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Near-infrared light-triggered release of small molecules for controlled differentiation and long-term tracking of stem cells *in vivo* using upconversion nanoparticles

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ABSTRACT

Human mesenchymal stem cells (hMSCs) hold considerable potential for regenerative medicine, but their application is limited by the lack of an efficient method to control differentiation and track the migration of implanted cells *in vivo*. In this study, we developed a multifunctional nanocarrier based on upconversion nanoparticles (UCNPs) for controlling differentiation and long-term tracking of hMSCs. The UCNPs are conjugated with the peptide (Cys-Arg-Gly-Asp, CRGD) and the differentiation-inducing kartogenin (KGN) via a photocaged linker on the surface, and the obtained UCNP nanocarrier can be efficiently uptaken by hMSCs. Under the exposure of near-infrared (NIR) light, the upconverted UV emission from the UCNP nanocarrier leads to the photocleavage of the photocaged linker and intracellular release of KGN. The NIR-triggered release of KGN mediated by the UCNP nanocarrier efficiently induces chondrogenic differentiation of hMSCs *in vitro* with reduced KGN dosage compared to the conventional protocol of directly supplementing KGN in the media. Furthermore, NIR irradiation through the skin of living animals induces the chondrogenic differentiation of the subcutaneously implanted hMSCs treated with the KGN-laden UCNP nanocarrier, thereby enhancing neocartilage formation *in vivo*. Finally, the luminescent UCNP nanocarrier enables the long-term tracking of the labeled hMSCs *in vivo*. We believe that our UCNP nanocarrier is a promising tool for the remote control of triggered delivery of inductive agents to stem cells at the prescribed time points and the elucidation of the function and the fate of the transplanted stem cells *in vivo*.

1. Introduction

Human mesenchymal stem cells (hMSCs) residing in the bone marrow can differentiate various cell lineages, including chondrocytes, osteoblasts, and adipocytes [1]. This multipotency of hMSCs affords a promising potential for clinical applications in the regeneration of damaged and injured tissues [2]. For example, osteoarthritis (OA) is a degenerative joint disease that is characterized by the degeneration of articular cartilage and eventual disability. MSCs are a promising cell source for the repair and regeneration of degenerated cartilage because of their ability to differentiate into chondrogenic lineage in the presence of inductive factors [3,4]. Furthermore, molecules promoting the selective differentiation of MSCs into chondrocytes may stimulate the repair of damaged cartilage. Recent studies have identified a number of

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small molecules, such as kartogenin (KGN, Scheme S1A), that promotes the chondrogenic differentiation of hMSCs by targeting intracellular signaling factors *in vitro* and *in vivo* [5]. However, these small molecules are generally poorly soluble in water, thereby making their efficient intracellular delivery difficult without using potentially cytotoxic organic solvents. Furthermore, the sustained and locally restricted exposure of optimal dosage of the inductive agents is also necessary to effectively induce the differentiation of the stem cells and to minimize the off-target effect. Moreover, controlling the differentiation of MSCs in time and space is necessary in stem cell-based regeneration therapies [6–8]. Therefore, a cytocompatible drug delivery system that can efficiently deliver small molecules into hMSCs for enhanced differentiation efficacy and controlled release of small molecules intracellularly in a stimuli-responsive manner is highly desirable for clinical translation of stem cell therapy.

Controlled or triggered delivery of bioactive molecules has been attempted by many methods, and light has gained popularity as the trigger mechanism in the past decade [9–11]. The major problem in the light-mediated delivery is that photoactivatable molecules mostly respond to UV radiation, not to visible or near-infrared (NIR) light. Unfortunately, the use of UV irradiation is limited by its toxicity and low tissue penetration power. By contrast, NIR light has high tissue penetration power and is expected to cause minimal phototoxicity to the biological specimen involved. Consequently, NIR light has attracted significant attention. Conversion of low-energy NIR light into weak and locally restricted UV light, which has significantly reduced cytotoxicity, has become an effective strategy to achieved light-triggered controlled delivery of drugs [12,13]. Upconversion nanoparticles (UCNPs) made of host lattices of ceramic materials embedded with trivalent lanthanide ions [14] can absorb NIR light and convert the NIR region to weak UV or visible light because of the distinct ladder-like energy level structures of lanthanide ions [15]. Therefore, UCNPs have become an effective tool for mediating light-triggered delivery of drugs in biomedical applications [16–32].

