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RESEARCH ARTICLE

Dye-Doped Silica Nanoparticle with HIV-1 TAT Peptide for Bioimaging

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Multifunctional silica nanoparticles for bioimaging and drug delivery have progressively been developed as novel modalities for cancer diagnosis and treatment. In this study, we synthesized fluorescein isothiocyanate (FITC) doped silica nanoparticle coated with 3-aminopropyl trimethoxysilane (APTS). Additionally, the surface of this silica nanoparticle was modified with HIV-1 *trans*-activating transcriptional activator (HIV-1 TAT) peptide. The HIV-1 TAT peptide (cell-penetrating peptide) is able to deliver cargos such as oligonucleotides and proteins across the plasma membrane of living cells. This study demonstrates the feasibility of TAT peptide coated silica nanoparticle as a potential carrier for bioimaging and drug delivery.

Keywords: Dye-Doped Silica Nanoparticle, Cell Penetrating Peptide, HIV-1 TAT Peptide, Bioimaging, Fluorescein Isothiocyanate.

1. INTRODUCTION

Silica nanoparticle (SiNP) has been progressively used in fields of drug delivery system. The existence of silanol groups on the surface easily grants a functionalization.[1] There are several advantages of dye-doped SiNP for bioimaging.[2,3] The SiNP is not toxic to healthy cells and easy to be synthesized. And it can be easily modified with some biomaterials on the surface of particle. Moreover, dye-doped SiNP provides high luminescent signals due to the high quantum yield of the dye molecules and numerous dye molecules inside the particle.[4] Due to all these properties, the dye-doped SiNP is used as outstanding labeling agent for cell imaging and drug delivery vehicle.

As therapeutic drug/gene alone cannot penetrate the cell membrane, efficient vehicles are needed. HIV-1 TAT peptide is rich in cationic residues and specially, arginines. Interestingly, a multiplicity of arginines/guanidines is a shared factor among many other cell penetrating peptides.[5] Also HIV-1 TAT peptide has low cytotoxicity, high profit of delivery and in the hereafter might become a extensively used new tool in the area of biology and nanomedicine.[6] Efficient delivery of diagnostic and therapeutic reagents across the cell membrane is essential in developing novel cancer therapies. Membrane penetrating peptides, in particular HIV-1 TAT peptide, have enabled the delivery of cargos of different shapes, sizes and physical properties into the cell. A diversity of intracellular cargos has been delivered by HIV-1 TAT peptide, including nucleic acid, nanoparticles, liposome, anticancer drugs, imaging agents.

In this paper, we explore an effective imaging of human cell using HIV-1 TAT peptides conjugated FITC doped silica nanoparticle (TAT-FITC-SiNP) sized by 500 nm. This approach begins a new feasibility of using cell penetrating peptide-silica nanoparticle delivery systems for bioimaging and therapy.

2. EXPERIMENTAL DETAILS

2.1. Materials

All silane compounds, dimethylsulfoxide (DMSO), Fluorescein isothiocyanate (FITC) were purchased from Sigma Aldrich Chemical (USA). Ammonium hydroxide and Ethanol were purchased from Jin chemical (KOREA).

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HIV-1 TAT peptide (YGRKKRRQRRRGGGC) was custom synthesized from ANYGEN (KOREA). All glassware was cleaned with autoclave.

2.2. Synthesis of FITC Doped Silica Nanoparticle with TAT Peptides

FITC doped SiNPs were synthesized by a stober method.[7] For 2-pyridyl disulfide of surface of FITC doped SiNPs, N-succinimidyl 3-(2-pyridyldithio)propionate in DMSO was added to FITC doped SiNPs in PBS buffer. For conjugation of HIV-1 TAT peptides to the FITC doped SiNPs through disulfide linkage, the amine groups of APTS coated FITC doped SiNPs were activated with SPDP. (Fig. 1)

2.3. Analysis of Particles Characteristics

Morphology of the dry TAT-FITC-SiNPs was investigated by FE-SEM(JSM-7500F, JEOL). Fluorescence microscopy image was obtained using a Zeiss Axio Observer. The Zeta potential measurement of SiNPs with/without HIV-1 TAT peptides was performed in 100% alcohol (Zetasizer 2000, Malvern Instruments).

