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A smart fluorescence nanoprobe for the detection of cellular alkaline phosphatase activity and early osteogenic differentiation

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Abstract

In the past decades, biomaterials were designed to induce stem cell toward osteogenic differentiation. However, conventional methods for evaluation osteogenic differentiation all required a process of cell fixation or lysis, which induce waste of a large number of cells. In this study, a fluorescence nanoprobe was synthesized by combining phosphorylated fluoresceinamine isomer I (FLA) on the surface of mesoporous silica-coated superparamagnetic iron oxide (Fe_3O_4@$mSiO_2$) nanoparticles. In the presence of alkaline phosphatase (ALP), the phosphorylated FLA on the nanoprobe would be hydrolyzed, resulting in a fluorescence recovery of FLA. During early osteogenic differentiation, a high-level expression of cellular ALP was induced, which accelerated the hydrolysis of phosphorylated FLA, resulting in an enhancement of cellular fluorescence intensity. This fluorescence nanoprobe provides us a rapid and non-toxic method for the detection of cellular ALP activity and early osteogenic differentiation.

Key words: Fluorescence nanoprobe; Stem cell; Alkaline phosphatase; Osteogenic differentiation

Introduction

Mesenchymal stem cells (MSCs) are multi-potential stem cells that can differentiate into a variety of cells, such as osteocytes, chondrocytes, adipocytes and myoblasts.[1,2] Simultaneously, because MSCs can be readily isolated from a number of tissues including blood, liver, bone marrow, spleen, dental and adipose[3–6] and easily expanded *in vitro*,[7] they have attracted great attention for the applications in stem cell based therapy. The osteogenic differentiation of MSCs has been widely studied since the pioneering study by Friedenstein and coworkers[8,9] that MSCs could form osteoblasts and bone matrix *in vivo*. To induce osteogenic differentiation, physical or chemical stimuli were introduced to biomaterials for tissue engineering and regenerative medicine.[10–12] Generally, the osteogenic differentiation capacity of the biomaterials was evaluated by traditional methods such as alkaline phosphatase (ALP) staining for enzyme activity, von Kossa or Alizarin red S staining for mineralization of the extracellular matrix, Western blot analysis for the marker proteins: osteopontin (OPN), osteocalcin (OCN) and Collagen I, and reverse transcription polymerase chain reaction (RT-PCR) for the osteogenic-related gene expressions.[10] However, all these methods require cell lysis or fixation, which might induce usage of a large number of stem cells.

In recent years, new methods were proposed for the detection of osteogenic differentiation. Kuo et al[13] used surface plasmon resonance (SPR) biosensors to detect the osteogenic protein marker by measuring the refractive angle shift during the differentiation process. Erdem et al[14] used an electrochemical sensor to detect the expression of osteogenic differentiation protein markers. Choi et al[15] designed a gold@polydopamine nanoprobe for the detection of osteogenic-related microRNAs. However, the methods for the detection of osteogenic differentiation are still limited.

ALP, which is an early marker for osteogenic differentiation,[16–18] is also a monophosphate hydrolase that could catalyze the hydrolysis of monophosphate to the corresponding alcohol (or phenol) and phosphate ion.[19] Therefore, in this study, a phosphorylated fluoresceinamine isomer I (FLA) based nanoprobe was developed to detect the cellular ALP activity and early osteogenic differentiation (Figure 1). In the presence of ALP, the monophosphate could be rapidly hydrolyzed,[20] resulting in a fluorescence recovery of FLA.

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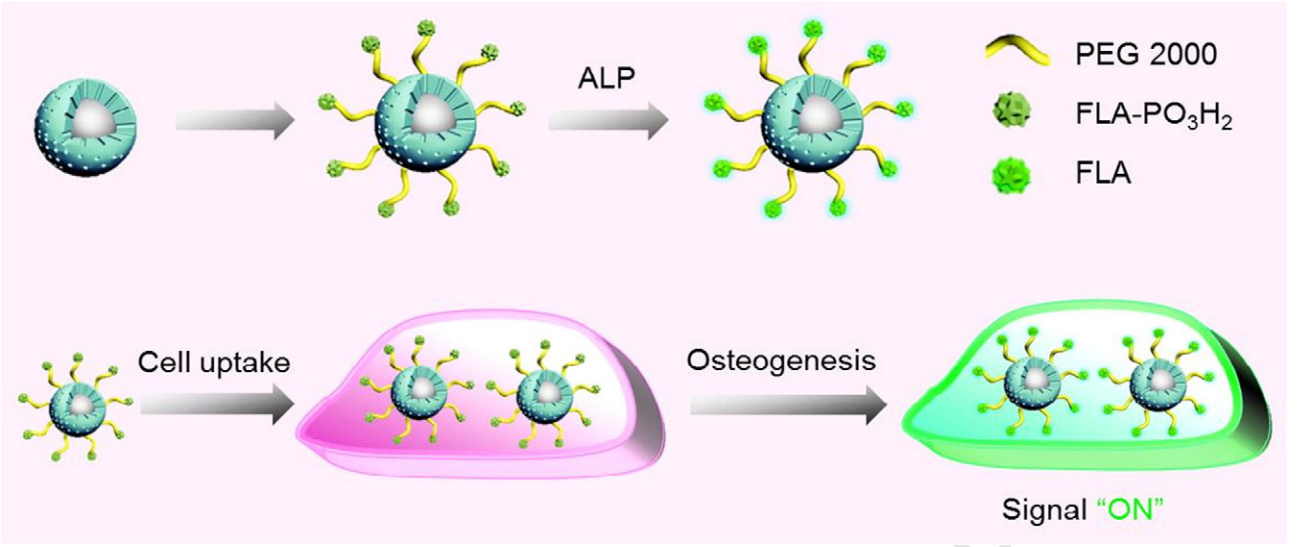

