Available online at www.sciencedirect.com
ScienceDirect
Biomaterials 28 (2007) 434–440
Biomaterials
www.elsevier.com/locate/biomaterials

The MR tracking of transplanted ATDC5 cells using fluorinated poly-L-lysine-CF_3

Junichi Maki[a,c], Chiaki Masuda[a,d], Shigehiro Morikawa[b], Masahito Morita[b], Toshiro Inubushi[b], Yoshitaka Matsusue[c], Hiroyasu Taguchi[d], Ikuo Tooyama[a,*]

[a]*Molecular Neuroscience Research Center, Shiga University of Medical Science, Setatsukinowa-cho, Otsu 520–2192, Japan*
[b]*Biomedical MR Science Center, Shiga University of Medical Science, Setatsukinowa-cho, Otsu 520–2192, Japan*
[c]*Department of Orthopedic Surgery, Shiga University of Medical Science, Setatsukinowa-cho, Otsu 520–2192, Japan*
[d]*Graduate School of Home and Economics, Kyoto Women's University, 35 Imakumano-Kitahiyoshi-cho, Higashiyama-ku, Kyoto 605–8501, Japan*

Received 1 May 2006; accepted 24 August 2006
Available online 15 September 2006

Abstract

Magnetic resonance (MR) imaging using super-paramagnetic iron oxides (SPIOs) is a powerful tool to monitor transplanted cells in living animals. However, since SPIOs are negative contrast agents it is difficult to track transplanted cells in bone and cartilage that originally display low signals. In this study, we examined the feasibility of tracking with fluorescein isothiocyanate (FITC)-labeled poly-L-lysine-CF_3 (PLK-CF_3) using mouse ATDC5 cells, a stem cell line of bone and cartilage cells. FITC-labeled PLK-CF_3 was easily internalized by ATDC5 cells by adding it into culture medium. No acute or long-term toxicities were seen at less than 160 μg/ml. Labeled cells transplanted into the cranial bone of mice were detected for at least 7 days by MR images. FITC-labeled PLK-CF_3 is a useful positive contrast agent for MR tracking in bone and cartilage.

Keywords: Bone; Cartilage; Cell therapy; Molecular imaging; Magnetic resonance; Transplantation

1. Introduction

Cell replacement therapy, using several kinds of cells including embryonic and adult stem cells, is applied to clinical and pre-clinical use [1,2], particularly in the field of orthopedics [3,4]. It is of great importance to be able to non-invasively track transplanted cells in vivo. Several current methods include positron emission tomography [5–7], fluorescent microscopy [8] and magnetic resonance (MR) imaging [9–13].

Magnetic labeling, commonly with super-paramagnetic iron oxides (SPIOs) [11,14] is a key step in MR tracking. One of its limitations is that although SPIOs have high sensitivity, MR signals in the surrounding areas are reduced [15]. Therefore, it is difficult to track cells in the bone and cartilage, which initially display low signals. In order to solve this issue, positive MR materials are needed. Gadolinium is often used as a positive contrast agent [16–19]. However gadolinium may be toxic and can be retained in tissues for a long time after cell death. More recently, perfluoropolyether has been used as a positive contrast agent [20]. ^{19}F displays positive signals but since it is present at a very low level in the body, the signal-to-noise ratio is very high. Therefore, we chose a candidate, *N*-4-(trifluoromethoxy)-benzylated poly-L-lysine (PLK-CF_3), as a positive contrast agent and examined the feasibility of using it for MR tracking of the cartilage stem cell line, ATDC5 cells.

2. Materials and methods

2.1. Synthesis of PLK-CF_3

We used three commercially available poly-L-lysines with molecular weights 1–4, 5–15 and 15–30 kDa. We prepared the fully PLK-CF_3 from poly-L-lysine of molecular weight 1–4 kDa, as follows. A preliminary experiment showed that the fully PLK-CF_3 was obtained as a sole product and the material was soluble in methanol, ethanol, ethyl acetate,

*Corresponding author. Tel.: +81 77 548 2328; fax: +81 77 548 2402.
E-mail address: kinchan@belle.shiga-med.ac.jp (I. Tooyama).

0142-9612/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2006.08.050

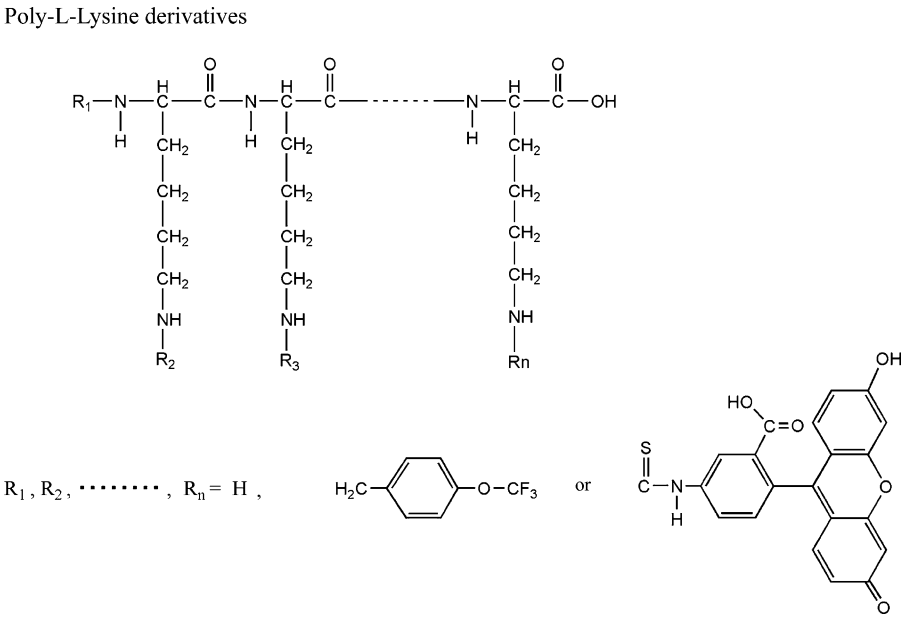

Fig. 1. Structure of PLK-CF_3. 4-(trifluoromethoxy-9-benzyl bromide and/or fluoresceinisothiocyanate are bounded to *n*-terminal residues (R_n) of poly-L-lysine.

