Development and evaluation of a dual-modality (MRI/SPECT) molecular imaging bioprobe

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Abstract

Specific bioprobes for single photon emission computed tomography (SPECT) and magnetic resonance imaging (MRI) have enormous potential for use in cancer imaging in near-future clinical settings. The authors describe the development of dual modality molecular imaging bioprobes, in the form of magnetic nanoparticles (NPs) conjugated to antibodies, for SPECT and MRI of mesothelin-expressing cancers. The bioprobes were developed by conjugating $^{111}$In labeled antimesothelin antibody mAbMB to superparamagnetic iron oxide NPs. Our experimental findings provide evidence that such bioprobes retain their magnetic properties as well as the ability to specifically localize in mesothelin-expressing tumors. It is anticipated that combining SPECT with MR will help obtain both functional and anatomical imaging information with high signal sensitivity and contrast, thereby providing a powerful diagnostic tool for early diagnosis and treatment planning of mesothelin-expressing cancers.

From the Clinical Editor: This team of investigators presents a dual-modality functionalized contrast material that enables tumor visualization both via MRI and SPECT. Similar systems are likely to be utilized in future cancer research.

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Combining two or more different imaging modalities using multimodal probes can be of considerable value in molecular imaging, especially for cancers that are difficult to diagnose and treat. This synergistic combination of imaging modalities, commonly referred to as image fusion, ensures enhanced visualization of biological targets, thereby providing information on all aspects of structure and function, which is difficult to obtain by a single imaging modality alone. Single photon emission computed tomography, SPECT, allows noninvasive determination of in vivo biodistribution of radiotracers at picomolar concentrations. Using specific radiolabeled probes, obtaining functional information with high sensitivity about molecular processes is possible. SPECT images, however, have limited spatial resolution and lack anatomical details for reference, making the precise localization of lesions difficult. Co-registration of SPECT with anatomical images, from either computed tomography (CT) or magnetic resonance imaging (MRI), has been commonly used in the clinic to address this problem. The use of MRI presents specific advantages in comparison with CT, including lack of ionizing radiation, high soft-tissue contrast, and sensitivity to tissue alterations.\textsuperscript{1,2} Further, imaging with gadolinium and iron oxide-based contrast agents extends MRI into the domain of molecular imaging, thus offering an excellent capability to examine soft tissues. Dual-modality imaging with SPECT and MRI thus provides anatomical references in an image while synergistically combining the high spatial resolution associated with MR with the high sensitivity of SPECT.\textsuperscript{3} Dual-modality SPECT/MR imaging bioprobes would allow for simultaneous dynamic imaging of structure and function, facilitate noninvasive monitoring of treatment, and directly provide information on pharmacokinetics and metabolism of drugs. Additionally, simultaneous dual-modality imaging can reduce the overall scan time, avoids multiple anesthesia sessions, and prevents errors associated with co-registration.\textsuperscript{4} Despite these promises, simultaneous SPECT/MRI dual-modality imaging technology is still in its infancy, due to the
technological challenges in operating a SPECT scanner within an MR instrument. With the development of gamma ray detectors based on avalanche photodiodes (APDs) as well as availability of fast scintillation materials like lutetium oxyorthosilicate (LSO), it has become possible to incorporate fully magnetic-field-insensitive, high-performance PET detectors within PET/MRI scanners. Similar design principles apply to the development of SPECT/MRI scanners, where semiconductor detectors (cadmium–zinc–telluride [CZT]) used in small-animal SPECT were shown to be insensitive to magnetic fields up to 7 T, leading to the development of a prototype for simultaneous SPECT/MRI systems. The feasibility of simultaneous SPECT and MRI data acquisition was demonstrated recently, justifying the further development of SPECT/MRI for small-animal and whole-body human imaging. We report on the development of a dual-modality SPECT/MR imaging agent for use with such dual-modality scanners.

