Micro-optical coherence tomography tracking of magnetic gene transfection via Au-Fe₃O₄ dumbbell nanoparticles

Shi, Wei; Liu, Xinyu; Wei, Chao; Xu, Zhichuan J.; Sim, Stanley Siong Wei; Liu, Linbo; Xu, Chenjie

2015

Shi, W., Liu, X., Wei, C., Xu, Z. J., Sim, S. S. W., Liu, L., et al. (2015). Micro-optical coherence tomography tracking of magnetic gene transfection via Au-Fe₃O₄ dumbbell nanoparticles. *Nanoscale*, 7(41), 17249-17253.

<https://hdl.handle.net/10356/80244>

<https://doi.org/10.1039/C5NR05459A>

© 2016 Royal Society of Chemistry. This is the author created version of a work that has been peer reviewed and accepted for publication by Nanoscale, Royal Society of Chemistry. It incorporates referee's comments but changes resulting from the publishing process, such as copyediting, structural formatting, may not be reflected in this document. The published version is available at: [<http://dx.doi.org/10.1039/C5NR05459A>].

Downloaded on 26 Apr 2023 17:42:01 SGT

Micro-Optical Coherence Tomography Tracking of Magnetic Gene Transfection via Au-Fe₃O₄ Dumbbell Nanoparticles

Wei Shi^{1,5}, Xinyu Liu², Wei Chao³, Zhichuan J. Xu³, Stanley Siong Wei Sim¹, Linbo Liu^{2,*}, Chenjie Xu^{1,4,*}

¹ School of Chemical and Biomedical Engineering, Nanyang Technological University, 70 Nanyang Drive, Singapore 637457

² School of Electrical and Electronic Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798

³ School of Materials Science and Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798

⁴ NTU-Northwestern Institute of Nanomedicine, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798

⁵ Key Laboratory of Flexible Electronics & Institute of Advanced Materials, Jiangsu National Synergetic Innovation Center for Advanced Materials, Nanjing Tech University, 30 South Puzhu Road, Nanjing, P.R. China 211816

* Correspondence to cjxu@ntu.edu.sg; liulinbo@ntu.edu.sg

Abstract: Heterogeneous Au-Fe₃O₄ dumbbell nanoparticles (NPs) are composed of Au NP and Fe₃O₄ NP that brings in optical and magnetic property respectively. This article reports the engineering of Au-Fe₃O₄ NPs as gene carriers for magnetic gene transfection as well as contrast agents for micro-optical coherence tomography (μ OCT). As a proof-of-concept, Au-Fe₃O₄ NPs are used to deliver green fluorescent protein to HEK 293T cells and their entrance into the cells is monitored through μ OCT.

Keywords: heterogeneous nanoparticles, dumbbell nanoparticles, magnetofection, gene delivery, micro-optical coherence tomography

Introduction

Heterogeneous nanoparticles are an important type of composite nanomaterial that has attracted growing interest.^{1,2} In this system, two or more nanocomponents with different properties are linked together, which allows the simultaneous realization of multiple functions.³ Au-Fe₃O₄ dumbbell nanoparticles (NPs) are one such representative material.^{4,5} They contain Au and Fe₃O₄ NPs, both of which are biocompatible and extensively used in biomedical application. These two components bring in magnetic properties as well as optical and electronic properties, which have triggered their multirole applications as carriers in targeted drug delivery^{6, 7}, contrast agents in multimodal imaging⁸⁻¹⁰, or enhancers in phototherapy¹¹.

Here we report a new application of Au-Fe₃O₄ NPs in gene transfection (**Figure 1**). Specifically, negatively charged plasmids can be conjugated with Au-Fe₃O₄ NPs pre-modified with cationic molecules (either cationic lipids or polymer). Given the presence of magnetic component (Fe₃O₄), under magnetic field, these complexes provided a high efficiency in transfecting adherent mammalian cells. More interestingly, Au-Fe₃O₄ NPs (especially the Fe₃O₄ core) enabled the visualization of this transfection process through micro-optical coherence tomography (μ OCT) technology, a new and high-resolution form of OCT with a resolution of 1-2 μ m¹²⁻¹⁵.

Figure 1. Schematic illustration of Au-Fe₃O₄ dumbbell nanoparticles for gene delivery and μ OCT tracking

Materials and methods

Oleic acid, oleylamine, 1,2-hexadecandiol, 1-octadecene (ODE), Fe(CO)₅, HAuCl₄•(H₂O)₃, branched

polyethylenimine (PEI, 25 kDa), chloroform were obtained from Sigma-Aldrich; Dulbecco's modified Eagle's medium (DMEM) fetal bovine serum (FBS) and penicillin-streptomycin solution (5 kU/mL) were purchased from Lonza. HEK 293T cells were from ATCC.

