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Modification of human BMSC with nanoparticles of polymeric biomaterials and plasmid DNA for BMP-2 secretion

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ABSTRACT

Background: Genetic modification of human bone marrow stem cells (hBMSCs) before administration to a patient is emerging as a viable approach to creating tailored cells that perform effectively in a clinical setting. To this end, safe delivery systems are needed that can package therapeutic genes into nanoparticles for cellular delivery.

Methods: We evaluated different plasmids on gene expression and compared the effective plasmids directly in hBMSCs. Then, we evaluated the transfection efficiencies of the polymeric carriers linoleic acid-substituted polyethylenimine (PEI-LA), polyethylenimine (PEI)-25, and PEI-2 using flow cytometry. We used 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to compare the toxicity of PEI-LA and PEI-25 on hBMSCs. We further assessed bone morphogenetic protein-2 (BMP-2) secretion and the osteogenic activity of hBMSCs transfected with the polymeric (PEI-LA and PEI-25) gWIZ-BMP-2 complex.

Results: Unlike the transformed cells that gave robust (>50%) transfection, only a few percent (<10%) of hBMSCs was transfected by the developed nanoparticles in culture. The plasmid DNA design was critical for expression of the transgene product, with the choice of the right promoter clearly enhancing the efficiency of transgene expression. Using the in-house designed PEI-LA, hBMSCs secreted BMP-2 in culture (~4 ng BMP-2/10^6 cells/d), which indicates the feasibility of using PEI-LA as a delivery system. Furthermore, we demonstrated an increased osteogenic activity *in vitro* for hBMSCs transfected with the PEI-LA containing the BMP-2 expression system.

Conclusions: These results provide encouraging evidence for the potential use of a low toxic PEI-LA to genetically modify hBMSC.

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1. Introduction

Human bone marrow stem cells (hBMSCs) are currently under intense investigation as the basis of cell-based therapies for a variety of orthopedic deficiencies [1,2]. These cells have been the focus of interest in the field of regenerative medicine, not only for their differentiation and self-renewal potential, but also for their high capacity of directional migration and immunomodulatory effects [3,4]. Genetic modification of hBMSCs before administration to a patient (i.e., *ex vivo* gene therapy) has further emerged as an effective approach to create tailored cells that perform more effectively in a clinical setting [5,6]. By acting as a sustained delivery vehicle for desired therapeutic proteins, hBMSCs can direct specific cellular processing, ultimately stimulating the healing process at a bone site [7,8]. The success of gene modification depends largely on the development of a carrier that can efficiently deliver a target gene to hBMSCs with minimal toxicity [9,10] To this end, nonviral gene carriers have received recent attention because of their safety, flexibility in chemical design, and large capacity for vector delivery [11,12]. Nonviral gene carriers enable condensation of long, string-like plasmid DNA (pDNA) into nanosize particles that allow their cellular internalization. Nonviral carriers are generally less effective for gene transfer to hBMSCs compared with viral systems, however, clinical application of nonviral carriers is safer and can be more widely practiced without concerns about immunogenicity and adverse reactions [13].

The hBMSCs are particularly difficult to transfect with exogenous pDNA, and viral transfections can give consistently high transfection efficiencies in the range of 50%–80% of the cell population [14,15] Careful optimization of physical delivery methods, such as electroporation, in which electric pulses temporarily allow pDNA entry into cells, has been shown to give similar transfection efficiencies (40%–80%) [16,17]. The cationic lipid Lipofectamine 2000 and the hydrophobic peptide palmitic acid-arginine$_{15}$ have also been used on hBMSCs; lower transfection efficiencies in the range of 2%–35% [18] and ~10% [19], respectively, have been obtained with these carriers. Although generally considered safer than viral carriers, both electroporation and cationic liposomes have both displayed significant toxicities in cells; therefore, it is necessary to develop new nonviral carriers that are both highly efficient and nontoxic to hBMSCs. We previously reported an amphophilic polymeric carrier, linoleic acid-substituted polyethylenimine (PEI-LA), which was derived from a nontoxic 2-kDa polyethylenimine (PEI) and the fusogenic lipid linoleic acid [20,21]. The PEI-LA gave superior transgene expression *in vitro* in rat BMSCs using the reporter gene, Green Fluorescent Protein (GFP) [22]. The pDNA/polymer nanoparticles were also effective in a rat subcutaneous implant model [23], in which a bone morphogenetic protein (BMP)-2 expression vector (BMP-2-IRES-AcGFP) was successfully expressed after implantation of the nanoparticles. The critical issue of the effectiveness of this nonviral delivery system for modification of hBMSCs with a therapeutically relevant transgene has not been explored to date.

