IV. Nano-particulate Systems and Other Novel Concepts in Drug Delivery

BUBBLE ASSISTED DRUG DELIVERY

M. Arora, C.D. Ohl
Institute for Biomedical Technology (BMTI), Physics of Fluids, Faculty of Science and Technology, University of Twente, P.O. Box 217, 7500AE Enschede, The Netherlands

Summary

Adherent epidermal cells are shown to take up fluorescent molecules (calcein and FITC-dextran) following the exposure to cavitation bubble activity created by a tensile acoustic pulse of $-4 \text{ MPa}$ peak amplitude. The geometrical uptake pattern of the fluorescent dye molecules allows to identify two mechanisms for drug uptake, both induced by the bubble dynamics close to cells: First, shear stress generated from non-spherically collapsing individual bubbles and second, the vivid flow field from the growth and collapse of a cavitation bubble cluster.

Introduction

In various studies, acoustic devices have been shown to stimulate the uptake of extracellular material into the cytoplasm without causing permanent damage to the cell [1–5], a process called sonoporation. Acoustic waves promise to be advantageous to certain drug delivery applications because they can be applied noninvasively, localized, and remotely controlled inside the patient’s body. However, the exact mechanisms causing the permeabilization of cell membranes are not yet clarified.

In this in vitro study, the poration of HeLa cells was investigated following exposure to cavitation bubble activity generated by a shockwave device. These shockwave devices are typically used for extracorporeal treatment of kidney stones. Cavitation bubbles generate and expand during the tensile part of the pressure pulse. When the pressure recovers to normal atmospheric pressure, cavitation bubbles shrink in an accelerated manner (also known as cavitation collapse). The collapse of these bubbles especially near boundaries or under influence of other bubbles can become aspherical leading to formation of liquid jets. Using fast imaging techniques and fluorescence microscopy, we show that these bubble collapse events not only cause detachment of the adhering cells but are also responsible for uptake of normally nonpermeable molecules into the cell’s cytoplasm.

Experimental methods

HeLa cells were grown at 37 °C and 5% CO$_2$ in Iscove’s Modified Dulbecco’s medium (Invitrogen, Breda Netherlands) and plated in polystyrol culture flasks. The flasks were filled up with medium prior to being exposed to cavitation bubble activity. Following exposure, cells were tested for viability using ethidium bromide/acridine orange (Fluka, Zwijndrecht, Netherlands) staining at final concentrations of 5 and 1.5 mg/ml, respectively.

Transient membrane permeabilization was checked by the uptake of fluorescein isothiocyanate dextran (FITC-dextran, 20 kDa) or calcein. Non-membrane-permeant FITC-dextran (1 mg/ml) or calcein (1 mg/ml) was added to the cell medium before shock wave exposure. Dye uptake of attached cells was determined by fluorescence microscopy after washing the cells four times with PBS and adding ethidium bromide at a final concentration of 5 mg/ml. Transiently porated cells, loaded with FITC-dextran or calcein appear green under fluorescence excitation. Permanently damaged cells appear orange due to ethidium bromide staining.

![Fig. 1. A typical pressure recording of the shock wave passage at 6 kV discharge voltage.](image-url)
Acoustic pulses were generated by a focused piezoceramic source, which was adapted from the commercial lithotripter Piezolith 3000 (Richard Wolf, Knittlingen, Germany). The lithotripter contains two piezoceramic layers with a diameter of 300 mm and a focusing angle of 94°. In our experiments, the operating voltage of the lithotripter was set between 3.5 and 7 kV and only the frontal piezoceramic layer was used. The pressure in the free field at the lithotripter focus was measured with a calibrated needle type hydrophone. A typical recording at 6 kV is presented in Fig. 1. The wave consists of a positive pressure pulse of about 1.5 µs with a steep front and amplitude between 10 and 40 MPa (depending on the discharge voltage) followed by a tensile pressure pulse of amplitudes around 4 MPa, which lasts for several microseconds.

Cells adhering on the flask surface are placed in the acoustic focus of the shockwave generator. For the optical observations, a long-distance microscope (K2 with CF4 objective, Infinity, USA) was used, which was attached to a dual frame CCD (charged coupled device) camera (Imager 3S, LaVision, Göttingen, Germany).

