Bacteria in biofilms sonoporated by oscillating microbubbles

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Background, Motivation and Objective

Treatment of bacterial infections is becoming progressively more challenging. Biofilm formation, a resistance mechanism where bacteria encase themselves in a protective matrix, hinders antibiotic effectiveness up to 1000-fold [Ito et al, Appl Environ Microbiol, 2009]. Ultrasound (US) induced microbubble (MB) oscillation can enhance drug uptake pathways in mammalian cells, such as by increased cellular permeability, i.e. sonoporation which can lead to cell death [Kooiman et al, Adv Drug Del Rev, 2014]. Therefore, we propose to harness these MB oscillations to sonoporate biofilm-associated bacteria and assess real-time responses.

Statement of Contribution/Methods

Staphylococcus aureus biofilms were grown for 24 h in OptiCells. MBs had a DSPC-based coating and C_4F_{10} gas core. Fluorophores SYTO 9 and propidium iodide (PI) were used to stain living and membrane-compromised bacteria, respectively. Biofilms were exposed to US (2 MHz, 50 kPa, 15x 1000 cycles) with either an antibiotic (oxacillin), MBs alone, or both antibiotic and MBs. Fluorescence microscopy (Fig. 1A) was used to visualize bacterial responses 2 min after US exposure and the ultra-high-speed Brandaris 128 camera for microbubble oscillation. Post-hoc image analysis for particle counting was performed in MATLAB to quantify sonoporation.

Results/Discussion

After US, antibiotic alone showed no enhancement of PI uptake (Fig. 1B). Directly following insonification, MBs alone resulted in ~2.4-fold increase of membrane-compromised bacteria than before US. After 2 min, the amount of sonoporated bacteria further increased by 44%. PI uptake in endothelial cells has been shown to increase over 3 min before stabilization [van Rooij et al, J Control Release, 2016]. When MBs and antibiotic were combined, minimal sonoporation was observed after insonification. Bacteria respond to antibiotics by ramping up their defense mechanisms, such as entering a dormant metabolic state or increasing exoprotein and toxin production [Hodille et al, Clin Microbiol Rev, 2017]. Changes in the bacterial defense responses alter the susceptibility to sonoporation. These findings demonstrate the potential of using MBs and US to aid the treatment of bacterial infections.

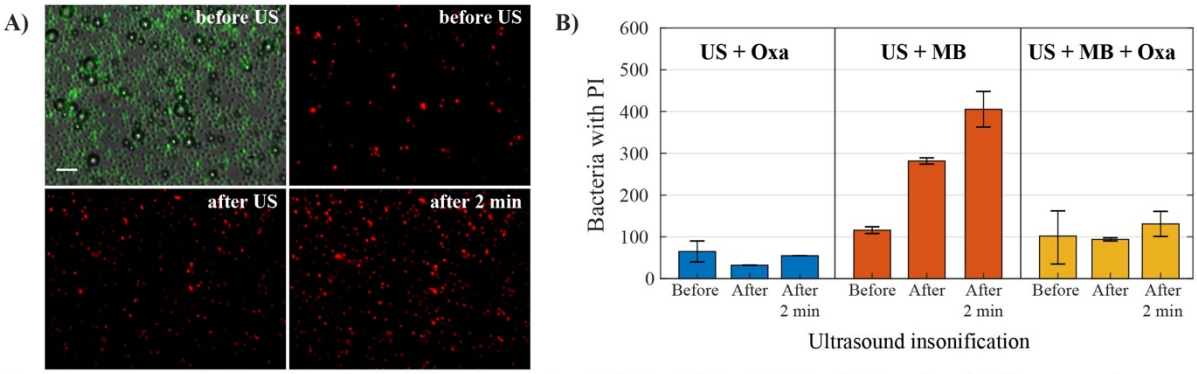

Figure 1. Effect of ultrasound (US) insonification of microbubbles (MB) at 50kPa, 1000 cycles. A) Microscopy images of a biofilm with MB alone before and after US; top left composite image showing MB and living (green, SYTO 9) bacteria. The remaining images are of membrane-compromised (red, propidium iodide (PI)) bacteria. Scale bar 5 µm. **B)** Amount of membrane-compromised bacteria stained with PI. Oxa = oxacillin.