Malignant mesothelioma and pancreatic and ovarian cancers are characterized by low survival rates, which can be attributed to late diagnosis and the cancers’ propensity for early metastasis, both of which significantly reduce the chance of a cure. Better imaging screening tools would facilitate the early identification of these tumors, thus improving chances of successful treatment or resection. Mesotheliomas, pancreatic adenocarcinomas, and ovarian cancers overexpress a cell-surface antigen known as “mesothelin”, which is a promising target for selective imaging of these difficult-to-diagnose tumors. Tumor-specific MRI contrast agents can be prepared by coating iron oxide nanoparticles (NPs) (T2 contrast) with monoclonal antibodies directed against tumor antigens. Studies have shown that such preparations retain their ability to bind tumor antigens as well as their relaxation properties. Using these antibody-coated iron oxide NPs, MRI image-contrast can be easily induced in a concentration range of 1–10 nM by spin-echo methods. In addition, Lijowski et al and Billotey et al have recently reported on the development of SPECT/MRI imaging agents by combining gadolinium (T1 contrast) with monoclonal antibodies directed against tumor antigens.

The purpose of the current study was to develop a dual-modality molecular imaging bioprobe by conjugating \(^{111}\text{In}\)-mAbMB to superparamagnetic iron oxide NPs (SPIIONS), which would allow combined SPECT/MR imaging of mesothelin-expressing cancers. The relaxation properties and cellular uptake of the \(^{111}\text{In}\) labeled mAbMB-SPION conjugates were investigated in vitro. Biodistribution and autoradiography studies in tumor-bearing mice were used to determine the suitability of these probes for SPECT imaging. Further, their properties as MR bioprosbes were examined in vivo using MRI.

**Methods**

**Materials**

All chemicals and reagents were purchased from Sigma-Aldrich (Oakville, Canada). The cell culture media and supplements were obtained from Invitrogen (Burlington, Canada). The mAbMB antibody was purchased from Rockland Immunoclonicals (Gilbertsville, Pennsylvania). \(^{111}\text{InCl}_3\) was obtained from MDS Nordion (Vancouver, Canada) and p-SCN-bn-DTPA from Macronetics (Dallas, Texas). SPIIONS (Fluidmag CMX) were received as a gift from Chemicell (Berlin, Germany). Activity measurements were carried out using a Packard Cobra II gamma counter (Perkin-Elmer, Waltham, Massachusetts).

**SPIIONS characterization**

SPIIONS (Fluidmag-CMX sized \(< 100 \text{ nm}\) have a magnetic core and are coated with carboxy methyl dextran, which provides the carboxylic acid groups for antibody conjugation. The core structure of SPIIONS was determined by transmission electron microscopy (TEM) to understand the general morphology of the magnetic core as well as to determine the particle size distribution. A drop of diluted solution of the SPIIONS in water was placed in carbon-coated copper TEM grid (150 mesh; Ted Pella Inc, Redding, California) and was allowed to air dry. The samples were imaged at 200 kV using a FEI Tecnai G2 TEM (Hillsboro, Oregon). The hydrodynamic diameter of NPs was determined by dynamic light scattering (DLS) measurements carried out on a Malvern 3000HS Zetasizer (Malvern Instruments Ltd., Malvern, United Kingdom) with a He–Ne laser (532 nm) and 90-degree collecting optics. Data was analyzed using CONTIN algorithms provided by Malvern Zetasizer software.

**Conjugation of \(^{111}\text{In}\)-mAbMB antibody with SPIIONS**

In labeling of mAbMB was accomplished as described previously. The carbodiimide coupling reaction was used to conjugate the \(^{111}\text{In}\)-mAbMB antibody to the SPIIONS. SPIONs (5 mg) were washed three times with 1 mL of 0.1M MES (2-(N-morpholino)ethanesulfonic acid) buffer, pH 4.5, suspended in a freshly prepared EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) solution containing 10 mg EDC in 0.25 mL MES solution and shaken in an Eppendorf thermomixer at 800 rpm for 10 minutes at room temperature (20 to 25°C), followed by magnetic washing three times with 1 mL phosphate buffered saline (PBS, pH 7.4). After resuspending the particles in 0.25 mL of PBS, about 50 \(\mu\)g of \(^{111}\text{In}\)-mAbMB (9.25 MBq) solution was mixed with the activated particles for 2 hours at room temperature (20 to 25°C). The antibody-conjugated NPs (\(^{111}\text{In}\)-mAbMB-SPIONs) were collected and washed three times with PBS. The amount of conjugated antibody was determined by measuring the activity in the washes and the SPIION suspension.

