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Research paper

A comparison of the effectiveness of cationic polymers poly-L-lysine (PLL) and polyethylenimine (PEI) for non-viral delivery of plasmid DNA to bone marrow stromal cells (BMSC)

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Abstract

Bone marrow stromal cells (BMSC) represent an important cell phenotype for pursuit of successful gene therapy. Non-viral methods to enable expression of exogenous genes in BMSC will accelerate clinical application of gene therapy, without the concerns associated with the viral means of gene transfer. Towards this end, this study investigated the potential of cationic polymers poly-L-lysine (PLL) and branched polyethylenimine (PEI) as gene carriers for modification of BMSC. Both polymers rapidly (~30 min) condensed a 4.2 kb Enhanced Green Fluorescent Protein (pEGFP-N2) plasmid into 100–200 nm particles. PLL and PEI were both readily internalized with BMSC with >80% of BMSC exhibiting polymer uptake by flow cytometric analysis. The relative uptake of PEI, however, was significantly higher as compared to the PLL. The majority of the BMSC (>60%) exhibited nuclear presence of the polymers as analyzed by fluorescent microscopy. Although both polymers were able to deliver the pEGFP-N2 into the cells under microscopic evaluation, only a small fraction of the cells (<10%) displayed nuclear localization of the plasmid. Consistent with better uptake, PEI gave a higher delivery of pEGFP-N2 into the BMSC, which resulted in a more sustained expression of the model gene EGFP in short-term (7-day) culture. We conclude that both PLL and PEI readily displayed cellular uptake, but PEI was more effective in delivering plasmid DNA intracellularly, which was likely the underlying basis for a more sustained gene expression.

Keywords: Non-viral gene delivery; Cationic polymers; Bone marrow stromal cells; DNA binding; DNA uptake; Flow cytometry

1. Introduction

Cells derived from the bone marrow are being actively pursued in human gene therapy protocols [1,2]. Bone marrow stromal cells (BMSC) are readily harvested from patients, obviating the ethical concerns associated with the use of embryonic stem cells in clinics. The harvested and modified BMSC can be grafted into the host without systemic immunosuppressants, an important consideration that limits application of the therapy to specific patient populations. Significant efforts were recently devoted to BMSC expansion *ex vivo* [3], and it is now a practical procedure to achieve a clinically sufficient cell mass using the current cell expansion technologies. Moreover, BMSC can be manipulated during *ex vivo* culture to direct them into multiple lineages, including osteoblastic, chondrogenic, adipogenic, and myogenic phenotypes [4]. Such a manipulation of BMSC enables construction of functional tissues suitable for tissue replacement in a host. Un-differentiated BMSC, due to their plasticity, may also augment

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the functioning of intact but diseased tissues, such as the case of BMSC injections to alleviate cardiac failure [5].

The modification of BMSC has been primarily achieved with viral vectors. Some studies reported almost complete modification of BMSC with reporter genes, such as Green Fluorescent Protein (GFP) genes, by using retroviral vectors on mouse-derived (96–98%, [6]), and rat-derived BMSC (90–95%, [7]). Other studies reported lower extent of GFP transfections (based on flow cytometric analysis) by retroviral vectors, ranging from relatively low levels of ~5% for mouse BMSC [8], to intermediate levels of 20–30% for mouse BMSC [9,10], to high levels of 60–80% for mouse, canine and human BMSC [10–12]. Other viral vectors, such as lentiviral and adenoviral vectors, similarly exhibited a wide range of (25–80%) transfection efficiencies in BMSC [13,14]. Our experience with an adenoviral vector delivery was lower than these reported values (10–15%) [15]. Unlike the viral vectors, non-viral vectors offer better safety profiles in a clinical setting, but little quantitative information was reported on the transfection efficiency of non-viral vectors in BMSC. A ~5% efficiency was reported for a lipid-based (FUGENE™) carrier in one report [16] and a higher transfection efficiency (5–17%) was reported for the liposomal Lipofectamine™ in rat BMSC [17]. A cationized gelatin carrier was not effective for modification of rat BMSC, but cationic polymer polyethylenimine (PEI) provided 4–12% cell modification after 48 h of transfection [17]. These results were indicative of lower effectiveness of the non-viral methods of gene transfer; however, due to heavy reliance on transgene expression as an end-point, these studies yielded little information about the limitations of non-viral methods.

