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1 Real-time sonoporation through HeLa cells

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9

10 Abstract. The purpose of this study was to investigate the physical mechanisms of  
11 sonoporation, to understand and ameliorate ultrasound-assisted drug and gene delivery.  
12 Sonoporation is the tran-sient permeabilisation of a cell membrane with help of ultrasound  
13 and/or an ultrasound contrast agent, allowing for the trans-membrane delivery and cellular  
14 uptake of macromolecules between 10 kDa and 3 MDa. We studied the behaviour of  
15 ultrasound contrast agent microbubbles near can- cer cells at low acoustic amplitudes. After  
16 administering an ultrasound contrast agent, HeLa cells were subjected to 6.6-MHz  
17 ultrasound with a mechanical index of 0.2 and observed with a high- speed camera.  
18 Microbubbles were seen to enter cells and rapidly dissolve. The quick dissolution after  
19 entering suggests that the microbubbles lose (part of) their shell whilst entering. We have  
20 demonstrated that lipid-shelled microbubbles can be forced to enter cells at a low  
21 mechanical index. Hence, if a therapeutic load is added to the bubble, ultrasound-guided  
22 delivery could be facilitated at diagnostic settings. However, these results may have  
23 implications for the safety regulations on the use of ultrasound contrast agents for  
24 diagnostic imaging.

25 Keywords: Sonoporation, Ultrasound, Microbubbles, HeLa Cancer cells, Drug delivery, Low  
26 Mechanical Index

27 PACS: 3.25.Ywm, 43.25.Nm, 43.30.Lz, 43.35.Ty

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## 35 INTRODUCTION

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37 In medical-diagnostics, guidelines state an  $MI < 0.3$  can be considered safe for pregnant  
38 women and neonatals, but yet diagnostic imaging machines allow the use of MI up to 1.9.  
39 Sonoporation is the transient permeabilisation and resealing of a cell membrane with the  
40 help of ultrasound and/or an ultrasound contrast agent, allowing for the trans- membrane  
41 delivery and cellular uptake of macromolecules between 10 kDa and 3 MDa [1]. Previous  
42 studies on non-invasive, ultrasound-induced therapeutics used acoustic amplitudes  
43 corresponding to mechanical indices between 0.2 and 7.0 [2, 3]. Many studies have  
44 demonstrated increased drug and gene uptake of sites under sonication [4, 5, 6]. These  
45 studies presumed that a physical membrane disruption mechanism, i.e. sonoporation,  
46 caused the increased uptake, as opposed to naturally occurring active uptake processes,  
47 such as endocytosis, which are controlled by the system biology. Here we examin the effect  
48 of low MI ultrasound ( $MI=0.2$ ) in combination with lipid shelled, gas filled microbubbles on  
49 HeLa cancer cells. Figure 1 shows six possible mechanisms of sonoporation.

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52 METHODS

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54 An overview of the experimental setup is shown in Figure 2. We have described our  
55 experimental setup extensively in Delalande et al. [7]. In short, a signal consisting of 40  
56 cycles with a centre frequency of 6.6 MHz and a pulse repetition frequency of 10 kHz, was  
57 generated by an AFG3102, dual channel arbitrary function generator (Tektronix, Inc.,  
58 Beaverton, OR), amplified by a 2100L, 50-dB RF amplifier (Electronics & Innovation Ltd.,  
59 Rochester, NY) and fed to a custom-built 6.6-MHz ultrasound transducer. The peak-negative  
60 acoustic pressure corresponds to an MI of 0.2. The transducer was placed in a custom-built,  
61 260×160×150 (mm)<sup>3</sup> Perspex sonication chamber, in which an OptiCell cell culture chamber  
62 (Nunc GmbH & Co. KG, Langenselbold, Germany) was placed. One side of the cell culture  
63 chamber contained a monolayer of HeLa cells. A customised BXFM-F microscope unit with  
64 an LCAch N 20×/0.40 NA PhC (OlympusDeutschland GmbH, Hamburg, Germany) and a  
65 LUMPlanFL 60×/0.90 NA water- immersion objective (Olympus) was placed on top of the  
66 sonication chamber with the objective lens immersed in the water. The colour charge  
67 coupled device (CCD) of a PHOTRON FastCam MC-2.1 high-speed camera (VKT Video  
68 Kommunikation GmbH, Pfullingen, Germany) was connected to the microscope.

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71 RESULTS AND DISCUSSION

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73 Lipid-shelled microbubbles were forced into cells using pulsed ultrasound at  $MI=0.2$  at  
74 transmit frequencies of 1.0 MHz and 6.6 MHz. This phenomenon typically takes 2 s from the  
75 moment a bubble contacts the cell membrane, to complete dissolution of the gas inside the  
76 cell. Most bubble-cell penetration occurred within 8 s from the start of sonication. These  
77 results were easily reproducible, independent of the setup geometry. We are the first to  
78 observe the translation of entire microbubbles into cells. Figure 3 shows a 3D reconstruction  
79 of the results seen. Targeted drug delivery down to the cellular level, with the use of  
80 encapsulated bubbles will allow the use of high-toxicity drugs to be injected into the body,  
81 but only delivered to a specific area. Thus, leaving healthy tissue unaffected. Our  
82 sonoporation observations could be attributed to the long pulse lengths used. Although cells  
83 themselves are acoustically active, this acoustic activity is probably negligible to that of  
84 microbubbles in high concentrations. Therefore, we expect bubble-cell interaction to be  
85 more likely in very low bubble concentrations.

