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MECHANICAL RESPONSE OF A MEGAKARYOCYTE ACOUSTICALLY COUPLED WITH A TETHERED MICROBUBBLE

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ABSTRACT

In a context of ultrasound therapies, the mechanical energy of acoustically-driven microbubbles can be used to facilitate the uptake of molecules or genes into biological cells. If not controlled, their possibly stochastic and violent mechanical behavior can be responsible for the rupture of the cell membrane and hence for cell lysis. Therefore, controlling stably oscillating microbubbles close to biological materials, with the view of reducing the negative effects and enhancing the therapeutic action, can be challenging. We propose thence an experimental study to investigate the dynamics of a wall-attached bubble in the vicinity of a biological cell and their mechanical interaction, at the microscale level.

1. INTRODUCTION

It is widely accepted that ultrasound contrast agents used as acoustic resonators help in enhancing the delivery of genes and drugs into biological cells. Their mechanical energy allows then to permeabilize cell membranes, through a process called sonoporation. Because of its stochastic and rapidly-evolving behavior, the displacement of thousands of oscillating microbubbles interacting with cells is a phenomenon hard to capture and predict. This is why several experimental studies investigate sonoporation either post-ultrasound exposure especially by flow cytometry measurement [1] or during ultrasound exposure by patch-clamp techniques that allow the monitoring of abnormal changes in ions Ca^{2+} [2]. In such works where the microbubbles are clearly identified as main actor in the sonoporation, their time-resolved dynamics is left to the side, while it might actually play an important role. Two distinct mechanisms caused by the bubble oscillation may be responsible for sonoporation. First, the direct deformation of the cell by the oscillating bubble, occurring at the acoustic time scale, may create pores in the membrane, when sufficiently stretched [3]. Second, due to a nonlinear response of the bubble interface at high pressure, the surrounding fluid may be subject to microstreaming, occurring at a slower time scale, which is likely to generate shear stresses on the cell membrane [4].

The present paper investigates the mechanical response of a biological cell, at the acoustic time scale, to the dynamics of a nearby oscillating wall-attached microbubble that triggers a nonspherical sectoral mode of degree $n = 4$. The cell response is evidenced by the fluctuating displacement of the close-to-bubble (CTB) cell's point with respect to the displacement of the close-to-cell (CTC) bubble's point, as defined in Fig. 2.

2. METHODOLOGY

2.1 Experimental setup.

Our experimental setup represented in Fig. 1 consists of a tank made of polymethyl methacrylate, filled with culture medium (DMEM) and disposed under a Nikon Eclipse-Ti microscope with a 20x magnification. At the bottom tank are positioned a couple of one microbubble and one biological cell, which are respectively sizing in the range [30 80] µm and [30 60] µm. The bubble results from a reduction reaction, occurring at the cathode of an electrolysis actuator, while the cell is a human megakaryocyte issued from MEG-01 cell lines (ATCC, CRL-2021™). The bubble is driven by an 85 kHz acoustic field modulated by a slowly varying triangle shape envelope of modulation frequency $f_m = 25$ Hz, which is generated by a signal generator (Agilent 33210A) and transmitted by a Langevin transducer (Reson, 30 kHz nominal frequency) located at one edge of the tank and coupled with ultrasound transmission gel (Aquasonic, Fisher ThermoScientific). The bubble interface dynamics is observed by transmission imaging, using a high-speed camera (177 kfps) Phantom V12.1 Vision Research. At that rate, the frames are limited to a resolution of 128 x 136 pixels, where one pixel corresponds to 1 µm.

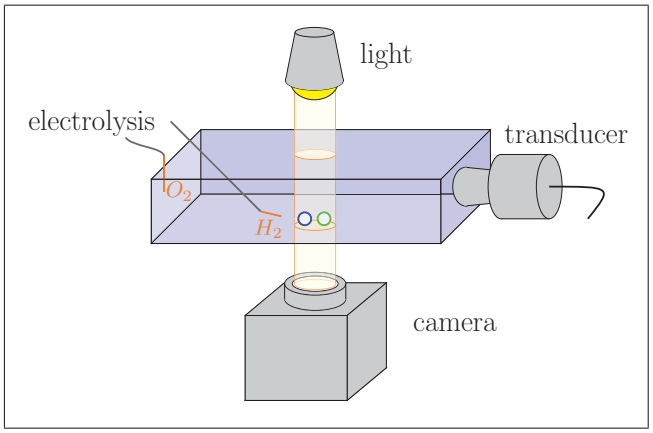

Figure 1. Schematics of the experimental setup.

2.2 Image processing.

With the aim to correlate the modal deformations of a bubble with the biological cell's response, a two-stages image process is performed. First, the modal amplitudes of the bubble are assessed by using a spatiotemporal Fourier analysis of the top-viewed bubble contour, as described in a previous work [5]. This results in modal decomposition such are the blue and black curves in Fig. 3. Secondly, from the bubble and cell contours are extracted two points: The CTC bubble's point and the CTB cell's point. Respectively, they correspond to the point of the bubble (cell) contour that belongs to the center-to-center axis, as illustrated in Fig. 2. This means that the CTC bubble's point does not necessarily correspond to an antinode of the bubble shape deformation, even if experimentally, that's what is aimed.

