Sensitive and Specific Detection of Breast Cancer Lymph Node Metastasis Through Dual-Modality Magnetic Particle Imaging and Fluorescence Molecular Imaging: a Preclinical Evaluation

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Research Article

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Abstract

Purpose: A sensitive and specific imaging method to detect metastatic cancer cells in lymph nodes (LNs) to detect the early-stage breast cancer is urgently needed. The purpose of this study was to investigate a novel breast cancer-targeting and tumour microenvironment ATP-responsive superparamagnetic iron oxide (SPIOs) imaging probe that was developed to detect lymph node metastasis (LNMs) through fluorescence molecular imaging (FMI) and magnetic particle imaging (MPI). The imaging nanoprobe comprised of SPIOs conjugated with breast cancer-targeting peptides (CREKA) and an ATP-responsive DNA aptamer (dsDNA-Cy5.5), abbreviated as SPIOs@A-T.

Methods: SPIOs@A-T was synthesised and characterized for its imaging properties, targeting ability and toxicity *in vitro*. Mice with metastatic lymph node (MLN) of breast cancer were established to evaluate the FMI and MPI imaging strategy *in vivo*. Healthy mice with normal lymph node (NLN) were used as control group. Histological examination and biosafety evaluation were performed for further assessment.

Results: After injection with SPIO@A-T, the obvious high fluorescent intensity and MPI signal were observed in MLN group than those in NLN group. MPI could also complement the limitation of imaging depth from FMI, thus could detect MLN more sensitively. The combination of the imaging strengths of FMI and MPI ensured the detection of breast cancer metastases with high sensitivity and specificity, thereby facilitating the precision differentiation of malignant from benign LNs. Besides, the biosafety evaluation results showed SPIO@A-T had good biocompatibility.

Conclusion: Due to the superior properties of tumour-targeting, detection specificity, and biosafety, the SPIOs@A-T imaging probe in combination with FMI and MPI can provide a promising novel method for the early and precise detection of LNMs in clinical practice.

Introduction

Worldwide, breast cancer, with approximately 2.3 million new cases (11.7% of all cancers) and 0.7 million deaths (15.5% of all cancers) in 2020, is the commonest cancer and the primary cause of cancer mortality in women in 185 countries (1). At initial diagnosis, 10–40% of breast cancer patients have lymph node metastasis (LNMs) (2), which are considered the first step toward distant metastases and are related to poor outcomes for patients (3–5). Therefore, the early, sensitive, and precise detection of LNMs plays a crucial role in guiding breast cancer treatment and improving prognosis. Various clinical imaging approaches, such as computerised tomography (CT), magnetic resonance imaging (MRI), and ultrasound (US) have been used for detecting LNMs. However, due to limitations in imaging sensitivity and spatial resolution, these imaging modalities do not achieve sufficient diagnostic sensitivity and specificity (6). Furthermore, CT has an associated risk of radiation exposure, and the contrast agent could potentially damage the patients' kidneys and impair renal function. Lymph node biopsy (LNB) is the gold standard for diagnosing breast cancer LNMs (7–9). However, LNB is an invasive procedure, is liable to sampling errors, and may cause several adverse effects, such as paraesthesia, lymphoedema, and shoulder

abduction deficits (9, 10). Currently, due to dramatic technological advances, molecular imaging techniques have been used to visualise the breast cancer-related metastatic lymph node (MLN) *in vivo*, and fluorescence imaging is most frequently used among these techniques. A recent study proposed a pH-amplified nanoparticle for visualising nodal metastases through near-infrared fluorescence imaging (NIRF) (11). Another study described an application for a dual-targeting nanoparticle, which could differentiate tumour metastases from benign lymph nodes (LNs) by using fluorescence molecular imaging (FMI) and photoacoustic imaging (PAI) (12). However, both FMI and PAI show limited imaging depth, which may influence the detection sensitivity and accuracy of these techniques. Furthermore, only 0.7% of the engineered nanoparticles could reach the tumour site on account of the enhanced permeation and retention (EPR) effect, which results in less penetration and accumulation at tumour areas (13, 14). Thus, there is an urgent need for an active-targeting, sensitive, specific, and non-invasive imaging method to detect breast cancer LNMs.

