Redox-Responsive Controlled Gene Transfection Based on Polymer-Conjugated Magnetic Nanoparticles

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Abstract

Gene transfection is a non-viral therapy on gene-based diseases by delivering nucleic acids into the nucleus of target cells[1]. The efficiency of gene transfection may be enhanced by magnetofection which involves magnetic nanomaterials (MNPs) under a magnetic field[2]. To combine nucleic acids with nanoparticles as well as protect them from degradation after endocytosis, MNPs are usually modified with cationic compounds, such as 25 kDa branched polyethylenimine (PEI) [3,4]. After cationic adsorption of plasmid DNAs on the surface of negative charged MNPs, addition of extra free PEI is often required to form a ternary complex for magnetofection[5]. It is because only the cationic compounds could transfer the nucleic acids into the cell nucleus, while the MNPs stay only in the perinuclear region. In this work, a redox-responsive disulfide bond is used to link 25 kDa PEI to MNPs, generating detachable PEIs for both DNA protection and nuclear entry.

The as-synthesized MNPs were first wrapped in silica with thiol groups on the surface. After thiol-exchanging with 2-carboxyethyl-2-pyridyl disulfide, PEI was linked to the carboxyl groups with EDC/NHS. The results of agarose gel electrophoresis indicated that 10 μg Fe_3O_4 nanoparticles could condense 250 ng pRL-CMV, *i.e.*, renilla luciferase control reporter vectors (**Fig. 1**). Repeated experiments indicated that the condensing nanoparticles were stable for at least one month. The magnetic gene carrier exhibited efficient gene transfection in both Hela and HepG2 cells lines (**Fig. 2**). After addition of 10 mM glutathione (GSH) in phosphate buffer solution (pH=7.4), plasmid DNA was released from the nanoparticles, confirming the redox-responsive property of the modified magnetic nanoparticles (**Fig. 3**).

The confocal microscopy images showed the labeled plasmid DNA located in the nucleus 3h post-transfection, which was more obvious 24 h after transfection (**Fig. 4**). In addition, the yellow colored area indicated a significant portion of PEI co-localized with the red florescent lysotracker, suggesting that the magnetic nanoparticles were taken into the cells via the endocytosis pathway. The co-localization of PEI and plasmid DNA in the nucleus confirmed the nucleic acids were taken in with the help of PEI, while nanoparticles were still in the perinuclear region.

References

[1] Feldman, A. L.; Libutti, S. K. **Cancer** (2000), 89, 1181.
[2] Plank, C.; Schillinger, U.; Scherer, F.; Bergemann, C.; Rémy, J. S.; Krötz, F.; Anton, M.; Lausier, J.; Rosenecker, J. **Biological Chemistry** (2003), 384, 737.
[3] Wang, X.; Zhou, L.; Ma, Y.; Li, X.; Gu, H. **Nano Research** (2009), 2, 365.
[4] McBain, S. C.; Yiu, H. H. P.; El Haj, A.; Dobson, J. **Journal of Materials Chemistry** (2007), 17, 2561.
[5] Ma, Y.; Zhang, Z.; Wang, X.; Xia, W.; Gu, H.; **International Journal of Pharmaceutics** (2011), 419, 247.

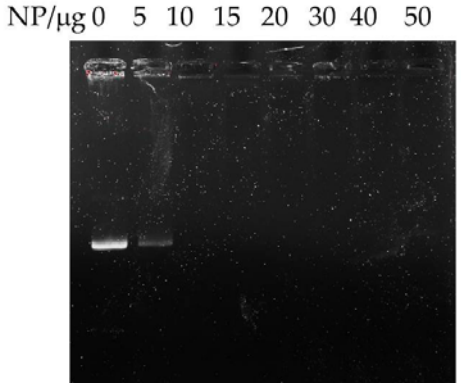

Fig1. Evaluation of the DNA condensing ability of **sFe_3O_4@SiO_2-SS-PEI** with agarose gel electrophoresis gel assay. Different weight of nanoparticles was incubated with **250 ng pRL-CMV** for **20 min** at room temperature.

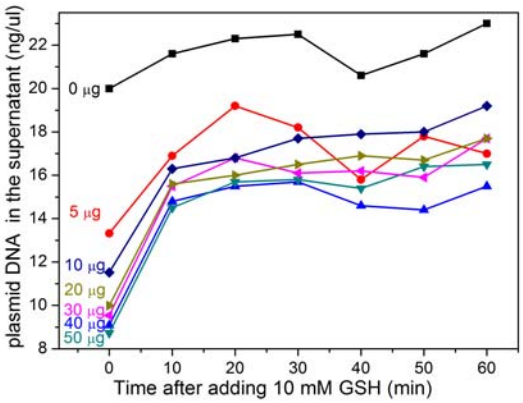

Fig 2. pRL-CMV released at different endpoints after addition of 10mM GSH. Plasmid DNA was mixed with different amount of **sFe_3O_4@SiO_2-SS-PEI**, and magnet was used to separate the condensed DNA on nanoparticles with the DNA in the supernatant.