chloroform and dimethyl sulfoxide, but was insoluble in water. Thus, we prepared benzylated poly-L-lysine with about half of the amino groups in the molecule (Fig. 1).

For the synthesis of PLK-CF_3, 100 mg of poly-L-lysine hydrobromide was dissolved in a mixture of water (0.5 ml) and methanol (0.5 ml). To the mixture was added a solution of 72 mg of 4-(trifluoromethoxy)benzyl bromide in methanol (0.5 ml) and 130 mg of solid potassium carbonate, in turn, and the mixture stirred for 19 h at room temperature. Ethanol (15 ml) was added to the mixture and the insoluble material removed by filtration. Ethanolic hydrogen chloride was added to the filtrate and the mixture concentrated to about 2 ml. The required PLK-CF_3 was precipitated out by adding 50 ml of diethyl ether to the solution.

For labeling of PLK-CF_3 with fluorescein isothiocyanate (FITC), a mixture of PLK-CF_3 (1 mol), FITC (0.1 mol) and tributylamine (1.2 mol) in methanol was stirred for 5 h at room temperature. The FITC labeled compound was obtained by addition of diethyl ether to the mixture. In our FITC-labeled PLK-CF_3, half of the amino groups were 4-(trifluoromethoxy)benzylated and 10% of the amino groups were combined with FITC (Fig. 1). Some PLK-CF_3 was labeled with Cy5.5 (Amersham Biosciences UK Limited, UK) instead of FITC because Cy5.5 displayed good permeability in tissues.

2.2. Fluorescent labeling and analysis of cultured cells

Murine ATDC5 cells were obtained from the RIKEN cell bank (Tsukuba, Japan). ATDC5 cells were cultured in maintenance medium consisting of a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F-12 medium (Sigma, St. Louis, MO) consisting of 5% fetal bovine serum (Sigma), antibiotic–antimycotic (Nacalai Inc., Osaka, Japan) 100 units/ml penicillin, 100 μg/ml streptomycin, 10 μg/ml human transferrin (Roche Applied Science, Mannheim, Germany). Cells were maintained at 37 °C in humidified 5% CO_2, 95% air atmosphere.

Just before cells reached confluency, we added FITC-labeled PLK-CF_3 into the culture medium at 5, 10, 20, 40, 80, 160 or 320 μg/ml. The incubation time also varied from 2 to 48 h. After incubation, we washed the culture dishes with 10 mM phosphate buffered saline (PBS), pH 7.4 and fixed cells for 10 min with 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer, pH 7.4, at room temperature. After washing the culture dishes with PBS, fluorecent signals were observed by fluorecent microscopy (IX 70, OLYMPUS Co., Tokyo, Japan). The images were analyzed by the Meta Morph imaging system (OLYMPUS Co., Tokyo, Japan).

The labeling intensity was calculated using the following formula: Labeling intensity = (fluorescent intensity) × (labeling area).

Cell toxicity was investigated using trypan blue staining after incubation for 24 h with different doses of FITC-labeled PLK-CF_3. The ratio of living cells to total cells was counted. For measuring long-term toxicity, we incubated ATDC5 cells for 24 h with different doses of FITC-labeled PLK-CF_3. After washing with PBS, cells were recovered using 0.25% trypsin into Flacon tube, and plated in 60 mm culture dish. We counted cell numbers in each dish at days 4 and 7 of incubation.

2.3. MR imaging of PLK-CF_3 in the cranial bone of living mouse

Experimental procedures were approved by the Committee on Animal Care of the Shiga University of Medical Science. Five male ICR mice (40–50 g) were used. ATDC5 cells were labeled for 24 h with 80 μg/ml of FITC-labeled PLK-CF_3. After washing with PBS, cells were harvested using 0.05% trypsin (Nacalai Inc., Osaka, Japan). Labeled cells (2×10^6–6×10^6) were resuspended in a mixture of Cellmatrix (Nitta-gelatin Co., Osaka, Japan) and culture medium at a ratio of 8:2 at a final concentration of 1×10^7 labeled cells/ml of the gel mixture.

Cranial skins of mice were opened under anesthesia (sodium pentobarbital 50 mg/ml, i.p.). The cranial bone of each mouse was defected with a reamer and the defected area was covered with Cellmatrix containing labeled cells. ATDC5 transplanted in the cranial bone was confirmed by fluorescent microscope (Lightools Macro-Imaging System plus cooled, Lightools Research Co., Encinitas, CA, USA), then applied to MR imaging measurements.

On days 1, 7 and 21 after transplantation, MR images were acquired with a 7 T Unity Inova MR scanner (Varian, Palo Alto, CA). A surface coil 20 mm in diameter, which can be tuned to both ^1H and ^{19}F frequencies (300 and 282 MHz), was used for signal acquisition. First, a gradient echo ^1H image of the mouse brain was acquired in the axial plane. MR imaging parameters were with 100 ms repetition time (TR), 5 ms echo time (TE),