Cell Medicine, Vol. 6, pp. 83–90, 2013
Printed in the USA.

2155-1790/13 $90.00 + .00
DOI: http://dx.doi.org/10.3727/215517913X674270
www.cognizantcommunication.com

Induced Pluripotent Stem Cell Labeling Using Quantum Dots

Hiroshi Yukawa,* Kaoru Suzuki,* Yuki Kano,† Tatsuya Yamada,† Noritada Kaji,*‡
Tetsuya Ishikawa,† and Yoshinobu Baba*‡§

*FIRST Research Center for Innovative Nanobiodevices, Nagoya University, Furo-cho, Chikusa-ku, Nagoya, Japan
†Department of Medical Technology, Nagoya University, Graduate School of Medicine, Daikominami, Higashi-ku, Nagoya, Japan
‡Department of Applied Chemistry, Nagoya University, Graduate School of Engineering, Furo-cho, Chikusa-ku, Nagoya, Japan
§Health Technology Research Center, National Institute of Advanced Industrial Science and Technology (AIST), Hayashi-cho, Takamatsu, Japan

Induced pluripotent stem (iPS) cells have received remarkable attention as the cell sources for clinical applications of regenerative medicine including stem cell therapy. Additionally, labeling technology is in high demand for tracing transplanted cells used in stem cell therapy. In this study, we used quantum dots (QDs), which have distinct fluorescence abilities in comparison with traditional probes, as the labeling materials and investigated whether iPS cells could be labeled with QDs with no cytotoxicity. iPS cells could not be labeled with QDs alone but required the use of cell-penetrating peptides such as octaarginine (R8). No significant cytotoxicity to iPS cells was confirmed by up to 8 nM QDs, and the iPS cells labeled with QDs maintained their undifferentiated state and pluripotency. These data suggest that QDs can be used for fluorescence labeling of iPS cells.

Key words: Induced pluripotent stem (iPS) cells; Quantum dots (QDs); Octaarginine (R8)

INTRODUCTION

Induced pluripotent stem (iPS) cells have recently been reported as being generated by the retroviral transfection of only four genes [v-myc myelocytomatosis viral oncogene homolog (c-Myc), Krüppel-like factor 4 (Klf4), octamer-binding transcription factor 4 (Oct4), and sex-determining region Y box 2 (Sox2)] and that this would be sufficient to induce a state of pluripotency from mouse fibroblasts (13,14,17). The iPS cells were confirmed to have self-renewal ability and pluripotency and could be obtained from mice and humans, including patients with genetic diseases (4,19). Recently, safe methods of iPS cell generation without retroviral transfection have been studied in many laboratories all over the world to reduce the possibility of cancerous change of generated iPS cells (18–20,24–27). The developments of their methods are thus thought to be important for the clinical application of regenerative medicine.

On the other hand, the applications of nanotechnology such as nanomaterials, nanostructures, and nanodevices to regenerative medicine have been increasingly recognized. Nanomaterials have some especially unique properties as compared with traditional biomaterials, meaning that their application in regenerative medicine including stem cell therapies is likely (9,10). In fact, the importance of nanomaterials in stem cell therapies for injuries and diseases have been reported (1,7,11,16,21). The nanomaterials known as quantum dots (QDs) generally consist of a cadmium selenide (CdSe) core, zinc sulfide (ZnS) shell, and polymer coating and have several distinctive fluorescence advantages such as high quantum yields, narrow emission bandwidths, and resistance to photobleaching, so they have been widely used for ultrasensitive sensing of small functional molecules and cells (2,3). In our laboratories, we have confirmed that the application of QDs and Western blot technology enables us to detect cancer proteins related to diseases (5).

We have already revealed that QDs are available for stem cell labeling and in vivo fluorescence imaging (28, 29,31). Mouse adipose tissue-derived stem cells (mASCs) can be labeled frequently and without cytotoxicity using less than or equal to 8 nM QDs in combination with cell-penetrating peptides such as octaarginine (R8) (28). Moreover, transplanted mASCs combined with heparin in the liver was successfully monitored by fluorescence imaging of QDs (31). However, we have not yet investigated whether iPS cells can be labeled with QDs, and so far,

Received April 30, 2013; final acceptance October 23, 2013. Online prepub date: October 29, 2013.
Address correspondence to Hiroshi Yukawa, FIRST Research Center for Innovative Nanobiodevices, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan. Tel: +81-52-789-5654; Fax: +81-52-789-3560; E-mail: hiroshiy@med.nagoya-u.ac.jp

there are currently no articles to show the fluorescence labeling of iPS cells using QDs. In addition, few reports are closely associated with the use of nanomaterials except QDs to label iPS cells for long-term imaging of their in vivo distribution and development.

In this study, we examined whether QDs can consistently label mouse iPS cells and whether QD-labeled iPS cells retain their undifferentiated state and pluripotency. Then, we examined the optimal concentration of QDs for labeling iPS cells without cytotoxicity.

