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比較噴霧及冷凍乾燥過程影響陽電性高分子爲載體之DNA複合體生物活性

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Abstract summary: The possibilities to preserve the transfection potential of PEI/DNA complexes by freeze-drying were studied. Several polymeric protectants were assessed on the spray-drying of plasmid DNA with or without a cationic polymer. With protective agents, we found the topology of plasmid DNA complexed with PEI was remained unchanged but loss of their transfection potential.

Introduction:

The spray-drying process was found to have adverse effects on DNA topology in presence of PEG4000 and PEG10000. With protective agent, PEG35000, the topology of spray-dried DNA was remained unchanged. Spray- drying of PEI/DNA complexes in presence of all studied PEGs preserved their DNA structure integrity. However, these spray-dried PEI/DNA complexes showed no transfection efficiency.

Introduction: Cationic polymer-based gene delivery systems have advantages of giving transient gene expression, non-restrictive for inserted DNA size, relatively cheap in preparation and without concerns of safety risks.[1] In addition to polymeric systems for delivering DNA, ultrasound is an another interesting tool to deliver macromolecules through non-intrusive ultrasonic transmission. Concerning the possible synergism of two methods, bioeffects of ultrasound on mammalian cells (293 cell line) with respect to transfection efficiency and cytotoxicity were investigated in this study.

Experimental methods: Cells were successfully transfected with cationic polymer complexed plasmid DNA particles (polyplexes) after ultrasound (1.5~2.2MHz, 3W/cm^2) transmitted through the well bottom of cell culture plates. A cationic polymer, poly-2(dimethyl amino)ethyl methacrylate (pDMAEMA), were complexed with pCMV-LacZ DNA and ONPG assay was conducted to examine the transfection potential of polymer/DNA particles before and after ultrasonic treatments.

Results and discussion: Results showed that transfection efficiency of pDMAEMA/DNA particles without ultrasonic treatment is dependent to the polymer to DNA ratios. Optimum in transfection efficiency at ratio around 3/1 to 5/1 was observed in agreement of previous findings.[2] Gene expression was increased of 5 folds higher in those cells treated with ultrasound at optimal ratio of 3/1 and 5/1(Fig. 1). Cell viability studies showed that there were no cytotoxic effects toward 293 cells from pulsed ultrasound signals. Also electrophoresis results indicate that tertiary structure of DNA was not affected by ultrasound signal exposed within 90 seconds. The possible cause of the increase in cell transfection might be due to cavitation effects by ultrasound.

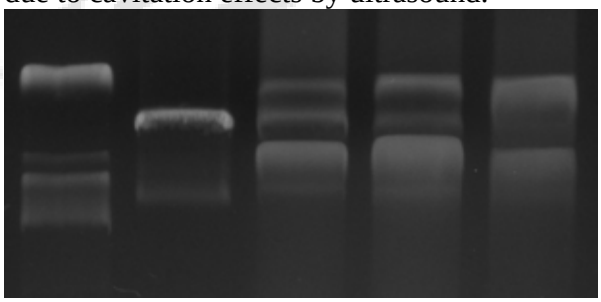
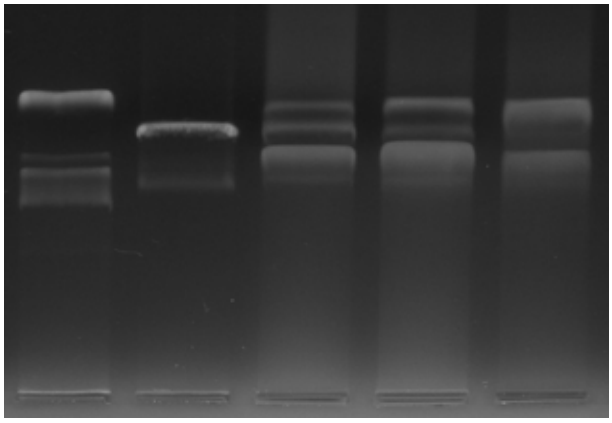


Fig.1 Gene expression of pDMAEMA/DNA complexes before (open column) and after (solid column) ultrasound treatment



Conclusion: This transfection method was verified to be a safe, simple and efficient implementation for an application in clinic (in vivo).

References:

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