In this contribution, we designed and synthesized multifunctional UCNPs for the controlled differentiation and long-term *in vivo* tracking of hMSCs (Scheme 1). The UCNPs was coated with silica first (UCNP@SiO_2) and then was conjugated with the peptide (Cys-Arg-Gly-Asp, CRGD) via the MAL-$(PEG)_4$-NHS linker (Scheme S1C) and the chondrogenic small molecule (KGN) via a photocaged linker (4-(hydroxymethyl)-3-nitrobenzoic acid) (ONA, Scheme S1B) on the surface to form the multifunctional UCNPs (RGD-KGN-UCNP@SiO_2, Scheme 1A) for tissue penetration of NIR-triggered release of KGN to induce the chondrogenic differentiation of hMSCs (Scheme 1B). The RGD-KGN-UCNP@SiO_2 shows efficient cell uptake in hMSCs by RGD peptide conjugation. The absorption band of ONA overlapped with the upconverted emission of UCNPs at the UV region; hence, excitation of UCNPs caused by the NIR could trigger the photocleavage of ONA and intracellular release of KGN from the nanoparticles to induce the chondrogenic differentiation of the hMSCs *in vitro* and *in vivo* efficiently. Furthermore, the excitation of the UCNPs via the tissue-penetrating NIR allows the long-term tracking of hMSCs *in vivo*, which helps elucidate the behaviors of the implanted hMSCs in the recipient animals.

2. Materials and methods

2.1. Synthesis of UCNPs and UCNP@SiO_2

$NaYF_4$:Yb/Tm (30/0.5 mol%) NPs (UCNPs) and UCNP@SiO_2-NH_2 were synthesized in accordance with a previously reported method [25]. UCNPs: In brief, $RECl_3$ (0.2 M, RE = Y, Yb, and Tm) in methanol was added to a 50 mL flask containing 3 mL of oleic acid and 7 mL of 1-octadecene. The solution was heated to 160 °C for 30 min and cooled down to room temperature. Subsequently, about 5 mL of NH_4F (1.6 mmol) and NaOH (1 mmol) methanol solution was added, and the solution was stirred for 30 min. After the evaporation of methanol, the solution was heated to 300 °C under argon for 1.5 h and cooled to room temperature. The resulting NPs were precipitated by the addition of ethanol, collected by centrifugation, washed with methanol and ethanol several times, and redispersed in cyclohexane. UCNPs@SiO_2: In brief, UCNPs (4 mg) were dissolved in cyclohexane (16 mL) and added with Triton X-100 (4 mL), 1-hexnol (4 mL), and DI water (680 μL). Tetraethyl orthosilicate (TEOS, 10 μL) and (3-aminopropyl)triethoxysilane (APTES, 2 μL) were added to the mixture. After 6 h of stirring, $NH_3{}^*H_2O$ (28.0–30.0% NH3 basis, 200 μL) was added. UCNPs@SiO_2 was obtained via centrifugation and washed thoroughly with ethanol. The as-prepared UCNPs@SiO_2 particles were characterized by TEM. The fluorescence emission spectrum of the upconversion particles was tested by a 980 nm laser.

2.2. Conjugating KGN with UCNP@SiO_2

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC-HCl) (0.002 mmol) and Hydroxybenzotriazole (HOBt) (0.001 mmol) were added to a solution of ONA (0.001 mmol) in DMF (500 μL), and the mixture was stirred for 4 h. Subsequently, UCNP@SiO_2-NH_2 (10 mg) and diisopropylethylamine (DIPEA, 1 μL) were slowly added. The reaction mixture was allowed to stir overnight at room temperature. UCNP@SiO_2-ONA was collected via centrifugation and washed with ethanol three times. KGN (0.001 mmol) was reacted with N,N′-dicyclohexylcarbodiimide (DCC, 2 mM) and 4-(dimethylamino)pyridine (DMAP, 1 mM) in DMF for 4 h to activate the carboxyl group. Subsequently, about 10 mg of UCNP@SiO_2-ONA was added into the solution to react with KGN for 24 h to form KGN-UCNP@SiO_2. KGN-UCNP@SiO_2 was collected via centrifugation and washed with ethanol three times.

2.3. Conjugating RGD peptide with KGN-UCNP@SiO_2

KGN-UCNP@SiO_2 (10 mg) was dissolved in DMF (500 μL) and added to MAL-$(PEG)_4$-NHS (0.001 mmol) and DIPEA (1 μL). The following day, the MAL-$(PEG)_4$-UCNP@SiO_2 NPs were collected via centrifugation and washed with ethanol three times. The MAL-$(PEG)_4$-UCNP@SiO_2 NPs were dissolved in CRGD peptide solution (0.001 mmol in DMF) and DIPEA (1 μL) overnight to form RGD-KGN-UCNP@SiO_2 NPs. RGD-KGN-UCNP@SiO_2 was obtained via centrifugation (8000 rpm, 5 min) and washed with ethanol three times.

2.4. NIR-triggered release of KGN from RGD-KGN-UCNP@SiO_2

The RGD-KGN-UCNP@SiO_2 NPs in PBS (1 mg/mL) were exposed to a 980 nm laser (2 W/cm^2) for different irradiation times (0, 30, 60, 120, and 240 min) at room temperature. The irradiated RGD-KGN-UCNP@SiO_2 solution was centrifuged, and the supernatant was collected to measure the release of KGN from the NPs via UV–Vis.

2.5. Cell culture

hMSCs (Lonza, Gampel, Valais, Switzerland) were expanded to passage 3 in growth media consisting of a-MEM with 16.7% FBS and 1% pen/strep (FBS, Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) at 37 °C in a humidified atmosphere with 5% CO_2 concentration, and a 35 mm diameter plastic bottom dish was used