Stem-cell transplantation is a proven and efficient method for regenerative medicine, especially for tissues and organs that are difficult to reconstruct due to their complicated structures and functions.\(^1\)–\(^3\) Adipose tissue-derived stem cells (ASCs) have received a great deal of attention in recent years as multipotent somatic stem cells. ASCs have the ability to self-renew and to multilineage differentiate in various types of mesenchymal cells, including adipocytes and osteocytes.\(^4\),\(^5\) Moreover, large amounts of ASCs could be prepared by a minimal invasive procedure that is relatively easy to perform, such as lipoaspiration under local anesthesia. ASCs have shown a curative effect in preclinical studies.\(^6\)–\(^10\) However, the clinical application of stem-cell transplantation for many internal organs has been restricted due to a lack of sufficient technologies to trace the transplanted stem cells and to evaluate their growth and migration in vivo.\(^11\) The development of labeling and in vivo imaging technologies for stem cells has therefore become important in their clinical applications.

Magnetic resonance (MR) imaging has already been used in clinical practice. The image contrast in MR imaging is higher than that in computed tomography (CT) imaging.\(^12\),\(^13\) Magnetic nanoparticles are known to be a contrast media for MR imaging, and are expected to be useful for stem-cell labeling and in vivo imaging.\(^12\)–\(^15\) We recently developed six kinds of positively charged nanoparticles [dextran hydroxypropyltrimethyl ammonium chloride-coated magnetic iron oxide nanoparticles (TMADM-01-06)], and found that some kinds of cells that include stem cells could be efficiently labeled with TMADM-03.\(^16\),\(^17\) We have already reported that TMADM-03 could label hepatocellular carcinoma cells (HepG2; liver hepatocellular carcinoma-derived cell line)\(^18\) and ASCs.\(^23\) TMADM-03 can transduce into cells through the cell membrane with high efficiency, and is therefore expected to serve as a transduction agent for various kinds of functional molecules.

QDs are inorganic fluorescence probes that have many distinctive advantages in comparison to organic or protein fluorescence probes, including high luminance, superior photostability, high quantum yields and wide excitation wavelengths.\(^19\),\(^20\) Thus, QDs have recently received a great deal of attention as potential fluorescence probes for biomolecules and live cells, including stem cells.\(^21\),\(^22\) In this study, we investigated whether TMADM-03 can serve as a transduction agent for stem-cell imaging with QDs. Qdot ITK carboxyl quantum dots 655 (QDs655: shows a high quantum yield in the NIR region) was used as a candidate for the QD.

**Transduction Function of a Magnetic Nanoparticle TMADM for Stem-Cell Imaging with Quantum Dots**

**Yusuke OGIHARA,†1,2 Hiroshi YUKAWA,†1,2† Daisuke ONOSHIMA,‡2,‡3 and Yoshinobu BABA†1,2,‡3,‡4,‡5**

†1 Department of Applied Chemistry, Graduate School of Engineering, Nagoya University, Furo-cho, Chikusa, Nagoya 464–8603, Japan

†2 ImPACT Research Center for Advanced Nanobiodevices, Nagoya University, Furo-cho, Chikusa, Nagoya 464–8603, Japan

†3 Institute of Innovation for Future Society, Nagoya University, Furo-cho, Chikusa, Nagoya 464–8603, Japan

†4 Health Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), 2217-14 Hayashi-cho, Takamatsu 761-0395, Japan

†5 College of Pharmacy, Kaohsiung Medical University, 100, Shin-Chuan 1st Rd., Kaohsiung, 807, Taiwan, R. O. C.

We investigated the transduction function of a cationic dextran hydroxypropyltrimethyl ammonium chloride-coated magnetic iron oxide nanoparticle (TMADM-03) for transducing quantum dots (QDs) into adipose tissue-derived stem cells (ASCs). As a result, the fluorescence intensity of ASCs labeled with QDs using TMADM-03 was much higher than that of QDs only labeling. These data suggest that TMADM-03 can be useful as a transduction agent for QDs in stem-cell imaging.

**Keywords** Bioimaging, stem cell, quantum dots, magnetic nanoparticle, transduction agent

(Received January 24, 2017; Accepted January 27, 2017; Published February 10, 2017)

---

\(^1\) To whom correspondence should be addressed.
E-mail: h.yukawa@nanobio.nagoya-u.ac.jp

---

Fig. 1 Schematic illustration of TMADM-03.

