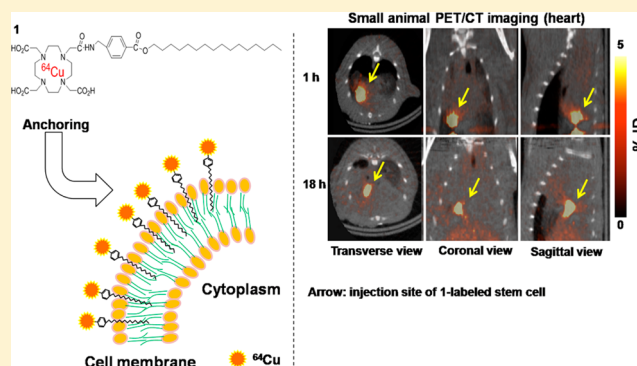


Simple Methods for Tracking Stem Cells with  $^{64}\text{Cu}$ -Labeled DOTA-hexadecyl-benzoateMin Hwan Kim,<sup>†,‡</sup> Sang-Keun Woo,<sup>†</sup> Kwang Il Kim,<sup>†</sup> Tae Sup Lee,<sup>†</sup> Chan Wha Kim,<sup>‡</sup> Joo Hyun Kang,<sup>†</sup> Byung Il Kim,<sup>†</sup> Sang Moo Lim,<sup>†</sup> Kyo Chul Lee,<sup>\*,†</sup> and Yong Jin Lee<sup>\*,†</sup><sup>†</sup>Molecular Imaging Research Center, Korea Institute of Radiological and Medical Sciences, Seoul 139-706, Republic of Korea<sup>‡</sup>School of Life Sciences and Biotechnology, Korea University, Seoul 136-701, Republic of Korea

## S Supporting Information

**ABSTRACT:** The purpose of this study was to evaluate  $^{64}\text{Cu}$ -labeled hexadecyl-1,4,7,10-tetraazacyclododecane-tetraacetic acid-benzoate ( $^{64}\text{Cu}$ -DOTA-HB) (**1**) as positron emission tomography (PET) radiotracer for stem cell imaging. Hexadecyl-DOTA-benzoate (DOTA-HB) (**2**) was efficiently labeled with  $^{64}\text{Cu}$  (>99%), and cell labeling efficiency with adipose-derived stem cells (ADSCs) was over 50%. Labeling with **1** did not compromise cell viability. In the PET imaging, intramuscularly transplanted **1**-labeled ADSCs were monitored for 18 h in normal rat heart. These results indicate that **1** can be utilized as a promising radiotracer for monitoring of transplanted stem cells.

**KEYWORDS:** Adipose-derived stem cells, copper-64, hexadecyl benzoate, radiotracer, PET



Longitudinal monitoring of transplanted stem cells is important for assessment of cell therapeutic effect. Molecular imaging technique allows noninvasive monitoring of the fate of transplanted stem cells in living bodies.

The molecular imaging modalities using radionuclide with positron emission tomography (PET) or single photon emission computed tomography provide high sensitivity ( $10^{-8}$  to  $10^{-9}$   $\mu\text{m/L}$ ) and spatial resolution (1–2 mm).<sup>1,2</sup>

$^{64}\text{Cu}$  has relatively long half-life (12.7 h) and its decay property ( $\beta^-$ , 0.573 MeV [38.4%];  $\beta^+$ , 0.655 MeV [17.8%];  $\gamma$ , 0.511 MeV [35.6%]) allows longitudinal PET imaging and tracking for transplanted cells.<sup>3,4</sup>  $^{64}\text{Cu}$  can react with a wide variety of chelator systems including 1,4,7,10-tetraazacyclododecane-tetraacetic acid (DOTA) or 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) due to its well-established coordination chemistry.<sup>5,6</sup> DOTA can bind with many different metal ions; thus, it leads to a decrease *in vivo* stability. Nevertheless, DOTA is currently used in  $^{64}\text{Cu}$ -diacetyl-bis(N4-methylthiosemicarbazone) ( $^{64}\text{Cu}$ -ATSM), approved by the U.S. Food and Drug Administration (FDA) in the United States. So, DOTA chelator is better clinically available and has a simple synthetic process.<sup>7</sup>  $^{64}\text{Cu}$  can potentially be linked to target including peptide, antibody, or nanoparticle via specified chelator system.