2.4. Cell Culture

HeLa cell was purchased from Korean cell line bank. The cell was cultivated in Dulbecco modified Eagle medium high glucose (GIBCO, USA) supplemented with 10% FBS (GIBCO, USA), 1% penicillin and streptomycin at 37 °C and 5% CO_2 atmosphere.

2.5. Fluorescence Microscopy Imaging of Human Cell Treated with SiNPs with/Without TAT Peptides

FITC doped SiNPs (200 μg/ml) with/without HIV-1 TAT peptides were added to human cells cultured in 30 mm dish for 2 hrs at 37 °C under serum free medium. Cells were washed three times with PBS buffer (pH 7.4) and fixed 4% paraformaldehyde. The fixed cells were imaged by confocal microscopy (488 nm excitation, Nikon, EZ-C1, JAPAN).

2.6. Flow Cytometry

Human cells were seeded onto 6 well plate at a density of 2×10^5 cells per well in 5 ml medium (Dulbecco modified Eagle medium supplemented with 10% FBS, 1% penicillin and streptomycin) and cultured for 24 hrs. After the cells were treated with FITC doped SiNPs with/without HIV-1 TAT peptides for 2 hrs at 37 °C in serum free medium, the cells were carefully washed with PBS buffer (pH 7.4) three times and then were trypsinized and resuspended in PBS buffer. The fluorescence intensity of the cells was measured by FACS analysis (BD FACS Calibur). Untreated cells were used as a negative control.

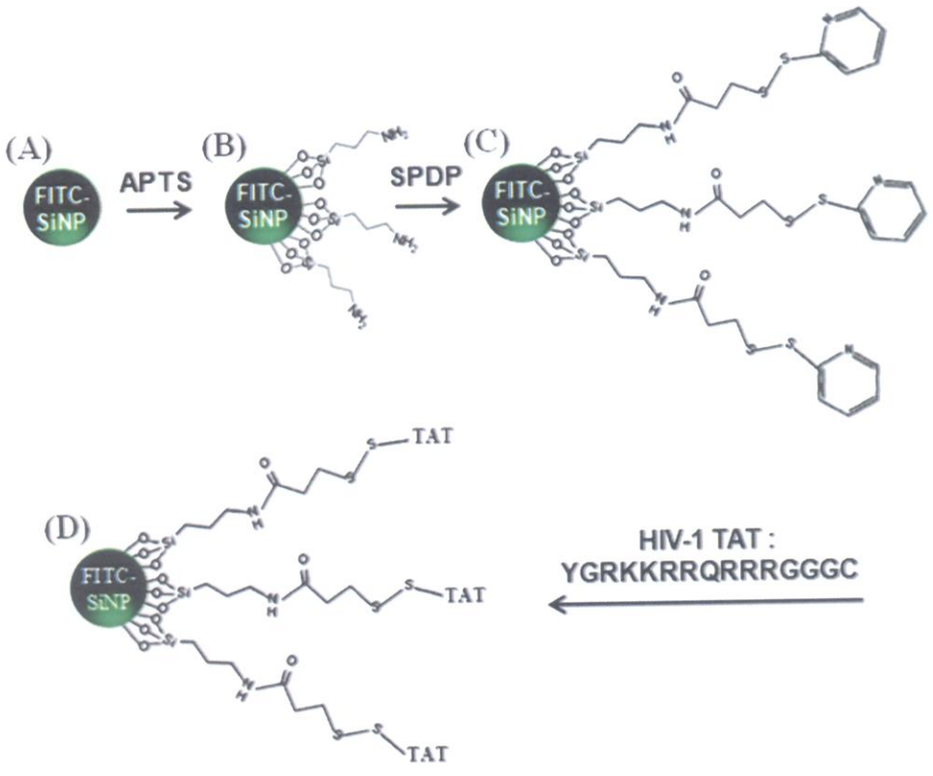

Fig. 1. Schematic diagram of TAT-FITC-SiNP synthesis.