MATERIALS AND METHODS

Materials

QDs (Qdot ITK Carboxyl Quantum Dots; Invitrogen, Life Technologies, Grand Island, NY, USA) and R8 (Sigma-Genosys Japan, Hokkaido, Japan) were used in this study. Dulbecco's modified Eagle medium (DMEM), antibiotics (penicillin, streptomycin), minimum essential medium, and nonessential amino acids solution (NEAA) were purchased from GIBCO BRL, Life Technologies (Grand Island, NY, USA). Fetal bovine serum (FBS, BIOWEST) was purchased from Funakoshi Co., Ltd. (Tokyo, Japan). Phosphate-buffered saline (PBS), 2-mercaptoethanol (M6250), glycerol, dimethyl sulfoxide (DMSO; D2650), and formaldehyde were obtained from Sigma-Aldrich (St. Louis, MO, USA). Leukemia inhibitory factor (LIF, Chemicon) was from Dainippon Sumitomo Pharma (Osaka, Japan). Cell Counting Kit-8 (CCK-8) was purchased from Dojindo Laboratories (Kumamoto, Japan). Hematoxylin and eosin (H&E) were purchased from Muto Pure Chemicals (Tokyo, Japan). Mitomycin C was purchased from Kyowa Hakko Kirin (Tokyo, Japan). Costar plate was purchased from Corning Incorporated (Corning, NY, USA). All chemicals were reagent grade and used as received without further purification.

Cells

Mouse iPS cells (iPS-MEF-Ng-20D-17) established by Prof. S. Yamanaka in Kyoto University from male embryonic fibroblasts were purchased from Cell Bank of Riken Bioresource Center (Tsukuba, Japan). These cells exhibit green fluorescence in the undifferentiated state because of green fluorescent protein (GFP) expression being under the control of the undifferentiated state gene, Nanog. Mouse embryonic fibroblast (MEF) feeder cells (Chemicon International Lot 70915-9) were purchased from Dainippon Sumitomo Pharma (Osaka, Japan).

Mice

Four 6-week-old, male nude mice (BALB/cA Jcl-nu) were purchased from Clea Japan (Osaka, Japan). The mice were housed in a controlled environment (12-h light/dark cycles at 21°C) with free access to water and a standard chow diet before sacrifice. All conditions and handing of animals in this study were conducted with protocols approved by the Nagoya University Committee on Animal Use and Care.

MEF Feeder Cell Culture

MEF cells were cultured at 37°C with 5% CO_2 in MEF culture medium (DMEM with 10% FBS and 1% penicillin–streptomycin) and maintained until they reached confluence. The cells were treated with 50 µl of 100×mitomycin C and incubated for more than 2 h. Thereafter, they were cultured in 0.1% w/v gelatin-coated flasks [noncoated 25-cm^2 flask (Iwaki, Tokyo, Japan) treated with 3 ml of StemSure 0.1% w/v gelatin solution (Wako, Osaka, Japan) at 37°C, overnight] for more than 5 h.

iPS Cell Culture

iPS cells were maintained at 37°C with 5% CO_2 in iPS culture medium (DMEM containing 15% FBS, 1×NEAA, 2 mM 2-mercaptoethanol, 1% penicillin–streptomycin, and 1,000 U/ml mouse LIF) on feeder layers of mitomycin-treated MEF feeder cells. The medium was changed to a fresh iPS culture medium every day, and passaging was conducted every 3–4 days (17,22,23).

Transduction of R8–QDs Complex

QDs are made from nanometer-scale crystals of a semiconductor material (CdSe), which are shelled with an additional semiconductor layer (ZnS) to improve their chemical and optical properties (6,8). In addition, the polymer coating has COO^- surface groups. QDs655 (2.0 nM) (emission peak at 655 nm) and 2.0 µM R8 were mixed for 20 min at room temperature at the optimal ratio (QDs655:R8 = 1:10,000) and R8–QDs655 complexes were prepared. Next, iPS cells were incubated with the R8–QDs655 complexes in a transduction medium (DMEM/F12, 2% FBS, 100 U/ml penicillin/streptomycin) in a 15-ml centrifugation tube (Corning Incorporated) at 37°C for 1 h. After 1 h of incubation, the transduction of QDs655 into iPS cells was confirmed by conventional fluorescence microscopy.

Dose-Dependent Transduction of QDs

To check whether separated iPS cells without MEFs could be labeled with QDs effectively in comparison with clustered iPS cells, not including MEFs, trypsin-treated and separated clusters were seeded on gelatin-coated flasks and incubated at 37°C for 30 min. Supernatant was collected and used for separated iPS cells. They were incubated in a 15-ml centrifugation tube (Corning Incorporated) at 37°C for 1 h and then in a 24-well plate (BD Falcon, Franklin Lakes, NJ, USA) for 1 h.

Cytotoxicity of QDs to iPS Cells

iPS cells (2×10^4 cells) were seeded in 96-well plates (BD Biosciences, Tokyo, Japan) with 100 µl

