Temporal stability of therapeutic microbubbles

Antonios N. Pouliopoulos[1], Daniella A. Jimenez[1], Alexander Frank[1], Alexander Robertson[1], Lin Zhang[1], Alina R. Kline Schoder[1], Vividha Bhaskar[1], Mitra Harpale[1], Elizabeth Caso[1], and Elisa E. Konofagou[1,2], [1]Department of Biomedical Engineering, [2]Department of Radiology, Columbia University, NY, USA

Background, Motivation and Objective

Non-invasive blood-brain barrier (BBB) opening using focused ultrasound (FUS) requires intravenous injection of pre-formed microbubbles. Although microbubble behavior during exposure to imaging sequences has been studied extensively, microbubble stability within a therapeutic field remains relatively unexplored. Here, we studied the temporal stability of microbubbles during therapeutic FUS exposure over two timescales: the short time scale (i.e., μs of low-frequency ultrasound exposure) and the long time scale (i.e., days post-activation). Our objective was to test whether in-house lipid-shelled microbubbles maintained their capacity to produce similar BBB opening for a period of up to 3 weeks following activation.

Statement of Contribution/Methods

In-house manufactured lipid-shelled microbubbles (DSPC:DSPE-PEG2000 molar ratio 9:1, C_4F_{10} gas core) were first characterized in terms of their size and concentration using optical microscopy. They were then channeled to flow through a 4-mm vessel within a tissue-mimicking phantom (5% gelatin) and were exposed to therapeutic pulses (fc: 0.5 MHz, peak-negative pressure: 300 kPa, pulse length: 1 ms, pulse repetition frequency: 1 Hz, n=10). We recorded and analyzed the microbubble acoustic emissions with concentration-matched samples (10^7 microbubbles/ml) on day 0, 7, 14, and 21 after activation. *In vivo* experiments were conducted in mice (n=3) using the same parameters and at the same time-points in order to examine the therapeutic efficacy of microbubbles in BBB opening over time.

Results/Discussion

Microbubbles had a concentration decay constant of 0.02 d^{-1} but maintained a stable size distribution for up to 3 weeks (< 10% variation). Temporal stability decreased while inertial cavitation increased over time both in vitro and in vivo, possibly due to changes in the lipid shell. BBB opening volume in mice (n=3) measured through T_1-weighted contrast-enhanced MRI was equal to 19.1 ± 7.1 mm^3, 21.8 ± 14 mm^3, 29.3 ± 2.5 mm^3, and 38 ± 20.1 mm^3 on day 0, 7, 14, and 21, respectively, showing no significant difference over the long time scale (p-value: 0.49). In conclusion, microbubbles maintain their capacity to produce similar therapeutic effects over a period of 3 weeks after activation, as long as the natural concentration decay is accounted for.

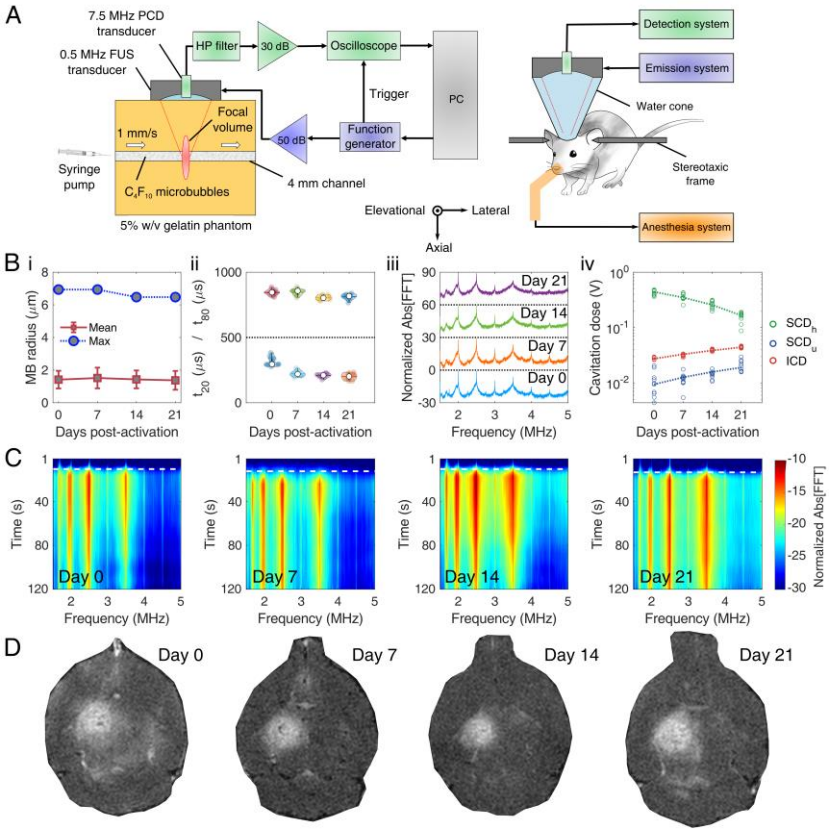

Figure: A) *In vitro* and *in vivo* experimental setup. B) *In vitro* microbubble stability. i) Mean and maximum microbubble size evolution. ii) Microbubble lifetime over time, expressed as the time required for 20% or 80% of the total energy to be emitted (i.e., t20 and t80). iii) Normalized spectra over time. iv) Cavitation doses evolution. SCDh: harmonic stable cavitation dose, SCDu: ultraharmonic cavitation dose, ICD: inertial cavitation dose. C) *In vivo* microbubble stability. Spectrogram evolution, showing increase of broadband emissions with storage time. D) BBB opening evolution over time.