3. RESULTS AND DISCUSSION

3.1. Characterization of TAT-FITC-SiNP

FITC doped SiNPs (dia. 500 nm) coated with APTS were synthesized and the surface of these SiNPs was modified with HIV-1 TAT peptides as shown in Figure 1. The HIV-1 TAT peptide is cell-penetrating peptide and it is able to deliver cargos such as oligonucleotides and proteins across the plasma membrane of living cells.

Prior to evaluating bioimaging scheme of TAT-FITC-SiNP, we characterized the TAT-FITC-SiNP through FE-SEM and fluorescence microscopy, Figures 2(A) and (B). The SEM image allows one to clearly visualize uniform spherical shape of TAT-FITC-SiNPs with 500 nm diameter, Figure 2(A). Fluorescence microscopy image shows that TAT-FITC-SiNPs exhibit strong fluorescence property by FITC incorporated in the SiNPs, Figure 2(B).

Zeta potential measurement of SiNPs with/without HIV-1 TAT peptides was carried out in alcohol. The zeta potential value of SiNPs without HIV-1 TAT peptides indicated a negative value, −52.2 mV and that of SiNPs with HIV-1 TAT peptides indicated a positive value, +11.5 mV. The positive zeta potential value of TAT peptides modified SiNPs results from nine amine group residues in one HIV-1 TAT peptide molecule. From these data, it could be confirmed that the SiNPs were well-modified with HIV-1 TAT peptides.

3.2. Bioimaging Characteristic and Cytotoxicity of TAT-FITC-SiNP

To investigate the bioimaging potential of TAT-FITC-SiNP for human cells, the cells were incubated with 200 μg/ml of TAT-FITC-SiNPs for 2 hrs. SiNPs without HIV-1 TAT peptides were used as a negative control. Bioimaging potential of TAT-FITC-SiNP for human cell was characterized through confocal microscopy, Figure 3. The confocal microscopy images show clearly that TAT-FITC-SiNPs were penetrated into human cells, Figures 3(A), (C), (E). However, SiNPs without HIV-1 TAT were not able to penetrate into cytoplasm of human cells (Figs. 3(B), (D), (F)).

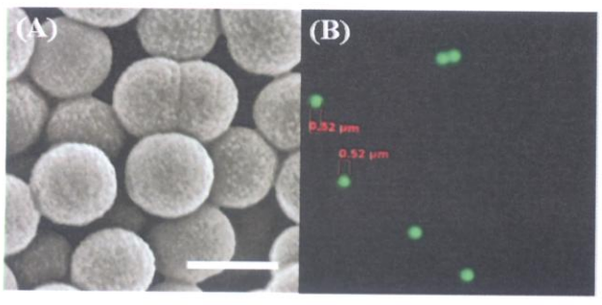

Fig. 2. (A) SEM image of TAT-FITC-SiNPs (scale bar: 500 nm), (B) fluorescence microscopy images of TAT-FITC-SiNPs.