Instruments for the particle characterization. The hydrodynamic diameter and zeta potential were examined with Zetasizer Nano ZS (Malvern instruments). UV-Vis spectra were acquired with UV-vis spectroscopy (SHIMADZU UV 2450). Au- Fe₃O₄ NP morphology was examined using a transmission electron microscope (TEM) JEOL 2010 operated at 200 kV. Plasmid concentration was determined by NanoDrop 2000 (Thermo Scientific) at 260 nm.

Synthesis and modification of Au-Fe₃O₄ NPs. Au-Fe₃O₄ NPs were prepared according the protocol published previously.⁸ Modification of Au- Fe₃O₄ NPs with PEI was achieved by adding 1 mL Fe₃O₄-Au NPs solution (1 mg/mL in chloroform) into the 1 mL chloroform containing 2.4 mg PEI under stirring. Chloroform was the dried under a nitrogen flow to obtain the Au-Fe₃O₄/PEI film, which was dissolved in 2 mL sterile deionized water. The Au-Fe₃O₄/PEI complex was separated from free PEI through high speed centrifugation, and re-dispersed in PBS. The concentrations of Au and Fe of the Au-Fe₃O₄/PEI complex were quantified through inductively coupled plasma mass spectrometry (ICP-MS, Perkin-Elmer Elan DRC-e).

Synthesis of Au-Fe₃O₄/PEI/DNA complex. Au-Fe₃O₄/PEI NPs were coupled to plasmid pEAK12-GFP via charge interactions. In brief, 10 µL Au-Fe₃O₄/PEI solution with a concentration of 0.5 mg/mL Au-Fe₃O₄ NPs was mixed with 40 µl plasmid (50µg/mL) in FBS-free DMEM, and incubated for 15 min at room temperature. The free plasmid was removed by high speed centrifugation. Immediately after centrifugation of Au-Fe₃O₄/PEI/DNA complex, supernatants were recovered and subjected to a standard protocol of ethanol precipitation of nucleic acids.¹⁶ The amount of precipitated DNA in the sample was measured by NanoDrop. DNA binding efficiency was calculated as percentage of conjugated DNA to total amount of DNA added. The concentrations of Au and Fe were quantified through ICP-MS.

***In vitro* magnetofection with Au-Fe₃O₄/PEI/DNA complex.** 24 hours before the transfection, HEK 293T cells were seeded onto 6-well plates at the density of 6×10⁵ cells per well. During transfection, the original full medium was removed and cells were first washed twice with PBS (pre-warmed to

37°C). Then 2 mg Au-Fe₃O₄/PEI/DNA complexes containing 2 µg of plasmid pEAK12-GFP in 2 mL serum-free DMEM was added. For the positive control, 12 µg of PEI was used to conjugate with 2 µg of plasmid pEAK12-GFP in 2 ml serum-free DMEM. The plate was later transferred to the cell incubator with a magnet (Super Magnetic Plate, OZ Biosciences, San Diego, CA) placed under the bottom of the plate for 0, 5, 10, 30, or 60 min. 6 hours later, the medium containing Au-Fe₃O₄/PEI/DNA complexes was replaced with full medium. 24 hours after the experiment, GFP expression in HEK 293T cells was observed with fluorescence microscope (Olympus IX71). The transfection efficiency was examined by flow cytometry (BD Accuri™ C6 flow cytometer) using a fluidics rate of medium (Flow = 35µl/min; Core = 16 µm). 100,000 gated events were collected per sample.

Monitoring the magnetofection with µOCT. The µOCT imaging setup used in this study was reported in a previous study (Supporting Figure S1).¹⁷ In brief, the light source produced broadband illumination from 755 nm -1105 nm, which was guided to the reference arm and the sample arm through a 50:50 non-polarizing cube beam splitter (BS). An objective lens (Mitutoyo Plan Apo NIR, 20×, NA 0.4, ~70% transmission) was used to focus the light beam to the sample, providing a lateral resolution of 2.5 µm. The backscattered interference signal was recombined with the cube beam splitter and directed to the spectrometers. The measured axial resolution of the system is ~1 µm in tissue (refractive index = 1.36). A three-dimensional µOCT was composed of 100 cross-sectional image frames with 1024 axial line (A-line) per frame. The imaging speed was 10 K A-line per second so that it took 10 seconds to acquire a three-dimensional image of 0.87 mm by 0.1 mm by 1 mm (Width by Height by Depth) in size. The optical power on the sample was ~ 1 mW and the illumination was turned off when scanning was finished to avoid unnecessary exposure.