**In vitro cell binding and specificity**

A431K5 and A431 cells were cultured and plated overnight in 24-well plates, followed by incubation with 0.05 mg iron per mL equivalent of \(^{111}\text{In}\)-mAbMB-SPIONs at 37°C and the cell uptake was determined after 3, 6, and 24 hours. In a separate experiment, one set of A431K5 cells was incubated with 0.05 mg/mL iron equivalent of \(^{111}\text{In}\)-mAbMB-SPIONs for 3 hours, and the second set was incubated with the same quantity of \(^{111}\text{In}\)-mAbMB-SPIONS in the presence of unlabeled mAbMB. Next, the cell-bound activity was determined. For confocal microscopy studies A431K5 cells were incubated with 0.05 mg/mL iron of \(^{111}\text{In}\)-mAbMB-SPIONs at 37°C for 3 hours,
stained with propidium iodide and mounted on microscopic slides and observed using a Leica DM2500 confocal microscope. Similarly, a control experiment was carried out with SPIONs (without antibody) at 37°C. The images were processed with ImageJ software (NIH, Bethesda, Maryland), and semiquantitative analysis was performed using the "RGB Measure" plug-in. Method details of in vitro cell binding experiments have been provided in the Supplementary Materials, Section M1, available online at http://www.nanomedjournal.com.

Relaxivity measurement and in vitro MRI

Phantoms of SPIONs and 111In-mAbMB-SPIONs in agar gel were prepared in the concentration range 0.0 to 0.55 mM and imaged using a 7 Tesla Bruker Biospec 70/30 USR MRI scanner (Bruker, Ettlingen, Germany). Inversion recovery images and multi-echo CPMG images were used to calculate the R1 relaxation rate and R2 relaxation rate of each sample, respectively. The R1 and R2 were plotted against contrast agent concentration to respectively determine the $r_1$ and $r_2$ relaxivities from the slope of the linear fit (method details in Supplementary Materials, Section M2).

Tumor uptake and biodistribution

Biodistribution of 111In-mAbMB-SPIONs was evaluated in male C.B-17 SCID mice (Taconic, New York). Mice were subcutaneously injected with 5 x 10⁶ A431K5 cells (mesothelin positive) in the upper back and 5 x 10⁶ A431 cells (mesothelin negative) in the lower back. When tumors exceeded a size of 0.5 cm in diameter, the mice were injected intravenously with 74 kBq of 111In-mAbMB-SPIONs at a dose of 15 mg/kg bodyweight equivalent of iron, based on previously reported studies. Animals were euthanized at 24 and 72 hours post injection, and major organs harvested. The radioactivity associated with each organ was analyzed with a γ-counter to obtain the biodistribution data. The ratio of radioactivity in the tumor or normal tissue to that in blood was determined by dividing the activity per weight of tissue by activity per weight of total blood. Animal experiments were carried out in accordance with the guidelines of the Canadian Council on Animal Care with approval of the University of British Columbia’s Animal Care Committee.

Prussian blue staining of tissue specimens

Tissue specimens were frozen in OCT compound (Sakura Finetek Inc., Torrance, California) with liquid nitrogen and 10 µm sections were made using a cryotome (CM1850; Leica Microsystems GmbH). At the time of staining, sections were washed with 5% formaldehyde in PBS, and then washed twice with PBS before applying Pearl’s Prussian Blue staining for 20 minutes. Pearl’s Prussian Blue solution is obtained by mixing equal volumes of 4% potassium ferrocyanide solution and 4%...
HCl. Slides were then counterstained for 10 minutes with neutral red stain. Slides were examined by optical microscopy using a Leica DMLB microscope (Leica Microsystems Inc, Buffalo Grove, Illinois) with attached Retiga 2000R camera and processed using ImageJ software.