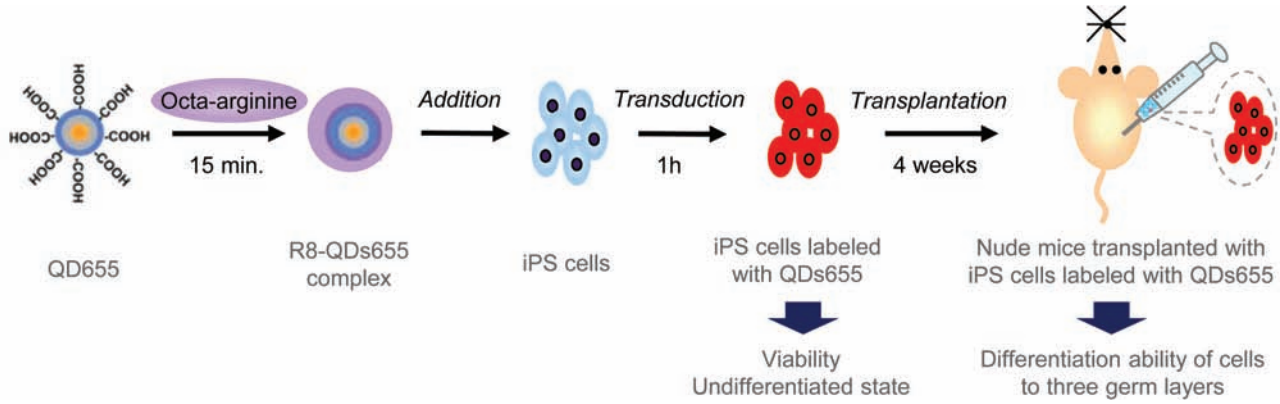

Figure 1. Experimental scheme of this study. QDs655 [emission peak at 655 nm of quantum dots (QDs)] were mixed with octaarginine (R8) for 15 min, and R8–QDs655 complexes were obtained. These complexes were then added to induced pluripotent stem (iPS) cells, and the iPS cells were labeled with QDs655. Next, the maintenance of undifferentiated state was checked in the iPS cells labeled with QDs655 by the detection of green fluorescent protein (GFP) expression under the control of the undifferentiated gene marker, Nanog. Finally, the multilineage differentiation ability of iPS cells labeled with QDs655 was confirmed in the mice by teratoma formation.

of iPS culture medium for 24 h, and then they were replaced with 100 µl of ES culture medium at 37°C. R8–QDs655 complexes formed in the optimal ratio were added to iPS cells, and after 1 h the medium was changed to new ES culture medium. Viable cells were then counted using CCK-8. CCK-8 reagent (10 µl) was added to each well, and the reaction was allowed to proceed for up to 4 h. The absorbance of the sample at 450 nm was measured against a background control using a microplate reader (POLARstar; BMG LABTECH, Offenburg, Germany).

Embryoid Body (EB) Formation

iPS cells were harvested with 0.25% trypsin–EDTA (Sigma-Aldrich), and the dissociated cells were suspended in iPS culture medium. The iPS cells were labeled with

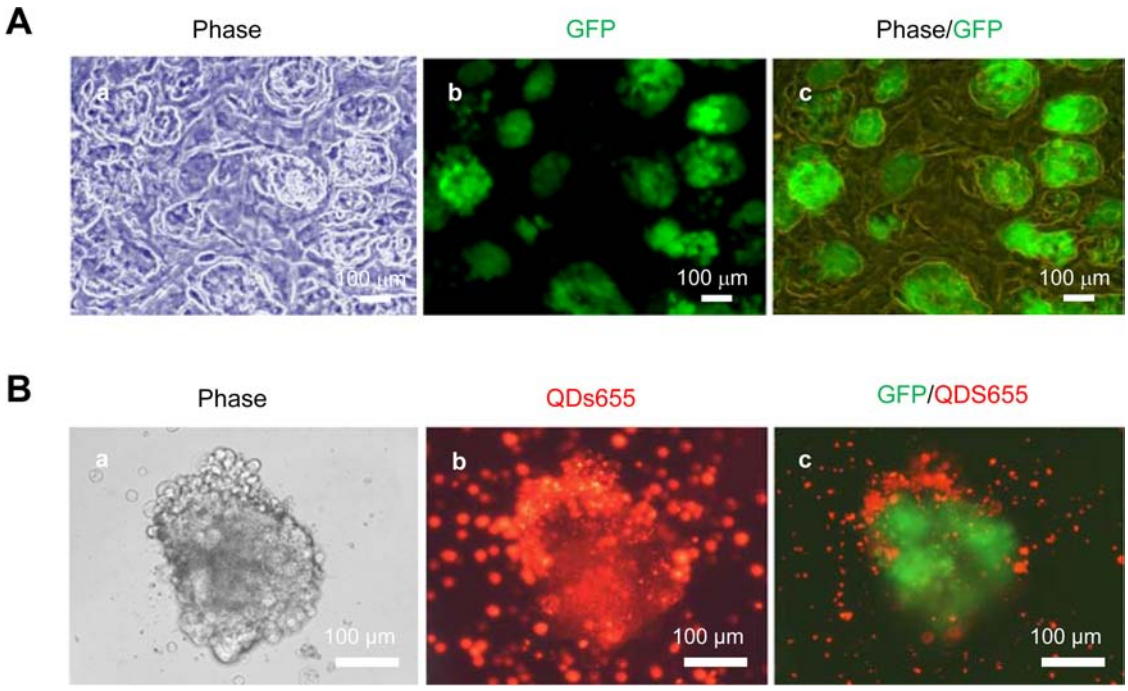

Figure 2. Morphology of the clusters of iPS cells labeled with QDs655. (A) The morphologies of nonlabeled iPS cells [phase contrast (a), fluorescence (b), merge (c)] are shown. The green fluorescence derived from GFP showing the nondifferentiated state was confirmed from the clusters of iPS cells. (B) The morphology of iPS cells labeled with QDs655 [phase contrast (a), fluorescence (b), merge (c)] are shown. The red fluorescence of the QDs was confirmed from the clusters of iPS cells. Scale bars: 100 µm.