HEK 293T cells were incubated with 2 mg Au-Fe₃O₄/PEI/DNA complexes containing 2 µg of plasmid pEAK12-GFP in 2 ml serum-free DMEM. The neodymium magnet (10 Oe) was placed right under the plate for 0, 4, 30, or 60 min before the cells were imaged with µOCT. All the cross-sectional µOCT images were acquired at a 20 frames/sec with the illumination power on the sample 8.3 mW. The standard image processing procedures were used to generate µOCT images.¹⁷ The boundary of the cell culture in each cross-sectional µOCT image was detected manually, followed by the measurement of the mean image intensity and the intensity standard deviation using

ImageJ.

Results and Discussions

Au-Fe₃O₄ NPs were synthesized according to the reported protocol⁶ and had a dumbbell structure in which Au and Fe₃O₄ NPs were ~ 6 nm and ~15 nm respectively (Figure 2A). The as-synthesized NPs were hydrophobic and made hydrophilic through conjugation with cationic polymer, PEI. The free PEI post surface modification could be removed during the purification through high speed centrifugation.

It is well known that PEI is cytotoxic.¹⁸ Thus it is reasonable to suspect that PEI coated Au-Fe₃O₄ NPs had some cytotoxic effects. To identify a concentration threshold for the safe use of Au-Fe₃O₄/PEI NPs, 1mg Au-Fe₃O₄ NPs were modified with different amounts of PEI ranging from 1.2 to 24 mg. After the removal of free PEI, the cytotoxicity assay was carried with these NPs in the HEK 293T cell culture by taking free PEI as the control. As shown in Supporting Figure S2, the higher the PEI concentration during the modification, the lower the cell viability. To minimize the cytotoxicity while providing a higher positive surface charge, 2.4mg PEI was chosen for the rest experiments to modify 1mg Au-Fe₃O₄ NPs. It also deserves to point out that the PEI concentration of Au-Fe₃O₄/PEI NPs in Supporting Figure S2 was the initial PEI concentration during the synthesis of Au-Fe₃O₄/PEI NPs. As free PEI was removed post the modification, the actual PEI concentration of Au-Fe₃O₄/PEI NP solution is expected lower than the theoretic one, which explains cells incubated with Au-Fe₃O₄/PEI NPs had a higher viability than free PEI.

After PEI modification, the Au-Fe₃O₄/PEI NPs dispersed well in aqueous solution and could respond to the magnet attraction (Figure 2B). The coating of cationic PEI also provided a positive surface charge (i.e. + 63 mV) for Au-Fe₃O₄/PEI NPs in PBS, which allowed the complexation with anionic plasmids via charge interactions with a loading capacity of 1-5 µg plasmid per milligram of Au-Fe₃O₄ NPs (Figure 2C).

Accordingly, the zeta potential dropped down from +63mV to +36mV while DNA bound onto Au-Fe₃O₄ NPs /PEI. The prepared Au-Fe₃O₄ NPs, Au-Fe₃O₄ NPs /PEI, Au-Fe₃O₄ NPs /PEI/DNA had a hydrodynamic diameter of about 21, 43, 59 nm, respectively (Figure 2D). The PEI coating increased the hydrodynamic diameter of Au-Fe₃O₄ NPs from 21 to 43 nm.¹⁹ And the further

increase from 43 to 59 nm was due to the absorption of plasmid.

Figure 2. Characterization of Au-Fe₃O₄ NPs: (A) a representative TEM image of Au-Fe₃O₄ NPs before modification. Scar bar: 20 nm. (B) Photos of Au-Fe₃O₄/PEI NP aqueous solution and its response to magnetic manipulation. (C) Zeta potentials of Au-Fe₃O₄/PEI and Au-Fe₃O₄/PEI /DNA NPs. (D) Hydrodynamic diameters of Au-Fe₃O₄, Au-Fe₃O₄/PEI, Au-Fe₃O₄/PEI/DNA.

Magnetofection with Au-Fe₃O₄ NPs

The efficiency of magnetofection with Au-Fe₃O₄ NPs was examined with the delivery of plasmid pEAK12-GFP to HEK 293T cells by using PEI as a positive control. As shown **Figure 3A&3B**, Au-Fe₃O₄/PEI complex without the aid of magnetic field already provided similar transfection efficiency, where the average fluorescence intensity per cell was 14 times of the negative control (**Figure 3C**). When the magnet was placed under the cell culture plate for more than 30 min, the transfection efficiency was further improved (**Figure 3B&3C**). With the aid of magnet, the average fluorescence intensity increased at least 2.5 times than those incubated with Au-Fe₃O₄/PEI/DNA complex only.