In this study, we focused on this issue and explored the utility of PEI-LA to modify hBMSCs obtained from patients undergoing orthopedic (arthroplasty) surgery. We first assessed the transfection efficiency of several expression vectors in immortal 293T cells and hBMSCs. We then investigated the feasibility of BMP-2 expression and osteogenic differentiation in hBMSCs *in vitro* using a custom-tailored expression vector (gWiz-BMP-2) delivered by PEI-LA.

2. Materials and methods

2.1. Materials

We obtained Dulbecco's modified Eagle's medium (DMEM; high glucose with L-glutamine), Lipofectamine 2000, Optimem Hank's balanced salt solution (HBSS), and penicillin-streptomycin (10,000 U/mL to 10,000 μg/mL) from Invitrogen (Grand Island, NY). Fetal bovine serum (FBS) was obtained from Atlanta Biologics (Lawrenceville, GA). We purchased the CyQUANT cell proliferation kit for DNA assay from Molecular Probes (Portland, OR). We also obtained 3-(4,5-dimethylthiazol-2-*yl*)-2,5-diphenyltetrazolium bromide (MTT), alkaline phosphatase (ALP) substrate *p*-nitrophenol phosphate, 8-hydroxyquinoline, *o*-cresolphthalein, 2-amino-2-methyl-propan-1-*ol*, dexamethasone, glycerolphosphate, calcium standards kit and ascorbic acid from Sigma (St. Louis, MO). We reconstituted the BMP-2 stock solution (Wyath Pharmaceutics, Inc.) in ddH_2O. Recombinant human basic fibroblast growth factor (bFGF) was obtained from the Biological Resource Branch of NCI-Frederickton (Bethesda, MD). The human BMP-2 enzyme-liked immunosorbent assay (ELISA) kit was from R&D Systems (Minneapolis, MN). We prepared the polymeric carrier PEI-LA (2.1 LA substitution per PEI) in house, as described previously [21].

2.2. Expression vectors

We obtained the blank (control) gWIZ plasmid and its GFP-expressing derivative gWIZ-GFP from Aldevron (Fargo, ND). Commercially available BMP-2 expression vector pCMV6-XL4-BMP-2 was from Origene (Rockville, MD). Our first BMP-2 expression plasmid (BMP-2-IRES-AcGFP) was derived from the commercially available pIRES2-AcGFP (Clontech, Mountain View, CA), described previously [23]. The PCAG-emerald GFP (emGFP) plasmid, in which the emGFP was subcloned into a plasmid with chicken β-actin promoter and cytomegalovirus (CMV) enhancer, was a kind gift from Dr. Peter Kwan (University of Alberta, Canada). We constructed a new BMP-2 expression vector, gWIZ-BMP-2 (Fig. 1), by subcloning the BMP-2 fragment from pCMV6-XL4-BMP-2 into gWIZ. This is done by restriction digest of both plasmids with the NotI; digested gWIZ was treated with Antarctic phosphatase (New England Biolab, Ipswich, MA) to remove 5′-phosphate and minimize self-re-ligation. Both the vector and insert DNA fragments were purified with the QIAquick PCR Purification Kit (Qiagen, Ontario, Canada) from the enzymatic reactions before ligation with T4 DNA ligase (New England Biolab). We then transformed ligated plasmids into competent *Escherichia coli* cells (DH5α; Life Technologies, Ontario, Canada), according to the manufacturer's protocol, and then grew them onto Luria broth plates supplemented with 30 μg/mL kanamycin.