In this study, the potential of cationic polymers to deliver exogenous genes to BMSC was further investigated. The use of polymers as gene carriers, as opposed to lipid-based carriers, might offer an important advantage if one wishes to engineer the carrier properties to further control the intracellular trafficking of polymers, for example, to facilitate endosomal escape or to protect against intracellular nucleases. Two cationic polymers commonly used for delivery of plasmid DNA, namely PEI [18] and poly-L-lysine (PLL) [19], were used in this study as the polymeric carriers. Both polymers have a high cationic charge density necessary for DNA condensation, but PEI additional exhibits membrane-perturbing activity necessary for escape of internalized DNA from endosomal compartment. Despite their relatively long history of use for modification of immortal cells, only one study reported the use of PEI on BMSC in a short-term study [17], and no studies reported the use of PLL on BMSC. This study systemically compared the cellular internalization of both polymers, as well as their ability to transport a model plasmid (pEGFP-N2) into the BMSC. The relative effectiveness of PEI and PLL to enable EGFP expression was compared in short-term (~7-day) culture. Our results indicated a more preferential uptake of PEI by BMSC, as well as a better delivery of plasmid DNA intracellularly, resulting in a more sustained expression of the model gene EGFP.

2. Materials and methods

2.1. Materials

Branched PEI (25 kDa), PLL hydrobromide (25 kDa), 5% (w/v) 2,4,6-trinitrobenzosulfonic acid (TNBS), Hanks' balanced salt solution (HBSS), and trypsin/EDTA were obtained from SIGMA (St. Louis, MO). Dulbecco's modified Eagle's medium (DMEM; high glucose with L-glutamine), penicillin (10,000 U/mL), streptomycin (10,000 μg/mL) and Lipofectamine-2000™ were from Invitrogen (Carlsbad, CA). Dialysis tubing with a MW cut-off of 12–14 kDa was purchased from Spectrum Laboratories (Gardena, CA). Fetal Bovine Serum (FBS) was obtained from Atlanta Biologicals (Lawrenceville, GA). A succinimide ester of Cy5.5 (Cy5.5-NHS) used for labeling of plasmid DNA was obtained from AMERSHAM (St. Laurence, QC). Fluorescein isothiocyanate (FITC) and AlexaFluor-350 (AF-350) were purchased from PIERCE (Rockford, IL) and Molecular Probes (Portland, OR), respectively. A 4.7 kb plasmid incorporating an enhanced green fluorescent protein (pEGFP-N2) and a kanamycin resistance gene was obtained from BD Biosciences, and replicated in kanamycin resistant DH5-α *E. coli* strain grown in Luria–Bertani medium [15]. The purified plasmid was dissolved in ddH_2O at 0.4 mg/mL.

2.2. Atomic force microscopy (AFM)

The MultiMode scanning probe microscope (Digital Instruments Inc., Santa Barbara, CA) was used for all AFM studies. A large-area scanner (J type) with a maximum *xy* scan range of 125×125 μm and a *z* vertical range of 5 μm was used, except for imaging naked plasmid DNA, where a small-area scanner (A type) with a maximum *xy* scan range of 0.4×0.4 μm and a *z* vertical range of 0.4 μm was used for higher resolution. Single crystal silicon cantilevers were cleaned by exposure to high intensity UV light for 3 min before use. The oscillation amplitude of the scanning tip was registered at 0.5 V and the frequency of the oscillation was in the range of 200–400 kHz. All AFM imaging was conventional ambient tapping mode AFM. The scan rate was typically 1.0–2.0 Hz and the data collection was at 512×512 pixels. Images were processed and analyzed using the Nanoscope III software (V5.12).

To visualize naked pEGFP-N2, the plasmid solution at 0.4 mg/mL was diluted to 2.5 μg/mL with 3 mM NaCl (in ddH_2O), vortexed for 10 s, and 10 μL of this solution was transferred onto freshly cleaved mica. After 3 min, the mica disk was dipped into water and excessive water was removed by filter paper. After drying at room temperature for 30 min, the surface was blow-dried with N_2 (if necessary). To visualize pEGFP-N2/polymer complexes, the plasmid DNA and the polymers were dissolved separately in 3 mM NaCl solution. Calculated amount of plasmid DNA and PEI solutions was transferred to a tube main-