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Gadolinium-chelate nanoparticle entrapped human mesenchymal stem cell via photochemical internalization for cancer diagnosis

Kyoung Sub Kim, Wooram Park, Kun Na*

Department of Biotechnology, The Catholic University of Korea, 43 Jibong ro, Wonmi-gu, Bucheon-si, Gyeonggi do 420-743, Republic of Korea

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ABSTRACT

To improve the gadolinium (Gd) internalization efficiency in stem cells, gadolinium-chelate nanoparticles were prepared from a pullulan derivative (pullulan-deoxycholic acid (DOCA)-diethylene triamine pentaacetic (DTPA)-Gd conjugate; PDDG) and then the PDDG was entrapped into human mesenchymal stem cells (hMSCs) by the photochemical-internalization (PCI) method for cancer diagnosis via the cancer homing property of hMSCs. The internalization efficiency of Gd in hMSCs was significantly increased to 98 ± 4 pg Gd/cell from 32 ± 2 pg Gd/cell via the PCI method. Moreover, the Gd-entrapped hMSCs revealed a low exocytosis ratio of gadolinium-chelate nanoparticles during cell division *in vitro* and a high cellular labeling efficiency for at least 21 days *in vivo*. The cancer-targeting and diagnosis effect of the Gd-entrapped hMSCs were confirmed in a small CT26 tumor-bearing mice model. The stem cells detected an early tumor (~3 mm^3) within 2 h using 4.7-T MR and optical imaging. The results demonstrated that the PCI-mediated internalization of Gd-incorporated nanoparticles into hMSCs is a promising protocol for efficient cell labeling and tracking.

1. Introduction

Stem cells have offered great promise as a regenerative medicine for various diseases, such as cardiovascular disease and neurodegenerative diseases, and for the repair of various damaged tissues [1–3]. Notably, human mesenchymal stem cells (hMSCs) have been found to improve cancer therapy and diagnosis due to their inherent tumor-homing properties, which allow them to serve as a vehicle/platform for the tumor-targeted delivery of agents [4–10]. To monitor the homing of stem cells to tumor tissues, the *in vivo* noninvasive imaging of stem cells after systemic administration with high sensitivity and accuracy is a critical issue in this field [11].

As a result, several stem cell engineering techniques to label stem cells with organic or inorganic probes, including gadolinium (Gd)-based contrast agents [12], iron oxide nanoparticles [13–15], silica nanoparticles [16], quantum dots (QDs) [17] and upconversion nanoparticles (UCNPs) [11], have been developed for *in vivo* stem cell tracking. Among them, Gd-based contrast agents have been extensively explored as T1-weighted contrast agents for stem cell labeling and tracking because of their clear, bright, positive signal intensity [18]. However, because Gd-based contrast agents cannot pass though the cellular membrane easily due to their hydrophilic properties [19], this technique often suffers from low cellular internalization [12,20]. Therefore, long-term incubation and high concentrations of Gd were required for efficient stem cell labeling, which can lead to undesirable side effects [12].

To overcome the limitation of the low cellular internalization efficiency in Gd-based contrast agents in stem cells, we introduce a photochemical internalization (PCI)-based stem cell engineering technique in hMSCs. Recently, PCI, an innovative approach to extended photodynamic therapy (PDT), has been used for light-induced intracellular drug delivery [21–23]. The mechanism of PCI is based on the disintegration of the cellular membrane by reactive oxygen-induced lipid peroxidation [23,24]. According to our previous reports, a gene-delivery system utilizing the PCI effect exhibited a significantly enhanced transfection efficiency in hMSCs by enhanced cellular internalization [25–27]. Inspired by this result, we hypothesized that the PCI effect would induce the enhanced cellular internalization of Gd-chelate nanoparticles into hMSCs without undesirable side effects (Scheme 1). Thus, Gd-entrapped hMSCs using the PCI effect enable accurate cancer diagnosis with high-signal intensity via T1-weighted magnetic resonance (MR) imaging. To prove the concept, we designed and synthesized polysaccharide-based Gd-chelate nanoparticles as representative Gd-based contrast agents. Additionally, pheophorbide-a (pheoA), which is a decomposition production of chlorophyll-a, was used as a photosensitizer with a continuous-wave laser (675 nm) [28].