ATP is a biomolecule that is essential for cellular energy supply and signal transmission (15, 16). ATP concentrations are higher in tumour cells than in normal cells. Therefore, the ATP level is distinctly higher in the tumour microenvironment (TME) due to the metabolism and proliferation of tumour cells (16–18). This indicates that an ATP-responsive imaging probe can be used for the imaging of the TME (19–22). Furthermore, DNA aptamers labelled with fluorescence dyes can exhibit the smart “turn on and off” fluorescence property by sensing specific molecules (e.g. protein) or TME characteristics (e.g. pH or hypoxia) (22–25). Therefore, FMI with an ATP-responsive aptamer can be applied for the specific detection of the TME.

In order to resolve the restriction with regard to imaging depth of FMI, magnetic particle imaging (MPI), an emerging and novel imaging method, is introduced in this study. MPI uses superparamagnetic iron oxide nanoparticles (SPIOs), which are also utilized for T2-weighed MRI, as the tracer. Compared with T2-weighed MRI, MPI possesses obvious advantages, such as high sensitivity and a positive signal (26–29). Importantly, MPI obviates the limitation of imaging depth, and this benefit enables the use of MPI as a promising sensitive method for detecting breast cancer LNMs.

In order to detect breast cancer LNMs, we designed and evaluated a tumour-targeting and TME ATP-responsive imaging probe, mainly comprising SPIOs conjugated with ATP-responsive aptamer double-stranded DNA-Cy5.5 (dsDNA-Cy5.5) and breast cancer targeting peptide Cys-Arg-Glu-Lys-Ala (CREKA). The CREKA peptide binds to the fibrin–fibronectin complexes that are highly expressed on breast cancer cells and interstitial cells and possesses good tumour-targeting capacity (30–36). The ATP-responsive fluorescence aptamer has a characteristic that fluorescence only emerges in the presence of ATP. Therefore, the ATP-responsive design could improve the detection specificity and signal-to-background ratio (SBR) of MLN. Moreover, SPIOs are suitable for MPI-based detection, which can facilitate the visualisation of metastatic cancer cells with high sensitivity and without image-depth limitation. This study aimed to explore the feasibility of the application and the superiority of breast cancer-cell-targeting ATP-responsive nanoparticles (NPs), abbreviated as SPIOs@A-T, to visualise the MLN of breast cancer using FMI and MPI in order to enable a more precision detection of LNMs (Scheme 1).

Materials And Methods

The detailed description is available in the supplementary information.

Results

FMI of the ATP-responsive property of SPIOs@A-T NPs

A Cy3-labeled double-stranded DNA (dsDNA-Cy3) probe was first designed and incubated with a series concentration of ATP (0, 0.5, 1, 2, 3, 4, 6, 8 and 10 mM) to verify the ATP-responsive ability. Fluorescence intensity was nearly undetectable in the absence of ATP, but fluorescent signals gradually increased with increasing ATP concentrations (Fig. S1a, S1b). Control groups with CTP, GTP, or UTP exhibited weak responses to the DNA probe even with increasing ATP concentrations. The results suggest that the dsDNA-Cy3 probe can specifically respond to ATP. The fluorescence signal changes showed a linear correlation with the concentration of dsDNA-Cy3 probe (from 0.1 to 2.0 μM) in response to 5 mM ATP (R^2=0.997, Fig. S1c).

Thereafter, the ATP-responsive aptamer (dsDNA-Cy5.5) and targeting peptide were conjugated onto the SPIOs (SPIOs@A-T NPs). The transmission electron microscopy (TEM) image showed that SPIOs@A-T NPs were mono-dispersed and homogeneous (Fig. 1a), without any obvious change in the shape compared to unmodified SPIOs (Fig. S2a). The ultraviolet-visible (UV-vis) absorption spectra showed that SPIOs@A-T had an absorption peak at 260 nm (Fig. 1b) and a positive potential of 4.24 ± 0.83 mV. After modification with CREKA and dsDNA, the zeta potential changed to −9.36 ± 2.04 mV (Fig. 1c). The hydrodynamic sizes of SPIOs and SPIOs@A-T were 51.63 ± 16.30 and 58.58 ± 19.34 nm, respectively (Fig. S2b). The abovementioned results verified the successful synthesis of SPIOs@A-T NPs. Both targeted, ATP-nonresponsive nanoparticles (SPIOs@nA-T NPs) and non-targeted, ATP-responsive nanoparticles (SPIOs@A-nT NPs) were synthesised as control groups.

