Biomaterials 33 (2012) 4370–4378

Contents lists available at SciVerse ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Long-term multimodal imaging of tumor draining sentinel lymph nodes using mesoporous silica-based nanoprobes

Xinglu Huang [a,1], Fan Zhang [a,1], Seulki Lee [a], Magdalena Swierczewska [a], Dale O. Kiesewetter [a], Lixin Lang [a], Guofeng Zhang [b], Lei Zhu [a], Haokao Gao [a], Hak Soo Choi [c], Gang Niu [a,*], Xiaoyuan Chen [a,**]

[a] Laboratory of Molecular Imaging and Nanomedicine (LOMIN), National Institute of Biomedical Imaging and Bioengineering (NIBIB), National Institutes of Health (NIH), Bethesda, MD 20892, USA
[b] Laboratory of Cellular Imaging and Macromolecular Biophysics, National Institute of Biomedical Imaging and Bioengineering (NIBIB), National Institutes of Health (NIH), Bethesda, MD 20892, USA
[c] Division of Hematology/Oncology, Department of Medicine, Beth Israel Deaconess Medical Center, Boston, MA 02215, USA

ARTICLE INFO

Article history:
Received 10 February 2012
Accepted 27 February 2012
Available online 16 March 2012

Keywords:
Mesoporous silica nanoparticles
Multimodality imaging
Tumor metastasis
Magnetic resonance imaging
Positron emission tomography
Near-infrared fluorescence imaging

ABSTRACT

The imaging of sentinel lymph nodes (SLNs), the first defense against primary tumor metastasis, has been considered as an important strategy for noninvasive tracking tumor metastasis in clinics. In this study, we report the development and application of mesoporous silica-based triple-modal nanoprobes that integrate multiple functional moieties to facilitate near-infrared optical, magnetic resonance (MR) and positron emission tomography (PET) imaging. After embedding near-infrared dye ZW800, the nanoprobe was labeled with T_1 contrast agent Gd^{3+} and radionuclide ^{64}Cu through chelating reactions. High stability and long intracellular retention time of the nanoprobes was confirmed by *in vitro* characterization, which facilitate long-term *in vivo* imaging. Longitudinal multimodal imaging was subsequently achieved to visualize tumor draining SLNs up to 3 weeks in a 4T1 tumor metastatic model. Obvious differences in uptake rate, amount of particles, and contrast between metastatic and contralateral sentinel lymph nodes were observed. These findings provide very helpful guidance for the design of robust multifunctional nanomaterials in SLNs' mapping and tumor metastasis diagnosis.

Published by Elsevier Ltd.

1. Introduction

Sentinel lymph node (SLN) is the hypothetical first lymph node or group of nodes reached by metastasizing cancer cells from a primary tumor [1–3], and continues to be used as an important parameter in tumor staging and therapeutic decision-making. Thus, lymph node imaging can be applied to evaluate the metastatic status of a tumor. SLN imaging is based on an injected contrast agent near the primary tumor that is taken up by the adjacent lymphatic system and then transported to the SLN. Currently, vital dyes and radionuclide-labeled sulfur colloids are the most common imaging agents for SLN imaging [4]. However, these methods have a number of drawbacks. For instance, SLNs need to be dissected to observe the blue dye staining and lymphoscintigraphy requires radiation exposure with relatively low resolution. Therefore, various groups have performed studies to develop lymphatic imaging probes and imaging methods that would exceed the capabilities of the established "blue dye" procedure, and to improve identification and mapping of lymph nodes, especially sentinel lymph nodes during surgery [5–7].

With the emergence of nanotechnology, several categories of nanoprobes have been developed to locate SLNs in living organisms, including quantum dots (QDs) [5,8–11], iron oxide [12], gold nanoparticles (NPs) [13], rare-earth-based NPs [14], carbon nanotubes [15], and perfluorocarbon-based NPs [16]. Based on their inherent properties, QDs and iron oxide can be detected and visualized by optical imaging or magnetic resonance imaging (MRI), respectively. When labeled with positron emitting radioisotopes, nanoprobes can be imaged with positron emission tomography (PET). However, each imaging modality has its own strengths and limitations. For example, MRI can provide three-dimensional tomography but is limited by low target sensitivity, whereas PET and optical imaging have good sensitivity but suffer from low spatial resolution or tissue penetration. To harness the strengths of

* Corresponding author.
** Corresponding author. Tel.: +1 301 451 4246.
E-mail addresses: gang.niu@nih.gov (G. Niu), shawn.chen@nih.gov (X. Chen).
[1] These authors contributed equally to this work.

