

CHARACTERIZATION OF NOVEL PLL-g-PEG-DNA NANOPARTICLES FOR LOCAL AND CONTROLLED DNA RELEASE

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INTRODUCTION: Deficient angiogenesis is a major clinical incidence and affects wound healing especially in elderly persons and diabetes patients. Many studies and different technologies aim to locally increase blood perfusion and improve the endogenous wound healing capacity and thereby ameliorate the patient's life quality. Since viral-based gene delivery systems are still associated with severe security problems many non-viral gene delivery vehicles were developed [1]. Unfortunately, non-viral DNA delivery vehicles often affect cell viability; therefore our project aimed to design novel DNA-containing-nanoparticles using grafted copolymers of PLL and PEG to increase their biocompatibility and stealth properties.

METHODS: The PLL-g-PEG polymer used in this study was synthesized as described previously [2] and for all experiments a pEGFP-N1-plasmid encoding for green fluorescence protein was used. The DNA-PLL-g-PEG condensates were formed at different P : N ratios and characterized as follows: a) particle size using dynamic light scattering, b) shape and homogeneity using negative staining transmission electron microscopy (TEM), c) *in vitro* transfection efficiency. The PLL-g-PEG-DNA nanoparticles were tested for their *in vitro* gene expression capabilities and cytotoxicity using WST-1 proliferation assay in COS-7 cells.

RESULTS: The PLL-g-PEG-DNA condensates formed particles with a wide range of polymer concentrations (P:N ratios = 1:1 to 1:25), indicating stable particle formation at room temperature. Dynamic light scattering revealed an average hydrodynamic diameter of about 100 nm (Fig. 1) of the PLL-g-PEG-DNA condensates.

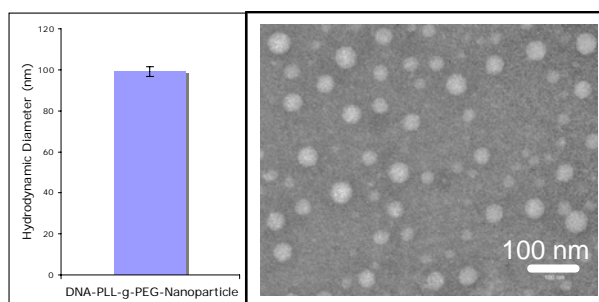


Fig.1: Hydrodynamic diameter of DNA-PLL-g-PEG-nanoparticles obtained by DLS measurement

(left). Negative staining TEM micrograph of the DNA-PLL-g-PEG-nanoparticles (right).

Negative stained TEM micrographs demonstrated that the DNA-PLL-g-PEG nanoparticles have a narrow size distribution and a spherical shape (Fig. 1). Moreover, the DNA-PLL-g-PEG nanoparticles do not aggregate in solution.

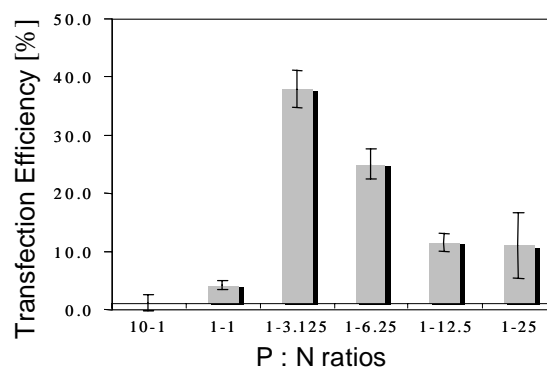


Fig.2: DNA-PLL-g-PEG nanoparticles were formed with different P:N ratios and *in vitro* transfection efficiency was determined on COS-7 cells after 2 days.

The here used DNA-PLL-g-PEG condensates displayed transfection efficiencies comparable with standard transfection agents (DEAE transfection efficiency is about 50 %, not shown). Cytotoxicity was found to be less than 5 % for DNA-PLL-g-PEG nanoparticles formed with P: N ratios between 10:1 and 1:6.25 (not shown).

DISCUSSION & CONCLUSIONS: These novel DNA-PLL-g-PEG condensates are promising candidates for future local and controlled release studies in the treatment of impaired wound healing. As PLL-g-PEG successfully condensed the plasmid DNA into non-aggregated, nano-sized particles that is suitable for cellular uptake [3]. Additionally, PLL-g-PEG-DNA nanoparticles seem to be biocompatible and stealth. All these features are a prerequisite for future somatic gene therapy.

REFERENCES: ¹ Itaka, et al., (2007) Mol. Ther., June 5 (Epub ahead of print). ² VandeVondele, et al., (2003) Biotechnol. Bioeng. Jun 30; 82(7): 784-90. ³ Tiera, et al., (2006) Curr. Gene Ther. Feb; 6(1):59-71.

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