**In vivo MRI studies**

SCID mice (n = 3) bearing A431K5 tumor xenografts implanted at their neck were injected via tail vein with 74 kBq of 111In-mAbMB-SPIONs, 15 mg/kg body weight equivalent of iron. At 24 and 72 hours post injection, the mice were anesthetized using isoflurane and underwent in vivo MR imaging (gradient echo scan) using a 7 Tesla Bruker Biospec 70/30 USR MRI scanner. For image acquisition, a quadrature birdcage volume coil of 7 cm inner diameter (Bruker Biospin, Ettlingen, Germany) was used. Axial images were taken with the following parameters: FOV = 4 x 4 cm²; matrix size = 256 x 256; slice thickness = 1 millimeter; TE = 6 milliseconds; TR = 700 ms. For image analysis of A431K5 tumors, the freely downloadable three-dimensional Slicer program version 3.6 (www.slicer.org) was used. MR images were co-registered based on manually selected fiducial markers (affine registration with 12 degrees of freedom). To determine quantitatively the change in MR contrast in A431K5 tumors, intensity histograms were constructed within manually delineated tumor region of interest (ROI) and normalized into a probability density plot.

**Autoradiography**

Ex vivo autoradiography of major organs was carried out after biodistribution studies by exposing a 12.5 x 25 cm² phosphor screen (Perkin-Elmer, Waltham, Massachusetts) to the excised organs, followed by visualization using a phosphor imager (Cyclone storage phosphor imager; Perkin-Elmer) with OptiQuant software. To verify the distribution of 111In-mAbMB-SPIONs in the tumors, 20 μm sections of A431K5 tumors were cut on a cryotome and exposed on a phosphor screen for 24 hours.

**Results**

**Particle characterization and antibody conjugation**

The average hydrodynamic size of SPIONs measured using a DLS method was 69.6 nm (Figure 1, D), and the average magnetic core size determined using TEM was 15.6 nm (Figure 1, B and C). The DLS measurements carried out on 111In-mAbMB-SPIONs showed that the size increased to 76.6 nm (Figure 1, E) and the polydispersity index increased from 0.16 to 0.18. By measuring the radioactivity associated with the NPs, we determined that 76.5 ± 4.9% of 111In-mAbMB was conjugated to the SPIONs after the carbodiimide coupling reaction. The specific activity obtained was 129 MBq/mMol of iron.
In vitro cell binding and specificity

For cell binding studies we used A431K5 cells, which are human epidermoid A431 cells stably transfected with pCD3CAK1-9, a plasmid encoding the mesothelin gene and producing mesothelin levels similar to those reported in patients.19 The addition of $^{111}$In-mAbMB-SPIONs to these mesothelioma cells was not cell toxic (see Supplementary Materials sections M3 and F1 for details). We then examined the cell uptake and binding specificity of $^{111}$In-mAbMB-SPIONs with the help of cell binding assays as well as confocal microscopy. The cell uptake of $^{111}$In-mAbMB-SPIONs by A431K5 cells (mesothelin expressing) was significantly higher than that of A431 cells (control cells that do not express mesothelin) at all the time points, indicating the immunoconjugates’ ability to bind specifically to mesothelin expressing cells (Figure 2, A). Furthermore, the covalent attachment of the $^{111}$In-mAbMB to the SPIONs through EDC coupling did not significantly damage the active binding site of the antibodies. The ratios of specific uptake to nonspecific uptake at 3, 6, and 24 hours post incubation were 4.5, 3.4, and 8.0, respectively. As shown in Figure 2, B, the binding of $^{111}$In-mAbMB-SPIONs to A431K5 cells was inhibited by mAbMB, which demonstrated that cellular uptake of $^{111}$In-mAbMB-SPIONs was mediated by antibody binding. The cell uptake of $^{111}$In-mAbMB-SPIONs by A431K5 cells was 4.5 times higher than the cell uptake in presence of excess of unlabeled mAbMB (Figure 2, B). The specific binding of $^{111}$In-mAbMB-SPIONs and SPIONs (control) to A431K5 cells was also studied qualitatively by confocal microscopy (Figure 2, C). Incubated at 37°C for 3 hours at concentrations of 0.05 mg iron/mL, $^{111}$In-mAbMB-SPIONs (Figure 2, C1) showed 2.3 times higher (semiquantitative analysis) cell binding to A431K5 cells in comparison with SPIONs (Figure 2, C2). Evidence from all three cell-binding studies thus confirmed the specific binding ability of $^{111}$In-mAbMB-SPIONs to mesothelin-expressing cells.

