

## A Non-Viral Gene Delivery System for Tissue Engineering

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**INTRODUCTION:** The combination of gene delivery with tissue engineering can offer a new approach to growing new tissue. A plasmid DNA can be incorporated into a polymer scaffold and a sustained release may lead to the transfection of a large number of cells which can enhance tissue development<sup>1</sup>. The success of gene therapy is dependent on the efficient and safe delivery of the encoding DNA. In this study DNA was complexed with a polyamidoamine cationic polymer of the methylene-bisacrylamide/dimethylethylenediamine (MBA-DMEDA) series and then the non-viral gene delivery device is embedded in a scaffold. Both freeze dried complex & polymer were placed in an autoclave and the bioactive scaffold was made by using supercritical fluid technology. Assessment of transfection & physico-chemical properties was carried out before and after lyophilising, in order to investigate whether the delicate 3-D architecture of the complex is preserved.

**METHODS:** The cationic polymer was synthesised following the method described by Ferruti<sup>2</sup>. Gel electrophoresis & Photon Correlation Spectroscopy (PCS) were used to look at the physico-chemical properties. Transfection activities of the complexes were assessed on A549 cells using a plasmid that contains the firefly luciferase reporter gene (gWIZLuc). Luciferase detection was performed 48h after transfection. Recoverable cellular protein was measured using the Bradford assay and results were expressed as Arbitrary Light Units (ALU) per mg recoverable cellular protein.

**RESULTS:** The results of the transfection activity are displayed in Fig 1. Transfection levels of the complex MH1 (poly(MBA-DMEDA)) – gWIZLuc in a 5:1 ratio were greater than the negative control (naked DNA). Levels were still lower than the commercial cationic lipid Lipofectamine®. Following freeze-drying a significant loss of transfection efficiency was observed (MH1 0%). Increasing concentrations (5% - 15% w/v) of the cryoprotectant trehalose were added to the complex-solution prior to freeze-drying<sup>3</sup>. After re-hydration transfection activities were measured. Transfection levels could be restored.

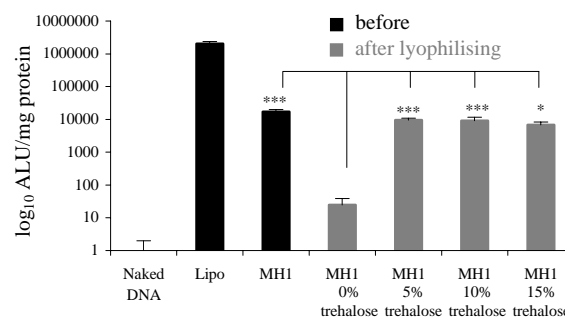


Fig. 1: Level of luciferase activity in A549 cells 48h after exposure to MH1/gWIZLuc complexes. (Statistical significance using the Tukey–Kramer multiple comparison post-test \*\*\* $p < 0.001$ , \* $p < 0.05$ )

The physico-chemical properties were investigated with PCS & gel electrophoresis and correlated to transfection efficiency. The average particle size of the polymer-DNA complex is  $\pm 120$ nm (Fig 2). After freeze-drying a significant increase in size was observed. The size following lyophilising could be reduced by the addition of trehalose.

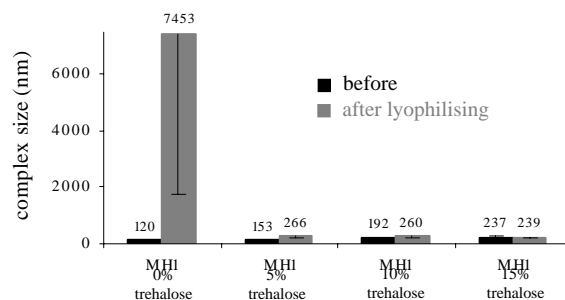


Fig. 2: Particle diameter of MH1/plasmid complexes before & after lyophilising with or without trehalose

**DISCUSSION & CONCLUSIONS:** Dried complexes that exhibited good transfection activity upon re-hydration had sizes comparable to non-lyophilised controls. The polymer/DNA complex required trehalose, a cryoprotectant, to maintain efficient levels of transfection after lyophilisation.

**REFERENCES:** <sup>1</sup>L.D. Shea et al. (1999) *Nat Biotechnol* **17**:551-554. <sup>2</sup>J. Franchini et al. (2004) *J Bioact Compat Polym* **19**:221-236. <sup>3</sup>T.J. Anchordoquy et al. (1997) *Arch Biochem Biophys* **348**(1):199-206.

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