0142-9612/$ – see front matter Published by Elsevier Ltd.
doi:10.1016/j.biomaterials.2012.02.060

different imaging methods, multimodality imaging has become attractive for both small animal and human studies [17,18]. It has emerged as a strategy that combines the strengths of different modalities and yields a hybrid imaging platform with characteristics superior to those of any of its constituents considered alone [19,20]. Development of imaging probes with multiple functions is the key for successful multimodal imaging.

With large surface area-to-volume ratios, unique mesoporous structure and excellent biocompatibility, mesoporous silica nanoparticles (MSNs) are considered an ideal matrix to integrate imaging tags for the development of either single functional [21,22] or multi-functional nanoprobes [23–25]. In several recently reported studies, silica-based nanoprobes have been designed to image the SLNs [26–28]. However, very few studies on the multimodal imaging of tumor metastatic SLNs (T-SLNs) have been reported so far. Herein, we designed a mesoporous silica-based triple-modal imaging nanoprobe (MSN-probe) that possesses the long-term imaging ability to track tumor metastatic SLNs. In this system, three imaging tags including near-infrared (NIR) dye ZW800, T_1 contrast agent Gd^{3+} and positron emitting radionuclide ^{64}Cu were integrated into MSNs by different conjugation strategies. We also applied these MSN-probes to visualize T-SLNs in a 4T1 tumor metastasis model. Due to their high stability and long intracellular retention time, signals from tumor draining SLNs are detectable up to 3 weeks. More importantly, obvious differences in uptake rate, amount of particles and contrast between metastatic and normal contra-lateral SLNs (N-SLNs) were observed.

2. Materials and methods

2.1. Materials

Cetyltrimethylammonium bromide (CTAB), tetraethyl orthosilicate (TEOS), aqueous ammonia, 3-aminopropyltriethoxysilane (APTES), bromoacetic acid, 3-(trimethoxysilylpropyl) diethylene triamine, and fluorescein isothiocyanate (FITC) were obtained from Sigma. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco. The centrifugal filter (30k cutoff) was bought from Millipore. RAW 264.7 cells were purchased from ATCC. Phallotoxins and 4′, 6-diamidino-2-phenylindole (DAPI) were obtained from Invitrogen and Vector Laboratories, respectively.

2.2. Synthesis and characterization of dye doped MSNs

Dye (FITC or ZW800) doped MSNs were synthesized according to previous reports [29,30]. Briefly, 3-aminopropyl triethoxysilane-dye (APTES-dyes) was firstly conjugated by stirring dyes in ethanolic APTES solution ($w_{APTES}/w_{dye} = 50$) for 4 h. Separately, CTAB (0.27 mmol) was dissolved in 70 ml of H_2O, and 14.29 mmol $NH_3 \cdot H_2O$ (28%–30%) was added with magnetic stirring for 10 min at room temperature. Half of TEOS (0.72 mmol) was then added with vigorous stirring for 30 min. 50 μl APTES-dye in ethanol solution ($v_{APTES}/v_{ethanol} = 1:3$) was added, and the additional half of TEOS was added with vigorous stirring for 4 h. The resulting particles were collected by centrifugation and then washed three times with deionized water and ethanol. The mesoporous structures of MSNs were obtained by removing CTAB in acidic ethanol (1 ml of concentrated HCl in 50 ml of ethanol) for 24 h. The particles were washed three times with deionized water and then stored at 4 °C. After sonication with a probe sonicator, the final particles were obtained, including FITC doped MSNs (MSN-FITC) and ZW800 doped MSNs (MSN-ZW800). The resulting particles were observed by transmission electron microscopy (TEM). The UV–Vis and fluorescence spectra of particles were recorded on a Genesys 10s UV–Vis spectrophotometer (Thermo, IL) and a F-7000 fluorescence spectrophotometer (HiTachi, Japan), respectively.