Relaxivity measurement and in vitro MRI

The $^{111}$In-mAbMB-SPIONs had a relaxivity of $r_2 = 469.57$ mMs$^{-1}$ and $r_1 = 0.59$ mMs$^{-1}$. In comparison, the unconjugated SPIONs had $r_2 = 397.33$ mMs$^{-1}$ and $r_1 = 0.54$ mMs$^{-1}$ (Figure 3, B and C). The higher $T_2$ relaxation indicated that $^{111}$In-mAbMB-SPIONs could be used for $T_2$-weighted MRI imaging. Iron oxide cores in $^{111}$In-mAbMB-SPIONs produced a characteristic darkening ($T_2$ contrast) at increasing iron concentrations, as seen in Figure 3, A. The equivalent contrast between $^{111}$In-mAbMB-SPIONs and SPIONs could be observed qualitatively from the signal intensity images at different concentrations (0.0 to 0.55 mM) (Figure 3, A).

Figure 3. (A) T2-weighted MR image at different iron concentrations for SPIONs and $^{111}$In-mAbMB-SPIONs in phantom agar gel. (B) Reciprocal of $T_1$ vs. concentration using inversion recovery measurements. (C) Reciprocal of $T_2$ vs. concentration using CPMG measurements. Relaxivity values $r_1$ and $r_2$ were obtained from slopes of linear fits of experimental data.
Tumor uptake and biodistribution

$^{111}$In-mAbMB-SPIONs exhibited significantly higher ($P < 0.05$) localization in the A431K5 tumors in comparison with A431 tumors at 24 and 72 hours post injection (Figure 4, A). The activity uptake into the other evaluated organs (lungs, heart, intestine, stomach, muscle, and brain) was relatively low and constant at both time points. As seen in Table 1, the tumor-to-blood ratios were greater than 1 at both 24- and 72-hour time points. These results correlated well with the autoradiographic images of the organs, where spleen, liver, kidney and A431K5 tumors showed the highest uptake (Figure 4, B).

**Prussian blue staining**

Microscopic examination of tumor sections stained with Prussian blue showed the presence of iron NPs in A431K5 tumor tissue (Figure 5, B). The red color indicates nuclei, and the pink is cytoplasm, whereas the SPIONs are stained blue. Strong blue color can be seen in the cytoplasm of the tumor cells. A431 tumors, though to a lesser extent, also showed the presence of iron oxide (Figure 5, A), which indicates nonspecific uptake due to the enhanced permeation and retention (EPR) effect.20 As expected on the basis of biodistribution data, higher iron oxide staining was observed in the spleen of mice (Figure 5, C).

