Ex Vivo Magnetofection with Gene/magnetic-nanoparticles Complex Enveloped with Liposome: A Novel Platform For non-viral Tissue Engineering

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INTRODUCTION

Bone tissues lost affect many individuals and account for enormous associated costs. Several gene therapy approaches were developed aiming to achieve bone tissue regeneration, most of them were based on viral gene delivery of osteogenic factors. The limitations of viral-based approaches have been extensively reported mostly dealing with the humoral immune response generated against the viral proteins, mutagenesis and carcinogenesis. [Scherer F, et al. 2002; Huth S, et al. 2004]. One of the promising methods is magnetofection, which associates genes/DNA with magnetic particles coated with cationic molecules. Green fluorescence proteins (GFPs) are responsible for the green bioluminescence from the jellyfish Aequorea victoria. In this study, we assessed the method of gene transfer to osteoblast using the Fe3O4 nanoparticles carrying a gene encoding green fluorescence protein (GFP).

MATERIALS AND METHODS

The hydrophilic vector (hereafter referred as liposome) is 1,2-Dioleoyl-3-trimethylammonium-propane: 1,2-dioleoyl-3-sn-phosphatidyl-ethanolamine (DOTAP:DOPE), which is first mixed in organic solution and then dried. The dried DOTAP: DOPE is added into water-based magnetic fluid and is sonicated. Thus, the magnetic nanoparticles Fe3O4 exactly in the water are enveloped with liposome. The water-based magnetic fluid used here is synthesized via chemical co-precipitation method [Jiang WQ, et al. 2004]. Then, the transfected gene/DNA is added into the solution having liposome coated magnetic nanoparticles. The gene/DNA can transport through the liposome shell and be with magnetic nanoparticles. Un-enveloped gene/DNA is separated from the liposome-enveloped gene/DNA-Fe3O4 via magnetic separation. By using suitable vectors, gene/DNA can be released from the enveloping vectors in cells. The magnetic nanoparticles are Fe3O4, and the transfected genes are Lac Z and enhanced green fluorescent protein (EGFP) gene. An ELISA reader is used to detected the fluorescence of EGFP in transfected cells. An excitation light of 485 nm in wavelength is radiated onto cells, the intensity of the emitted light of 528 nm in wavelength from EGFP in cells of each well is detected. Equal-amount of cells are laid on the bottoms of culture wells and emerged with medium. Each osteoblasts cells culture well is added with 80-µl liposome-enveloped gene-Fe3O4 complex, in which there is 1-µg Lac Z gene. Then, these wells are divided into two groups. Group I is located above 3500-Gauss magnets for 5 minutes, while Group II is under zero magnetic field. After 10 days’ culture, the images of cells were taken. Another example given here is to magnetofect enhanced green fluorescent protein (EGFP) gene (BD, PT3465-5, Catalog#6342-1, USA) into human HE299 cells. The magnetic-particle-gene/DNA complexes are transported into cells under external magnetic fields. It has been demonstrated that magnetofection is nontoxic, high-efficiency, and versatile. Therefore, the studies on magnetofection attract much attention of scientists, engineers, and medical doctors. In summary, we synthesize the magnetic vectors for magnetofecting gene/DNA into cells with an aid of magnetic force. The vector consists of hydrophilic shells, which are electrically opposite to the gene/DNA to be magnetofected, e.g. DOTAP:DOPE liposome. Instead of exposing gene/DNA outward in the solution, the gene/DNA and magnetic nanoparticles are enveloped inside the vectors. The vectors release gene/DNA when transported into cells, and then the genes are magnetofected to the chromosomes in nuclei. Under a gradient magnetic field, the magnetofection efficient of the gene/DNA into cells is enhanced as compared to that under zero field. Examples of magnetofection on osteoblasts using the synthesized magnetic vectors.

REFERENCES


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RESULTS

The diameter distribution of synthesized liposome shell is analyzed by using dynamic laser scattering method (Microtrac Nanotrac-150). The result found that the diameter of liposome shell is 64.0 ± 8.4 nm. When magnetic nanoparticles, which mean diameter is around 25 ± 5.7 nm, are inserted into liposome shell via sonication, the diameter of liposome coated magnetic nanoparticles becomes 66.2 ± 9.5 nm. Finally, for the liposome-enveloped gene/DNA-magnetic-nanoparticle complex, the diameter is measured as 67.5 ± 9.3 nm. The complex shows almost the same size as the original liposome shell. The Group I exhibits a much higher efficiency for magnetofecting Lac Z into osteoblasts cells. This evidences the validity of magnetofection using the synthesized composition of liposome-enveloped Lac Z-Fe3O4 complex. It was observed that the sets added with home-made liposome-enveloped EGFP gene-Fe3O4 complex (iii) in Group I shows a higher transfection rate than that of the same set (iii) in Group II. This reveals that, under zero field, the transfection can be enhanced with magnetic nanoparticles because the liposome bring magnetic nanoparticle in set (iii) shows a larger specific gravity than that of liposome only for the sets added with commercial liposome-enveloped EGFP gene (ii). Under the action of gravity force, the vectors possessing magnetic nanoparticles are much more distributed around the bottom of wells, where cells are. Thus, a higher possibility of magnetofection is achieved. When a magnetic force is applied to push these magnetic vectors downward the well bottom, the transfecting rate is further speed.

DISCUSSIONS

The magnetic-particle-gene/DNA complexes are transported into cells under external magnetic fields. It has been demonstrated that magnetofection is nontoxic, high-efficiency, and versatile. Therefore, the studies on magnetofection attract much attention of scientists, engineers, and medical doctors. In summary, we synthesize the magnetic vectors for magnetofecting gene/DNA into cells with an aid of magnetic force. The vector consists of hydrophilic shells, which are electrically opposite to the gene/DNA to be magnetofected, e.g. DOTAP:DOPE liposome. Instead of exposing gene/DNA outward in the solution, the gene/DNA and magnetic nanoparticles are enveloped inside the vectors. The vectors release gene/DNA when transported into cells, and then the genes are magnetofected to the chromosomes in nuclei. Under a gradient magnetic field, the magnetofection efficient of the gene/DNA into cells is enhanced as compared to that under zero field. Examples of magnetofection on osteoblasts using the synthesized magnetic vectors.

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