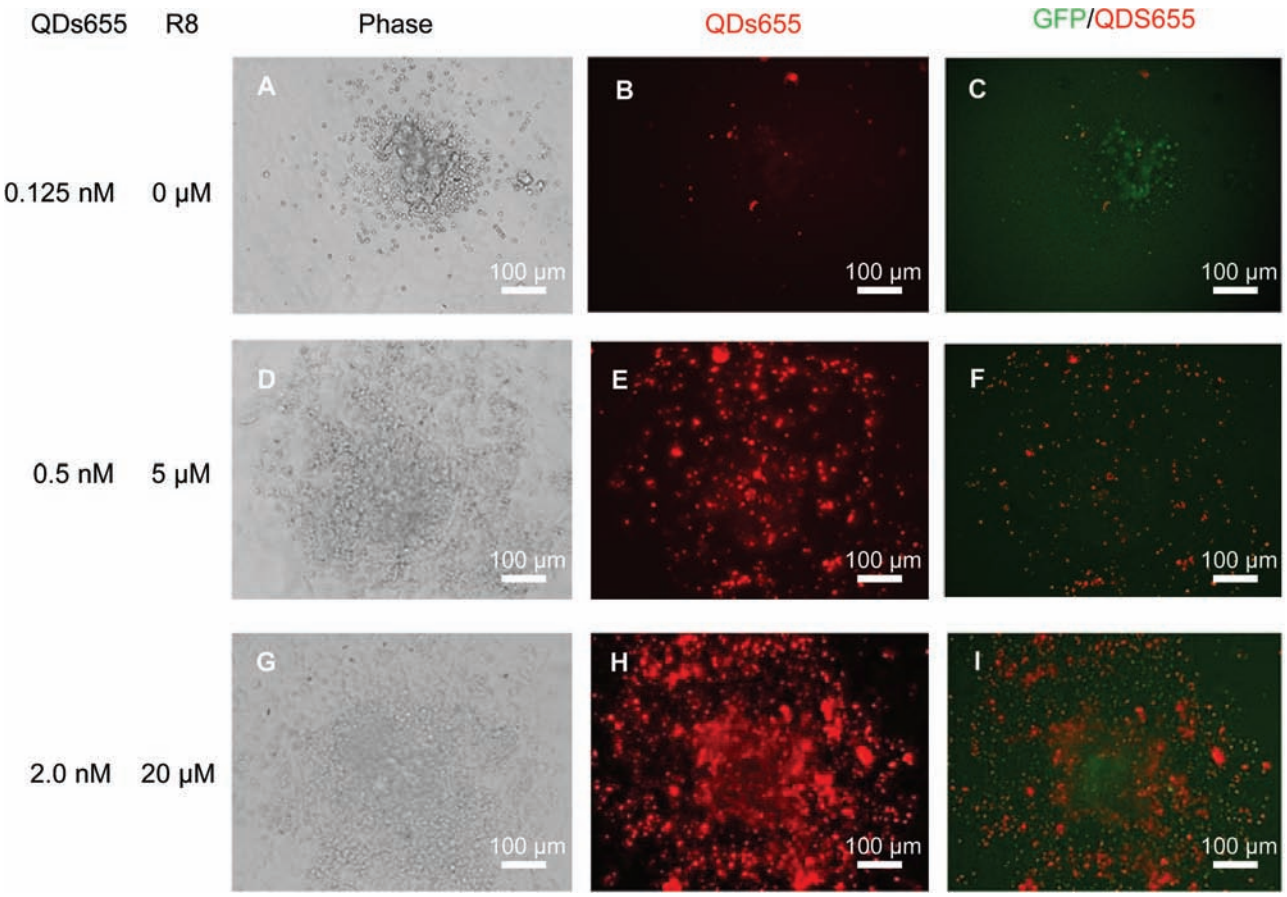

Figure 3. Morphology of the separated iPS cells labeled with QDs655. The morphologies of separated iPS cells labeled with QDs655 using R8 [phase contrast (A, D, G), fluorescence (B, E, H), merge (C, F, I)] are shown. The red fluorescence could be observed under the condition of R8 usage. Scale bars: 100 μm.

1.0 nM QDs655 using R8 for 1 h and washed once with PBS. For EB formation, iPS cells suspended in medium at 2×10^4 cells/droplet per well were applied to each well of 96-well round-bottomed low attachment Costar plates and incubated for 1 day. The EB morphology, GFP, and QDs655-related fluorescence were monitored under a fluorescence microscope.

Teratoma Formation and Histological Analysis

iPS cells labeled with 4.0 nM QDs655 were suspended at 1×10^7 cells/ml in PBS. A total of 100 μl of the iPS cell suspension (1×10^6 cells) was injected subcutaneously into the dorsal flank of nude mice (Fig. 1). Four weeks after the injection, the tumors were surgically dissected from the mice. The samples were fixed in PBS containing 4% formaldehyde and were embedded in paraffin. The embedded paraffin samples were sectioned by 5-μm thickness with a microtome (Leica, Wetzlar, Germany). The paraffin sections were stained with H&E and mounted.

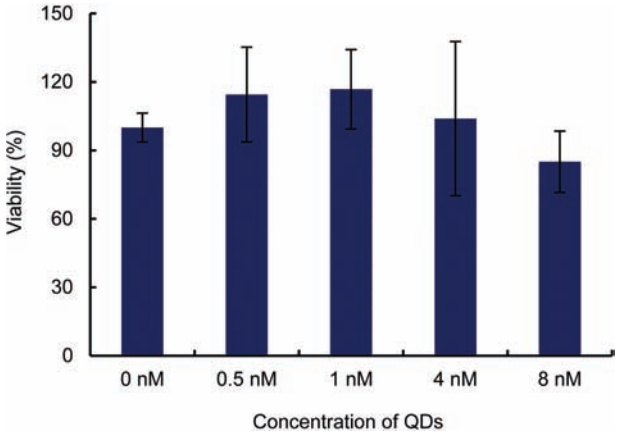

Figure 4. Cytotoxicity of QDs655 to iPS cells. Survival rate of iPS cells labeled with QDs after 24 h of labeling is shown. iPS cells were transduced with QDs655 using R8 at various concentrations for 24 h. The viability of iPS cells transduced with QDs655 using R8 was compared with nonlabeled iPS cells. No significant differences were detected at any concentration. The data, derived from triplicates, are shown as the mean ± SD values.

