Sonoporation of Cultured Cells in the Rotating Tube Exposure System

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Suspensions of Chinese hamster ovary cells were exposed to ultrasound in the presence of large fluorescent dextran molecules. Ten percent of Albunex^{R}, a gas-body-based ultrasound contrast agent, was added to assure cavitation nucleation. Sterile 4.5 ml exposure chambers normally were rotated at 60 rpm to promote cavitation activity during the 1 min exposures. Ultrasound was continuous wave at frequencies of 1.0, 1.68, 2.25, 3.3, 5.3 and 7.15 MHz. After exposure cells were tested for sonoporation by counting fluorescent cells, and for cell lysis by counting cells stained by Trypan blue. Sonoporation was noted for spatial peak pressure amplitudes as low as 0.1 MPa up to 3.3 MHz, increasing to 0.39 MPa at 7.15 MHz. Significant lysis occurred for 0.14 MPa exposures at 1.0 MHz, but not for the other frequencies for the relatively low-pressure amplitudes of exposure. Sonoporation decreased slightly if the tube was not rotated, and increased for increasing Albunex concentration. The plating efficiency of cells exposed to 0.28 MPa at 2.25 MHz and sorted by a flow cytometer were 19 % (3.6% standard deviation) for fluorescent cells, compared to 67% (1% s. d.) for non-fluorescent exposed cells and 62% (6% s. d.) for sham-exposed cells. When gas bodies are supplied to the suspension, membrane damage and sonoporation can be accomplished at pressure amplitudes, which are relatively low, for example compared to inertial cavitation thresholds.

USTUR-0115-98