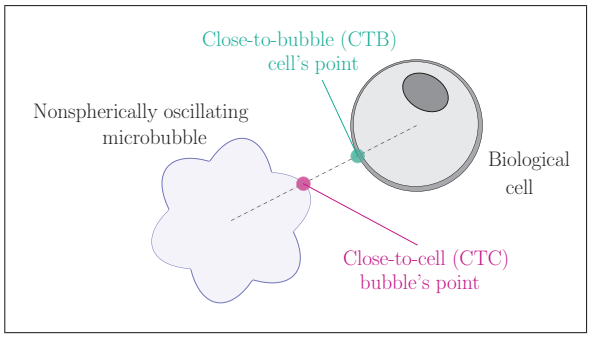

Figure 2. Example of a top-viewed bubble-cell disposition and locations on bubble and cell contours chosen as mechanical indexes: close-to-cell (CTC) bubble's point in purple and close-to-bubble (CTB) cell's point in green.

2.3 Cell culture.

The human megakaryocytes employed in this study are issued from MEG-01 cell lines (CRL-2021, American Type Culture Collection, Manassas, Virginia). They were chosen because of their nature and their likeliness to be targeted for sonoporation in clinical application, and because of their very large size. They were grown in $15\,\mathrm{mL}$ Gibco Dulbecco modified Eagle medium (DMEM) suplemented with 10% foetal calf serum (FCS) and 1% L-glutamin, then incubated at $37°\mathrm{C}$ and 5% CO_2.

3. CELL RESPONSE TO AN OSCILLATING MICROBUBBLE

When immersed in DMEM and excited at $85\,\mathrm{kHz}$ at sufficient acoustic pressure, a microbubble sizing in the range [30 80] $\mu\mathrm{m}$ preferentially displays modal shape deformations of degree $n = 3$ and $n = 4$. The onset of shape instabilities, which lead to nonspherical modes, occurs when a critical acoustic pressure is reached. This is why a slowly varying acoustic pressure field is employed, in order to explore different bubble modal configurations and associated cell deformations at quasi-static acoustic pressure. In this study, we focus on the particular case of a bubble triggering sectoral modes, since they emerge easily when the bubble radius is around the resonant radius [5]. Since both cell and bubble are laying at the tank bottom, the interest of using similarly sizing cells and bubbles is to ensure that the maximal bubble deformations will occur nearby the cell membrane in term of elevation, which is not always straightforward in a microscope top-view.

Figure 3 depicts an experimental case of bubble-cell mechanical interaction. The bubble's modal decomposition is illustrated along the increasing part of a complete modulation period that lasts $\frac{1}{2}f_m^{-1} = 20\,\mathrm{ms}$. The radial mode displays a linearly increasing amplitude, while the sectoral mode emerges only later when the radial mode amplitude is sufficient to non-linearly trigger it. A zoom over two different particular times, highlighted by colored areas in Fig. 3, allows to explore bubble-cell dynamics at key moments, when there is uniquely strong radial oscillations and when a sectoral mode exists. Their duration is chosen in a way to ensure quasi-static amplitudes. This allows then to display the dynamics refolded over two acoustic periods in order to artificially increase the temporal resolution. In the illustrated case, we are very confident in the modal amplitudes since sectoral modes, which have no nodal line in elevation, unlike other non-sectoral shape modes, are fully resolved in a top-view configuration. It worths mentioning that Fig. 3 evidences a synchronisation between radial and sectoral modes. Indeed, the moment when the subharmonically-excited nonspherical deformation attains a value of zero displacement recurrently synchronises with the moment when the radial mode has a zero displacement amplitude. This experimental case of degree $n = 4$ actually differs from previous results where the coexistence between zonal and sectoral was investigated in pure water [5]. Unsurprisingly, since the surrounding fluid in the present study consists of cell culture instead of pure water, changes in surface tension at the bubble interface are to be expected, thence differences in contact line dynamics and modal interplay.

When regarding at the cell response, we chose to investigate the oscillations of the CTB cell's point with respect to the oscillations of the CTC bubble's point, as studied in similar works [6]. When the sectoral emerges, the amplitude displacement of the CTB cell's point reaches $6\,\mu\mathrm{m}$ peak-to-peak while it reached barely $3\,\mu\mathrm{m}$ peak-to-peak when the bubble was purely oscillating radially.