Figure 3. Magnetofection with Au-Fe₃O₄ NPs: (A) Fluorescence images and (B) Flow cytometry analysis of HEK293T cells 24 hours post transfection, in which cells were incubated with blank, PEI/DNA, Au-Fe₃O₄/PEI or Au-Fe₃O₄/PEI/DNA (1 μ g plasmid per milligram Au-Fe₃O₄ NPs) plus 30 min magnet attraction. (C) Flow cytometry analysis of HEK293T cells transfected with Au-Fe₃O₄/PEI/DNA NPs under different exposure times to magnet attraction.

Au-Fe₃O₄ NPs enhance detectability by μ OCT

Au-Fe₃O₄ NPs were firstly compared with Au NPs for their enhancement of μ OCT signal. As shown in Figure 4A, the signal intensity steadily improved when the NP concentration increased for both samples. However, the improvement of μ OCT signals in the solution containing Au-Fe₃O₄ NPs was much stronger compared to the solution containing Au NPs. Given the Au core of Au-Fe₃O₄ NPs had the same size as Au NPs, it is clearly that the Fe₃O₄ core caused this difference. In another words, Fe₃O₄ core was the main contributor to the μ OCT signal. To test this hypothesis, Au-Fe₃O₄ NPs (6 nm-15 nm) were then compared with Fe₃O₄ NPs (15 nm) for their enhancement of μ OCT signal (Figure 4B). Under the same iron concentration, both types of NPs provided the same amount of

μ OCT signal, which proved that Fe_3O_4 core contributed more in μ OCT imaging that might because the Fe_3O_4 core (15 nm) is larger than the Au core (6 nm).

The image contrast of μ OCT is originated from the refractive index inhomogeneity of the sample.²⁰ We hypothesize that internalized NPs may alter the refractive index distribution within the cell body which is reflected in the intensity uniformity of cell images acquired by μ OCT. Image intensity uniformity is defined as the ratio between the mean image intensity and the standard deviation of the image intensity. We found that the image of control cell culture demonstrated the lowest intensity uniformity as many low intensity areas, presumably nuclei (Figure 4C, green arrows), were surrounded by the high intensity scatters, possibly cell cytoplasm. The image intensity uniformity was significantly higher ($P < 0.05$) in the image of the cell culture with internalized NPs, and positively correlated with the magnetic field exposure from 0 -30 min (Figure 4D). This data supports that image intensity uniformity may be an indicator of magnetofection efficiency.

Figure 4. Au- Fe_3O_4 NPs act as contrast agents for μ OCT imaging: (A) Scattered light intensity from the aqueous solutions of Au and Au- Fe_3O_4 NPs as a function of concentration. (B) Scattered light intensity from the aqueous solutions of Fe_3O_4 and Au- Fe_3O_4 NPs as a function of concentration. (C) Representative cross-sectional μ OCT images of the cell cultures with various exposure times to magnetic field. Scale bars: 25 μm . (D) Image intensity uniformity of the cells in C as a function of the exposure time to magnetic field.

Dynamic monitoring the cellular uptake of Au- Fe_3O_4 /PEI/DNA NPs with μ OCT

Finally, μ OCT imaging was used to track the cellular uptake of Au- Fe_3O_4 /PEI/DNA NPs under the

magnet in real time. Following the addition of Au-Fe₃O₄/PEI/DNA NPs, three-dimensional μ OCT images were acquired every 5 minutes over 20 minutes around the same volume of the cell culture (Figure 5). The change in the scattering uniformity of the cells over time was clearly visualized (Figure 5A), presumably due to NP uptake by the cells. The intensity of the cells treated with Au-Fe₃O₄/PEI/DNA increased from 0 minute to 10 minute followed by a gradual decrease (Figure 5B). The relative high overall scattering intensity and uniformity at 10 minutes may indicate that the NP uptake reached a maximum which was not observed in the control group.

Figure 5. Dynamic monitoring the cellular uptake of Au-Fe₃O₄/PEI/DNA NPs under the magnet with μ OCT imaging: (A) Representative μ OCT image of control cells or cells transfected with Au-Fe₃O₄/PEI/DNA NPs under magnet attraction (Scale bars: 25 μ m); (B) Image intensity uniformity of the single cell in A as a function of the exposure time to magnetic field.

