Transfection of a Reporter Plasmid into Cultured Cells by Sonoporation In-Vitro

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Cultured Chinese hamster ovary cells were exposed to 2.25-MHz ultrasound in sterile 4.5-mL polyethylene chambers and tested for cell lysis, sonoporation and DNA transfection. Ten percent of Albunex^{R,} a gas-body-based ultrasound contrast agent, was added to ensure cavitation nucleation, and the chambers were rotated at 60 rpm to promote cavitation activity during the 1-min exposures. Uptake of large fluorescent dextran molecules by some cells was observed for spatial peak pressure amplitudes as low as 0.1 MPa, which indicates transient permeabilization and resealing, *i.e.*, sonoporation, of these cells during exposure. Significant loss of membrane integrity occurred for 0.2 MPa, and increased rapidly for exposures above the apparent cavitation threshold (using the H_20_2 production test) of about 0.4 MPa spatial peak pressure amplitude. For the DNA transfection tests, 20 ug/ml luciferase reporter plasmid was added to the suspension during exposure, and cells were assayed for proliferation ability and luciferase gene expression 2 days after exposure. Cell proliferation was greatly reduced above the cavitation threshold. Luciferase production was significant for 0.20-MPa exposure, and reached 0.33 ng per 10^6 cells at 0.8-MPa exposure. The luciferase production was greater fort cells exposed in medium supplemented with serum than for cells exposed in serum-free medium. Cells harvested for exposure either in the log phase or in the stationary phase of culture gave similar proliferation and transfection results. The effects essentially disappeared when the Albunex^R was omitted from the suspension and the tube was not rotated. Thus, sonoporation by ultrasonic cavitation in the rotating tube exposure system yields plasmid transfection with subsequent gene expression.

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