Remote manipulation of cells with ultrasound and microbubbles

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Abstract - Ultrasound in combination with contrast microbubbles has been shown to alter the permeability of cell membranes. This permeabilization feature is used to design new drug delivery systems using ultrasound and contrast agents. Although the exact underlying mechanisms are still unknown, one hypothesis is that oscillating microbubbles cause cell deformation resulting in enhanced cell membrane permeability. In this paper we show the actions of oscillating microbubbles on cultured cells under a microscope recorded with a fast framing camera at 10 million frames per second.

Optical observations of microbubbles and cultured cells is possible through the use of a microscope mounted in front of the fast framing camera Brandaris128. The Brandaris128 is capable of recording a sequence of 128 images with a frame rate up to 25 million frames per second. Pig aorta endothelial cells were grown on the inside of an Opticell^{TM} container. A diluted suspension of experimental agents BR14 (Bracco Research, Geneva, Switzerland) was added. Ultrasound exposure consisted of one burst of 6 cycles at a frequency of 1 MHz and a P_ of 0.5 MPa. During ultrasound transmission, the interactions between BR14 microbubbles and cultured cells were recorded using a frame rate of 10 million frames per second.

Cell deformation as a result of vibrating microbubbles is studied. Cell deformation is quantified through measuring the displacement of the cells. Microbubble vibration is quantified by measuring its initial, maximal, and minimal radii. We observed that, upon ultrasound arrival and microbubble oscillations, the cell membrane deforms up to a few micrometers in length as a result of the oscillation of the microbubble. The membrane deformation rate changes with the oscillation strength of the microbubble. During the sonication, changes in the cross-sectional distance of the cultured cells were observed due to microbubble vibrations. Depending on the maximal vibrations of the microbubble and the distance between the microbubble and the cell, the displacement of the cells varied form 0 to 20% of the cell size.

This study reveals the action of oscillating microbubbles on cells. It is known that living cells sense mechanical forces thus there is no doubt that perturbation of the oscillating microbubbles results in profound alterations in the cellular content. This study is the beginning of revealing the functional relationships that lie beyond the remote manipulation of cells and ultrasound microbubble induced permeabilization of the cell membrane.

INTRODUCTION

Several groups have studied the use of diagnostic ultrasound (0.5-5MHz) with or without microbubbles for local drug delivery as shown in the recent reviews by Unger [1]. Studies of influx of membrane impermeable dye have confirmed that the action of ultrasound-irradiated microbubbles, using diagnostic ultrasound levels, on the cell membrane is to alter its permeability [2]. It is generally accepted that diagnostic ultrasound and microbubbles can increase cell membrane permeability, although the mechanisms behind it are unknown. However, it is suspected that the cellular-mechanism behind diagnostic ultrasound permeabilisation is the formation of pores [3]. The permeabilisation action of ultrasound and microbubbles on cells lies in the fact that microbubbles oscillate while irradiated with ultrasound resulting in an increased cell membrane

permeability [3,4]. This study shows for the first time the relation between oscillating microbubbles and nearby cells using the recently developed high-speed camera Brandaris128 [5]. With this camera it is possible to record 128 frames with a frame rate between 1-25 MHz and therefore recording the interaction between oscillating microbubbles and cells is possible over a long acquisition period. In this study endothelial cells were used to investigate the mechanical effects of oscillating microbubbles on nearby cells.

MATERIALS & METHODS

Cell culture and ultrasound microbubbles

Endothelial cells, isolated from pig aorta and cultured *in-vitro,* were used for this study. Primary pig aortic endothelial cell cultures were grown in Opticell units (Biocrystal, Westerville, Ohio, USA) and maintained in 10 ml DMEM supplemented with 5% foetal calf serum (FCS) (Gibco BRL, Invitrogen, Groningen, The Netherlands) and antibiotics in a humified incubator at 37°C and 5% CO_2. BR14 (Bracco Research SA, Geneva) is an experimental lipid-shelled contrast agent.

Ultrasound exposure protocol

The experimental acoustic set up consisted of a 1-MHz unfocused single-element transducer (Panametrics, Waltham, MA) mounted in a water tank. The cell culture monolayer was positioned in front of the ultrasound beam. The peak negative acoustic pressure generated at the region of interest was 0.5 MPa. This acoustic pressure corresponds to a mechanical index of 0.5. The microbubbles and cells were exposed to only one single burst of ultrasound. The transmitted ultrasonic wave consisted of one single sine wave burst of 6 cycles, generated by an arbitrary waveform generator and amplified by a 60-dB linear power.