Conclusion

In summary, this report described the usage of Au-Fe₃O₄ NPs in gene transfection as well as μ OCT imaging. After the conjugation of plasmids on PEI modified surface, Au-Fe₃O₄ NPs provided a high efficiency in transfecting adherent mammalian cells under the magnetic attraction. More interestingly, Au-Fe₃O₄ NPs (especially the Fe₃O₄ core) enabled the visualization of NP internalization through μ OCT technology. Considering the multirole applications of Au-Fe₃O₄ NPs already demonstrated in

targeted drug delivery and contrast agents in multimodal imaging, the properties reported here are expected to trigger more cross-disciplinary research.

Acknowledgments

This work was supported by Northwestern University-Nanyang Technological University Nanomedicine Institute. We would also like to thank you Dr. Lifeng Zhang (School of Biological Science, Nanyang Technological University) for providing plasmid DNA pEAK12-EGFP.

References

1. C. Wang, C. Xu, H. Zeng and S. Sun, *Advanced materials*, 2009, 21, 3045-3052.
2. A. Walther and A. H. Müller, *Chemical reviews*, 2013, 113, 5194-5261.
3. T. H. Shin, Y. Choi, S. Kim and J. Cheon, *Chem Soc Rev*, 2015, 44, 4501-4516.
4. H. Yu, M. Chen, P. M. Rice, S. X. Wang, R. White and S. Sun, *Nano letters*, 2005, 5, 379-382.
5. N. Lee, D. Yoo, D. Ling, M. H. Cho, T. Hyeon and J. Cheon, *Chem Rev*, 2015, DOI: 10.1021/acs.chemrev.5b00112.
6. C. Xu, B. Wang and S. Sun, *Journal of the American Chemical Society*, 2009, 131, 4216-4217.
7. J. H. Lee, J. W. Kim and J. Cheon, *Mol Cells*, 2013, 35, 274-284.
8. C. Xu, J. Xie, D. Ho, C. Wang, N. Kohler, E. G. Walsh, J. R. Morgan, Y. E. Chin and S. Sun, *Angewandte Chemie International Edition*, 2008, 47, 173-176.
9. J. Zhu, Y. Lu, Y. Li, J. Jiang, L. Cheng, Z. Liu, L. Guo, Y. Pan and H. Gu, *Nanoscale*, 2014, 6, 199-202.
10. D. Kim, M. K. Yu, T. S. Lee, J. J. Park, Y. Y. Jeong and S. Jon, *Nanotechnology*, 2011, 22, 155101.
11. D. K. Kirui, D. A. Rey and C. A. Batt, *Nanotechnology*, 2010, 21, 105105.
12. M. Kashiwagi, L. Liu, K. K. Chu, C.-H. Sun, A. Tanaka, J. A. Gardecki and G. J. Tearney, *PLoS ONE*, 2014, 9, e102669.
13. L. Liu, K. K. Chu, G. H. Houser, B. J. Diephuis, Y. Li, E. J. Wilsterman, S. Shastri, G. Dierksen, S. E. Birket and M. Mazur, *PloS one*, 2013, 8, e54473.
14. L. Liu, J. A. Gardecki, S. K. Nadkarni, J. D. Toussaint, Y. Yagi, B. E. Bouma and G. J. Tearney, *Nature Medicine*, 2011, 17, 1010-1014.
15. L. Liu, S. Shastri, S. Byan-Parker, G. Houser, K. K. Chu, S. E. Birket, C. M. Fernandez, J. A. Gardecki, W. E. Grizzle and E. J. Wilsterman, *American journal of respiratory cell and molecular biology*, 2014, 51, 485-493.
16. V. Cebrián, F. Martín-Saavedra, C. Yagüe, M. Arruebo, J. Santamaría and N. Vilaboa, *Acta Biomaterialia*, 2011, 7, 3645-3655.
17. D. Cui, X. Liu, J. Zhang, X. Yu, S. Ding, Y. Luo, J. Gu, P. Shum and L. Liu, *Optics letters*, 2014, 39, 6727-6730.
18. O. Boussif, F. Lezoualc'h, M. A. Zanta, M. D. Mergny, D. Scherman, B. Demeneix and J.-P. Behr, *Proceedings of the National Academy of Sciences*, 1995, 92, 7297-7301.
19. M. Hasanzadeh Kafshgari, M. Alnakhli, B. Delalat, S. Apostolou, F. J. Harding, E. Makila, J. J. Salonen, B. J. Kuss and N. H. Voelcker, *Biomaterials Science*, 2015, DOI: 10.1039/C5BM00204D.
20. W. Drexler, J. G. Fujimoto and SpringerLink (Online service), in *Biological and medical physics, biomedical engineering,, Springer Berlin Heidelberg,, Berlin, Heidelberg*, 2008, pp. xxix, 1346 p.