4. CONCLUSION

In a medical context of localized drug delivery, oscillating microbubbles facilitate the sonoporation of biological cells, or reversible opening of their membrane by acoustic excitation. Because of the stochastic and complex behavior of microbubbles interacting with biological cells, the success of sonoporation is usually investigated either post-ultrasound exposure or using patch-clamp techniques. Neither of them go into details of the microbubble dynamics and its impact on cells, while this could play an important role in the cell response. In the present paper, an original experimental setup allows to capture the bubble-cell interaction, at the acoustic time scale, with the aim to improve

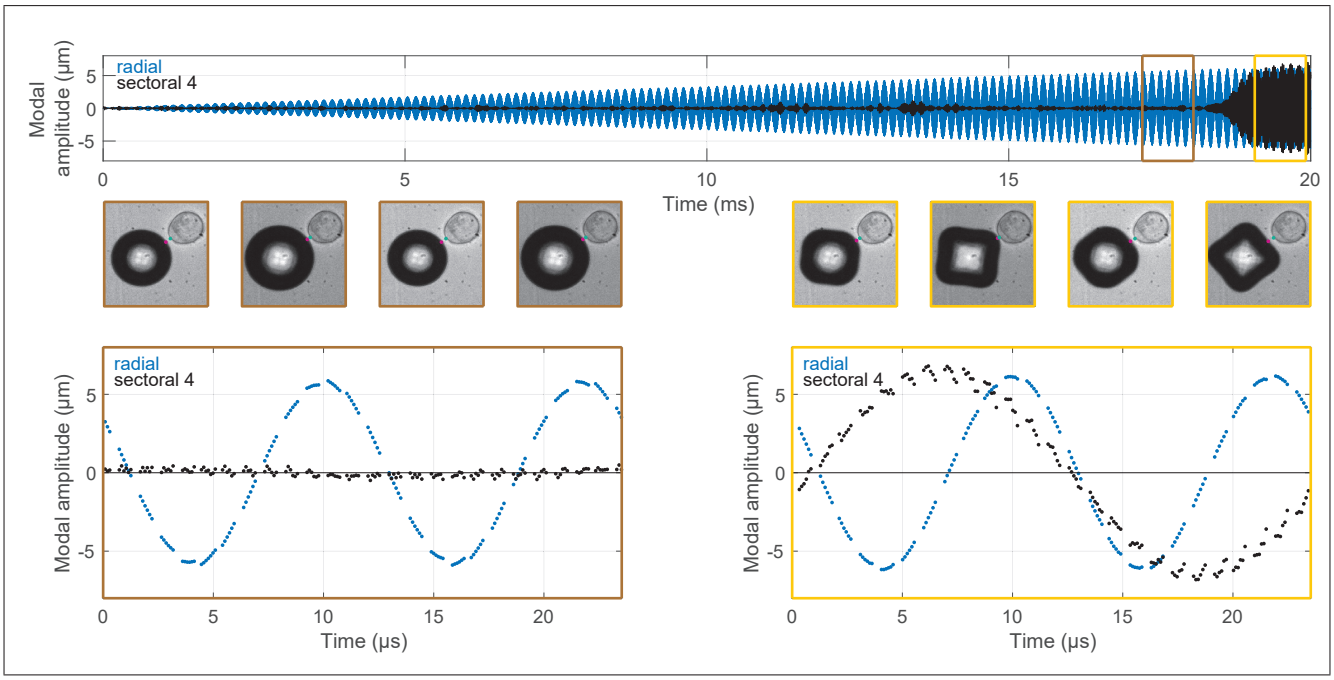

Figure 3. Bubble of equilibrium radius $38\,\mu\mathrm{m}$ mechanically interacting with a megakaryocyte of equilibrium radius $21.5\,\mu\mathrm{m}$. The modal decomposition of the bubble dynamics is depicted along a complete pressure ramp (above) and at particular times when the bubble oscillates only radially (middle-left) and when a sectoral mode 4 exists (middle-right), and refolded over two acoustic periods. Below, the bubble-cell dynamics is represented, at the same particular times, following two mechanical indexes, the CTC bubble's point and the CTB cell's point, which are located on the associated snapshots series, for the sake of clarity.

the prediction and control of their mechanical interaction. Its visualization at the micro-scale level evidences important amplitude of the cell displacement when stresses are applied on its membrane by nonspherical shape modes.

5. REFERENCES

[1] J. M. Escoffre, K. Kaddur, M. P. Rols and A. Bouakaz. In vitro gene transfer by electrosonoporation. *Ultrasound in Med. Biol.*, 36(10):1746–1755, 2010.

[2] T. An Tran, J.-Y. Le Guennec, P. Bougnoux, F. Tranquart and A. Bouakaz. Characterization of cell membrane response to ultrasound activated microbubbles. *IEEE Trans. Ultrason. Ferroelect. Freq. Contr.*, 55(1):44–49, 2008.

[3] A. Van Wamel, K. Kooiman, M. Harteveld, M. Emmer, F. J. ten Cate, M. Versluis and N. de Jong. Vibrating microbubbles poking individual cells: Drug transfer into cells via sonoporation. *J. Control. Release*, 112:149–155, 2006.

[4] P. Marmottant and S. Hilgenfeldt. Controlled vesicle deformation and lysis by single oscillating bubbles. *Nature*, 423:153–156, 2003.

[5] M. Fauconnier, J.-C. Béra and C. Inserra. Nonspherical modes nondegeneracy of a tethered bubble. *Phys. Rev. E*, 102(3):033108, 2020.

[6] M. Wang, Y. Zhang, C. Cai, J. Tu, X. Guo and D. Zhang. Sonoporation-induced cell membrane permeabilization and cytoskeleton disassembly at varied acoustic and microbubble-cell parameters. *Scientific Reports*, 8:3885, 2018.