TMR

COMMENT

Biomedical Engineering Communications 2023;2(2):7. https://doi.org/10.53388/BMEC2023007

Ultrasound-responsive microbubbles in antibacterial therapy

Xiao-Ye Li[1], Wei-Jun Xiu[2], Dong-Liang Yang[3], Heng Dong[1*]

[1]Nanjing Stomatological Hospital, Affiliated Hospital of Medical School, Nanjing University, Nanjing 210008, China. [2]Key Laboratory for Organic Electronics and Information Displays, Jiangsu Key Laboratory for Biosensors, Institute of Advanced Materials, Jiangsu National Synergetic Innovation Centre for Advanced Materials, Nanjing University of Posts and Telecommunications, Nanjing 210023, China. [3]School of Physical and Mathematical Sciences, Nanjing Tech University (NanjingTech), Nanjing 211800, China.

[*]**Corresponding to:** Heng Dong, Nanjing Stomatological Hospital, Affiliated Hospital of Medical School, Nanjing University, No.30 Zhongyang Road, Nanjing 210008, China. E-mail: dongheng90@smail.nju.edu.cn.

Microbubbles (MBs) are gas-filled micrometer-scale spheres that are commonly formed by the gas core encapsulated with stabilizing shells, including polymers, surfactants, proteins, or liposomes shells. Clinically, MBs were originally used as contrast agents for enhanced ultrasound (US) imaging and diagnostics. Nowadays, MBs were given expectations that they can be alternative platforms for drug delivery owing to their unique acoustic properties. MBs can respond to the US by cavitation effect which refers to a series of complex dynamic processes, such as oscillation, expansion, contraction, and implosion [1]. Drug molecules or therapeutic agents can be associated with the MB shells by means of van-der-Waals forces, electrostatic or hydrophobic interactions, or merely by physical encapsulation [2]. Therefore, strategies are emerging which take advantages of US-mediated MBs drug delivery systems, mainly focusing on sonothrombolysis, cancer therapy and central nervous system (CNS) pathologies [3]. Nevertheless, several researchers have apperceived the promising potential of US-responsive MBs in antibacterial therapy. Here, we aimed to paint an overview of the latest published papers on MBs for antibacterial therapy, hoping to help understand the perspectives that the field may offer emerging generations of antibacterial agents.

When assisted with the US, MBs not only play the role of drug carriers, the cavitation effects of MBs can create pores in cell membranes temporarily and lead to an efficient increase of cell membrane permeability. This results in enhanced tissue distribution and intracellular delivery of antibacterial agents, which is rather important for breaking through biological barriers and treat some obstinate infections [4]. For example, Horsley et al. developed novel US-activated lipid MBs for urinary tract infection (UTI) [5]. During acute UTI, the common uropathogenic bacteria can invade the urothelial wall and form dormant reservoirs within cells where they may escape luminal antibiotics and the immune system. This is one possible explanation for high UTI recurrence rates after oral antibiotic treatments which have difficulty in penetrating the bladder wall and accumulating to an effective concentration. Therefore, Horsley and co-workers developed US-activated sulfur hexafluoride-filled MBs whose shells were constructed from lipids and decorated with liposomes and gentamicin. Confocal results showed that US-activated intracellular delivery of MBs in the human urothelial organoid model was over 16 times greater than the control group and double that of liposomes without MBs. The authors then infected human urothelial organoids with a patient-isolated strain of *E. faecalis*, and the results confirmed the effectiveness of killing and clearing uropathogenic bacteria by US-activated microbubble therapy. Besides intracellular infection like UTIs, some CNS infections also challenge traditional antibacterial therapy and threaten human life in a clinic because of the blood-brain barrier (BBB). BBB is established by the neurovascular unit (NVU), which helps to regulate influx and efflux transport, thus maintaining brain homeostasis and protecting the brain from harm [6]. On the other hand, BBB limits the transport of most therapeutic agents and entities into CNS when neurological diseases happen [7]. Therefore, research for blood-brain-barrier disruption (BBBD) methods is crucial, whose goal is to compromise the BBB transiently to allow the circulating therapeutic molecules and biomarkers to pass into the parenchyma and, less frequently, out of it [8]. Focused-US with MBs is an attractive noninvasive approach for permeabilizing the BBB, owing to its adjustable and transient impact on the vasculature, as well as a significant number of tunable parameters that can influence its safety and efficacy. Xu et al. prepared a tigecycline-loaded, US-activated MBs for the treatment of CNS infectious disease caused by the multidrug-resistant *Acinetobacter baumannii* (AB) [9]. The results indicated that the US improves the anti-AB performance of tigecycline-loaded MBs *in vitro*. The authors expected to apply pulsed US in conjunction with the intravenous infusion of tigecycline-loaded MBs to treat intracranial AB infections in animals. While the authors have not published the work yet, it is worth paying attention to subsequent progress. In conclusion, US-activated MBs may have great potential in crossing biological barriers for the treatment of infectious diseases, including but not limited to cell walls and BBB.