Membrane anchoring methods using lipophilic long alkyl chain or hydrophobic oleyl group is widely used for tight anchoring with lipid bilayer of cell membrane.<sup>8–11</sup> Hexadecyl benzoate (HB) has lipophilic property, so it easily and tightly anchors with cell membrane. Moreover, it is free from effects

mediated by other receptors that exist in the cell. Ma et al. developed hexadecyl-4- $^{18}\text{F}$ -fluorobenzoate ( $^{18}\text{F}$ -HFB), a long chain fluorinated benzoic acid ester, and labeled with rat mesenchymal stem cell with approximately 25% labeling efficiency. They reported that intravenously injected  $^{18}\text{F}$ -HFB-labeled mesenchymal stem cells showed persistent accumulation of radioactivity in the lungs by PET imaging.<sup>12</sup> However, half-life of  $^{18}\text{F}$  (110 min) is too short to longitudinally monitor the transplanted stem cells *in vivo*. Here, we developed HB conjugated DOTA bifunctional chelator and labeled it with  $^{64}\text{Cu}$  (**1**), then labeled it with rat adipose-derived stem cells (ADSCs). Next, we evaluated the efficiency of longitudinal monitoring of transplanted **1**-labeled ADSCs in rat normal heart.

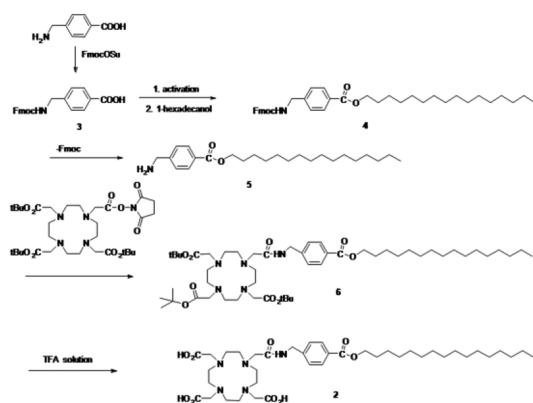
The precursor of **2** was prepared in five steps from *p*-amino methyl benzoic acid. Hexadecyl ester (**5**) was prepared in high yield by the condensation of 1-hexadecanol and triphenyl phosphite at 0 °C for 1 h. Compound **2** was prepared by conjugation of hexadecyl ester (**5**) and DOTA-mono-NHS-tris(*t*Bu ester) using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and then hydrolysis using trifluoroacetic acid (TFA)/DCM/ $\text{Et}_3\text{SiH}$  = 8:2:1 solution, giving the desired products with 59% yields (Scheme 1 and Supporting Information Figures 1S–6S).

Scheme 2 shows the chemical structure of  $^{64}\text{Cu}$ -labeled DOTA-HB, **1**.  $^{64}\text{Cu}$  and HB were conjugated via DOTA

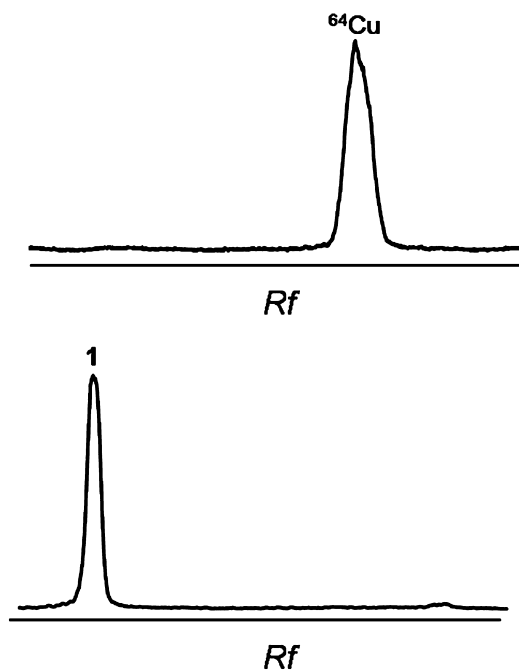
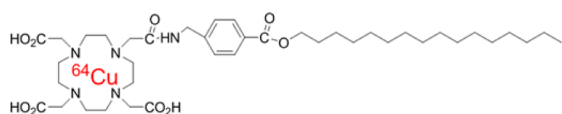
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Scheme 1. Synthesis of Precursor of  $^{64}\text{Cu}$ -Labeled DOTA-HB, 1