Statistical Analysis

Numerical values are presented as the mean ± SD. Each experiment was repeated three times. Statistical significance was evaluated using unpaired Student's *t* test with Bonferroni correction for multiple comparisons. A value of $p < 0.05$ was considered to be statistically significant. All statistical analyses were performed using the SPSS software package (IBM, Armonk, NY, USA).

RESULTS

Morphology of *iPS Cells and Transduction of QDs Into iPS Cells*

We confirmed that iPS cells cultured on MEF feeder cells show GFP staining under an undifferentiated state (Fig. 2A).

To check whether iPS cells can be labeled with QDs, iPS cells were incubated in MEF-treated 24-well microplates at 37°C with R8–QDs655 complexes for 1 h. The red fluorescence derived from QDs655 could be observed from iPS cells on MEF feeder cells using fluorescence microscopy. However, the red fluorescence could not be observed from all iPS cells (Fig. 2B). These results suggest that iPS cells could be labeled with QDs but that the labeling efficiency was not high when iPS cells formed a cluster state on the MEFs.

Transduction of QDs in a Dose-Dependent Manner

Separated iPS cells could be labeled with QDs (Fig. 3A–C). However, iPS cells were more effectively labeled by QDs in the presence of R8, and the labeling

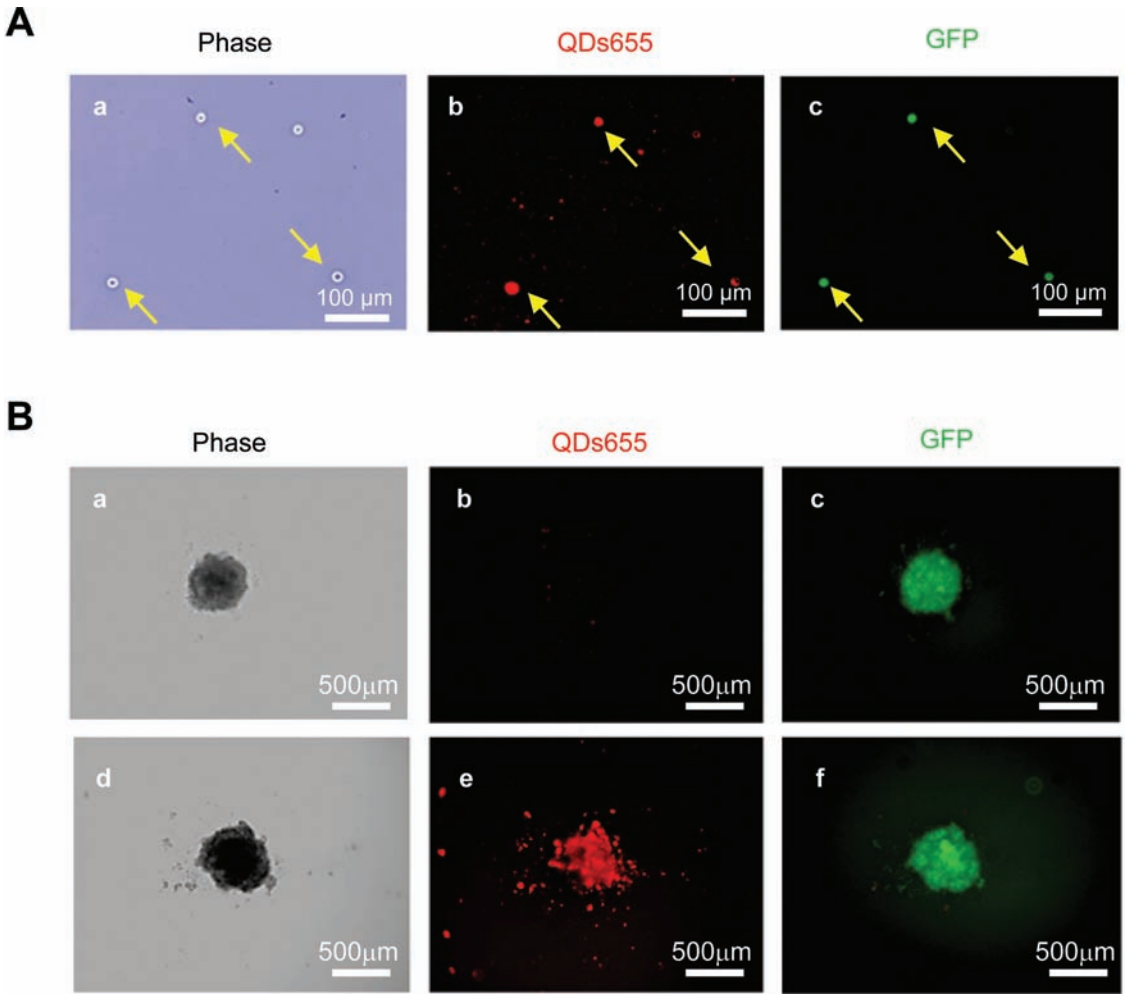

Figure 5. Maintenance of undifferentiated state and multipotency of iPS cells labeled with QDs. (A) The morphologies of iPS cells labeled with QDs655 [phase contrast (a), red fluorescence derived from QDs655 (b), the green fluorescence derived from GFP (c)] are shown. The labeled iPS cells are noted by yellow arrows. (B) The morphologies and fluorescence images 1 day after EB formation of iPS cells nonlabeled (a–c) or labeled (d–f) with QDs using R8 [phase contrast (a, d), red fluorescence derived from QDs655 (b, e), the green fluorescence derived from GFP (c, f)]. Scale bars: 100 μm in (A) and 500 μm in (B).

efficiency was dependent on the concentration of the R8–QDs655 complex (Fig. 3D–I).