Results and conclusions

A typical example of the interaction of cavitation bubbles with cells is depicted in Fig. 2. The time data is given with respect to the shock wave impact on the substrate. Shortly after that impact, we see two bubbles appearing due to the tensile pressure pulse following the shock wave. Between the second and third frame of Fig. 2, both bubbles will develop a jet flow directed towards the substrate (rigid boundary) [6], which subsequently results into an outward radial spreading flow. This flow pattern is sufficiently strong to detach cells located in the vicinity of the bubbles. Cells only become detached at the positions where the bubble dynamics was visible. The initial detachment of cells could be directly correlated to the positions of cavitation bubbles. No detachment was observed when the strength of the lithotripter generated tensile pressure was below the threshold for the nucleation of cavitation bubbles.

Fig. 3 depicts attached cells after shock wave exposure with the lithotripter operating at 6 kV. Prior to shock wave treatment, the cell medium was supplemented with FITC-dextran. It can be seen that the substrate is partially cleared of cells caused by cavitation activity. Cells, which have not been washed away but line the border between occupied and vacated regions, emit a strong green fluorescent light upon excitation originated by the uptake of FITC-dextran. Only slight emission of green fluorescence is detected for cells further away from the region of cavitation-induced detachment. Dead cells appear red or brown in the image due to the intercalation of ethidium bromide into the DNA (frame size 0.72×0.60 mm).

Results and conclusions

A typical example of the interaction of cavitation bubbles with cells is depicted in Fig. 2. The time data is given with respect to the shock wave impact on the substrate. Shortly after that impact, we see two bubbles appearing due to the tensile pressure pulse following the shock wave. Between the second and third frame of Fig. 2, both bubbles will develop a jet flow directed towards the substrate (rigid boundary) [6], which subsequently results into an outward radial spreading flow. This flow pattern is sufficiently strong to detach cells located in the vicinity of the bubbles. Cells only become detached at the positions where the bubble dynamics was visible. The initial detachment of cells could be directly correlated to the positions of cavitation bubbles. No detachment was observed when the strength of the lithotripter generated tensile pressure was below the threshold for the nucleation of cavitation bubbles.

Fig. 3 depicts attached cells after shock wave exposure with the lithotripter operating at 6 kV. Prior to shock wave treatment, the cell medium was supplemented with FITC-dextran. It can be seen that the substrate is partially cleared of cells caused by cavitation activity. Cells, which have not been washed away but line the border between occupied and vacated regions, emit a strong green fluorescent light upon excitation originated by the uptake of FITC-dextran. Only slight emission of green fluorescence is detected for cells further away from the region of cavitation-induced detachment. Dead cells appear red or brown in the image due to the intercalation of ethidium bromide into the DNA (frame size 0.72×0.60 mm).

Results and conclusions

A typical example of the interaction of cavitation bubbles with cells is depicted in Fig. 2. The time data is given with respect to the shock wave impact on the substrate. Shortly after that impact, we see two bubbles appearing due to the tensile pressure pulse following the shock wave. Between the second and third frame of Fig. 2, both bubbles will develop a jet flow directed towards the substrate (rigid boundary) [6], which subsequently results into an outward radial spreading flow. This flow pattern is sufficiently strong to detach cells located in the vicinity of the bubbles. Cells only become detached at the positions where the bubble dynamics was visible. The initial detachment of cells could be directly correlated to the positions of cavitation bubbles. No detachment was observed when the strength of the lithotripter generated tensile pressure was below the threshold for the nucleation of cavitation bubbles.

Fig. 3 depicts attached cells after shock wave exposure with the lithotripter operating at 6 kV. Prior to shock wave treatment, the cell medium was supplemented with FITC-dextran. It can be seen that the substrate is partially cleared of cells caused by cavitation activity. Cells, which have not been washed away but line the border between occupied and vacated regions, emit a strong green fluorescent light upon excitation originated by the uptake of FITC-dextran. Only slight emission of green fluorescence is detected for cells further away from the region of cavitation-induced detachment. Dead cells appear red or brown in the image due to the intercalation of ethidium bromide into the DNA (frame size 0.72×0.60 mm).

From our experiments, we can conclude that cell detachment is caused by the generation of cavitation. This statement is based on the fact, that the detachment on cells was only observed when cavitation bubble dynamics took place. Also, it is shown that the violent
bubble activity leads to permeabilization of the cell membrane causing uptake of the molecules which are normally nonpermeable to the cell membrane. The geometrical pattern of uptake reveals that not only individual bubble activity leads to membrane permeabilization but that also the flow field generated by the multiple bubble formation can lead to similar scenarios.

Acknowledgements

The experimental work is supported by FOM (The Netherlands) under grant 00PMT04. R. Ikink (University of Twente) and B. Wolfrum (Third Physical Institute, Germany) are acknowledged for their support during this work.

References


Fig. 4. Fluorescence image showing large-scale uptake pattern of calcein.