To verify the ATP-responsive fluorescence imaging property of SPIOs@A-T NPs, the same experiments were performed to test whether SPIOs@A-T can respond to ATP, with SPIOs@nA-T in the control group. As shown in Fig. 1d and 1e, SPIOs@A-T manifested a significant increase in fluorescence signal intensity with increasing ATP concentrations, whereas they showed no change in the response with the addition of CTP, GTP or UTP. In contrast, SPIOs@nA-T showed a weak fluorescence response to ATP as well as to CTP, GTP, and UTP under the same experimental condition (Fig. S3a, S3b). SPIOs@A-T showed a significantly higher fluorescence intensity than SPIOs@nA-T at the same ATP concentration (****p< 0.0001 in Fig. 1f). Furthermore, fluorescence intensity maintained a good linear relationship with the SPIOs@A-T concentration at 5 mM ATP (R^2=0.990, Fig. 1g). Thus, our results revealed that SPIOs@A-T possesses excellent ATP-responsive ability.

MPI imaging property of SPIOs@A-T NPs

SPIOs@A-nT and Vivotrax (Magnetic Insight Inc., USA) were used as two controls to examine the MPI-based imaging property of SPIOs@A-T. We tested MPI signals of SPIOs@A-T, SPIOs@A-nT, and Vivotrax within an Fe concentration range of 0.0625–1.0000 mM. In Fig. 1h, there is no obvious difference of MPI signal between SPIOs@A-T and SPIOs@A-nT, and the quantitative value of MPI signals were both linearly related to the Fe concentration (Fig. 1i). Moreover, the MPI signal of SPIOs@A-T was relatively higher than that of commercial Vivotrax at the same Fe concentration (Fig. 1h, 1i), suggesting that SPIOs@A-T possess good MPI performance. We examined the MRI imaging property of SPIOs@A-T, which may provide *in vivo* anatomical information about lymph nodes. With increasing Fe concentrations, the T2-weighted MRI images gradually darkened (Fig. S4a). Furthermore, the r2 relaxation time of SPIOs@A-T was higher than that of Vivotrax in the same Fe concentration (Fig. S4b). Thus, we successfully developed SPIOs@A-T and proved that SPIOs@A-T have good ATP-responsive fluorescence and MPI/MRI imaging characteristics.

Characterization of cellular targeting and cytotoxicity of SPIOs@A-T NPs

The specific expression of fibronectin in malignant lymph node (MLN) tissues was examined using Western blotting analysis of 4T1 breast tumour-bearing mice. Normal lymph node (NLN) from healthy mice were used as the control. MLN showed a 1.8-fold higher expression of fibronectin compared to NLN (Fig. 2a, 2b, $*p < 0.05$), suggesting that fibronectin is highly expressed in metastatic tumour cells within LN.

To further evaluate the cellular uptake of targeted SPIOs@A-T, 4T1 cells were incubated with SPIOs@A-T for confocal microscopic imaging. The activation of Cy5.5 red fluorescence represents an ATP-responsive reaction of SPIOs@A-T in the presence of 4T1 tumour cells, whereas a blue colour represents the cellular nucleus (DAPI staining). An obvious red fluorescence signal was observed in the SPIOs@A-T group, and the merged confocal images indicated good SPIOs@A-T uptake by 4T1 cells (Fig. 2c). Non-targeted SPIOs@A-nT and ATP-nonresponsive nanoparticles (SPIOs@nA-T) were used as controls. The red fluorescence signals were weak for both SPIOs@nA-T and SPIOs@A-nT due to their non-ATP-responsive and non-targeting properties, respectively (Fig. 2c).