Cytotoxicity of QDs to iPS Cells

QDs655 was transduced into iPS cells using R8 at various concentrations in a transduction medium for 24 h at 37°C. No significant cytotoxicity was observed in iPS cells labeled with less than or equal to 8 nM QDs655 (Fig. 4). The morphology and fluorescent images were confirmed by conventional fluorescent microscopy, and no abnormalities could be confirmed with any of the tested concentrations of QDs655 (0.0–8.0 nM). These data suggest that iPS cells could be labeled with at least 8.0 nM QDs using R8.

Maintenance of Undifferentiated State of iPS Cells Labeled With QDs

To ascertain whether the undifferentiated state of iPS cells can be maintained after labeling with QDs, the red and green fluorescence of iPS cells labeled with QDs655 was checked. Both the red and the green fluorescence derived from QDs655 and GFP, respectively, could be observed in the iPS cells labeled with 1.0 nM QDs (Fig. 5A). Moreover, after EB formation of iPS cells labeled with QDs655 using R8, the red and green fluorescence was again checked. The green fluorescence derived from GFP alone could be observed in the nonlabeled iPS cells (Fig. 5B, a–c). On the other hand, both red and green fluorescence derived from QDs655 and GFP could be observed in the iPS cells labeled with QDs using R8 (Fig. 5B, d–f). The GFP staining suggests that labeling with QDs does not affect the maintenance of the undifferentiated state of iPS cells.

Teratoma Formation of iPS Cells Labeled With QDs

To evaluate the pluripotency of iPS cells labeled with 2.0 nM QDs, we transplanted the iPS cells labeled with QDs into the dorsal flank of nude mice. The labeled iPS cells formed teratomas after 4 weeks of transplantation (Fig. 6A). Moreover, the teratoma could be ascertained to contain various tissues, such as nerves, cartilage, adipose, gut epithelium, and glomerulus of the kidney belonging to the three germ layers (endoderm, mesoderm, and ectoderm) by H&E staining (Fig. 6B–F). These results raise the possibility that the pluripotency of the iPS cells labeled with QDs may be maintained.

DISCUSSION

We challenged the labeling of iPS cells using QDs without cytotoxicity for the application to regenerative medicine including stem cell therapy and in vivo imaging of stem cells. iPS cells could not be labeled with QDs alone but were labeled with QDs efficiently in combination with R8. We previously revealed that cell-penetrating peptides such as R8 and transactivator of transcription peptides can effectively and rapidly penetrate iPS cell membranes with no cytotoxicity, and the iPS cells transduced with cell-penetrating peptides maintained their undifferentiated state (30). R8 was confirmed to be able to serve as the transfection agent of QDs to iPS cells in this study. However, when iPS cells were in the cluster state on the feeder cells, the transduction efficiency of QDs with R8 was comparatively low. This result is considered to be caused by the cluster state of iPS cells and the existence of feeder cells. The cluster state of iPS

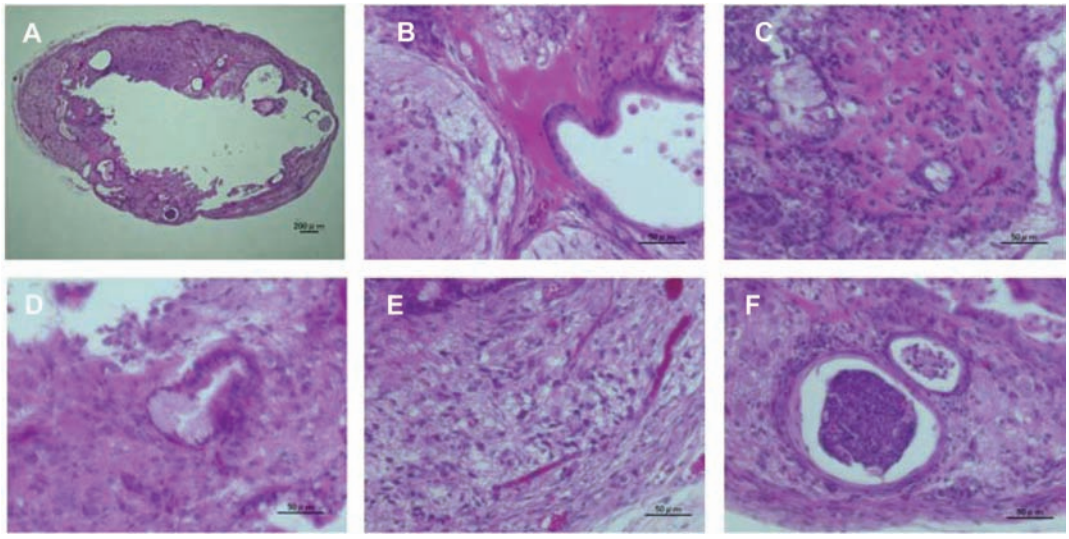

Figure 6. Teratoma formation of iPS cells labeled with QDs is shown. Four weeks after injection of labeled iPS cells into the nude mouse, the teratoma was surgically dissected from the mouse. Various tissues were present in the teratoma. Obtained histological sections were stained with H&E (A). Nerve-like structures (B), cartilage-like structures (C), gut epithelium-like structures (D), adipose-like structures (E), and glomerulus of the kidney-like structures (F) are shown. Scale bars: 200 μm in (A) and 50 μm in (B–F).

cells decreases the cell membrane area, which can interact with QDs, and the existence of feeder cells diverts the transfection of QDs into feeder cells. Therefore, iPS cells were cultured in a nonfeeder state, and then QDs with R8 were transduced into the separated iPS cells.