The above-mentioned studies are all about the treatment for planktonically growing, suspended bacteria, while bacterial infections are mostly caused by bacteria in an adhering and biofilm mode of growth [10]. Biofilms are structured communities of microbial cells where bacteria are embedded in the matrix, which is composed of extracellular polymeric substances (EPS) [11, 12]. EPS functions as physical barrier to enable bacteria within biofilm to escape the host immune response and treatment of anti-bacterial agents [13]. Therefore, it is important to destroy the biofilm structural barrier which protects the bacteria [14]. US-assisted microbubble therapy may perform well due to its ability to break through barriers. To be specific, under US stimulation, MBs can contract in an oscillatory manner and generate local micro-streams by cavitation, which can disrupt the structures of biofilms and produce holes and channels within biofilms to enhance the penetration of drug molecules [15]. Kouijzer et al. decorated lipid MBs with vancomycin as a therapeutic agent against *Staphylococcus aureus* biofilms [16]. The authors confirmed the microbubble oscillation and biofilm disruption upon ultrasound exposure using time-lapse confocal microscopy combined with the ultra-high-speed camera. Results showed that upon US in sonification, biofilm area was reduced by up to 28%. Moreover, microbubbles can eliminate biofilms not only by physical destruction and the release of antibiotics but also by chemical degradation and the activation of the host immune response for pathogen clearance when MBs are well-designed. Xiu et al. designed self-assembly microbubbles composed of Fe_3O_4 nanoparticles (NPs) loading piperacillin (MB-Pip) to enhance biofilm elimination and immune activation to treat chronic lung infections (Figure 1) [15]. Besides the physical disruption of *Pseudomonas aeruginosa* biofilms and penetration of Pip, the released Fe_3O_4 NPs with peroxidase-like catalytic activity can catalyze H_2O_2 to generate hydroxyl radicals (•OH) and chemically degrade the biofilm matrix. Also, both *in vitro* and *in vivo* results indicated that the Fe_3O_4 NPs released from MB-Pip can induce proinflammatory macrophage (M1-like phenotype) infiltration and polarization which improved the aberrant state of the host inflammatory response. In addition, some studies have found that perfluoro pentane (PFP) or perfluorocarbon (PFC) liquid can be made as nanodroplets and ultrasound-induced pressure variations can cause them to vaporize due to the low boiling

point of PFP or PFC, thereby converting these droplets into gas bubbles [4, 17, 18]. Compared to pre-prepared MBs, those transformed from microdroplets are more stable and controllable and possess a longer half-life in vivo [19]. Xin et al. developed such US-activatable phase-shift microdroplets by a double emulsion approach, where the PFP and antibiotic meropenem (MEM) were encapsulated within poly (lactic-co-glycolic acid) (PLGA) NPs [20]. Also, to target and eradicate *Pseudomonas aeruginosa* (*P. aeruginosa*) biofilms, the surface was conjugated to a *P. aeruginosa*-specific monoclonal antibodies via 1-(3-(dimethylamino)propyl)-3-ethyl carbodiimide hydrochloride/N-hydroxysuccinimide (NHS/EDC) chemistry. Results demonstrated a satisfactory antibiofilm effect when microbubbles were triggered by the US. And no changes in zeta potential and dynamic light scattering (DLS) analyses for microdroplets stored in PBS over a 21 days period indicated great stability under physiological conditions. Overall, MBs present a promising sonobactericidal approach to biofilm elimination, thereby fighting against infections and relieving inflammation.