* Corresponding author.
E-mail address: kna6997@catholic.ac.kr (K. Na).

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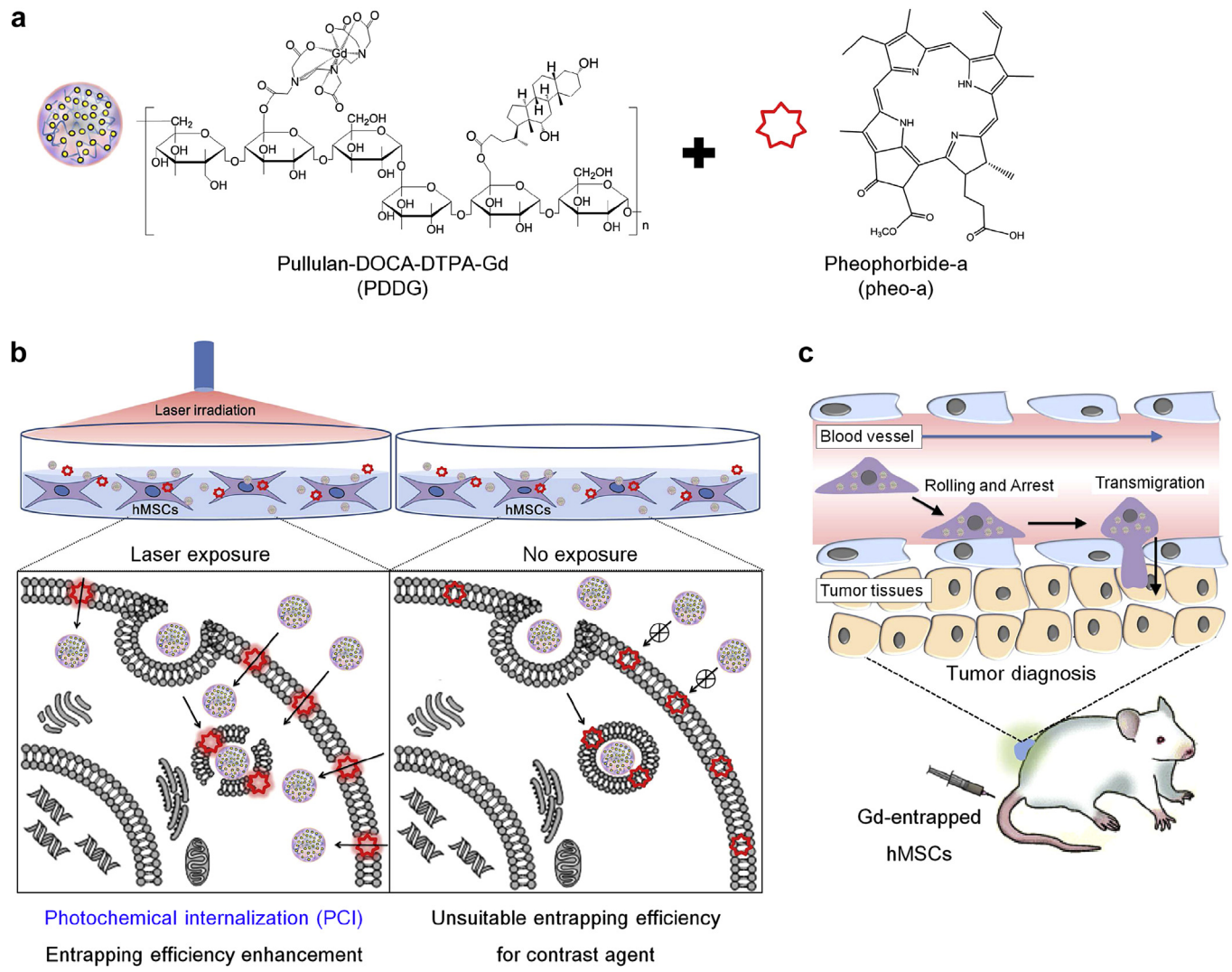

Scheme 1. a) Schematic representation of the chemical structure of the PDDG nanoparticles and pheoA. b) Schematic representation of hMSCs engineering via PCI with PDDG and pheoA. c) Schematic representation of the tumor-accumulation behavior of Gd-entrapped hMSCs.

