CELL PERMEABILISATION AND TRANSPORT FOCUSED AROUND OSCILLATING MICROBUBBLES

Philippe Marmottant*, Sascha Hilgenfeldt*, Michel Versluis*, Han Gardeniers**, Albert van den Berg** and Detlef Lohse*

**Department of Applied Physics, University of Twente, PoBox 217, 7500AE Enschede, The Netherlands*

***MESA+ Research Institute, University of Twente, PoBox 217, 7500AE Enschede, The Netherlands,*

Summary The ultrasound-driven oscillation of a microbubble drives a steady streaming focused around the bubble. The study of individual bubbles attached to a wall shows vivid recirculations. When cells are in the vicinity of these bubbles, also used in medecine as contrast agent for ultrasound echography, they experience considerable shear rates. We introduce in the flow giant unilamelar lipid vesicles, acting as artificial cells. Rupture of the lipidic membrane with the opening of pores is revealed by high-speed camera recordings. A reversible permeation of the membrane wall can also be obtained, demonstrating at the micron scale the efficiency of microbubbles to deliver drugs in cells. The streaming flow of bubble on a surface can be further controlled, with the adjunction of a solid obstacle nearby: the flow turns to be directed. We will present a microfluidic device using the principle of bubble/obstacle doublets to locally transport small objets such as cells.

INTRODUCTION

Under an acoustic pressure a bubble can undergo large amplitude oscillations of its volume. This property is exploited in ultrasound echography to enhance the constrast: small micron size bubbles are injected in the blood flow and scatter back the sound. The small scale oscillation of the liquid around the bubble exerts forces on its surrounding. We will be interested in two effects of the microbubble oscillations on neighbouring small, cell-like, objects: shear stress forces and directed transport.

We will describe in a first part the intense acoustic streaming circulations around a single microbubble siting on a wall, and the effect on soft vesicles, mimicking cells. The observations help to understand a recent therapeutic application: the increased permeability of cell walls for drug delivery, in the presence of microbubble [1].

The focusing power of bubbles can be incorporated in small microfluidic applications. We will show in a second part how the streaming can be oriented, which gives the principles for a micron scale analysis device designed to transport cells without the specific use of channels.

DEFORMATION AND PERMEABILISATION OF BIOMIMETIC VESICLES

To study the interaction of a bubble with a single cell, we designed a set-up allowing direct microscopic observation. We consider air bubbles (10 to 100 microns radius) fixed by capillarity to a wall, and excited by an ultrasound standing wave (20 to 200 kHz).

We observe fast liquid recirculations around the bubble, even for low amplitudes of vibration. The origin of the motion is a steady acoustic streaming, a non-linear effect driven by the oscillation of the bubble [2]. The steady flow pattern near a wall can be derived analytically [3]. The streaming presents strong velocity gradient, which exerts shear stress on objects

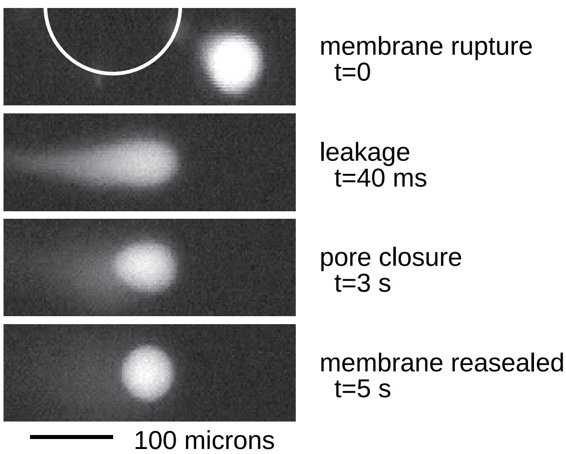

Figure 1. Deformation and rupture of a unilamellar lipid vesicle, near an oscillating microbubble (white circle) for a short instant of time. Leakage of the fluorescent inside liquid, and resealing of the membrane.

embedded in the neighbouring liquid. To study the effect of shear stress on cell membranes, we introduced biomimetic lipid vesicles in the flow: the vesicles are vividly deformed, which can lead to membrane rupture and the opening of pores

(see figure 1). Reversible poration is observed when the stress is applied for a small time, a process that can be used in the context of drug delivery.
Direct imaging of the bubble oscillation is achieved with the ultra-high frequency camera Brandaris 128 [4], that can record sequences of 128 images at frequencies up to 25 million frames per second.

