Overcoming the biofilm barrier by cell-like microbubbles for the treatment of biofilm-associated implant infections

Yan-Ling Hu\textsuperscript{1,3}, Jin-Gai Jiang\textsuperscript{1}, Hui-Juan Cheng\textsuperscript{2}, Wei Shi\textsuperscript{3}, Yan-Ni Song\textsuperscript{2}, Min Zhang\textsuperscript{2}, Dong-Liang Yang\textsuperscript{2,3}

\textsuperscript{1}College of Life and Health, Nanjing Polytechnic Institute, Nanjing 210048, China. \textsuperscript{2}Hangzhou Huasu Industry Co., Ltd., Hangzhou 310012, China. \textsuperscript{3}Key Laboratory of Flexible Electronics (KLOFE) and Institute of Advanced Materials (IAM), School of Physical and Mathematical Sciences, Nanjing Tech University (NanjingTech), Nanjing 211816, China.

Corresponding to: Wei Shi, Dongliang Yang, Key Laboratory of Flexible Electronics (KLOFE) and Institute of Advanced Materials (IAM), School of Physical and Mathematical Sciences, Nanjing Tech University (NanjingTech), No. 5, Xinmofan Avenue, Nanjing 211816, China. E-mail: iamwshi@njtech.edu.cn, yangdl1023@njtech.edu.cn; Min Zhang, Hangzhou Huasu Industry Co., Ltd., No. 90, Wensan Avenue, Hangzhou 310012, China. E-mail: 11229018@zju.edu.cn.

Although the advent of antibiotics has significantly improved the quality of life of infected patients, bacterial infections continue to pose a serious threat to public health \cite{1, 2}. According to a recent report, within the next 30 years, bacterial infections are projected to surpass cancer in terms of lethality rates, resulting in an alarming 10 million deaths annually by 2050 due to the development of bacterial resistance \cite{3}. Moreover, the formation of bacterial biofilms hampers the penetration of antibacterial agents and inhibits the host immune response, making biofilm infections extremely challenging to treat \cite{4-7}. Hence, the development of innovative antimicrobial biofilm therapeutics is imperative. Under this scenario, Xiu et al.'s innovative exploration of biofilm infection treatment involved the combination of microbubble-mediated ultrasound (US) therapy; nitric oxide (NO), reactive oxygen species (ROS), and immune therapy for drug delivery within bacterial biofilms; and the removal of bacterial biofilms, which is a promising strategy (Figure 1) \cite{8}.

The landscape of bacterial and biofilm infection treatment has changed and mainly consists of nanotechnology and new therapeutic strategies \cite{9, 10}. To date, a range of nanodelivery technologies and novel therapeutic strategies have demonstrated promising therapeutic effects, garnering significant attention from clinicians \cite{11, 12}. Nevertheless, the relentless development of infectious diseases necessitates continuously exploring innovative and effective treatment

Figure 1 Use of EMB-Hu for enhancing biofilm penetration and in combination with microbubble-mediated US therapy, ROS, NO, and immune therapy. NO, nitric oxide; ROS, reactive oxygen species; Fe\textsubscript{3}O\textsubscript{4}, NP, ferriferous oxide nanoparticle; Hu, hydroxyurea; MRSA, methicillin-resistant \textit{Staphylococcus aureus}. 

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to combat a diverse range of clinical infections. Recently, microbubble (MB), which serves as nanocarrier and therapeutic have been subjected to extensive attention from researchers and medical workers, and has exhibited excellent therapeutic efficacy in the treatment of disease [13]. While MB-mediated US therapy utilizes the physical shear force generated by MBs to destroy the biofilm matrix barrier, ROS-mediated antibacterial therapy can eliminate bacteria within biofilms, and immunotherapy changes the immunosuppressive of biofilms and activates autimmunotherapy to achieve bacterial clearance. In addition, the emergence of MB-mediated US therapy, which overcomes bacterial sanctuaries to boost the therapeutic efficacy of immune therapy and ROS-mediated antibacterial therapy, has led to breakthroughs in physical destruction, NO, ROS, and immune combined therapy.

First, ultrasonic stimulation can trigger the exocytosis-like behavior of erythrocyte membrane-costed hydroxyurea-loaded Fe₂O₃ nanoparticle-fabricated MB (EMB-Hu), and the formation of a microstream is conducive to the delivery of antibacterial drugs to bacterial biofilms, thereby enhancing therapeutic penetration. Concurrently, the addition of H₂O₂, hydroxyurea (Hu), and peroxidase-like Fe₂O₃ NPs released from EMB-Hu can generate NO gas. During this process, the author employed low-intensity US to trigger stable oscillation of the MBs for slow therapeutic release while minimizing the adverse effects caused by cavitation. In addition, US-induced cavitation facilitates the therapeutic crossing of the bacterial biofilm matrix barrier.