The cytotoxicity of the nanoparticles on 4T1 cells was examined by co-culturing the cells with serial concentrations of SPIOs@A-T, SPIOs@nA-T, and SPIOs@A-nT for 24 h. The data demonstrated that cell viability was 90–100% for these three groups, indicating that nanoparticles have good biosafety (Fig. S5).

In vivo FMI/MPI dual-modality imaging of orthotopic breast tumour

First, to evaluate the specific and targeted imaging of SPIOs@A-T *in vivo*, an orthotopic 4T1 breast cancer murine model was created and imaged after intratumoural injection of SPIO@A-T, SPIOs@A-nT, and SPIOs@nA-T (n = 3 mice in each group), respectively. FMI image acquisition was carried out sequentially at time points both before and after the injection of nanoparticles. The results showed that the SPIOs@A-T group showed a higher fluorescence intensity than the non-targeted SPIOs@A-nT for a 24-h post-

injection observation period, indicating that, in addition to the EPR effect *in vivo*, the targeting CREKA peptide could effectively facilitate the targeted binding of nanoparticles to the tumour site (Fig. 3a, 3b). The fluorescence intensity of the SPIOs@A-T group at tumour sites was significantly stronger than that of the SPIOs@nA-T group at all time points, which suggested that these novel nanoparticles could activate fluorescence signalling in the presence of ATP in breast tumours *in viv*o. Moreover, after the *in vivo* observation, the tumours were dissected for *ex vivo* FMI observation (Fig. 3a), and the results suggested that mice injected with SPIOs@A-T exhibited the highest fluorescence signal intensity among the three groups (Fig. 3c).

Furthermore, the MPI was further undertaken on small breast tumours in order to test the feasibility of sensitive imaging of small breast tumours (diameter < 4 mm) (37). As shown in Fig. 3d and 3e, the MPI signal was detected at the tumour site 4 h after the injection of SPIOs@A-T, SPIOs@A-nT, and Vivotrax, and the signal intensity peaked at 8 h after the injection. The MPI signal of SPIOs@A-T was notably higher than those of SPIOs@A-nT and Vivotrax at all time points (n = 3 mice). The *ex vivo* tumour images from MPI revealed that mice treated with SPIOs@A-T showed significantly higher signal intensities than mice in the other groups (Fig. 3d, 3f).

In vivo FMI/MPI dual-modality imaging of MLNs of breast cancer

Based on the results of the above-described experiment, we conducted FMI of MLNs in the breast tumour model in four groups that received an injection of SPIOs@A-T, SPIOs@nA-T, and SPIOs@A-nT into the left hind paws of breast-tumour-bearing mice as well as the NLN mice which were injected with SPIOs@A-T. After the intradermal injection of SPIOs@A-T in MLN mice, the fluorescence intensity augmented notably and peaked at 12 h, and the signal was detectable for 24 h (Fig. 4a). In contrast, only a very weak fluorescence signal was detected in the remaining three groups, suggesting that SPIOs@A-T augment the targeted and specific metastatic breast tumour cell detection capability. Due to the lack of ATP-responsive fluorescence in the TME, mice injected with SPIOs@nA-T presented almost no fluorescence signal at every time point. Similarly, due to the lack of the CREKA targeting peptide, mice injected with SPIOs@A-nT showed a relatively lower signal intensity than those treated with targeted SPIOs@A-T. Quantitative analysis showed that the average SBR of the SPIOs@A-T group was 222.02 ± 9.33, which was 14.3- and 1.96-fold higher than those of the SPIOs@nA-T (15.51 ± 1.22) and SPIOs@A-nT (113.01 ± 13.84) groups, respectively, at 12 h post-injection. To verify the specific detection of SPIOs@A-T for MLN, the mice with NLN were utilized as controls. The NLN group did not manifest an observable fluorescence signal, further demonstrating that SPIOs@A-T could specifically detect malignant LN (Fig. 4a). The average SBR of SPIOs@A-T (222.02 ± 9.33) was 4.99-fold higher than that of the group with NLN mice (44.47 ± 3.53) 12 h after FMI *in vivo* (Fig. 4b). Moreover, 24 h after injection of different NPs, the LN were dissected out for FMI *ex vivo*. As expected, MLN in the SPIOs@A-T group demonstrated the most obvious fluorescence signal compared to the other two groups. In the SPIOs@A-T group, the corresponding quantitative fluorescence intensity of LNs *ex vivo* was $4.5 \pm 0.2 \times 10^8$, which was 16.7- and 1.93-fold higher than that of the SPIOs@nA-T ($0.27 \pm 0.06 \times 10^8$) and SPIOs@A-nT ($2.33 \pm 1.30 \times 10^8$) groups (Fig. 4c). The sizes of MLN and NLN are shown in Fig. S6a. Histological examination of LN further revealed