DIRECTED STREAMING: MICROFLUIDIC TRANSPORT OF SMALL OBJECTS

The presence of a solid particle nearby the bubble breaks the axisymmetry of the streaming flow (a particle like the bump of figure 2a). The particle, placed in the oscillating flow of the bubble, exerts an acoustic streaming as well, directed away from the bubble [5]. The far-field velocity of the solid particle is the one generated by a point force (Stokeslet) parallel to the wall. The flow field of the ensemble of bubbles/particle doublets is easily computed in the low Reynolds number approximation (figure 2b), showing streamline going from bubble to bubble.

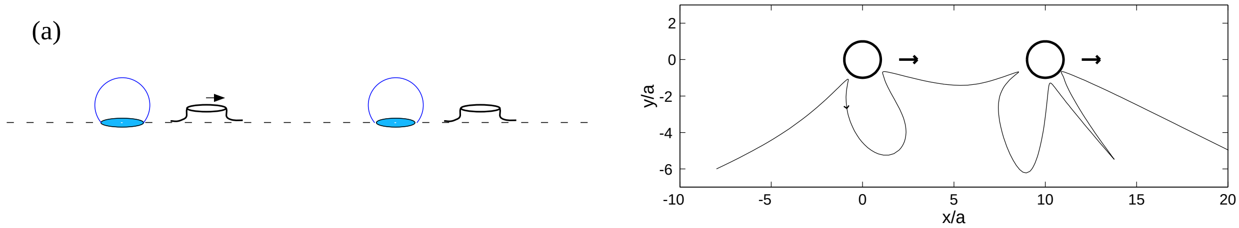

Figure 2. (a) Schematic structural elements for a microfluidic device: solid bumps are placed near the bubbles to direct the flow. (b) Predicted streaming.

Through a collaboration with the MESA+ Institute, we could prepare substrates, aligning pairs of holes and bumps on a PDMS substrate. From the gas pockets enclosed in the holes, bubbles are grown by warming up slightly the liquid, thus reducing the solubility of dissolved air. Upon ultrasound emission the acoustic streaming does not show a symmetric pattern around the bubbles any more. Tracer particles introduced in the fluid show the transport to be directed by the bubble/particle doublet (see figure 3), as predicted.

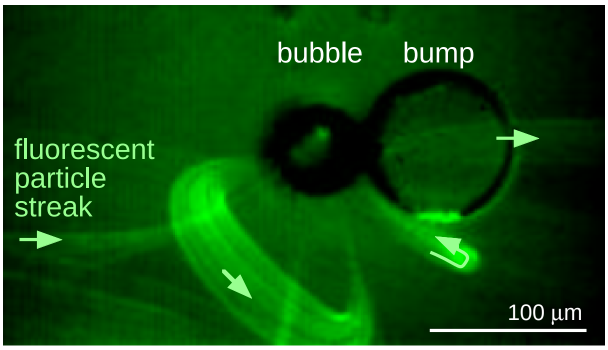

Figure 3. Transport of fluorescent tracer particle (2 micron bead) by a bubble/bump element.

CONCLUSIONS

The oscillation of a microbubble generates fast local streaming motions, concentrated on micron scale. The shear stress induced deforms and ruptures lipid vesicles, whose membrane is identical to the one of a cell. The permeabilisation is reversible when the shear is vanishing after the rupture.
An other application of bubble is found in the microfluidic field. A directed motion of the fluid can be impressed when an obstacle is nearby. The acoustic streaming of the obstacle allows to realise a focused small scale transport.

References

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