With the in-depth study of the authors, it is clear that, under US stimulation, EMB-Hu can eliminate bacterial biofilms effectively ex vivo and in biofilm-related implanted infections (in vivo). After encapsulation in the erythrocyte membrane, the surface charge of EMB-Hu changes from positive (MB-Hu) to negative. The mature biofilm can be disrupted in vitro after treatment with both EMB-Hu and US stimulation (0.5 W/cm²). Moreover, upon exposure to the US, EMB-Hu significantly enhanced NO and ROS permeability compared with erythrocyte membrane-encapsulated Fe₂O₃ NPs (EFe). The antibiofilm assay indicated that the MRSA biofilm was still intact in the EFe + Hu + H₂O₂ treatment; however, 70.35% of the biofilm structure was disrupted, and approximately 99.99% bacteria within the biofilm were killed in the EMB-Hu + US + H₂O₂ treatment. These results confirmed that the ROS generated from the Fenton-like catalytic reaction and the NO gas produced from the reaction of ROS and Hu exhibit excellent antibiofilm performance when US is used.

In addition, Xiu et al. investigated the activation effect of EMB-Hu + US on M1-type macrophages. The results of confocal microscopy and flow cytometry indicate that the M1-type marker CD86 increases significantly. Cytokine analysis revealed that the Fe₂O₃ NPs released from EMB-Hu can enhance the expression of M1-type associated cytokines (IL-1β) while inhibiting the expression of M2-type associated cytokines (TGF-β). Using Fe₂O₃ NPs as “signaling molecules”, the dilemma that biofilms inhibit immune activation can be effectively solved. In biofilm-associated implant infection, US-stimulated enhanced therapeutic penetration can also be observed via histopathological analysis. Immunofluorescence and ELISA analysis of infected tissue confirmed the immunoactivation effect of EMB-Hu + US. For example, the expression of CD86 and TNF-α (M1-type cell biomarkers) increased markedly, while that of CD206 and arginase-1 (Arg-1) (M2-type cell biomarkers) decreased significantly in the EMB-Hu + US-treated group. This analysis fully illustrated the feasibility of utilizing EMB-Hu for immunoactivation of biofilm-infected tissue.

This study reveals a novel therapeutic strategy for future biofilm infection research. MB-mediated US therapy effectively disrupts the biofilm matrix barrier primarily through physical action originating from the microstream effect, which significantly enhances the antibiofilm performance of therapeutics, whereas immunotherapy clears surviving pathogenic organisms, which can effectively prevent the recurrence of infection. By leveraging MB-mediated US therapy, combination therapy with NO, ROS, and immunotherapy significantly enhances the therapeutic efficacy of biofilm-associated infectious diseases. However, using this strategy on a large scale still leads to other issues, including mass synthesis of EMB-Hu, long-term therapeutic stability, and side effects from gas blasting. The limited H₂O₂ concentration in the infected tissue may also limit its antibacterial performance.

Based on the results of previous studies, MBs have been shown to achieve favorable theranostic effects as contrast agents and therapeutic reagents. Currently, there are two FDA-approved MBs for contrast imaging, Definity, DuPont Pharmaceuticals Co., North Billerica, MA, and Mallinckrodt, San Diego, CA. [14]. The Fe₃O₄ NP component of EMB-Hu also exhibits great potential for clinical use; for example, some Fe₃O₄ NP-based therapeutics (e.g., Feraheme® and Feridex®) have been approved for clinical use. These results are beneficial for the clinical translation of the EMB-Hu. In the present study, the authors confirmed the therapeutic effect of EMB-Hu on only MRSA biofilms. In the future, it is crucial to evaluate the antibiofilm activity of EMB-Hu against gram-negative/positive bacteria and fungi. In addition, the potential and long-term toxicity of EMB-Hu must be considered before clinical trials.

In summary, Xiu et al.'s research significantly improved the therapeutic effect of biofilm-related implanted infections through US-mediated exocytosis for enhanced biofilm penetration, resulting in enhanced biofilm penetration and NO/ROS-immune combination antibiofilm therapy. We urgently anticipate further advancements, including clinical trials. In this field. Such an overcome bacterial biofilm barrier and combination therapy strategy has the potential to revolutionize the treatment of biofilm-associated implant infections.

References


Hu YL wrote the draft. Jiang JG, Cheng HJ, and Song YN collected relevant information. Shi W, Zhang M and Yang DL revised and edited the manuscript. All authors have reviewed and agreed to the final draft.

**Competing interests**

The authors declare no conflicts of interest.

**Acknowledgments**

This work was supported by the Natural Science Foundation of Jiangsu Province (BK20230117), and the Natural Science Research Project of Nanjing Polytechnic Institute (NIPI-2023-04). We would also like to thank Heng Dong from Nanjing University for their valuable input in revising the manuscript.

**Abbreviations**

NO, nitric oxide; ROS, reactive oxygen species; Fe3O4 NP, ferriferous oxide nanoparticle; Hu, hydroxyurea; MB, microbubble; US, ultrasound.

**Citation**


**Executive editor:** Na Liu.

**Received:** 19 December 2023, **Accepted:** 28 March 2024, **Available online:** 01 April 2024.

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