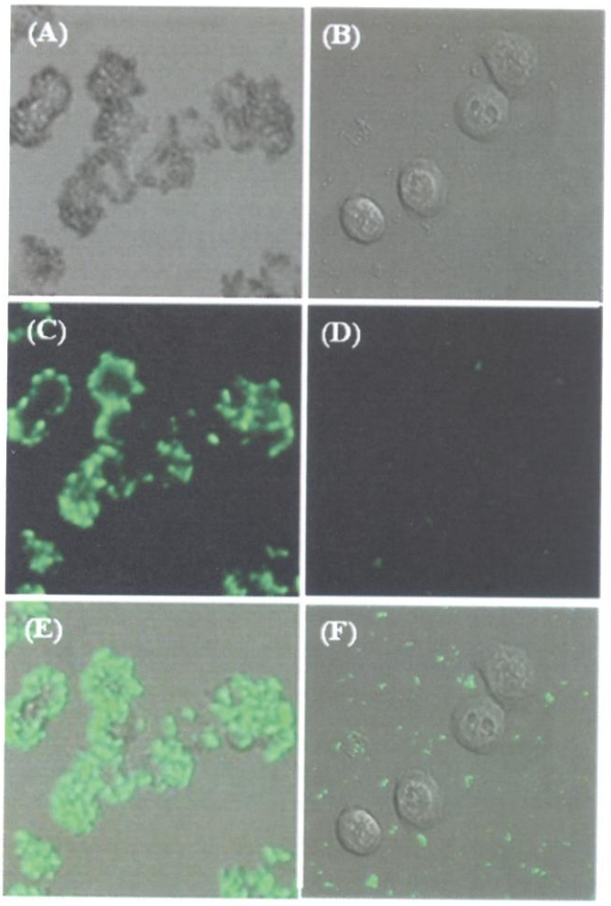

Fig. 3. Confocal microscopy images. (A) optical microcopy image of human cells treated with TAT-FITC-SiNPs, (B) optical microcopy image of human cells treated with SiNPs without HIV-1 TAT, (C) fluorescence microcopy image of human cells treated with TAT-FITC-SiNPs, (D) fluorescence microcopy image of human cells treated with SiNPs without HIV-1 TAT, (E) merged image of (A) and (C), (F) merged image of (B) and (D).

For quantitative evaluation of cell penetration event of SiNPs by the presence of HIV-1 TAT peptide, flow cytometry experiment was carried out with human cells treated with SiNPs with/without HIV-1 TAT (data now shown). It could be observed that cell penetration event of 500 nm SiNPs for human cells were improved over four times by HIV-1 TAT peptides on the surface of SiNPs. From these data, it could be confirmed that the penetration of TAT-FITC-SiNPs through cell membrane took place with high efficiency. Also, it was observed that TAT-FITC-SiNPs were not able to penetrate cell nucleus due to size effect of SiNPs. (data not shown)

For evaluating the cytotoxicity of TAT-FITC-SiNP for human cells, cell viability was measured after treating them with SiNP with/without HIV-1 TAT. As shown in Figure 4, MTT cell viability assay indicated that SiNPs without HIV-1 TAT reduced the cell viability sharply at 200 μg/ml, while TAT-FITC-SiNPs were nearly non-toxic

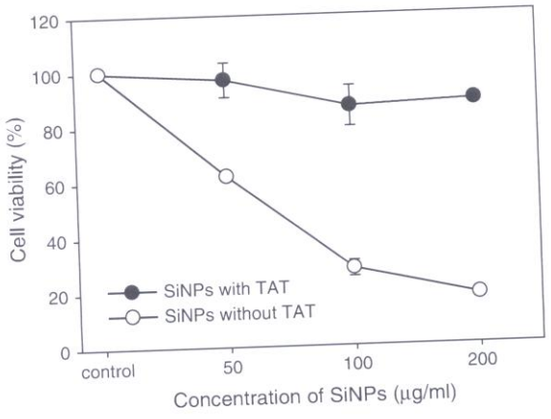

Fig. 4. Biocompatibility evaluation of TAT-FITC-SiNP for human cell.

for human cells by cationic charge of HIV-1 TAT peptides on the surface of SiNPs.

4. CONCLUSION

In conclusion, we synthesized the TAT-FITC-SiNP that has strong fluorescence intensity and good biocompatibility, and demonstrated its feasibility as a potential carrier for bioimaging. HIV-1 TAT peptide relatively increased the cellular uptake of SiNPs and localized them in the perinuclear zone. So, the SiNP might have potential for drug/gene delivery as an intracellular delivery carrier.

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