In this study, iPS cells could be labeled with QDs with high efficiency using R8, and no significant cytotoxicity to iPS cells could be confirmed at less than or equal to 8 nM QDs. These results showed the similar tendency to the case of labeling mASCs (28,30). The iPS cells labeled with QDs also maintained their undifferentiated state and pluripotency. GFP expression from iPS cells labeled with QDs was observed, and the differentiation of QD-labeled iPS cells to various germ layers in a teratoma was detected. These data suggested that the QDs can be used to label iPS cells without adverse effects. However, at concentrations higher than 8 nM, QDs may show cytotoxicity to iPS cells due to free Cd^{2+} derived from QDs (5,28). To reduce the likelihood of this, the use of Cd-free QDs may be preferable. Actually, we have already addressed the development of Cd-free QDs for cell labeling applications (15). For effective clinical application, thus, we would like to use Cd-free QDs for the labeling of iPS cells in the future.

In conclusion, iPS cells could be labeled with QDs using R8 effectively without cytotoxicity at less than or equal to 8 nM. Moreover, the iPS cells labeled with QDs maintained their undifferentiated state and pluripotency. These results suggest that QDs are useful for labeling iPS cells to track their application in regenerative medicine including stem cell therapy and in vivo imaging of stem cell distribution.

ACKNOWLEDGMENT: This research is partially supported by the Japan Society for the Promotion of Science (JSPS) through its "Funding Program for World-Leading Innovative R&D on Science and Technology (FIRST Program)" and partially supported by the Japan Science and Technology Agency (JST) through its "Research Center Network for Realization of Regenerative Medicine." The authors declare no conflict of interest.

REFERENCES

1. Aniruddh, S.; John, D. K.; Ki-Bum, L. Nanotechnology for regenerative medicine: Nanomaterials for stem cell imaging. Nanomedicine 3(4):567–578; 2008.
2. Bakalova, R.; Ohba, H.; Zhelev, Z.; Ishikawa, M.; Baba, Y. Quantum dots as photosensitizers. Nat. Biotechnol. 22(11):1360–1361; 2004.
3. Bakalova, R.; Zhelev, Z.; Ohba, H.; Baba, Y. Quantum dot-based Western blot technology for ultrasensitive detection of tracer proteins. J. Am. Chem. Soc. 127(26):9328–9329; 2005.
4. Chiang, C. H.; Chang, C. C.; Huang, H. C.; Chen, Y. J.; Tsai, P. H.; Jeng, S. Y.; Hung, S. I.; Hsieh, J. H.; Huang, H. S.; Chiou, S. H.; Lee, F. Y.; Lee, S. D. Investigation of hepatoprotective activity of induced pluripotent stem cells in the mouse model of liver injury. J. Biomed. Biotechnol. 2011:219060; 2011.
5. Cho, S. J.; Maysinger, D.; Jain, M.; Röder, B.; Hackbarth, S.; Winnik, F. M. Long-term exposure to CdTe quantum dots causes functional impairments in live cells. Langmuir 23(4):1974–1980; 2007.
6. Dabbousi, B. O.; Rodriguez-Viejo, J.; Mikulec, F. V.; Heine, J. R.; Mattoussi, H.; Ober, R.; Jensen, K. F.; Bawendi M. G. (CdSe)ZnS Core–shell quantum dots: Synthesis and characterization of a size series of highly luminescent nanocrystallites. J. Phys. Chem. 101(46):9463–9475; 1997.
7. Dennig, J.; Duncan, E. Gene transfer into eukaryotic cells using activated polyamidoamine dendrimers. J. Biotechnol. 90(3–4):339–347; 2002.
8. Hines, M. A.; Guyot-Sionnest, P. Synthesis and characterization of strongly luminescing ZnS-capped CdSe nanocrystals. J. Phys. Chem. 100(2):468–471; 1996.
9. Jain, K. Applications of nanobiotechnology in clinical diagnostics. Clin. Chem. 53(11):2002–2009; 2007.
10. Jain, K. Role of nanotechnology in developing new therapies for diseases of the nervous system. Nanomedicine 1(1):9–12; 2006.
11. Ji, J.; Ruan, J.; Cui, D. Advances of nanotechnology in the stem cells research and development. Nano Biomed. Eng. 2:66–88; 2010.
12. Lee, C. H.; Kim, J. H.; Lee, H. J.; Jeon, K.; Lim, H.; Choi, H.; Lee, E. R.; Park, S. H.; Park, J. Y.; Hong, S.; Kim, S.; Cho, S. G. The generation of iPS cells using non-viral magnetic nanoparticle based transfection. Biomaterials 32(28):6683–6691; 2011.
13. Maherali, N.; Sridharan, R.; Xie, W.; Utikal, J.; Eminli, S.; Arnold, K.; Stadtfeld, M.; Yachechko, R.; Tchieu, J.; Jaenisch, R.; Plath, K.; Hochedlinger, K. Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. Cell Stem Cell 1(1): 55–70; 2007.
14. Meissner, A.; Wernig, M.; Jaenisch, R. Direct reprogramming of genetically unmodified fibroblasts into pluripotent stem cells. Nat. Biotechnol. 25(10):1177–1181; 2007.
15. Miyazaki, Y.; Yukawa, H.; Nishi, H.; Okamoto, Y.; Kaji, N.; Torimoto, T.; Baba, Y. Adipose tissue-derived stem cell imaging using cadmium-free quantum dots. Cell Med. 2013.
16. Nagesha, D.; Laevsky, G. S.; Lampton, P.; Banyal, R.; Warner, C.; DiMarzio, C.; Sridhar, S. In vitro imaging of embryonic stem cells using multiphoton luminescence of gold nanoparticles. Int. J. Nanomedicine 2(4):813–819; 2007.
17. Okita, K.; Ichisaka, T.; Yamanaka, S. Generation of germline-competent induced pluripotent stem cells. Nature 448(7151): 313–317; 2007.
18. Okita, K.; Nakagawa, M.; Hyenjong, H.; Ichisaka, T.; Yamanaka, S. Generation of mouse induced pluripotent stem cells without viral vectors. Science 322(5903):949–953; 2008.
19. Park, H. Y.; Noh, E. H.; Chung, H. M.; Kang, M. J.; Kim, E. Y.; Park, S. P. Efficient generation of virus-free iPS cells using liposomal magnetofection. PLoS One 7(9):e45812; 2012.
20. Soldner, F.; Hockemeyer, D.; Beard, C.; Gao, Q.; Bell, G. W.; Cook, E. G.; Hargus, G.; Blak, A.; Cooper, O.; Mitalipova, M.; Isacson, O.; Jaenisch, R. Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. Cell 136(5):964–977; 2009.
21. Svenson, S.; Tomalia, D. A. Dendrimers in biomedical applications: Reflections on the field. Adv. Drug Deliv. Rev. 57(15):2106–2129; 2005.
22. Takahashi, K.; Okita, K.; Nakagawa, M.; Yamanaka, S. Induction of pluripotent stem cells from fibroblast cultures. Nat. Protoc. 2(12):3081–3089; 2007.

