Acoustic Characterization of the CLINIcell for Ultrasound Contrast Agent Studies

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***Abstract*— Ultrasound contrast agents consist of gas-filled coated microbubbles that oscillate upon ultrasound insonification. Their characteristic oscillatory response provides contrast enhancement for imaging and has the potential to locally enhance drug delivery. Since microbubble response depends on the local acoustic pressure, an ultrasound compatible chamber is needed to study their behavior and the underlying drug delivery pathways. In this study, we determined the amplitude of the acoustic pressure in the CLINIcell, an optically transparent chamber suitable for cell culture. The pressure field was characterized based on microbubble response recorded using the Brandaris 128 ultrahigh-speed camera and an iterative processing method. The results were compared to a control experiment performed in an OptiCell, which is conventionally used in microbubble studies. Microbubbles in the CLINIcell responded in a controlled manner, comparable to those in the OptiCell. For frequencies from 1 to 4 MHz, the mean pressure amplitude was −5.4 dB with respect to the externally applied field. The predictable ultrasound pressure demonstrates the potential of the CLINIcell as an optical, ultrasound, and cell culture compatible device to study microbubble oscillation behavior and ultrasound-mediated drug delivery.**

***Index Terms*— Acoustic characterization, drug delivery, ultrahigh-speed imaging, ultrasound contrast agents (UCAs).**

I. Introduction

ULTRASOUND contrast agents (UCAs) are comprised of gas-filled coated microbubbles (1–10 μm in diameter). Upon ultrasound insonification, the microbubbles compress and expand due to the acoustic pressure. This oscillatory behavior is the characteristic microbubble response used in the clinic for contrast enhancement of diagnostic ultrasound imaging. More recently, their potential to locally enhance vascular drug delivery has been demonstrated [1], [2]. Microbubble oscillation upon ultrasound insonification generates local mechanical stress that can increase endothelial cell membrane permeability by pore formation (i.e., sonoporation), opening of cell–cell junctions, and stimulation of endocytosis [1]. However, the underlying mechanisms of these pathways remain unknown.

A better understanding of the microbubble's oscillation behavior can aid the design of UCAs for diagnostic ultrasound imaging. In addition, we need to better understand the microbubble–cell interaction to control different pathways of ultrasound-mediated drug delivery. Microbubble oscillation strongly depends on ultrasound insonification, composition of shell and gas core, targeting ligands, microbubble size, production method, and their local environment [1]. Therefore, we require a chamber in which microbubble behavior can be characterized experimentally, with or without cellular interaction, which is both ultrasound compatible and optically transparent. If we quantitatively know how the ultrasound pressure field transmits into the chamber, the microbubble can be insonified in a controlled manner. Optical transparency is essential to image microbubble behavior and cellular response with a microscope. Moreover, the chamber should be suitable for cell culture to study ultrasound-mediated drug delivery *in vitro*.

The OptiCell (Nunc, Thermo Fisher Scientific, Wiesbaden, Germany) has been extensively used for both microbubble characterization and drug delivery studies [3]. Since the OptiCell is no longer being manufactured, the CLINIcell (MABIO, Tourcoing, France) is an interesting alternative imaging and cell culture chamber. It has recently been used to characterize microbubbles [4], [5] and to study ultrasound-mediated drug delivery [6]. However, a thorough acoustic characterization has not been performed yet.

In this study, we therefore quantitatively assessed microbubble oscillation in the CLINIcell by performing microbubble spectroscopy using the Brandaris 128 ultrahigh-speed camera. The recordings were iteratively processed using our previously reported method [7].

II. Material and Methods

The CLINIcell (50 μm membrane, 25 cm^2 area) is a cell culture chamber consisting of two parallel gas permeable polycarbonate membranes, with a separation of 5 mm (Fig. 1).