findings that were consistent with *in vivo* and *ex vivo* FMI observation (Fig. S6a; tumour cells in LN are indicated by black arrows).

In order to evaluate the sensitivity for the detection of MLN, we performed an MPI with SPIOs@A-T, and used the non-targeted SPIOs@A-nT and Vivotrax as controls. As shown in Fig. 4d, at 4, 12, and 24 h after the injection, the MPI signal of SPIOs@A-T was localised to the popliteal LN, gradually peaked and remained stable, respectively. Quantitative assessment at 12 h of the non-targeted SPIOs@A-nT and commercial Vivotrax showed a value of 577.68 ± 19.99 for SPIOs@A-T, which was 1.52- and 1.61-fold stronger than those of SPIOs@A-nT (366.15 ± 9.25) and Vivotrax (359.13 ± 14.03), respectively (Fig. 4e). Moreover, the NLN group was used as the control group. MPI signal values of the SPIOs@A-T group was 1.87-fold higher at 12 h than that of the NLN group due to the specific targeting effect of CREKA (Fig. 4e). After 24 h *in vivo* observation, the lymph nodes were dissected out for further *ex vivo* MPI. The results were consistent with that of the *in vivo* observation (Fig. 4d, 4f). Moreover, T2-weighted MRI provided anatomical structural information of LN, which exhibited a darkening signal at the left popliteal LN site 2 h post-injection with SPIOs@A-T, and the location was nearly consistent with the FMI and MPI signal areas (Fig. S7).

To verify the *in vivo* observation, Prussian blue staining and histology were performed for LN to test the biodistribution of SPIOs@A-T NPs and showed more Fe-positive staining in the MLN of the SPIOs@A-T NPs group compared to the other groups (Fig. S6b), which was consistent with the findings of the *in vivo* observation.

Interestingly, MPI is well known for the superior performance with high sensitivity and the absence of an imaging-depth limitation and, therefore, MPI can be utilised for quantitative analysis. To validate this aspect in our study model, we performed the FMI and MPI on the same mice and the dual modality imaging scan on the same MLN both before and after skin incisions. Mice were injected with SPIOs@A-T NPs and scanned at 12 h post-injection. Compared to before exposure, the fluorescence signal was significantly higher after the skin exposure, although there was no remarkable difference for MPI signal intensities between the before and after skin incision evaluations (Fig. 5). This suggests that MPI is a promising method for the detection of metastatic breast cancer cells in LN or even in deep metastases to other organs.

To validate the specific and targeted binding of SPIOs@A-T to the metastatic breast cancer cells in LN, the MLN tissues were cryosectioned and fluorescence-stained for fibronectin. The confocal microscopy images showed that SPIOs@A-T displayed the strongest fluorescence intensity, compared to SPIOs@nA-T and SPIOs@A-nT. Moreover, SPIOs@A-T specifically bound to metastatic cancer cells, showing merged images (denoted by white arrows). In general, we concluded that SPIOs@A-T NPs can specifically discern and bind to the metastases of breast cancer in LN (Fig. S8).

In vivo biosafety assessment of SPIOs@A-T NPs

In order to estimate the biosafety of NPs *in vivo*, healthy mice were divided into four groups treated with injection of SPIOs@A-T, SPIOs@nA-T, SPIOs@A-nT NPs, and saline, respectively. There were no observable pathological changes for major organs, including the heart, liver, spleen, lungs, and kidneys, in all groups (Fig. S9). In addition, the serum biochemical indicators, including HDL-C as heart function biomarkers, BUN and CREA as kidney function biomarkers, and ALT and AST as liver function biomarkers were measured (Fig. S10). The data indicated no significant differences between nanoparticle-treated groups and the normal saline group. Thus, SPIOs@A-T, SPIOs@nA-T, and SPIOs@A-nT NPs showed good *in vivo* biocompatibility and biosafety.