---

Rapid Communications
655 nm fluorescence wavelength peak) and a Hank’s balanced salt solution were purchased from Life Technologies™ Japan (Tokyo, Japan). Fetal bovine serum (FBS) was purchased from Trace Scientific Ltd. (Melbourne, Australia). Collagenase Type II was purchased from Koken Co., Ltd. (Tokyo, Japan). TMADM-03 (Fig. 1) was synthesized and purchased from Meito Sangyo Co., Ltd (Aichi, Japan). Briefly, TMADM-03 was provided by coating magnetic iron oxide with a polysaccharide modified trimethylamine dextran.

Polysaccharide was synthesized by the following method. Dextran (100 g) was dissolved in water (100 mL); sodium hydroxide and diethylaminoethyl chloride were added to the dextran solution at approximately 30°C or less. The solution was stirred at approximately 60°C for 2 – 3 h. Hydrochloric acid was added to the solution to regulate pH 8. Methanol and acetone of 2.0-times the solution were added to the solution under stirring, and the reaction products were precipitated. The reaction products were redissolved to water (500 mL), filtrated by a glass filter, concentrated under reduced pressure, and lyophilized.

Cationic polysaccharide (15.0 g) was dissolved in water (40 mL), and underwent nitrogen substitution at room temperature while stirring. The solution was mixed with an iron salt solution (ferrous chloride (0.9 g) in 1 M ferric chloride solution (8.8 mL)), and a 1.5 M of sodium hydroxide solution was added to this solution to regulate pH 11. The provided solution was purified by ultrafiltration using boric acid buffer (pH 9) as a solvent. Acetone was added to the solution to precipitate TMADM-03, and then TMADM-03 was collected by filtering using a membrane filter (pore size, 0.2 μm).

The isolation and culture of ASCs were reported previously. Briefly, 7- to 14-month-old female C57BL/6 mice were killed by cervical dislocation, and then adipose tissue specimens were isolated from the inguinal groove and washed by Hank’s buffer to remove the blood cells. The adipose tissues were cut finely and digested with type II collagenase at 37°C in a shaking water bath for 90 min. Adipose tissue cells were suspended in the cell culture medium (Dulbecco’s modified Eagle’s medium (DMEM)/F12 containing 20% FBS and 100 U/mL penicillin/streptomycin). The cells were centrifuged at 1200 rpm for 5 min at room temperature to obtain a pellet containing the ASCs. The cells were washed three times by suspension and centrifugation in the culture medium. The primary cells were cultured for 4 – 5 days until they reached confluence, and were defined as passage “0”. The cells used in all of the experiments were between passages 2 and 5.

QDs655 (2 nM) and TMADM-03 (60 μg-Fe/mL) were mixed for 15 min at room temperature, and then added to ASCs in a medium for QDs transduction (transduction medium: DMEM/F12 containing 2% FBS and 100 U/mL penicillin/streptomycin) at 37°C. After 4 h of incubation, the ASCs were washed using a transduction medium. Then, the transduction of QDs655 into ASCs was observed by phase-contrast fluorescence microscopy (A1MP+/A1RMP+, Nikon, Tokyo, Japan).

ASCs (1 × 10⁵ cells) were seeded in a 12-well plate (BD Falcon; BD Biosciences) with 100 μL of the culture medium; the cells were then cultured for 24 h. QDs655 (0.8 nM) were added in a transduction medium. QDs655 (0.8 nM) and TMADM-03 (25 μg-Fe/mL) were mixed for 15 min at room temperature. ASCs were incubated with the QDs655 only, QDs655 and TMADM-03 in a transduction medium at 37°C. After 24 h of incubation, the cells were washed using a transduction medium. The transduction efficiency was evaluated by flow cytometry analysis (BD LSRFortessa™ X-20, Japan BD, Tokyo, Japan).
A schematic illustration of the cationic dextran hydroxypropyltrimethyl ammonium chloride-coated magnetic iron oxide nanoparticles (TMADM-03) is shown in Fig. 1. The diameter and zeta voltage of QDs655 and TMADM-03 were detected. The diameter of QDs655 was 20 nm, and the zeta voltage of QDs655 was –39 mV. Oishi et al. have already reported that the diameter of TMADM-03 was 51 nm, and the zeta voltage of TMADM-03 was +2.0 mV.17 When QDs655 and TMADM-03 were mixed, they were aggregated. The particle diameter was detected by dynamic light scattering, and the peak of the size frequency distribution was 107.3 ± 0.10 nm. This result was probably caused by the interaction of QDs655 with TMADM-03. In a previous study, TMADM-03 was proven to be an effective contrast agent.23 The transduction efficiency of TMADM-03 into ASCs was about four-fold more efficient than that of the alkali-treated dextran-coated magnetic iron oxide nanoparticle (ATDM), which is a major component of commercially available contrast agents, such as ferucarbotran. The labeled ASCs could be imaged with good contrast using a nanoparticle (ATDM), which is a major component of commercially available contrast agents, such as ferucarbotran. The labeled ASCs could be imaged with good contrast using a 1T MR imaging system in mice.23