To characterize the acoustic pressure transmitted into the CLINIcell *in situ* and noninvasively, microbubbles were used as pressure sensors, as previously described by us [7]. The CLINIcell was blocked prior to the experiment with 12 mL of 2% bovine serum albumin in phosphate-buffered saline (PBS) for 1 h, to prevent unspecific microbubble binding to the membranes. Next, it was rinsed three times with PBS before introducing 12 mL of 10^5 microbubbles/mL. We replicated the experimental setup, ultrasound insonification parameters, microbubble preparation, and experimental data analysis. Briefly, the 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC)-based microbubbles were insonified at 45° incidence angle with a single-element transducer (1-9 MHz bandwidth, 25-mm focal distance, −6 dB beamwidth at 1 MHz

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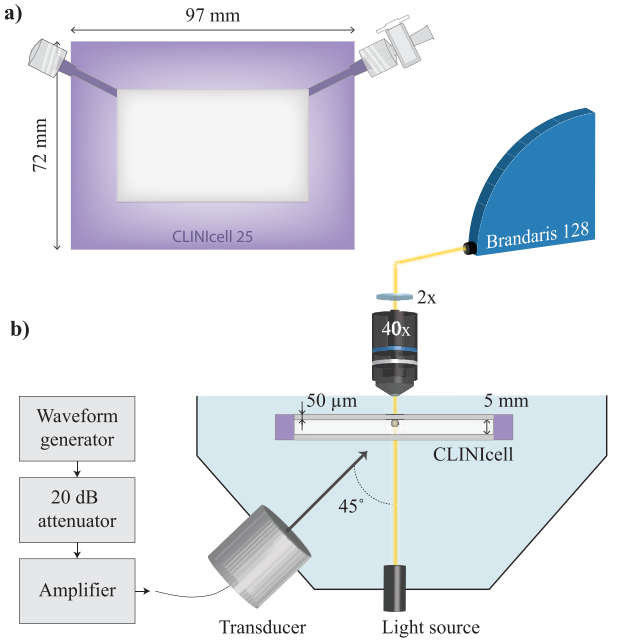

Fig. 1. (a) Sketch of a CLINIcell with a 25 cm^2 cell culture area. (b) Schematic representation (not drawn to scale) of the experimental setup to study microbubble oscillation upon insonification using the Brandaris 128 ultrahigh-speed camera. The two parallel membranes of the CLINIcell each have a thickness of 50 μm. The microscope was equipped with a 40× water immersion objective and a 2× lens (Olympus, Tokyo, Japan). The insonification scheme was generated by a waveform generator (Tabor 8026, Tabor Electronics, Tel Hanan, Israel), connected to a 20-dB attenuator (Mini-Circuits, Brooklyn, NY, USA) and a broadband amplifier (ENI A-500, Electronics & Innovation, Rochester, NY, USA).

of 1.3 mm, PA275, Precision Acoustics, Dorchester, U.K.). Microbubble spectroscopy [8] was performed by successively insonifying each individual microbubble while sweeping through a range of transmit frequencies (f_T) from 1 to 4 MHz (in steps of 300 kHz). An 8-cycle sine wave burst was applied at 20-kPa external peak negative pressure (PNP), as calibrated in a separate experiment using a 1-mm needle hydrophone. The microbubble oscillation behavior was recorded using the Brandaris 128 ultrahigh-speed camera [9] (17 million frames/s) combined with a microscope (80× magnification, BX-FM, Olympus, Tokyo, Japan), as depicted in Fig. 1(b).

Using custom-designed image analysis software, microbubble oscillation was quantified as the change in radius over time $R(t)$, as previously described [8]. Briefly, the relative excursion was defined as $x(t) = R(t)/R_0 - 1$, with resting radius R_0. The amplitude (x_0) of the relative excursion was defined as the maximum after bandpass filtering $x(t)$ (third-order Butterworth filter, 500-kHz bandwidth, centered at f_T). Next, we determined the acoustic pressure amplitude (P) experienced by the microbubbles by fitting x_0 in an iterative manner [7] to the harmonic oscillator model

$$x_0 = \frac{|P|/\left(4\pi^2 \rho R_0^2\right)}{\sqrt{\left(f_0^2 - f_T^2\right)^2 + (\delta f_T f_0)^2}} \tag{1}$$

with $\rho = 10^3$ kg/m^3 being the density of water. The eigenfrequency (f_0) of the microbubble is given as

$$f_0 = \frac{1}{2\pi}\sqrt{\frac{1}{\rho R_0^2}\left[3\gamma\, P_0 + \frac{2(3\gamma - 1)\sigma_w}{R_0} + \frac{4\chi}{R_0}\right]} \tag{2}$$