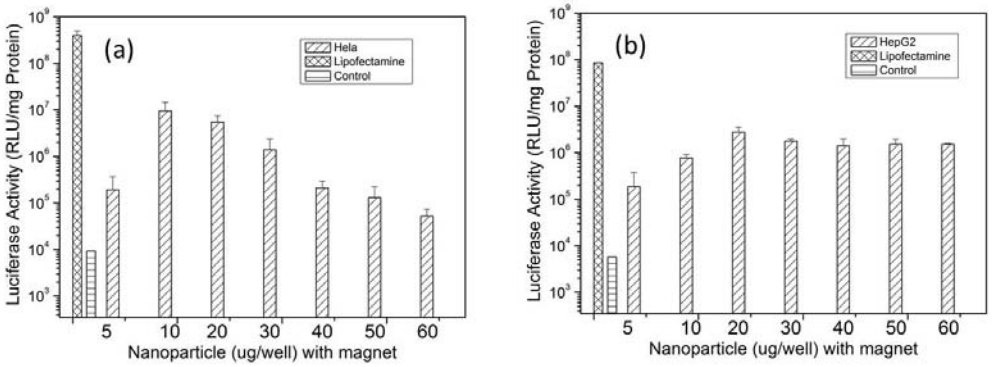

Fig 3. Evaluation of the transfection efficiency using luciferase assay: (a) in Hela cell line; (b) in HepG2 cell line. The activity was measured at 48h post-transfection.

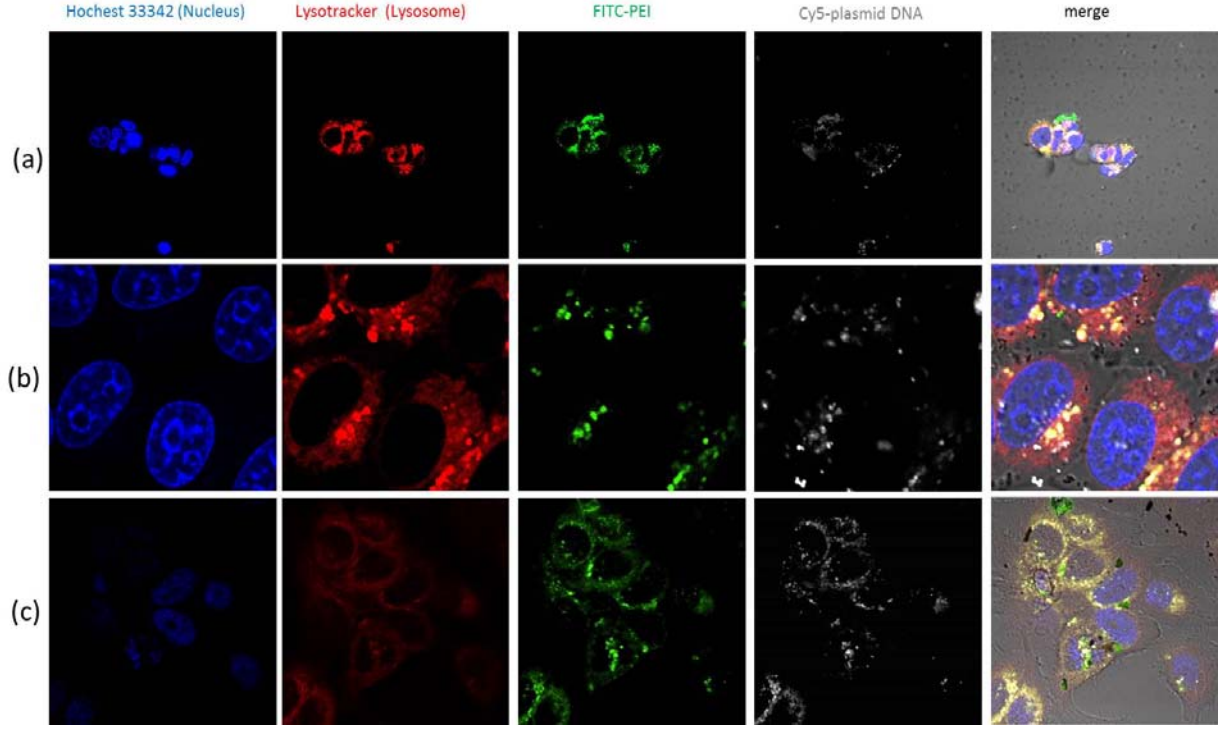

Fig 4. Tracking of different components in Hela cells 1 h (a), 3 h (b) and 24 h (c) post-magnetofection. Nucleus was labeled by Hochest 33342, lysosome with LysoTracker® Red DND-99, PEI with FITC, and plasmid DNA with Cy5.Merged images with bright field were also shown in the right coloumn.