Ultra-fast optical observations

Pig aorta endothelial cells were grown on the inside of an Opticell^{TM} container and the cell diameter ranged from 10 to 50 micrometers. A 1:1000 diluted suspension of BR14 (Bracco, Geneva, Switzerland) was added. Optical observations of microbubbles and endothelial cells is possible through the use of a standard BX-2 Olympus microscope mounted in front of the high frame rate camera Brandaris128. An extended description of the camera is written by Chin et al. [6]. The Brandaris128 is capable of recording a sequence of 128 images with a frame rate up to 25 million frames per second. The field of view of the recorded image has a total size of 90x68 μm^2. Ultrasound was applied as described above and the physical interactions between BR14 microbubbles and endothelial cells were recorded using a frame rate of 10 million frames per second. Perpendicular to the centre of the microbubble and on either side of the cell, two points (one fixed and one moving point) on the cell were selected to measure the displacement of cell. Further, the change in microbubble radii in the direction of the cell was measured.

RESULTS

The present study focused on oscillating microbubbles, during exposure to ultrasound, and its consequences when vibrating next to an endothelial cell with specific reference to the displacement of the cell. This study is meant to shed light on the mechanisms underlying cell membrane permeabilisation as a result of microbubble-cell interaction. Cell displacement in relation to expansion and contraction of the microbubbles was studied. In the expansion phase, the microbubbles pushed the cells inwards and in the contraction phase the microbubbles pulled the cells outwards. In figure 1 pushing and pulling is shown in a schematic manner.

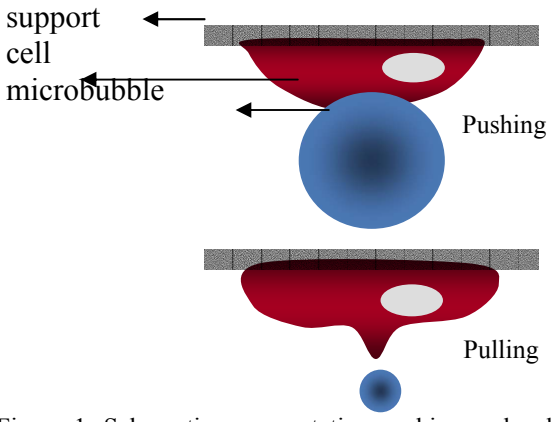

Figure 1: Schematic representation pushing and pulling of a microbubble on a cell.

The extension of the expansion and contraction is measured for 33 microbubbles with an initial radius between 0.7 and 4.5 µm and the results are plotted in figure 2.

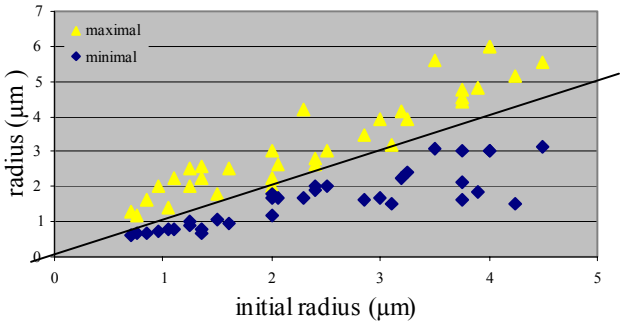

Figure 2: Maximal and minimal radii changes of microbubbles with sizes between 0.7-4.5 µm (floating against cells) as a response to ultrasound.

Observations revealed that during ultrasound sonication the cell membrane deforms up to a few micrometers in length as a result of the oscillation of the microbubble. The cell deformation changes with the oscillation behavior of the microbubble. The cell membrane deformation is studied through measuring the distance between two fixed points of the cell. The microbubble vibration is quantified by measuring the microbubble radius. Both curves of maximal pushing and pulling cell displacement are plotted as a function of the maximal microbubble vibrations in Fig. 3 and 4, respectively.

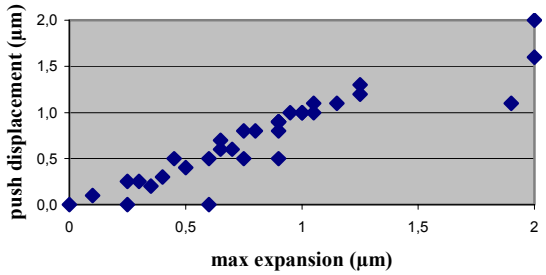

Figure 3: Maximal pushing displacement as function of the maximal radii of the microbubbles.

Cell displacement is linear to the bubble expansion until an expansion of ~ 1.3 µm and a maximum displacement of ~ 2 µm was measured. This is between 4-20% of the cell size.