bifunctional chelator. The labeling yield of **2** with  $^{64}\text{Cu}$  was  $99.7 \pm 0.2\%$  (Figure 1).

Scheme 2. Chemical Structure of  $^{64}\text{Cu}$ -labeled DOTA-HB, 1Figure 1. Radio-TLC of **1**.  $R_f$  = retention factor.

Isolated ADSCs highly expressed stem cell specific markers including CD44 (78.9%) and CD90 (82.5%), whereas expression levels of endothelial or hematopoietic cell markers including CD31 or CD45 were as low as 1.1% and 0.8%, respectively (Supporting Information Figure 7S).

Cell labeling efficiency was  $25.7 \pm 1.4\%$ ,  $53.0 \pm 10.2\%$ , and  $59.4 \pm 1.2\%$  at 0.5, 1, and 2 h, respectively (Figure 2A), which is higher than previously reported results using lipophilic cell labeling agent.<sup>12,13</sup> Cell labeling efficiency of **1** was high; thus, only the low dose of **1** is sufficient for labeling a number of stem cells.

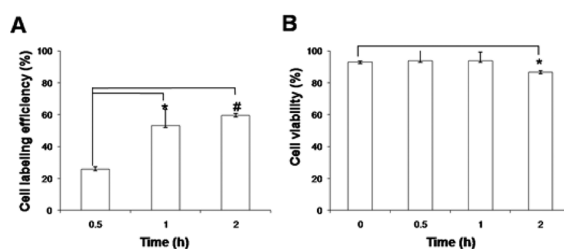


Figure 2. Cell labeling efficiency and cell viability with time. (A) Cell labeling efficiency of **1** with ADSCs for 2 h. (B) Cell viability of **1**-labeled ADSCs for 2 h. Error bars denote the standard deviation ( $n = 3$ ). \* $P < 0.05$ ; # $P < 0.001$ .

Cell viability of **1**-labeled ADSCs was  $92.9 \pm 0.6$ ,  $93.8 \pm 6.7$ ,  $93.7 \pm 5.6$ , and  $86.5 \pm 0.9\%$  at 0 min, 30 min, 1 h, and 2 h, respectively (Figure 2B). Adonai et al. reported that  $^{64}\text{Cu}$  labeled pyruvaldehyde-bis(N4-methylthiosemicarbazone) ( $^{64}\text{Cu}$ -PTSM)-labeled C6 glioma cells showed 85 and 80% of cell viability at 35 and 270 min, respectively.<sup>3</sup> Labeling with **1** showed slightly higher cell viability compared with  $^{64}\text{Cu}$ -PTSM,<sup>3</sup> and it did not significantly affect the viability of ADSCs (>85%) up to 2 h (Figure 2B). We labeled with **1** for 1 h to obtain optimal cell viability.

The *in vivo* distribution of ADSCs labeled with **1** was observed at 1 h postinjection by a small animal PET/computed tomography (CT) scanner. Radioactivity associated with ADSCs was deposited in the lungs by the injection of **1**-labeled ADSCs, whereas in the group of **1** injection most radioactivity was accumulated in the liver, then excreted via bladder (Supporting Information Figure 8S). Intravenously injected **1**-labeled ADSCs were efficiently trapped in the lungs, but intravenously injected labeling agent **1** did not react with cells in the mouse and was metabolized and excreted through liver and bladder. The high lung uptake of intravenously injected cells reported by many previous studies for investigation of *in vivo* stability in the cells labeled with radioactive agents is the same as the results in the current study.<sup>14–16</sup> To confirm the accumulation of **1**-labeled ADSCs in the lungs, digital autoradiography was performed at 1 h after cell injection. In the lungs, high radioactivity of ADSCs was observed; thus, this result showed that **1** was tightly anchored with cell membrane of ADSCs (Supporting Information Figure 9S).

