Targeted Gene Delivery Into Peripheral Sensorial Neurons Mediated by Self-Assembled Vectors Composed of Poly(ethylene imine) and Tetanus Toxin Fragment c

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Introduction
Peripheral nervous system (PNS) problems are common and encompass a large spectrum of traumatic injuries, diseases, tumors or iatrogenic lesions. The principle of gene therapy, which relies on the use of DNA as a pro-drug that can lead to the production of therapeutic proteins within specific cells, recently got much attention. A simple, safe and efficient system that can specifically transfect peripheral sensorial neurons can bring new answers to address peripheral neuropathies. Aiming at a therapeutic application, a multi-component non-viral gene delivery vector targeted to peripheral nervous system cells was developed, using poly(ethylene imine) (PEI) as starting material.

Materials and Methods
Branched 25 kDa PEI was thiolated1 (PEISH), which resulted in a 7.8% grafting with 2-iminothiolane. Ternary complexes with different molar ratios of primary amines to phosphate groups (N/P) were prepared by mixing equal volumes of a plasmid DNA (pCMVGFP) solution and PEISH solution in 5% (w/v) glucose pH 7.4. The optimal N/P for the preparation of the binary DNA/polymer complex based on PEISH was found to be 3, considering complex size and zeta potential and the ability to transfect a sensorial neuron cell line (ND7/23). Subsequently, different amounts of pegylated tetanus toxin fragment (HC-PEG; a bifunctional 5 kDa PEG bearing an NHS and a MAL group (JenkUSA)) were grafted on the surface of the DNA-PEISH complexes. ND7/23 or NIH3T3 (mouse embryonic fibroblast) cells were subcultured 24 hrs prior to internalization/transfection studies in supplemented DMEM. YOYO-1 (Invitrogen) labeled complexes were left in contact with cells at 37°C and processed for FACS. 48, 72 and 96 hrs post-transfection, GFP+ cells were determined by FACS. The best performing ternary complex was tested in primary cultures of dissociated dorsal root ganglia (DRG).

Results and Discussion
The 50 kDa non-toxic fragment from tetanus toxin (HC), which has been previously shown to interact specifically with peripheral neurons and to undergo retrograde transport, was grafted to the complex core via a bi-functional PEG (HC-PEG) reactive for the thiol moieties present in the complex surface. Several formulations of HC-PEG ternary complexes were tested for targeting, by assessing the extent of cellular internalization and levels of transfection, in both the ND7/23 and NIH 3T3 cell lines. Targeted gene transfer to the neuronal cell line was observed for the complex formulations containing 5 and 7.5 µg of HC-PEG (per 2 µg of plasmid DNA). The latter formulation was able to transfect primary cultures of DRG dissociated neurons in a targeted manner and elicit the expression of a relevant neurotrophic factor (BDNF - brain derived neurotrophic factor; Fig. 1). The testing of these systems in vivo is underway in the rat animal model.

Fig. 1. BDNF secretion (24-hr period) in dissociated DRG cultures mediated by PEISH complexes with or without HC-PEG (72 hrs post-transfection). Naked plasmid was used as control.

Reference
Carlisle, R. et al. J. Gen Med. 6:337-344, 2004

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