Figure 1. Illustration of the fluorescence nanoprobe for monitoring ALP activity and detection early osteogenic differentiation.

Methods

Fluorescence nanoprobe synthesis and characterization

The mesoporous silica-coated superparamagnetic iron oxide (Fe_3O_4@$mSiO_2$) nanoparticles were prepared according to previous report,[21] and the fluorescence nanoprobe was synthesized by surface modification on the Fe_3O_4@$mSiO_2$ nanoparticles (Figure S1). Expanded methods including reagent information, nanoparticle synthesis and surface modification procedures are in Supplementary Information. The nanoparticles were fully characterized using transmission electron microscopy (TEM), zeta potential measurement, Fourier-transform infrared (FTIR) spectroscopy, dynamic light scattering (DLS) elemental analysis and LS55 luminescence spectrometer. Full experimental details are listed in Supplementary Information.

Fluorescence response to ALP

To evaluate the fluorescence response to ALP, the nanoprobes were dispersed in phosphate buffer (10 mM, pH 8.0) and different concentrations of ALP (Sigma, USA) (1 U/mL, 0.5 U/mL, 0.1 U/mL and 0.01 U/mL) were added to the nanoprobe. The resulting concentration of the nanoprobe was 100 μg/mL. The fluorescence intensities were measured at an LS55 luminescence spectrometer (Perkin-Elmer).

Bone marrow mesenchymal stem cell (BMSC) culture

BMSCs were harvested from 5-week-old male Sprague-Dawley (SD) rats according to previously reported methods.[22] SD rats were purchased from Laboratory Animal Center of Zhongnan Hospital of Wuhan University (China). The animal experiments were approved by the Animal Research Committee of Zhongnan Hospital of Wuhan University and were in accordance with the guidelines of the Experimental Animals Management Committee (Hubei Province, China). The harvested cells were cultured in the maintenance medium (low glucose Dulbecco's modified Eagle's medium, 10% FBS, 1% penicillin–streptomycin) at 37 °C in a humidified atmosphere containing 5% CO_2. All cells used were from passage 2. For cell behavior studies, BMSCs were first cultured in the dish or plate for 24 h, then the medium was aspirated and the nanoprobe dispersed in the maintenance medium was added. After incubation for 2 h, the culture medium was suctioned and cells were washed with PBS for 5 times. In addition, the time point after cell internalized with the nanoprobe for 2 h was defined as 0 h.

Prussian blue staining for cellular uptake

To assess the endocytosis of the nanoprobe, 1 mL of BMSCs at a seeding density of 5×10^4/mL was cultured on a glass bottom dish. The nanoparticles at different concentrations (20, 40, 60, 80, 100 and 150 μg/mL) were studied. After incubation for 2 h, the cells were fixed with 4% paraformaldehyde. The fixed cells were stained with 10% $K_4Fe(CN)_6 \cdot 3H_2O$ solution for 5 min and the mixture of 10% $K_4Fe(CN)_6 \cdot 3H_2O$ and 20% HCl (1:1) for 30 min successively. The images were taken using a low magnification inverted microscope (OLYMPUS IX70, Japan). The percentage of BMSCs that acquired the nanoprobe was calculated by counting the Prussian blue stained and unstained cells, and for each sample, at least 300 cells were calculated.

Biocompatibility of the fluorescence nanoprobe

The cell viability and cell proliferation were firstly evaluated by MTT assay. BMSCs at a seeding density of 5×10^3/well were cultured in 96-well plate and the nanoprobes at different concentrations (20, 40, 60, 80, 100 and 150 μg/mL) were studied. After incubation for 2 h, 20 μL MTT (5 mg/mL) was added to each well and further cultured for 4 h. Then the MTT medium was removed and 150 μL DMSO was added. The optical density (OD) values were measured with a microplate reader (Bio-Rad, Model 550, USA) at 570 nm. The OD values after cell internalized with the nanoprobe and further cultured in the maintenance medium for 24 h and 48 h were also measured.