Degradable PEG Nanogels for Cytosol-specific Release of Plasmid DNA

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1. INTRODUCTION

Recently, DNA vaccines have received attention because plasmid DNA is believed to overcome many limitations of conventional vaccines. For delivery of plasmid DNA into cells, efficient and non-toxic carriers have been required.

Here, a new concept of gene delivery using biocompatible and biodegradable PEG nanogel was investigated. Plasmid DNA was molecularly collapsed in organic solvent by interacting with PEG and formed a nano-scale DNA/PEG complex. Thiol-functionalized six-arm branched PEG was used for condensing the plasmid DNA. The thiol groups were crosslinked to produce nano-sized PEG hydrogels. Plasmid DNA was encapsulated within the PEG nanogel and the release of DNA could be modulated by changing the concentration of external reducing agent. The formulated nanogels exhibited good intracellular uptake in HEK293 cell line. GFP plasmid released from the PEG nanogel was also expressed in the cell lines.

2. MATERIALS AND METHODS

2.1 Preparation of PEG nanogel:

Six-arm branched PEG-SH (7.5 mg) and plasmid DNA (0.5 mg) were mixed and freeze-dried. Then the PEG/DNA mixture was dissolved in 2 ml of DMSO and added with the thiol crosslinker, DTME, in 1 ml of DMSO, dropwise while stirring. After 5 hr, the reaction mixture was centrifuged at 14000 rpm for 1 hr to remove free DTME and uncrosslinked PEG/DNA. PEG nanogels were collected in a pellet which was resuspended with deionized distilled water. The PEG nanogels, along with DNA/PEG nano-complex in DMSO, and DNA in DW, were visualized by AFM(Atomic Force Microscopy)

2.2. Analysis of cleavable PEG nanogel

In order to mimic intracellular reducing conditions, GSH (glutathione) was used as the reducing agent. GSH was added to PEG nanogel in PBS at concentrations of 0, 0.1, 5 mM. At pre-determined time intervals, the supernatant was collected by centrifuging at 14000 rpm for 30 min. Picogreen assay was used to determine the amount of the released DNA in the incubation medium.

2.3. Intracellular uptake of PEG nanogel

HEK293 cells were seeded at a density of $2X10^5$ cells/well in a 4-well chamber slide and kept over-night. pEGFP-C1 plasmid DNA encapsulated within the PEG nanogel was stained with the YOYO-1 dye, and the DNA/PEG gels were treated to the HEK293 cells. The cells were stained with a cytoplasm-specific dye, Cell Tracker Orange CMRA (Molecular Probes). The PEG nanogels present within the cells were visualized under a confocal laser scanning microscope (LSM 510, CARL-ZEISS).

3. RESULTS and DISCISSION

DNA/PEG nanocomplexes were formed as a result of hydrogen bonding between PEG and DNA dissolved in the DMSO phase. The PEG/DNA mixture showed presence of compact, nano-sized complexes, compared to the relaxed state of DNA in water. Crosslinked PEG nanogels maintained structural stability in water, with a diameter about 315±91 nm (Figure 1). Plasmid DNA was intactly encapsulated within the PEG gel.

PEG nanogels crosslinked by disulfide bonds went through cleavage when treated with a reducing agent. The intracellular concentration of GSH is maintained at high levels (1-8 mM) in a variety of cells, while extracellular concentration is relatively low (0.1 mM). The results showed that DNA was released from the PEG nanogel at cytosolic GSH concentrations, but not at extracellular concentrations (Figure 2).

Intracellular DNA delivery of DNA/PEG nanogel was examined using a confocal microscope (Figure 3). It is shown that plasmid DNA (green) encapsulated within PEG nanogel was successfully delivered into the cytosolic region of cells (red staining).

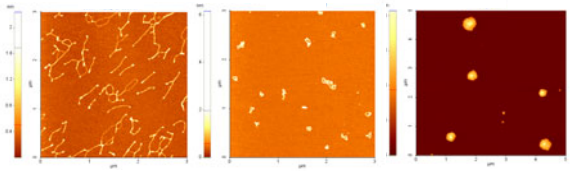

Figure 1. AFM image of plasmid DNA and DNA/PEG nanogel
(left panel: DNA in water, middle panel: PEG/DNA complex in DMSO, right panel: PEG nanogel in water)

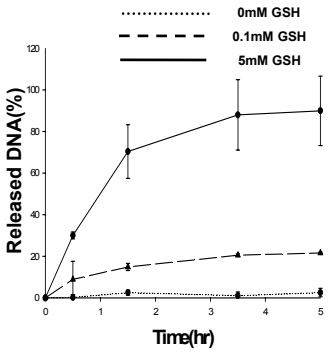

Figure 2. DNA release from DNA/PEG nanogel at different GSH (glutathione) concentrations.

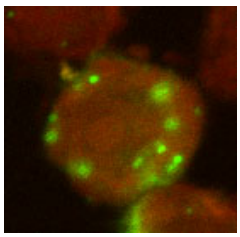

Figure 3. Intracellular uptake of DNA/PEG nanogel in HEK293 cell.

4. CONCLUSION

We have demonstrated that plasmid DNA can be solubilized in organic solvent without using cationic polymers. Nano-sized PEG hydrogel can be formulated in organic solvent from the PEG/DNA complex. Biocompatible and biodegradable PEG nanogel can be a good carrier system for plasmid DNA in gene therapy.

5. REFERENCES

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