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Gene delivery to human embryonic-derived stem cells using biodegradable Poly(β -amino esters) nanoparticles

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Introduction: Gene delivery to stem cells holds great potential for tissue regeneration and delivery of therapeutic Proteins. The major barrier is the lack of safe and efficient delivery methods. Non-viral vectors based on the use of cationic polymers appear to have promising potential, given the problems of safety encountered with viral vectors. . This study is a description of generating VEGF high-expressing stem cells using biodegradable polymer–DNA nanoparticles. Poly(β -amino esters) are a kind of biocompatible polymers that are able to interacted electrostatically with polyanionic plasmid DNA in water and buffer at physiological pH forming nanoparticles suitable for gene delivery applications..

Methods: Poly(β-amino esters) were synthesized after a two-step procedure, in which C32-Ac was first prepared by polymerization by using excess diacrylate over amine monomer, and C32-Ac was then reacted with various amine reagents to yield end-modified C32 polymers: C32-103, C32-117 and C32-122.

Passage 3 hESCds were seeded in 6-well plates at 300000 cells per well 24 h before transfection. Cells were transfected with DNA plasmid-encoding human *VEGF* 165 (pBLAST49-hVEGF).Transfection using end-modified C32 polymers (C32-103, 117 and 122) were performed using optimized condition (20w/w polymer/DNA weight ratio, 12 μg DNA per well in 6-well plate) in growth medium containing 10% serum. Cells were also transfected with VEGF using Lipofectamine 2000 for comparison. Following a 4-h transfection incubation time, the complexes were removed and refilled with 3 ml endothelial growth medium.Cells incubated with endothelial growth medium containing 2 ng/ml VEGF growth factor and untreated cells incubated in endothelial growth medium were included as controls.

Total RNA was extracted from cells and the cDNA was synthesized. Quantitative PCR was performed and Endothelial differentiations were examined using Taqman Gene Expression Assays including *PECAM* ، *Tie2* ، *vWF* .

Results: After 2 weeks of culture in endothelial growth medium, all hESCds transfected with VEGF using end-modified C32 polymers expressed significant higher levels of endothelial markers than the untreated control group. For the C32-103 transfected group, the expression levels of *Tie 2* and *vWF* were, respectively, 3.2- and 2.3-folds higher than the untreated controls. Compared to the untreated control, the gene expressions of *PECAM* and *vWF* in the C32-117 transfected group were, respectively, 4.7- and 4.9-folds higher; and gene expressions of PECAM and vWF in the C32-122 group were 6.5- and 7.4-folds higher. Lipofectamine 2000 transfected group showed a slight increase in the gene expressions of all three endothelial markers, and the differences were not statistically significant. The control group incubated with VEGF growth factor also showed some increase in the expressions of all three markers, with 83 and 62% increase in *PECAM* and *Tie-2*, and 1.3-fold increase in vWF expression compared with the untreated control.

Conclusion: we have shown that poly(β-amino esters), and end-modified C32 polymers Specifically, are a class of highly efficient nonviral transfection vectors for gene delivery to embryonic-derived stem cells.

Keywords: stem cells , polymeric vectors

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