Moreover, the intramuscularly transplanted **1**-labeled ADSCs were longitudinally monitored for 18 h in normal rat heart by PET/CT imaging (Figure 3). Zhang et al. reported that hexadecyl-4- $^{18}\text{F}$ -fluorobenzoate ( $^{18}\text{F}$ -HFB)-labeled human cir-

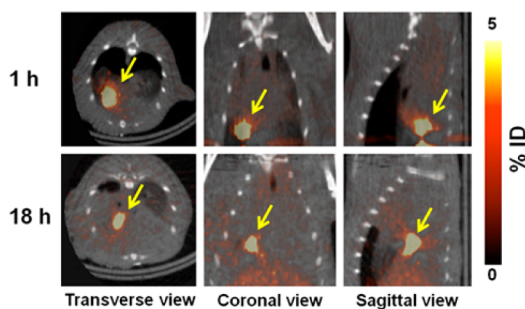


Figure 3. Small animal PET/CT image of intra-myocardially injected **1**-labeled ADSCs (1.8–3.7 MBq, 100  $\mu\text{L}$ ) in normal heart for 18 h. Arrow indicates the site of **1**-labeled ADSC injection.

culating progenitor cells were noninvasively monitored in a rat myocardial infarction model up to 4 h, but long-term monitoring is impossible due to the short half-life of  $^{18}\text{F}$ .<sup>13</sup> However, in the current study, we used  $^{64}\text{Cu}$ , which has a relatively long half-life; thus, 1-labeled ADSCs were monitored *in vivo* up to 18 h. Adonai et al. reported that a lipophilic agent,  $^{64}\text{Cu}$ -PTSM, was rapidly effluxed from C6 rat glioma cells; radioactivity decreased by  $\sim 80\%$  at 24 h.<sup>3</sup> Also, Park et al. reported that radioactivity of intravenously injected  $^{64}\text{Cu}$ -PTSM-labeled K562 (human erythromyeloblastoid leukemia cell line) cells rapidly decreased in the lungs from 2 to 24 h.<sup>17</sup> In the current results, however, the intramuscularly injected 1-labeled ADSCs were clearly visualized at the injection site for 18 h.

Although we did not investigate the effect of 1-labeling on the functionality of stem cells, through our previous study a lipophilic labeling agent similar to 1,  $^{124}\text{I}$ -HIB, did not affect stem cell differentiation including osteogenic, chondrogenic, adipogenic, and cardiomyogenic lineage.<sup>18</sup> Thus, labeling with 1 may not affect stem cell functionality.

In conclusion, we have designed and synthesized 1, a PET imaging agent, and evaluated the efficiency of monitoring transplanted stem cells in normal rat heart. Compound 1 was efficiently labeled with ADSCs, and 1-labeled ADSCs were successfully monitored *in vivo* by PET imaging. Cell labeling with 1 is a simple and suitable cell labeling agent for monitoring of *in vivo* distribution and deposition of transplanted stem cells.

## ■ ASSOCIATED CONTENT

### Supporting Information

Detailed experimental procedures including synthesis, ADSCs isolation, cell labeling with 1, small animal PET/CT imaging, and digital whole-body autoradiography. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### Notes

The authors declare no competing financial interest.

## ■ ABBREVIATIONS

ADSCs, adipose-derived stem cells; CT, computed tomography; DOTA, 1,4,7,10-tetraazacyclododecane-tetraacetic acid; HB, hexadecyl benzoate; PET, positron emission tomography; TLC, thin layer chromatography

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