23. Tateishi, K.; He, J.; Taranova, O.; Liang, G.; D'Alessio, A. C.; Zhang, Y. Generation of insulin-secreting islet-like clusters from human skin fibroblasts. J. Biol. Chem. 283(46): 31601–31607; 2008.
24. Wang, W.; Lin, C.; Lu, D.; Ning, Z.; Cox, T.; Melvin, D.; Wang, X.; Bradley, A.; Liu, P. Chromosomal transposition of PiggyBac in mouse embryonic stem cells. Proc. Natl. Acad. Sci. USA. 105(27):9290–9295; 2008.
25. Warren, L.; Manos, P. D.; Ahfeldt, T.; Loh, Y. H.; Li, H.; Lau, F.; Ebina, W.; Mandal, P. K.; Smith, Z. D.; Meissner, A.; Daley, G. Q.; Brack, A. S.; Collins, J. J.; Cowan, C.; Schlaeger, T. M.; Rossi, D. J. Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. Cell Stem Cell 7(5): 618–630; 2010.
26. Woltjen, K.; Michael, I. P.; Mohseni, P.; Desai, R.; Mileikovsky, M.; Hamalainen, R.; Cowling, R.; Wang, W.; Liu, P.; Gertsenstein, M.; Kaji, K.; Sung, H. K.; Nagy, A. piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. Nature 458(7239):766–770; 2009.
27. Yu, J.; Hu, K.; Smuga-Otto, K.; Tian, S.; Stewart, R.; Slukvin, I. I.; Thomson, J. A. Human induced pluripotent stem cells free of vector and transgene sequences. Science 324(5928):797–801; 2009.
28. Yukawa, H.; Kagami, Y.; Watanabe, M.; Oishi, K.; Miyamoto, Y.; Okamoto, Y.; Tokeshi, M.; Kaji, N.; Noguchi, H.; Ono, K.; Sawada, M.; Baba, Y.; Hamajima, N.; Hayashi, S. Quantum dots labeling using octa-arginine peptides for imaging of adipose tissue-derived stem cells. Biomaterials 31(14):4094–4103; 2010.
29. Yukawa, H.; Mizufune, S.; Mamori, C.; Kagami, Y.; Oishi, K.; Kaji, N.; Okamoto, Y.; Takeshi, M.; Noguchi, H.; Baba, Y.; Hamaguchi, M.; Hamajima, N.; Hayashi, S. Quantum dots for labeling adipose tissue-derived stem cells. Cell Transplant. 18(5):591–599; 2009.
30. Yukawa, H.; Noguchi, H.; Nakase, I.; Miyamoto, Y.; Oishi, K.; Hamajima, N.; Futaki, S.; Hayashi, S. Transduction of cell-penetrating peptides into induced pluripotent stem cells. Cell Transplant. 19(6):901–909; 2010.
31. Yukawa, H.; Watanabe, M.; Kaji, N.; Okamoto, Y.; Tokeshi, M.; Miyamoto, Y.; Noguchi, H.; Baba, Y.; Hayashi, S. Monitoring transplanted adipose tissue-derived stem cells combined with heparin in the liver by fluorescence imaging using quantum dots. Biomaterials 33(7):2177–2186; 2012.