

Approaches to improve the long-term circulation and tumor targeting of biotin inserted ^{89}Zr -biomimetic nanoparticles

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1. Introduction

Nano-medicines have been investigated for a long-term blood circulation and tumor targeting for over a decade [1,2]. Despite these efforts, there is still not solve the limitation of nanoparticles to overcome immune-barrier. CD47 is natural protein on the red blood cell (RBC) membrane (Rm) surface as a self-marker. The abundant natural proteins including glycan and sialic acid moieties presenting on RBC membrane play a critical role for avoiding immune-barrier. Particularly, CD47 protein can be interacted with the signal regulatory protein- α (SIRP- α) receptor [3]. This interaction send to macrophage with a "Don't eat me" signal, which can inhibit the phagocytosis of macrophage. Based on these interactions, we studied increasing tumor targeting by inserted target ligand on the cell membrane. Herein, we report for the synthesis of Rm-coated, hollow mesoporous silica nanosphere (HMSN) and biotin inserted Rm-HMSN with enhanced blood circulation capabilities and effective tumor accumulation as an imaging agent. In addition, long-term monitoring of Rm-HMSN and biotin-Rm-HMSN was enabled by incorporating the long-lived ^{89}Zr on the silanol groups of the HMSN without chelator.

2. Methods and Results

2.1 Preparation of hollow mesoporous silica nanosphere

0.8 mL of ammonia solution and 5 mL of D.W were added to 35.7 mL of ethanol and then 1 mL of TEOS was added rapidly. Mixture was stirred constantly at 37 °C for 1 hour following which it was centrifuged and thoroughly washed with ethanol and water. Mesoporous silica coated SNs were further synthesized as a template using 6 g of cationic surfactant and 0.18 g of triethylamine which was dissolved in 40 mL of D.W. following which 20 mL of TEOS was added with cyclohexane (10 v/v%). The mixture was dispersed in 120 mL of 0.2 M sodium bicarbonate with stirring it for 1 hour. The reactant was centrifuged, washed and re-dispersed in 250 mL of HCl/ethanol (1/10 v/v%) for removing CTAC. Finally, synthesized HMSN was characterized with a particle size of ~150 nm and surface charge of -18.6 mV.

2.2 Incorporating ^{89}Zr

HMSN was dispersed in a HEPES buffer (pH 7.5) at various concentrations. The pH of ^{89}Zr -chloride was adjusted to pH 8 using a sodium bicarbonate solution to which dispersed HMSN was added. After stirring at room-temperature for 2 hours, labelling efficiency was confirmed by radio-iTLC using 50 mM DTPA as the mobile phase (Fig. 1). ^{89}Zr labeling efficiency of >80% was measured within 2 hours and it increased depending on reaction time.

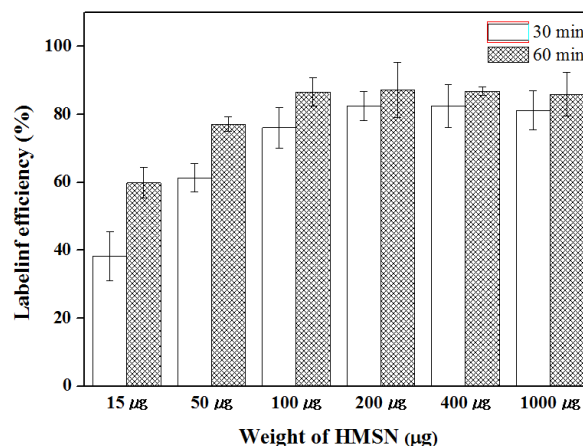


Fig. 1. Labeling efficiency of ^{89}Zr -HMSN using neutralized ^{89}Zr -chloride with chelator free method.