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88 CONCLUSION

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90 It has been demonstrated that lipid-shelled microbubbles can be forced to enter cells at a  
91 low MI. Hence, if a therapeutic load is added to the bubble, ultrasound guided delivery  
92 could be facilitated at diagnostic settings. In addition, these results may have implications  
93 for the safety regulations on the use of ultrasound contrast agents for diagnostic imaging.

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97

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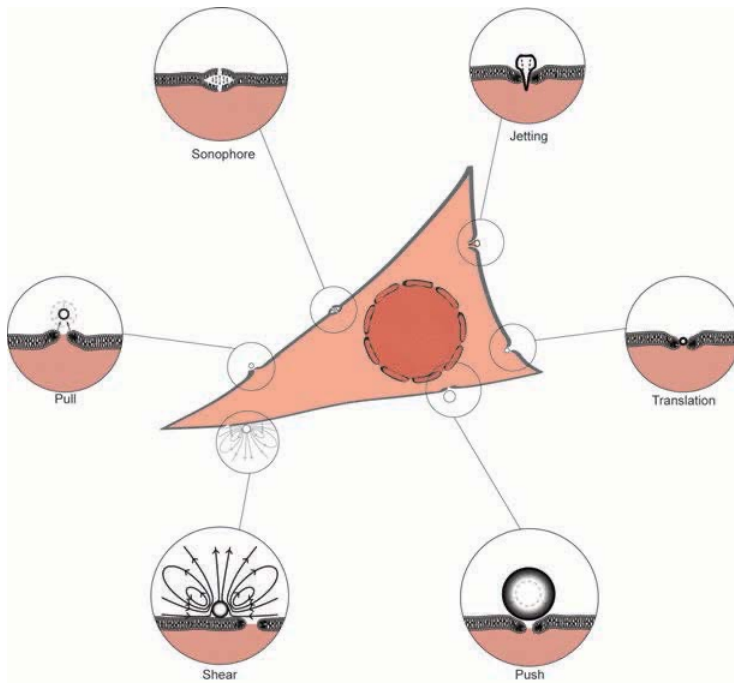
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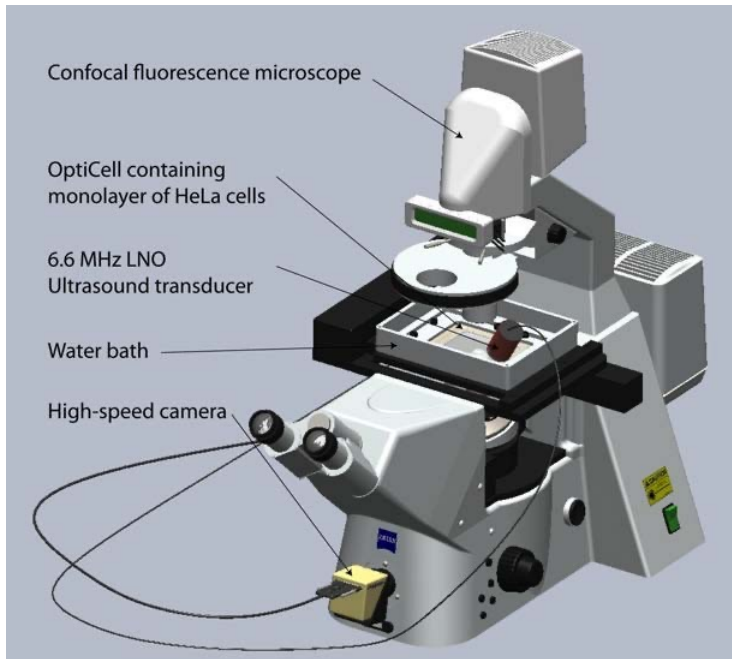


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123 Figure 1. Possible mechanisms of sonoporation.

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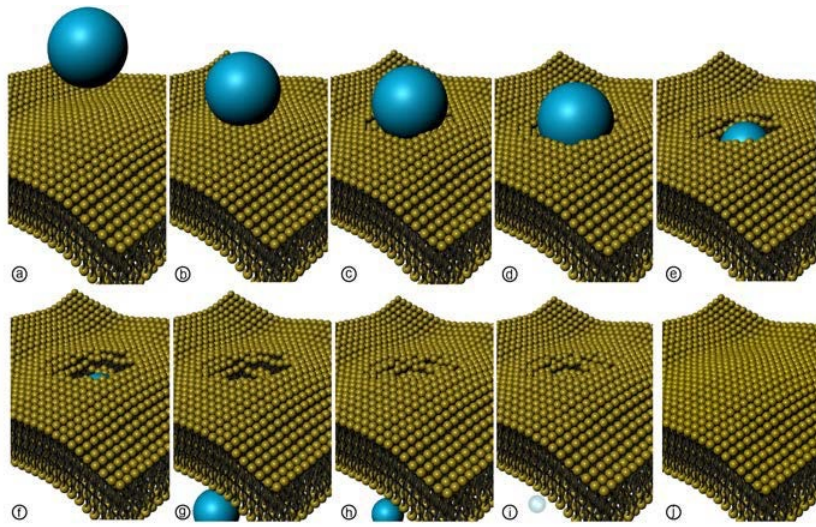
127 Figure 2. Confocal fluorescence configuration used to simultaneously observe and sonicate

128 HeLa cells in the presence of fluorescence coated microbubbles.

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133 Figure 3. 3D reconstruction of microbubble translation through a cell membrane. Once the  
134 microbubble has translated through the membrane, the pore re-seals and the microbubble  
135 is seen to dissolve.