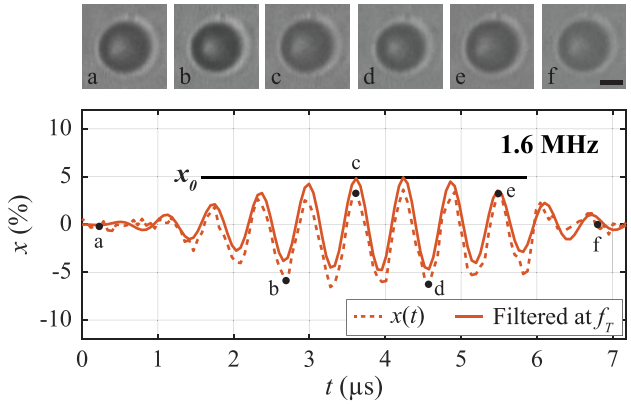

Fig. 2. Selected frames of a Brandaris 128 ultrahigh-speed recording of a single microbubble (R_0 = 2.4 μm) in the CLINIcell, insonified at f_T = 1.6 MHz (2 μm scale bar). Dashed line: microbubble's relative excursion x. Black dots: time points of the selected frames. Red solid line: x bandpass filtered around f_T and its maximum, defined as the relative excursion amplitude x_0 (see Fig. 3).

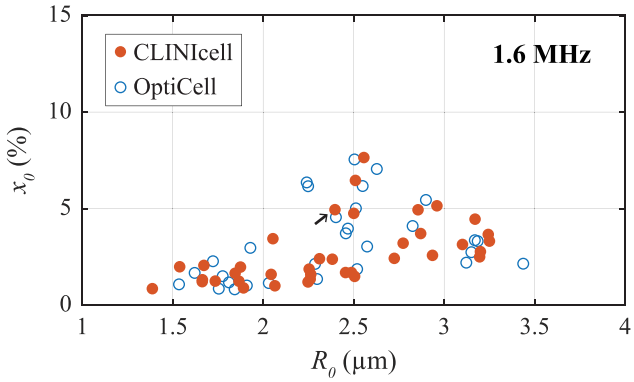

Fig. 3. Relative excursion amplitude (x_0) of single microbubbles in the CLINIcell (n = 38, red solid line) and OptiCell (n = 30, blue open line) as a function of resting radius (R_0) when insonified at f_T = 1.6 MHz. The solid data point indicated by the arrow is shown in Fig. 2.

with $\gamma = 1.07$ being the ratio of specific heats for C_4F_{10}, $P_0 = 10^5$ Pa being the ambient pressure, $\sigma_w = 0.072$ N/m being the surface tension in water, and χ being the microbubble shell elasticity [8]. The damping coefficient (δ) is given as

$$\delta = \frac{\omega_0 R_0}{c} + 2 \cdot \frac{4\mu}{R_0^2 \rho \omega_0} + \frac{4\kappa_s}{R_0^3 \rho \omega_0} \tag{3}$$

with c = 1500 m/s being the speed of sound in water, $\mu = 10^{-3}$ Pa · s being the viscosity of water, and κ_s being the microbubble shell viscosity [10]. Therefore, with the iterative scheme not only the pressure experienced by the microbubbles in the CLINIcell can be determined but also the microbubble shell elasticity and viscosity since they are incorporated in f_0 and δ. The results were compared to those of experiments previously performed in an OptiCell, using an identical experimental setup [10]. All analyses were performed using MATLAB (The MathWorks, Natick, MA, USA).

III. Results

Single microbubbles with resting radius (R_0) from 1.4 to 3.3 μm were recorded upon insonification in the CLINIcell (n = 38). Fig. 2 shows a typical example of recorded frames and illustrates how the relative excursion amplitude (x_0) was determined. This example also demonstrates that bandpass filtering $x(t)$ removes the low-frequency component of compression-only behavior [11], such that

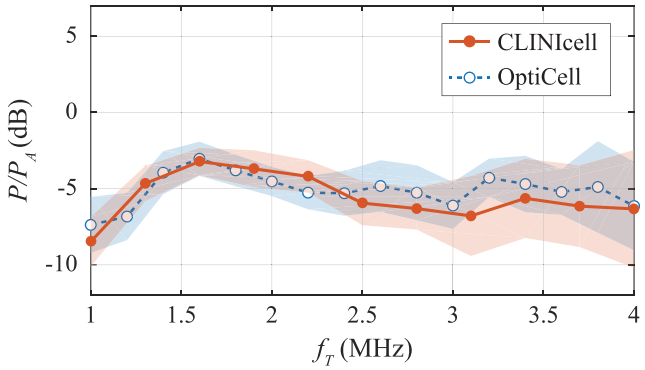

Fig. 4. Normalized median pressure amplitude derived from microbubble oscillations in the CLINIcell (red solid line) and OptiCell (blue open/dashed line). Shaded area: interquartile range.

