Letters

Influence of Microbubble Size on Postexcitation Collapse Thresholds for Single Ultrasound Contrast Agents Using Double Passive Cavitation Detection

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***Abstract*—For the first time, and using an acoustical method, it has been shown experimentally that the inertial cavitation threshold pressure of an albumin-shelled microbubble is significantly correlated with its initial size.**

I. Introduction

The primary clinical use of ultrasound contrast agents (UCAs) is their image enhancement capability for ultrasound imaging. More recently, the therapeutic applications of UCAs are being pursued. These therapeutic applications require a greater understanding of whether the UCA collapses, given that some applications require UCA collapse and others do not. Thus, this study's aim is to assess experimentally the role of UCA initial size on the inertial cavitation threshold pressure, a necessary step for further understanding of theoretical models of bubble dynamics and bubble collapse.

II. Materials and Methods

Four populations of UCAs were made based on the protocol by Borrelli *et al.* [1]. Briefly, the UCAs were produced from aqueous solutions of bovine serum albumin (BSA; Sigma-Aldrich Co., St. Louis, MO) and dextrose (Fisher Chemical, Fair Lawn, NJ) that were saturated with perfluorobutane gas (FluoroMed LP, Round Rock, TX). The solutions were sonicated with a 20-kHz Fisher 500 sonic dismembrator (ThermoFisher Scientific, Waltham, MA) using a 1.1-cm-diameter sonic horn.

Microscopic images (Olympus BX51, Tokyo, Japan) of the tested bubbles were acquired for each set of experiments and analyzed using a circle detection routine based on the Hough transform to evaluate the size distribution of the population.

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The double passive cavitation detection (DPCD) experiments involved a 4.6-MHz transmit transducer and 13.8- and 14.6-MHz receive transducers. Three-cycle tone bursts with a pulse repetition frequency of 10 Hz at the transmit center frequency were generated using a pulse-receive system (RAM-5000, RITEC Inc., Warwick, RI). Several thousand signals [8 series of 500 signals for each tested incident peak rarefactional pressure amplitude (PRPA)] were acquired. The complete setup including the transmit PRPA calibration procedures has been fully described elsewhere [2]. The DPCD analysis of collapsed UCAs involved categorizing the UCAs according to a classification scheme based on the presence or absence of postexcitation signals (PESs). PES is defined as a secondary, short, broadband response typically occurring between 1 and 5 μs after the principle response. In this study, the measurement criterion was based on the 5% and 50% postexcitation thresholds. This parameter is defined as the level at which a certain percentage of the total population of microbubbles transiently collapsed with PES for an applied PRPA. It is determined as the ratio between the total number of signals exhibiting PES and the total number of signals associated with the presence of a single bubble in the confocal region. Postexcitation curves were then obtained using a modified logistic regression using Matlab (The MathWorks Inc., Natick, MA) to fit the experimental data, allowing the evaluation of the 5% and the 50% postexcitation thresholds and their 95% confidence intervals [2].

An analysis of variance (ANOVA) was performed between the four UCA populations for the two parameters: the initial radius and the postexcitation threshold. For the collapse thresholds, the ANOVA was based on comparing the weighted means of the PRPA associated with the PES-determined UCA collapse for the four groups. Results were significant for p-value < 0.05. In addition to the ANOVA, we graphically compared the 95% confidence intervals of the four groups for the parameters initial radius and 5% and 50% postexcitation threshold. For the size parameter, the 95% confidence interval was based on the standard deviation for the UCA radius of the selected group. For the postexcitation parameters, the 95% confidence intervals were directly provided through the fitting of the logistic regression curve for the four groups. Results were significant when the confidence intervals did not overlap.

Previous experimental studies [3] compared PES-determined UCA collapse data for lipid-shell UCA with the Marmottant model [4] for large amplitude UCA behavior: good agreement between experimental measurements and simulated curves using the Marmottant equation was obtained using the criterion of maximum radial expansion to indicate the onset of postexcitation collapse. Simulations

