431e DNA and RNA Delivery and Transfection Mediated by Electroporation and Ultrasound

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Electroporation and ultrasound have been shown to facilitate the loading of drugs and genes into viable cells. Optimization of gene therapy and drug delivery applications in similar systems was systematically studied before. We previously identified physical parameters influencing transfection and viability of cells, and a range of optimal conditions was found. The transfection of cells with DNA is a complex multi-step phenomenon, which includes DNA delivery across the cell membrane, trafficking within the cell to the nucleus, and synthesis of mRNA and finally protein. The transfection of cells with cRNA does not involve traffick to the nucleus and thus may be more efficient. This study addresses the relations between DNA and RNA delivery and subsequent protein expression. The study was performed on the DU145 prostate cancer cells. For DNA transfection experiments we used gWiz-GFP plasmid. DNA plasmids were stained with fluorescent dyes. For electroporation, electric pulses produced by a high-voltage, exponential-decay pulser were applied to cell suspensions. Treatment with 500 kHz ultrasound was performed using a focused transducer in a water bath. After treatment, cells were washed and analyzed for DNA uptake and GFP expression by flow cytometry 1 and 24 h after treatment. Stained DNA and GFP produced signals at different wavelengths. For RNA transfection experiments we used T7-gwiz-GFP DNA. The needed amount of cRNA was cloned by mMessage mMachine kit (Ambion). Our data showed that DNA delivery and transfection after either electroporation or ultrasound treatment correlated with DNA uptake. However, only a small fraction of cells with DNA uptake expressed GFP, indicated an inefficient process with additional rate-controlling barriers. As a difference between the two transfection methods, electroporation was able to deliver more than an order of magnitude greater intracellular concentrations of DNA molecules inside cells than ultrasound, but DNA transfection by these methods were comparable. The possible explanation for this result could involve more efficient intracellular trafficking of DNA following ultrasound exposure. GFP protein expression from RNA transfection was a process with faster dynamics than DNA transfection. Maximum GFP expression was observed within several hours after transfection, in contrast to DNA transfection, which peak close to 24 h after treatment. Protein expression correlated with intracellular cRNA concentration. The percentage of cells expressing GFP due to RNA expression was lower than due to DNA expression. This may be partly explained by fast degradation of exogenous RNA by cellular enzymes. Conclusions from this study suggest that the bottleneck for DNA transfection by electroporation or ultrasound is DNA delivery to the nucleus after efficient delivery into the cytosol. This is also consistent with observations from the gene therapy literature that transport across the nuclear membrane also limits DNA transfection by other methods. This hypothesis suggests that further optimizing transfection by electroporation or ultrasound may require facilitating intracellular plasmid DNA trafficking. The lack of improvement of transfection efficiency with the exchange of DNA to RNA suggests that additional intracellular barriers may exist.