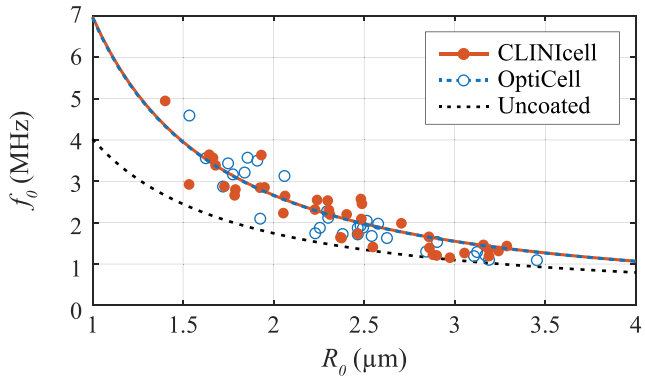

Fig. 5. Eigenfrequency (f_0) of microbubbles in the CLINIcell (red solid line) and OptiCell (blue open/dashed line) and the corresponding fit to obtain the shell elasticity. The f_0 of an uncoated microbubble is given by $\chi = 0$ N/m (black dashed line).

the fundamental amplitude is obtained. Fig. 3 shows the relative excursion amplitude (x_0) as a function of R_0 for every microbubble when insonified at $f_T = 1.6$ MHz. The microbubble response was similar in both the OptiCell and CLINIcell, with comparable excursion amplitudes and characteristic resonance behavior for $R_0 = 2.5$ μm at 1.6 MHz. The pressure experienced by the microbubble (P) was obtained by iteratively fitting x_0 to the harmonic oscillator model for 30 iterations. Fig. 4 shows the frequency dependence of P normalized to the applied external pressure (P_A = 20-kPa PNP). The mean normalized transmitted pressure amplitude from 1 to 4 MHz was similar in both systems: -5.4 dB in the CLINIcell and -5.1 dB in the OptiCell. Moreover, the transmitted pressure showed no clear frequency dependence.

The median shell elasticity (with interquartile range between brackets) obtained by fitting the eigenfrequency f_0 (Fig. 5) to (2) was similar, with $\chi = 0.32$ (0.27) N/m in the CLINIcell and $\chi = 0.32$ (0.25) N/m in the OptiCell. The median shell viscosity obtained from (3) was also similar in both systems, $\kappa_s = 1.1$ (0.5) $\cdot 10^8$ kg/s in the CLINIcell and $\kappa_s = 0.7$ (0.4) $\cdot 10^8$ kg/s in the OptiCell.

IV. Discussion

The acoustic pressure in the CLINIcell was successfully evaluated using microbubbles as noninvasive pressure sensors, confirming the applicability of our iterative processing method [7]. The pressure amplitude transmitted into the CLINIcell was similar to that of the OptiCell and no clear frequency dependence was observed. The microbubbles oscillated in a controlled manner and their shell parameters remained unchanged. We expect an unaltered focal region of the transmitted ultrasound field, since the CLINIcell has two parallel membranes similar to the OptiCell, in which the focal region remains unchanged [7]. We chose to characterize a CLINIcell with a 50-μm membrane since it is most comparable to the 75-μm membrane of the conventional OptiCell. Also, we assumed that the ultrasound propagation would be affected more by the CLINIcell design with a thicker membrane (125 μm). The surface area available in the CLINIcell (25 cm^2) for cell culture is smaller than in the OptiCell (50 cm^2) while the filling volume remains similar (12 mL versus 10 mL). Therefore, microbubble concentration and cell culture protocols might have to be adjusted.

V. Conclusion

The pressure amplitude inside the CLINIcell was -5.4 dB on average with respect to that of the externally applied pressure, similar to the OptiCell. Controlled microbubble behavior was achieved in the CLINIcell and the applied pressure can be corrected for the now quantified pressure drop. This demonstrates the potential of the CLINIcell as an alternative chamber to characterize microbubble behavior and to study ultrasound-mediated drug delivery in an optical, ultrasound, and biocompatible chamber.

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