2.3 Biotin-inserted RBC membrane with ^{89}Zr -HMSN

RBCs were isolated from the CT-26 (mouse colon cancer) bearing female Balb/c mouse withdrawn by cardiac puncture. Mouse blood was collected into the 500 µL syringe (27 G) containing 1 mg of EDTA as an anti-coagulation and whole blood was centrifuged at 180 x g for 10 min to separate the serum, buffy coat, WBC and platelet layers sequentially. Separated RBCs were washed with ice 1XPBS. The purified RBCs were hemolyzed by ice 0.25XPBS treatment for 30 min then washed with ice 1XPBS. The Rm and DSP-PEG-biotin were dispersed in 1XPBS. The mixture was sonicated for 1 min and then biotin inserted Rm fragments were extruded sequentially through 400 nm and 200 nm polycarbonate (PC) membrane filters. The prepared biotin-Rm and ^{89}Zr labeled HMSNs were mixed and

extruded by 200 nm of PC membrane for at least 7 passes. Biotin-Rm coated ^{89}Zr -HMSN was measured with hydrodynamic size which was increased from 180 nm to 200 nm.

2.4 *In vitro*; cellular uptake

An internalization assay was performed to check the affinity of Rm- ^{89}Zr -HMSN and biotin-Rm coated ^{89}Zr -HMSN on various cancer cell lines. The cancer cells were sub-cultured in 24 well-plate (1.0×10^5 cells/well) and incubated for 2 days. The results of biotin-Rm- ^{89}Zr -HMSN tends to increase over 2 days ($13.98 \pm 0.99\%$). However, Rm- ^{89}Zr -HMSN was not showed ideal uptake which was constantly maintained with $4.69 \pm 0.13\%$. There is no tendency to decrease uptake and it is maintained at a certain level.

2.5 *In vivo*; small animal PET images

CT-26 bearing mice were used to identify pharmacokinetic pathways and active tumor targeting. The mice were anesthetized by exposing it to 2% isoflurane in oxygen. Small-animal PET images were obtained at 0, 0.5, 1, 3 and 24 hours of interval after intravenous (i.v) injection through tail vein in mice with Rm- ^{89}Zr -HMSN and biotin-Rm- ^{89}Zr -HMSN. A post-injection, the Rm coating could prevent the phagocytosis of macrophage which was allowed long-term monitoring, and showed a certain level of tumor accumulation due to the EPR effect. The biotin inserted Rm for the active target was showed a more effective tumor accumulation (Fig. 2), compared to Rm- ^{89}Zr -HMSN.

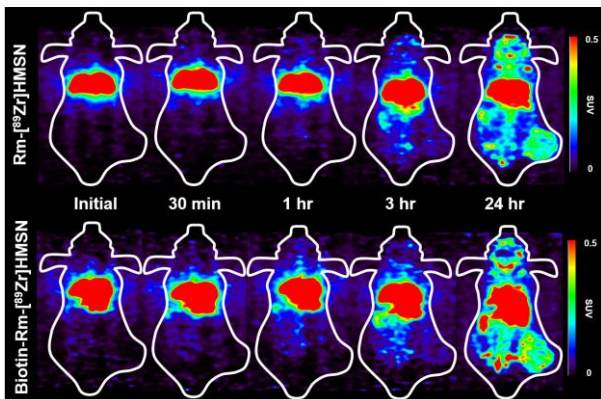


Fig. 2. Small-animal PET images of Rm- ^{89}Zr -HMSN and biotin-Rm- ^{89}Zr -HMSN

3. Conclusions

Rm- ^{89}Zr -HMSN and Biotin-Rm- ^{89}Zr -HMSN were successfully synthesized with having stability, long-term circulation ability and active tumor accumulation in the biological systems. We used chelator free method for incorporating ^{89}Zr within 1 hour with high labeling

efficiency (>80%). Rm played an important role in evading immune system and prevents release of free ^{89}Zr . Furthermore, “Don’t eat me” signal from CD47 and SIRP- α proteins on cell membrane enable long-term circulation. Biotin inserted on the Rm surface to confirm the high level of tumor accumulation due to the synergetic effect of active targeting.

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