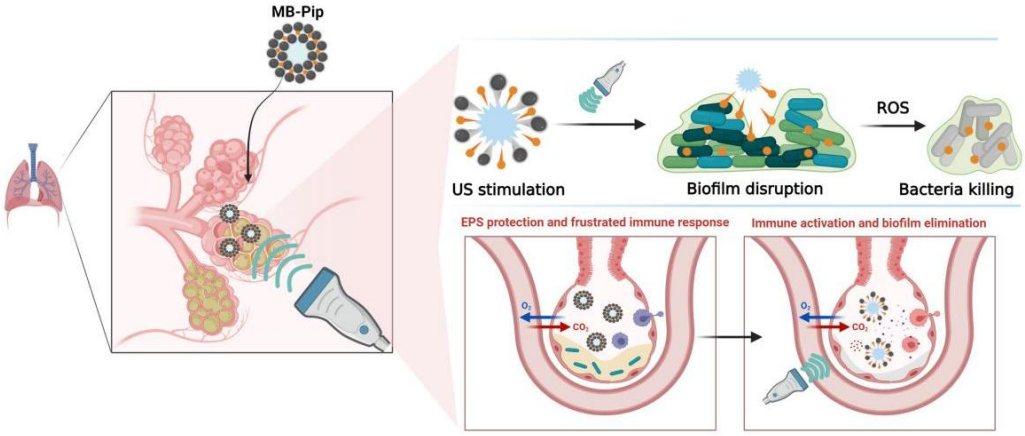


Figure 1 Schematic illustration of US-activated MBs for efficient treatment of chronic lung infections by promoting biofilm elimination and immune activation

Above all, US-activated microbubble therapy could be considered a promising method for bacterial infection. However, there are still many aspects that need to be studied and figured out. The formulations, size, composition, dose and pharmacokinetics of MBs, as well as ultrasound parameters, matter to effective and safe antibacterial behavior in different kinds of infectious diseases. For example, the balance between treatment effectiveness and BBBD safety has been a long-standing concern when it comes to the standardization of parameters for ultrasound and microbubbles [8]. MBs may induce leakage of the BBB vasculature and cause significant adverse effects like hemorrhages, sterile inflammation or neurotoxicity [21]. In summary, additional technical knowledge and fundamental research will be essential to allow the evolution of therapeutic US-activated MBs into a safe technique for antibacterial applications.

References

1. Wu J, Nyborg WL. Ultrasound, cavitation bubbles and their interaction with cells. *Adv Drug Delivery Rev* 2008;60(10):1103–1116. Available at: http://doi.org/10.1016/j.addr.2008.03.009
2. Tinkov S, Bekeredjian R, Winter G, Coester C. Microbubbles as ultrasound triggered drug carriers. *J Pharm Sci* 2009;98(6):1935–1961. Available at: http://doi.org/10.1002/jps.21571
3. Fournier L, de La Taille T, Chauvierre C. Microbubbles for human diagnosis and therapy. *Biomaterials* 2023;294:122025. Available at: http://doi.org/10.1016/j.biomaterials.2023.122025
4. Chowdhury SM, Abou-Elkacem L, Lee T, Dahl J, Lutz AM. Ultrasound and microbubble mediated therapeutic delivery: Underlying mechanisms and future outlook. *J Controlled Release* 2020;326:75–90. Available at: http://doi.org/10.1016/j.jconrel.2020.06.008
5. Horsley H, Owen J, Browning R, et al. Ultrasound-activated microbubbles as a novel intracellular drug delivery system for urinary tract infection. *J Controlled Release* 2019;301:166–175. Available at: http://doi.org/10.1016/j.jconrel.2019.03.017
6. Obermeier B, Daneman R, Ransohoff RM. Development, maintenance and disruption of the blood-brain barrier. *Nat Med* 2013;19(12):1584–1596. Available at: http://doi.org/10.1038/nm.3407
7. Wang J, Li Z, Pan M, et al. Ultrasound-mediated blood-brain barrier opening: An effective drug delivery system for theranostics of brain diseases. *Adv Drug Delivery Rev* 2022;190:114539. Available at: http://doi.org/10.1016/j.addr.2022.114539
8. Song KH, Harvey BK, Borden MA. State-of-the-art of microbubble-assisted blood-brain barrier disruption. *Theranostics* 2018;8(16):4393–4408. Available at: http://doi.org/10.7150/thno.26869
9. Xu Y, Ren Y, Zhu Y, et al. Preparation, characterization, and antibacterial activity of tigecycline-loaded, ultrasound-activated microbubbles. *Pharm Dev Technol* 2021;27(1):1–8. Available at: http://doi.org/10.1080/10837450.2021.2017967
10. Liu Y, Shi L, Su L, et al. Nanotechnology-based antimicrobials and delivery systems for biofilm-infection control. *Chem Soc Rev* 2019;48(2):428–446. Available at: http://doi.org/10.1039/C7CS00807D
11. Hu Y, Ruan X, Lv X, et al. Biofilm microenvironment-responsive nanoparticles for the treatment of bacterial infection. *Nano Today* 2022;46:101602. Available at: http://doi.org/10.1016/j.nantod.2022.101602
12. Lv X, Wang L, Mei A, et al. Recent Nanotechnologies to Overcome the Bacterial Biofilm Matrix Barriers. *Small* 2022;19(6):2206220. Available at: http://doi.org/10.1002/smll.202206220
13. Karygianni L, Ren Z, Koo H, Thurnheer T. Biofilm Matrixome: Extracellular Components in Structured Microbial Communities. *Trends Microbiol* 2020;28(8):668–681. Available at: http://doi.org/10.1016/j.tim.2020.03.016
14. Dong H, Xiu W, Wan L, et al. Biofilm microenvironment response nanoplatform synergistically degrades biofilm structure and relieves hypoxia for efficient sonodynamic therapy. *Chem Eng J* 2023;453:139839. Available at:

http://doi.org/10.1016/j.cej.2022.139839
15. Xiu W, Ren L, Xiao H, et al. Ultrasound-responsive catalytic microbubbles enhance biofilm elimination and immune activation to treat chronic lung infections. *Sci Adv* 2023;9(4):eade5446. Available at: http://doi.org/10.1126/sciadv.ade5446
16. Kouijzer JJP, Lattwein KR, Beekers I, et al. Vancomycin-decorated microbubbles as a theranostic agent for Staphylococcus aureus biofilms. *Int J Pharm* 2021;609:121154. Available at: http://doi.org/10.1016/j.ijpharm.2021.121154
17. Phillips LC, Puett C, Sheeran PS, Dayton PA, Wilson Miller G, Matsunaga TO. Phase-shift perfluorocarbon agents enhance high intensity focused ultrasound thermal delivery with reduced near-field heating. *J Acoust Soc Am* 2013;134(2):1473–1482. Available at: http://doi.org/10.1121/1.4812866
18. Fernandes DA, Fernandes DD, Li Y, et al. Synthesis of Stable Multifunctional Perfluorocarbon Nanoemulsions for Cancer Therapy and Imaging. *Langmuir* 2016;32(42):10870–10880. Available at: http://doi.org/10.1021/acs.langmuir.6b01867
19. Sridharan A, Eisenbrey JR, Forsberg F, Lorenz N, Steffgen L, Ntoulia A. Ultrasound contrast agents: microbubbles made simple for the pediatric radiologist. *Pediatr Radiol* 2021;51(12):2117–2127. Available at: http://doi.org/10.1007/s00247-021-05080-1
20. Xin L, Zhang C, Chen J, et al. Ultrasound-Activatable Phase-Shift Nanoparticle as a Targeting Antibacterial Agent for Efficient Eradication of Pseudomonas aeruginosa Biofilms. *ACS Appl Mater Interfaces* 2022;14(42):47420–47431. Available at: http://doi.org/10.1021/acsami.2c13166
21. Deprez J, Lajoinie G, Engelen Y, De Smedt SC, Lentacker I. Opening doors with ultrasound and microbubbles: Beating biological barriers to promote drug delivery. *Adv Drug Delivery Rev* 2021;172:9–36. Available at: http://doi.org/10.1016/j.addr.2021.02.015

Author contribution

Xiaoye Li wrote the draft and drew the figure. Dongliang Yang collected relevant information. Weijun Xiu and Heng Dong edited the text and finalized 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 financially supported by "3456" Cultivation Program for Junior Talents of Nanjing Stomatological Hospital, Medical School of Nanjing University (No. 0222R212), Natural Science Foundation of Jiangsu Province (No. BK20200710).

Abbreviations

MBs, microbubbles; US, ultrasound; CNS, central nervous system; UTI, urinary tract infection; BBB, blood-brain barrier; NVU, neurovascular unit; BBBD, blood-brain-barrier disruption; EPS, extracellular polymeric substances; NPs, nanoparticles; MB-Pip, microbubbles composed of Fe_3O_4 nanoparticles loading piperacillin; •OH, hydroxyl radicals; PFP, perfluoropentane; PFC, perfluorocarbon; PLGA, poly(lactic-co-glycolic acid); EDC, 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride; NHS, N-hydroxysuccinimide; DLS, dynamic light scattering.

Citation

Li XY, Xiu WJ, Yang DL, Dong H. Ultrasound-responsive microbubbles in antibacterial therapy. *Biomed Eng Commun*. 2023;2(2):7. doi: 10.53388/BMEC2023007.

Executive editor: Na Liu.

Received: 21 April 2023, **Accepted:** 25 April 2023, **Available online:** 28 April 2023.

© 2023 By Author(s). Published by TMR Publishing Group Limited. This is an open access article under the CC-BY license. (https://creativecommons.org/licenses/by/4.0/).