Discussion

Given the urgent need for early detection of breast cancer LNMs, we developed a novel breast tumour-targeting and smart detection-imaging probe with SPIOs@A-T NPs, which are SPIOs conjugated with the breast tumour-targeting peptide CREKA and an ATP-responsive aptamer. When used in combination with the imaging strengths of FMI and MPI, nodal metastases of breast cancer can be detected with high sensitivity and specificity to differentiate malignant LN from normal one. Thus, our study presents a novel and potential imaging method to provide guidance for the detection of breast tumour LNMs in the clinical setting.

Specific and sensitive visualisation of LNMs in a non-invasive manner in early-stage cancer can maximise the benefits of early tumour staging and effective guide treatment selection (38, 39). We developed a unique ATP-responsive and breast cancer-targeting imaging probe to visualise MLN in breast cancer in an actively targeted, specific, sensitive, and non-invasive manner. The CREKA peptide was used as the targeting element of NPs, and the results verified that the peptide showed excellent binding ability to metastatic breast cancer cells in LN, both *in vitro* and *in vivo*. Results of both FMI and MPI not only demonstrated that the signal intensity of the targeted SPIOs@A-T is quantitatively and qualitatively stronger than that of non-targeted SPIOs@A-nT, but also that SPIOs@A-T would prolong the retention time of the signal at the LN area. Furthermore, we adopted an ATP-responsive fluorescence element in our nanoprobe to further improve the detection specificity for metastatic cancer cells in LN. In our *in vivo* FMI experiment, we found that SPIOs@A-T NPs showed an obvious, more intense fluorescence signal at MLN regions compared to SPIOs@nA-T NPs. To our knowledge, this is the first study using an ATP-triggered response for the *in vivo* visualisation of LNMs by FMI that has obtained promising results.

In this study, we introduced a dual-modality imaging method combining FMI and MPI to visualise tumour MLN. This initial use of MPI to detect LNMs achieved precise detection when complemented with FMI. Using an ATP-responsive fluorescence design, FMI can specifically lighten MLN. Because the 4T1 tumor cells in MLN show high ATP concentration, it can activate the labeled Cy5.5 on the aptamer after SPIOs@A-T injection, so that MLN can be visualized by FMI. The principle underlying this technique and the significance of MPI for MLN detection in this experiment is mainly based on several characteristics, including: 1) no image-depth limitation; 2) high sensitivity, which is suitable for the early detection of small metastases; 3) no background interference, which can reduce false-negative and false-positive

results; and 4) safety, when compared with other imaging modalities, because MPI is safe and does not have radioactivity or half-life interference.

There are also some limitations in this study. First, FMI and MPI cannot provide anatomical information for subjects, though we applied MRI to manifest the structure of LN and validated the effectiveness of FMI and MPI to image MLN. Therefore, the combination of CT and FMI or CT and MPI imaging techniques is expected to be used in the future investigation. Second, SPIOs@A-T NPs with FMI and MPI to detect MLN in the present study are focused only on the preclinical mouse model. Because of its good biocompatibility and biosafety, clinical human tissues may be tested by SPIOs@A-T NPs in the following study.

In conclusion, a novel dual-modality molecular imaging probe, SPIOs@A-T NPs, was integrated with two complementary FMI and MPI techniques and manifested the feasibility for diagnostic evaluation to distinguish MLN from NLN in a preclinical breast cancer model. On the one hand, the unique ATP-responsive fluorescence design allows FMI to specifically light up the lymphatic metastasis. On the other hand, MPI can compensate for the limited imaging-depth defect of FMI and to ensure sensitive imaging of metastatic tumour cells. LNMs are associated with TNM stage and poor prognosis. Due to superior tumour-targeting, specificity, and biosafety, SPIOs@A-T NPs can be a powerful tool for non-invasive, early detection and accurate diagnosis of MLN in clinical practice following further evaluations in future investigations.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Experiments conduction, data collection and analysis were mainly performed by Guorong Wang. Guangyuan Shi, Yu Tian, Jing Lei, Lingyan Kong and Ning Ding participated the experiment performance. The first draft of the manuscript was written by Guorong Wang and Yang Du. Experimental direction was performed by Yang Du. Yang Du, Zhengyu Jin and Jie Tian supervised the experiment implementation and gave the funding support. All authors participated in the revision of the manuscript. All authors read and approved the final manuscript.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics Approval

All animal experiments were approved by the Institutional Review Board of Peking Union Medical College Hospital (Permit No: B371-1).