To investigate whether QDs655 can be transduced into ASCs using TMADM-03, ASCs were incubated with QDs655 using TMADM-03. Fluorescence images of ASCs incubated with QDs655 only and using TMADM-03 are shown in Figs. 2a – 2h. ASCs incubated with QDs655 only were confirmed to be labeled with QDs655; however, red fluorescence derived from QDs655 was found to be very weak (Fig. 2c). On the other hand, ASCs incubated with QDs655 using TMADM-03 was confirmed to be labeled with QDs655, and the red fluorescence derived from QDs655 was much stronger than that in QDs655 only labeling (Fig. 2g). In addition, QDs655 were confirmed to exist around the nucleus (Figs. 2b, 2d and 2f, 2h). In addition, 3D fluorescence image of the ASCs labeled with QDs655 using TMADM-03 is shown in Fig. 2i. This figure shows that almost all QDs655 were not on the cell membrane, but transduced into the cytoplasm of ASCs.

TMADM-03 is positively charged and can transduce into cells through the cell membrane with high efficiency.14 On the other hand, QDs655 appears to be negatively charged, so appeared to interact with TMADM-03 due to the electrostatic force. As a result, TMADM-03 seems to serve as a transduction agent for QDs655.

The transduction efficiency and fluorescence intensity of QDs655 in ASCs were analyzed using flow cytometry (Fig. 3). The threshold of the fluorescence intensity was determined to be 10 a.u. from the intrinsic fluorescence intensity of non-labeled ASCs (2.17%; Fig. 3a). By using the threshold value, the transduction efficiency can be calculated, as shown in Figs. 3b and 3c. The transduction efficiencies in QDs655 only and using the TMADM-03 conditions transduction were 97.8 ± 1.11 and 99.6 ± 0.10%, respectively (Figs. 3b and 3c). The peaks of the fluorescence intensity were 2.53 (non-labeled ASCs), 62.9 (QDs655 only labeling), and 473 (QDs655 using TMADM-03). The fluorescence intensity of QDs655 in ASCs using TMADM-03 was about seven-times higher than that of QDs655 only labeling. These data suggest that more QDs were transduced into ASCs by TMADM-03. Therefore, TMADM-03 could be useful as a transduction agent for QDs.

Acknowledgements

This research was mainly supported by the Japan Agency for Medical Research and Development (AMED) through its “Research Center Network for Realization of Regenerative Medicine”. This work was partially supported by JSPS KAKENHI Grant Numbers JP26790006. We appreciate the help of Yoko Tsutsui and Tomoko Arimoto (Nagoya University) for the treatment of ASCs.