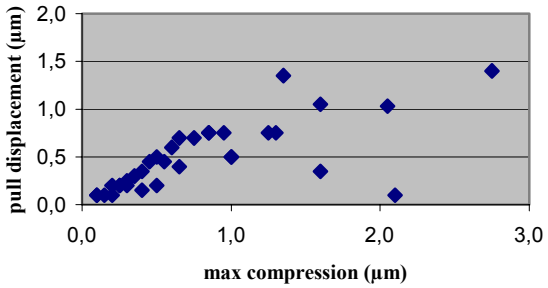

Figure 4: Maximal pulling displacement as function of the change in maximal compression of the microbubbles.

Cell membrane pulling displacement is linear to microbubble compression until a compression of ~ 0.7 µm, and a maximum displacement of ~ 1.4 µm was measured. This is between 3-14% of the cell size.

Under the rarefaction effect of ultrasound, the microbubble expands to a maximum whereby it presses against the cell. This maximal expansion is translated to a movement (i.e. displacement) of the cell membrane. The compression of the microbubble is also translated in a movement of the cell membrane. Thus, microbubble vibrations are translated into pushing and pulling of the cell membrane. These pushing and pulling effects are also seen in situations were the bubble is located at a small distance from the cell. A bubble with a initial radius of 1.5 µm or 4 µm can still induce pushing and pulling displacement at a distance of 2 to 5 µm respectively, although the displacements were smaller than the displacements seen when a bubble is in contact with the cell.

CONCLUSIONS

Ultrasound and microbubbles have the potential to lead to applications in molecular medicine (e.g. for the detection and treatment through targeted delivery of drugs and genes). To fully explore the use of this new opportunity it is necessary to

understand the mechanisms of microbubble action. This study provides preliminary insight in the action between microbubbles and cells under ultrasound exposure. Interaction of diagnostic ultrasound fields with microbubbles results in oscillating microbubbles that induce displacement of biological cell membranes of adhered cells. Despite the short exposure (single ultrasound burst), changes in microbubble diameter are translated into changes in fluid velocity, or 'pushing' events across the cell membrane whereby cells experience a mechanical strain. In-vitro data have shown that cells can sustain stresses up to an MI of 1.0 with addition of microbubbles [2]. Cell permeabilisation may be the consequence of the local interaction of ultrasound with the microbubble, which lead to a mechanical force on the cell membrane. Shear stress produced by oscillating microbubbles will most likely lead to cell membrane permeabilisation [4]. Further, oscillating microbubbles nearby the cell membrane will, with no doubt, initiate signaling cascades in endothelial cells that lead to other cellular responses. Deformations result in membrane apposition that on its turn results in reorganization of the cell cytoplasm [6]. The contact zone of several μm^2, containing thousands of adhesion receptors, will transfer a pushing and pulling signal through the cytoplasm to the nucleus resulting in effects similar to those caused by shear stress. These include increased activity of tyrosine kinase, protein kinase C, enhanced expression of IE (immediate early) genes and their related genes [6, 7]. Further research is still necessary to elucidate the precise influence of oscillating microbubbles on a cellular level.

REFERENCES

1) Unger E, Porter T, Culp W, Labell R, Matsunaga T, Zutshi R., "Therapeutic applications of lipid-coated microbubbles," *Adv. Drug Deliv. Rev.*, vol. 56, 1291-1314, 2004.
2) van Wamel A, Bouakaz A, Bernard B, ten Cate FJ, and de Jong N., "Radionuclide tumour therapy with ultrasound contrast microbubbles," *Ultrasonics*, vol. 42, pp. 903-906, 2004.
3) Y. Taniyama, K. Tachibana, K. Hiraoka, T. Namba, K. Yamasaki, N. Hashiya, M. Aoki, T. Ogihara, K. Yasufumi, and R. Morishita, "Local delivery of plasmid DNA into rat carotid artery using ultrasound," *Circulation*, vol. 105, pp. 1233-1239, 2002.
4) P. Marmottant and S. Hilgenfeldt, "Controlled vesicle deformation and lysis by single oscillating bubbles," *Nature*, vol. 423, pp. 153-6, 2003.
5) C. T. Chin, C. Lancee, J. Borsboom, F. Mastik, M. Frijlink, N. de Jong, M. Versluis, and D. Lohse, "Brandaris128: a 25 million frames per second digital camera with 128 highly sensitive frames," *Rev. Sci. Instru.*, vol.74, pp. 5026-5034, 2003.
6) S. Chien, S. Li, and Y. J. Shyy, "Effects of mechanical forces on signal transduction and gene expression in endothelial cells," *Hypertension*, vol. 31, pp. 162-169, 1998.
7) S. Lehoux and A. Tedgui, "Cellular mechanics and gene expression in blood vessels," *J. Biomech.*, vol. 36, pp. 631-643, 2003.

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