[PE3-1] [10/18/2002 (Fri) 13:30 - 16:30 / Hall C]

Effect of Polyethylenimine Type in Polylactic acid Nanoparticles/DNA Complex on the Transfection Efficiency

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Nanoparticles of polylactic acid (PLA) and polyethylenimine (PEI) as an effective gene delivery agent were prepared and characterized. As a model plamid DNA, PME185/β-gal, a mammalian expression vector, and fluorescence enhancing protein (pEGHP) were used. The effects of PEI type on the physical properties of nanoparticles and transfection efficiency were examined.
Nanoparticles prepared by a solvent evaporation method were shown as a discrete and spherical shape having an average diameter of about 250 nm. The PLA/PEI-25000 and PLA/PEI-750000 nanoparticles showed the positive zeta-potential value, while PLA and PLA/PEI-800 nanoparticles showed the negative value. Complex formation between nanoparticles and plasmid DNA was analyzed by gel electrophoresis. Transfaction efficiency was measured by the detection of β-gal activity and fluorescence image. Complex of PLA/PEI-25000 with DNA showed greater transfection efficiency than PLA/PEI-800 or PLA/PEI-75000 complex.

[PE3-2] [10/18/2002 (Fri) 13:30 - 16:30 / Hall C]

Thermo-sensitive lipid nanoparticles as a novel topical delivery system of retinol

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The purpose of this study was to prepare thermo-sensitive solid-lipid nanoparticles (SLNs) with a lipid melted at human body temperature and to evaluate physicochemical properties of SLNs containing retinol, anti-wrinkle agent, as a model drug. SLNs were prepared using a high pressure homogenizing method. The SLNs were composed of retinol as a model drug, thermo-sensitive lipid (DS-CBS) as a lipid core, and egg phosphatidylcholine and Tween 80 as surfactants. Manufacturing variables such as homogenization pressure, homogenization cycles and cooling temperature were optimized. The thermo-sensitivity of SLNs was evaluated by using a VP-DSC Micro Calorimeter. Drug encapsulation efficacy, particle size distribution and zeta potential were determined after preparation. The chemical stability of retinol incorporated in SLNs was evaluated by HPLC determination. The physical stability was evaluated by monitoring the changes in the mean size and zeta potential of SLNs. To confirm the thermo-sensitivity, the release of retinol from SLNs was compared at 4℃ and 37℃, respectively. By optimizing the manufacturing variables, retinol could be incorporated in SLNs at the concenration of 10 mg/ml. The mean particle size was 229 nm and zeta potential was -14.56 mV. The chemical stability of retinol could be significantly improved by SLNs formulation. The transition melting peak of retinol-loaded SLNs appeared at 33.6℃, close to human body temperature. The release of retinol from SLNs was minimal at 4℃, but greatly enhanced at 37℃, suggesting the thermo-sensitivity of SLNs. These results suggest that the use of thermo-sensitive SLNs could be potential for the topical delivery of retinol.

PE3-3] [10/18/2002 (Fri) 13:30 - 16:30 / Hall C]

In vitro and in vivo transfection efficiency of a cationic lipid containing sodium cholate

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Cationic lipids have been used as one of the major components for making most promising non-viral gene delivery systems, whereas sodium cholate, an edge activator has been used as a surfactant in making ultradeformable and ultraflexible liposomes called Transfersomes. Using both a cationic lipid, DOTAP and sodium

cholate, a newly formulated ultradeformable cationic liposome has been prepared. The average particle size of this formulation was approximately 80nm. The in vitro transfection efficiency of plasmid DNA was assessed by the expression of green fluorescent protein (GFP) in four cell lines, OVCAR-3 (human ovarian carcinoma cells), HepG-2 (human hepatoma cells), H-1299 (human lung carcinoma cells) and T98G (human brain carcinoma cells). The optimal ratio of DNA to liposome for maximal transfection efficiency was 1:14 (w/w) in all the cell lines except the human brain carcinoma cells. The same formulation was tested for in vivo transfection efficiency by complexing it with genetic material(GFP) and applying them on dorsal skin of mice non-invasively. It was found that genes were transported into several organs once applied on intact skin, especially kidney was the organ with the most GFP.

[PE3-4] [10/18/2002 (Fri) 13:30 - 16:30 / Hall C]

Polyethylene glycol (PEG)-modified cationic liposome mediated gene delivery

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In this study, we modified the cationic liposomes by polyethylene glycol (PEG)-grafted or PEG-added methods. The PEG-grafted transfection complexes were prepared by adding the plasmid DNA to the PEG-grafted cationic liposomes, composed of PEG and cationic lipids. PEG-added transfection complexes were prepared by adding the PEG to the mixture of cationic lipids and plasmid DNA. The particle sizes of PEG-modified transfection complexes did not change during storage compared to conventional transfection complexes. In the presence of serum, the expression of green fluorescent protein of conventional liposome/DNA decreased but PEG-modified transfection complexes maintained high transfection efficiency in the presence of serum. The transfection efficiency of conventional transfection complexes was significantly decreased in storage but the PEG-modified transfection complexes maintained their transfection properties after 2 weeks. After administration of the conventional, PEG-grafted and PEG-added liposome/DNA complexes into the mice by tail vein, the plasmid DNA in the blood was analyzed by PCR. PEG-added transfection complexes were showed even longer plasmid DNA circulation than PEG-grafted or conventional cationic liposomes. These results suggest that the PEG-added transfection complexes could be a promising nonviral vector because it is easy to make and has a high transfection efficiency and stability in vitro and in vivo.

[PE3-5] [10/18/2002 (Fri) 13:30 - 16:30 / Hall C]

Nonviral Vector for Efficient Gene Transfer to Human Ovarian Adenocarcinoma Cells

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Various strategies have been attempted to design efficient protocols for ovarian cancer gene therapy but there has been little progress in their clinical application. In this study, we formulated and evaluated a new cationic liposome composed of dioleoyltrimethylaminopropane (DOTAP), 1,2-dioleoyl-3-phosphophatidylethanolamine (DOPE), and cholesterol (Chol) (DDC) for plasmid DNA transfer into ovarian cancer cells. The DDC liposome was prepared by mixing DOTAP, DOPE, and Chol using extrusion method. Plasmid DNA (pEGFP-C1) and DDC were complexed at various ratios to find the optimum condition and the percentage of transfected cells was determined by flow cytometric analysis. The transfection efficiency of the DDC liposome was compared with 3 [N-(N,N-dimethylaminoethylene) carbamoyl] cholesterol (DC-Chol)/DOPE liposome and commercially available lifopectin. The optimal transfection of plasmid DNA was achieved at a 1:4 (w/w) ratio of DDC to DNA. The DDC/DNA complex exhibited higher transfection efficiency in human ovarian cancer cell lines compared to that in other types of cell lines. Flow cytometry revealed that of formulations, the DDC/DNA complex exhibited an over 4-fold increase in GFP expression levels in OVCAR-3 cells, further confirmed by confocal microscopy and RT-PCR. These results suggest that new cationic liposome could be a promising nonviral vector for treating ovarian adenocarcinoma because of its selective high gene transfer ability in ovarian cancer cells.

[PE3-6] [10/18/2002 (Fri) 13:30 - 16:30 / Hall C]

Enhancement of Emulsion-mediated Gene Expression by Using Chitosan as a Pre-Condensing Agent