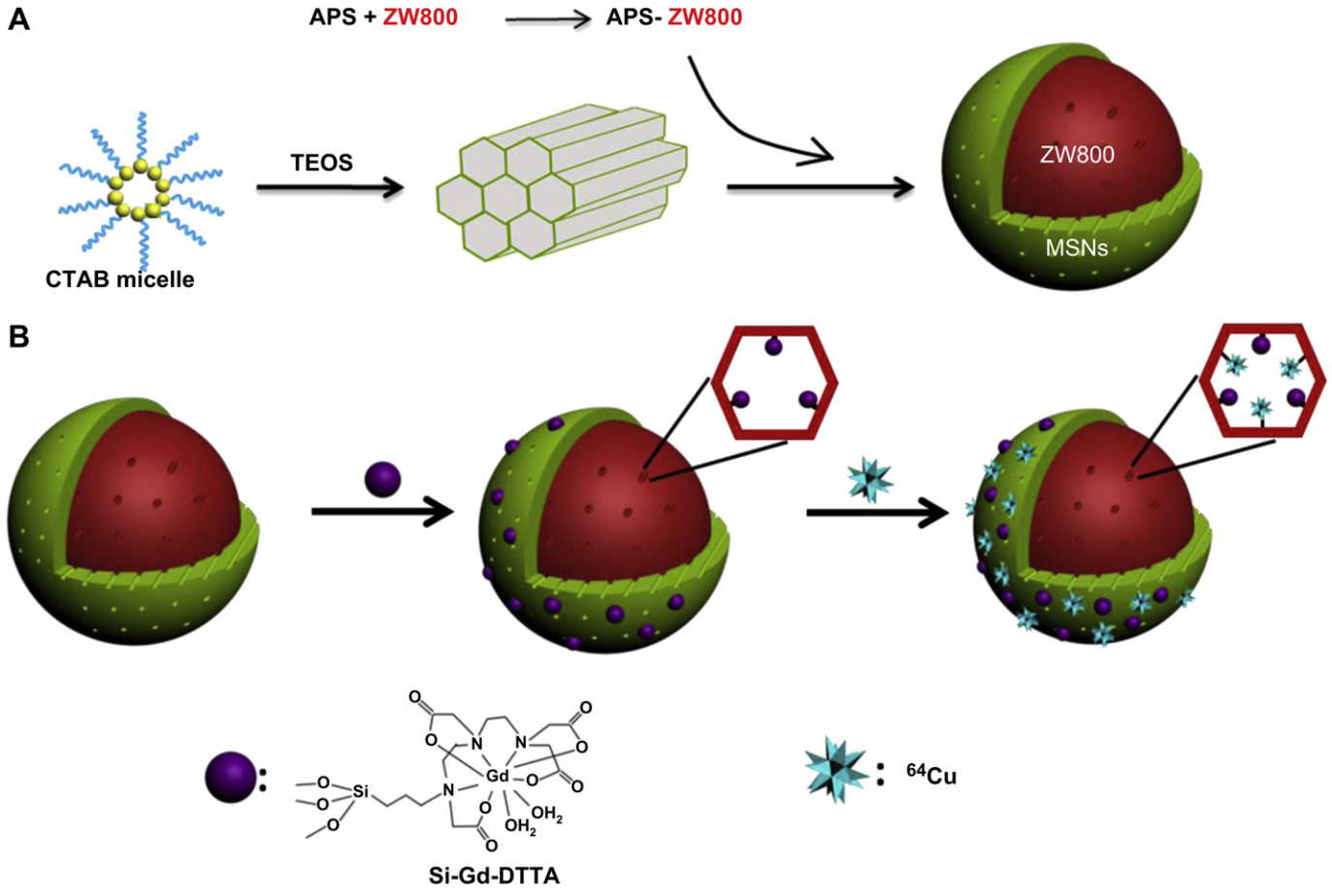

Fig. 1. Schematic illustration of tri-modal imaging MSN-probes. (A) Diagram of ZW800 doped MSN fabrication; (B) Diagram of Gd^{3+} and ^{64}Cu integration.