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Scheme

Schemes 1 is available in the Supplemental Files Section.

Figures

Figure 1

(a) TEM image of SPIOs@A-T NPs, (b) UV-vis absorption spectra and (c) zeta potential of SPIOs and SPIOs@A-T NPs. Fluorescence imaging of the SPIOs@A-T (d) and its corresponding quantitative analysis in response to different nucleoside triphosphates (e) (Analytes=100 nM). (f) Comparison of fluorescence intensity between SPIOs@A-T and SPIOs@nA-T at different concentrations of ATP. (Analytes=100 nM; ****, $p < 0.0001$). (g) Standard curve of the fluorescence signal in samples with serial concentrations of SPIOs@A-T in response to 5 mM ATP. (h) MPI images of SPIOs@A-T, SPIOs@A-nT, and Vivotrax at series different concentrations. (i) Plot of MPI signals of all samples

Figure 2

(a) The fibronectin protein expression level in lymph node metastases and normal lymph node in Western blotting analysis. (b) Densitometric analysis of fibronectin protein expression normalised to that of β-actin. Data represent the mean ± SD, n=3. Mets, metastases. *: $p < 0.05$. (c) Confocal microscopy images of 4T1 cells treated with different NPs. Blue fluorescence indicates nuclear staining, and red fluorescence indicates corresponding nanoparticles. Scale bars: 20 μm

Figure 3

(a) *In vivo* and *ex vivo* FMI of 4T1 orthotopic tumours (yellow circle) in mice treated with SPIOs@A-T, SPIOs@nA-T, and SPIOs@A-nT at multiple timepoints after injection (0, 2, 4, 8, 12, and 24 h). The comparison of fluorescence intensity among SPIOs@A-T, SPIOs@nA-T, and SPIOs@A-nT *in vivo* (b) and *ex vivo* (c). *: $p < 0.05$. (d) *In vivo* and *ex vivo* images from MPI of 4T1 orthotopic tumour (yellow circle) mice treated with SPIOs@A-T and SPIOs@A-nT and Vivotrax at multiple timepoints after injection (0, 2, 4, 8, 12, and 24 h). The comparison of MPI signal values among SPIOs@A-T and SPIOs@A-nT and Vivotrax *in vivo* (e) and *ex vivo* (f). *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$

Figure 4

(a) *In vivo* and *ex vivo* fluorescence images of metastatic lymph node (MLN) treated with SPIOs@A-T, SPIOs@nA-T and SPIOs@A-nT. Normal lymph node (NLN) treated with SPIOs@A-T is the control group. Images were acquired at multiple timepoints (0, 4, 8, 12 and 24 h) after injection. (b) The comparison of SBR among these groups *in vivo*. (c) The comparison of fluorescence intensity among these groups *ex vivo*. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$. (d) *In vivo* and *ex vivo* images from MPI of MLNs treated with SPIOs@A-T and SPIOs@A-nT and Vivotrax. NLN treated with SPIOs@A-T was seen as control group. Images were acquired at multiple timepoints (0, 4, 8, 12 and 24 h). The comparison of MPI signal values among the study groups *in vivo* (e) and *ex vivo* (f). *: $p < 0.05$, **: $p < 0.01$, ****: $p < 0.0001$

Figure 5

Comparison of the signal-intensity changes of FMI and MPI before and after skin incision. FMI images (a) and signal intensity (b) of lymph nodes (LNs; yellow circles) before and after skin incision. MPI images (c) of LNs (yellow circles) and signal distinction (d) before and after skin incision. **: $p < 0.01$. ns: no significant difference

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Scheme.1.png
- SI.docx