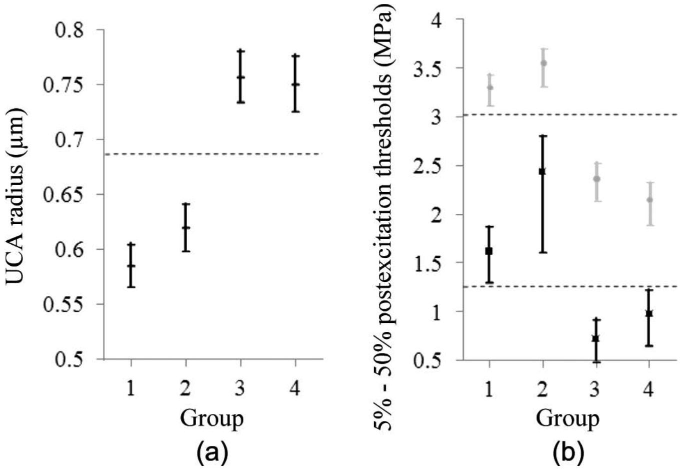

Fig. 1. (a) Mean radii and their 95% confidence intervals. (b) Mean 5% (black) and 50% (gray) collapse thresholds and their 95% confidence intervals. The absence of overlapping between the groups is represented by the dashed lines.

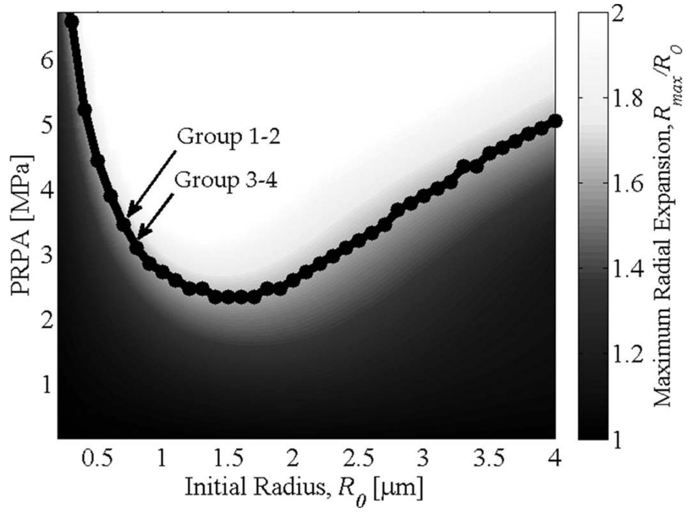

Fig. 2. Marmottant model simulations at 4.6 MHz showing maximum radial expansion, a predictor of postexcitation signal, as a function of R_0 and peak rarefactional pressure amplitude (PRPA).

indicated that the UCA size may have an effect on postexcitation curves. Thus, simulations using the Marmottant equation for a 3-cycle 4.6-MHz pulse were performed by adapting the parameters to Optison-like microbubbles with an albumin shell [5].

III. Results

The null hypothesis was rejected for the weighted mean size: there was at least one weighted mean size of the four UCA populations that was significantly different from the others. The 95% confidence interval graphical evaluation provided a better understanding of the behavior of the four tested groups: groups 1 and 2 exhibited similar radii, as did groups 3 and 4 [Fig. 1(a)].

The null hypothesis was rejected for the percentage postexcitation thresholds: there was at least one group where the postexcitation threshold was significantly different from the others. The graphical evaluation of the 95% confidence interval [Fig. 1(b)] showed the same significantly different two subgroups as for the initial size: groups 1 and 2 have similar postexcitation thresholds, as did groups 3 and 4.

Table I displays the measured and the predicted values of the 50% postexcitation thresholds of the two statistically determined subgroups.

The simulation using the Marmottant equation predicted the broad trend in the response of UCA; groups 3 and 4 were predicted to have lower 50% thresholds than groups 1 and 2 (Fig. 2).

IV. Discussion and Conclusion

The four UCA populations could be divided into two distinct and statistically significant subgroups based on their initial size. The 5% and 50% postexcitation thresholds showed the same statistically significant split between the four groups. These two subgroups were thus significantly different in size as well as postexcitation threshold.

In addition, the global trend of the UCAs was predicted by the Marmottant equation. The PRPA versus radius trend noted in Fig. 2 for the two subgroups was consistent with the experimental findings; however, quantitative agreement was not as good. One reason may be that the Marmottant-based simulations were performed for Optison-like microbubbles that may exhibit differences in shell properties with microbubbles made for this study. Therefore, additional work is likely needed to optimize the model so that it will better predict the postexcitation threshold values.

TABLE I. Measured and Predicted 50% Collapse Thresholds for the Two Subgroups.

Group	Mean radius [µm] + 95% confidence interval	Measured 50% postexcitation threshold [MPa] + 95% confidence interval	Predicted 50% postexcitation threshold [MPa]
1–2	0.63 [0.62–0.64]	3.29 [3.13–3.41]	3.81
3–4	0.76 [0.75–0.77]	2.22 [2.05–2.37]	3.32

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