In this study, the enhanced cellular internalization of the Gd-chelate nanoparticles into hMSCs by the PCI effect was investigated under both *in vitro* and *in vivo* experimental conditions. *In vivo* MR and optical imaging techniques were utilized to exploit to the cellular internalization efficiency, cancer targeting (homing) and diagnostic effect of the Gd-chelate nanoparticle-engineered hMSCs in a small CT26 tumor (~3 mm^3)-bearing mice model.

2. Materials and methods

2.1. Materials

The neutral polysaccharide pullulan (MW 100 kDa) was acquired from Tokyo Chemical Industry Co., Ltd. Dimethyl sulfoxide (DMSO), deoxycholic acid (DOCA), 4-di-methylaminopyridine (DMAP), *N,N*-dicyclohexylcarbodiimide (DCC), diethylene triamine pentaacetic (DTPA) dianhydride, and $GdCl_3 \cdot 6H_2O$ were purchased from Sigma (St. Louis, MO). Pheophorbide-a was purchased from Frontier Scientific (Logan, UT). A dialysis membrane with a molecular weight cut-off (MWCO, 12 ~ 14 kDa) was purchased from Spectrum Laboratories Inc. (Rancho Dominguez, CA). Fetal bovine serum (FBS), a 0.25% (w/v) trypsin–0.03% (w/v), and EDTA solution were purchased from Gibco (Uxbridge, UK). An MEM alpha modification medium was purchased from HyClone (Logan, UT). hMSCs were purchased from Lonza (Walkersville, MD). The gadolinium (Gd^{3+}) contents were estimated using inductively coupled plasma-optical emission spectrometry (ICP-OES) (Perkin–Elmer, Optima 4300 DV, Norwalk, CT, USA). The ^1H NMR spectra were recorded using a Bruker NMR Spectrometer (500 MHz). The samples were prepared by adding aliquots of products (10 mg) into a deuterated solvent. The ^1H NMR chemical shifts are reported in ppm and calibrated against TMS (δ 0).

2.2. Synthesis of pullulan-DTPA and pullulan-DOCA-DTPA

We synthesized pullulan-diethylene triamine pentaacetic (DTPA) according to our previous report [29]. Pullulan-deoxycholic acid (DOCA)-DTPA was synthesized using the following method (Figure S1). Briefly, Pullulan (1 g, 0.01 mmol), DOCA (23.9 mg, 0.06 mmol), DMAP (9.7 mg, 0.08 mmol) and DCC (16.4 mg, 0.08 mmol) were dissolved in 50 mL of DMSO and allowed to stand for 24 h at room temperature. The solution was dialyzed for three days and lyophilized. The synthesized pullulan-DOCA (the molar contents of DOCA per 1 mol of pullulan = 6.4) was analyzed by ^1H NMR (Figure S3). Subsequently, the pullulan-DOCA (150 mg, 0.0015 mmol) and DTPA dianhydride (300 mg, 0.84 mmol) were dissolved in 80 mL of anhydrous DMSO and allowed to stand for 24 h at room temperature. The solution was dialyzed for three days and lyophilized. The synthesized pullulan-DOCA-DTPA was characterized by FT-IR (Fourier transform infrared spectroscopy, Shimadzu FTIR-8700).

2.3. Gadolinium(III) chelation of pullulan-DOCA-DTPA

$GdCl_3 \cdot 6H_2O$ (37 mg, 0.0014 mmol) was added to pullulan-DOCA-DTPA (200 mg, 0.002 mmol) in 20 mL of deinized water (DW) at a 0.7:1 M ratio of Gd to DTPA. The pH of the solution was maintained between 6.0 and 6.5 with 0.1 N NaOH. The reaction mixture was stirred for 4 h 50 °C, after which the reactant mixture was dialyzed and lyophilized. The chelation of DTPA to Gd was confirmed by FT-IR analysis (Figure S4). We performed elemental analysis by inductively coupled plasma-optical emission spectrometry (ICP-OES) (Perkin–Elmer Optima 4300 DV).

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