References

1. I. Sakaida, S. Terai, N. Yamamoto, K. Aoyama, T. Ishikawa, H. Nishina, and K. Okita, Hepatology, 2004, 40, 1304.
2. A. Banas, T. Teratani, Y. Yamamoto, M. Tokuhara, F. Takeshita, M. Osaki, M. Kawamura, T. Kato, H. Okochi, and T. Ochiya, Stem Cells, 2008, 26, 2705.
3. V. S. Urbán, J. Kiss, J. Kovács, É. Góca, V. Vas, É. Monostori, and F. Uher, Stem Cells, 2008, 26, 244.
4. D. Ding, D. Mao, K. Li, X. Wang, W. Qin, R. Liu, D. S. Chiam, N. Tomczak, Z. Yang, B. Z. Tang, D. Kong, and B. Liu, ACS Nano, 2014, 8, 12620.
5. G. Constantin, S. Marconi, B. Rossi, S. Anqiari, L. Calderan, E. Anqlieri, B. Gini, S. D. Bach, M. Martinello, F. Bifari, M. Galié, E. Turano, S. Budui, A. Sbarbati, M. Krampera, and B. Bonetti, Stem Cells, 2009, 27, 2624.
6. A. Banas, T. Teratani, Y. Yamamoto, M. Tokuhara, F. Takeshita, M. Osaki, T. Kato, H. Okochi, and T. Ochiya, J. Gastroenterol. Hepatol., 2009, 24, 70.
7. L. Jackson, D. R. Jones, P. Scotting, and V. Sotille, J. Postgrad Med., 2007, 53, 121.
8. L. S. Meireles and N. B. Nardi, Front. Biosci., 2009, 14, 4281.
9. B. Puissant, C. Barreau, P. Bourin, C. Clavel, J. Corre, C. Bousquet, C. Taureau, B. Cousin, M. Abbal, P. Laharrague,
10. E. W. Choi, I. S. Shin, S. Y. Park, E. J. Yoon, S. K. Kang, J. C. Ra, and S. H. Hong, *Cell Transplant.*, 2014, 23, 873.
11. A. Crabbe, C. Vandeputte, T. Dresselaers, A. A. Sacido, J. M. Verdugo, J. Eyckmans, F. P. Luyten, K. Van Laere, C. M. Verfaillie, and U. Himmelreich, *Cell Transplant.*, 2010, 19, 919.
12. K. Nohroudi, S. Armhold, T. Berhorn, K. Addicks, M. Hoehn, and U. Himmelreich, *Cell Transplant.*, 2010, 19, 431.
13. M. Gerben, G. Kotek, P. A. Wielopolski, E. Farrell, P. K. Bos, H. Weinans, A. U. Grohnert, H. Jahr, J. A. Verhaar, G. P. Krestin, G. J. van Osch, and M. R. Bernsen, *PLoS One*, 2011, 6, e17001.
14. T. H. Kim, J. K. Kim, W. Shim, S. Y. Kim, T. J. Park, and J. Y. Jung, *Magn. Reson. Imaging*, 2010, 28, 1004.
15. X. He, J. Cai, B. Liu, Y. Zhong, and Y. Qin, *Stem Cell Res. Ther.*, 2015, 6, 207.
16. K. Oishi, H. Noguchi, H. Saito, H. Yukawa, Y. Miyamoto, K. Ono, K. Murase, M. Sawada, and S. Hayashi, *Cell Med.*, 2012, 3, 43.
17. K. Oishi, Y. Miyamoto, H. Saito, K. Murase, K. Ono, M. Sawada, M. Watanabe, Y. Noguchi, T. Fujiwara, S. Hayashi, and H. Noguchi, *Plos One*, 2013, 8, e57046.
18. Y. Miyamoto, Y. Koshidaka, H. Noguchi, K. Oishi, H. Saito, H. Yukawa, N. Kaji, T. Ikeya, S. Suzuki, H. Iwata, Y. Baba, K. Murase, and S. Hayashi, *Cell Medicine*, 2013, 9, 89.
19. J. H. Liu, L. Cao, G. E. LeCroy, P. Wang, M. J. Meziani, Y. Dong, Y. Liu, P. G. Luo, and Y. P. Sun, *ACS Appl. Mater. Interfaces*, 2015, 7, 19439.
20. H. S. Han, E. Niemeyer, Y. Huang, W. S. Kamoun, J. D. Martin, J. Bhaumik, Y. Chen, S. Roberge, J. Cui, M. R. Martin, D. Fukumura, R. K. Jain, M. G. Bawendi, and D. G. Duda, *Proc. Natl. Acad. Sci. U. S. A.*, 2015, 112, 1350.
21. J. S. Park, S. W. Yi, H. J. Kim, S. M. Kim, S. H. Shim, and K. H. Park, *Biomaterials*, 2016, 77, 14.
22. H. Yukawa, Y. Kagami, M. Watanabe, K. Oishi, Y. Miyamoto, Y. Okamoto, M. Tokeshi, N. Kaji, H. Noguchi, K. Ono, M. Sawada, Y. Baba, N. Hamajima, and S. Hayashi, *Biomaterials*, 2010, 31, 4094.
23. H. Yukawa, S. Nakagawa, Y. Yoshizumi, M. Watanabe, H. Saito, Y. Miyamoto, H. Noguchi, K. Oishi, K. Ono, M. Sawada, I. Kato, D. Onoshima, M. Ohayashi, Y. Hayashi, N. Kaji, T. Ishikawa, S. Hayashi, and Y. Baba, *PLOS One*, 2014, 9, e110142.