare no conflicts of interest.
Author contributions
Conceptualization: XW, DW, and ZZ; *Writing-original draft:* XW; *Writing–review & editing:* YH, DW, and ZZ. All authors read and approved the final manuscript.
Ethics approval and consent to participate
Not applicable.
Consent for publication
Not applicable.
Availability of data
Not applicable.

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Received: February 25, 2025
Revised: March 11, 2025
Accepted: March 12, 2025
Available Online: May 8, 2025