**In vivo MRI studies**

The potential of the $^{111}$In-mAbMB-SPIONs for MRI was investigated in a mouse xenograft model. Axial images were acquired at pre-injection and at the 24- and 72-hour post-injection time points. The change in MR signal for A431K5 tumors is apparent in axial images, especially in the region pointed out by the arrows in Figure 6. Confirmation of $^{111}$In-mAbMB-SPIONs uptake in this region was obtained from autoradiographs of the excised tumor sections (Figure 6). The MR intensity histograms (Figure 6, D) show the frequency of occurrence of particular image intensity within the ROI, indicated by the color plots. The suppression of tumor signal by $^{111}$In-mAbMB-SPIONs is mainly seen in the post 24-hour scan, as a right shift in the distribution toward lower intensities on the x axis. When comparing mean image intensities (Figure 6, D), a 33% and 9% decrease from the pre-injection time point was observed for the 24- and 72-hour time points, respectively.

**Discussion**

In an attempt to improve imaging in a few of the most challenging cancers, such as mesothelioma and pancreatic and ovarian cancers, we designed a dual-modality MRI/SPECT imaging bioprobe by conjugating $^{111}$In-mAbMB with SPIONs. The resulting $^{111}$In-mAbMB-SPIONs showed specific uptake into A431K5 tumors and produced a change in the MR signal when tested in MRI experiments. We used $^{111}$In as a SPECT motif to develop dual-modality bioprobes for imaging mesothelin-expressing tumors, as $^{111}$In provides more sensitivity (less scatter and internal shielding) than the two previously reported $^{125}$I-labeled antibody-magnetic NP conjugates. In the first study by Otsuji et al., $^{125}$I-labeled antibody-magnetic NP conjugates...
showed significantly higher uptake in colorectal tumors than in normal tissue and also observed reduced MR signal intensity in the treated tumors. In the second study, Liu et al demonstrated specific tumor uptake of their \(^{125}\)I-labeled antibody-magnetic NPs by SPECT and MRI. A few additional groups have also reported on the development of multimodality imaging agents with \(^{111}\)In and \(^{99m}\)Tc-labeled iron oxide conjugates using targeting agents such as peptides and bisphosphonates. Lewin et al demonstrated that TAT-peptide derivatized magnetic particles labeled with fluorescence and \(^{111}\)In could be used to track progenitor cells in vivo. Kaufner et al studied the in vivo biodistribution and MRI properties of polymer-coated maghemite labeled with \(^{111}\)In and compared it with Resovist for liver imaging by MRI. Recently, Torres et al developed dual-modality SPECT/MRI probes by conjugating bisphosphonates with \(^{99m}\)Tc and iron oxide (Endorem). In comparison with these and other clinically approved iron oxide contrast agents, our antibody-magnetic NP conjugates demonstrated higher molar relaxivity. Additionally, the ability of the particles to extravasate and bind to a tumor antigen makes them promising as multimodality imaging agents for tumor imaging.

SPIONs for MRI consist of numerous iron oxide crystals, have high molar relaxivities, and are nontoxic due to their dextran coating. For tumor-specific imaging, monoclonal antibodies specific for infracted myocardium, carcinoembryonic antigen, epidermal growth factor receptors, and human glioma cell-surface antigen have been attached to iron oxide NPs. Their efficacies have been established in ex vivo and animal studies. For our studies, we used SPIONs (Fluidmag-CMX) that were first characterized by TEM and DLS to ensure that the SPIONs were present as stable colloidal dispersions, without aggregation. Post antibody conjugation, the hydrodynamic diameter of the particles expectedly increased from 69.6 nm to 76.6 nm, accompanied by a change in the size-distribution profile leading to slight increase in the polydispersity index from 0.16 to 0.18. This size distribution is much better than the one seen in most clinically approved SPION formulations like Feridex, Endorem, and Resovist, whose mean size ranges between 60 nm to 250 nm.

A comparison of cell binding to mesothelin-expressing A431K5 and non-mesothelin-expressing A431 cells, over a period of 24 hours suggested that \(^{111}\)In-mAbMB-SPIONs retained their mesothelin reactivity and binding specificity. Higher molar relaxivity of a contrast agent implies that lower concentrations are required to achieve the characteristic darkening associated with MRI. Higher molar relaxivity is especially important for applications requiring extravasation of the contrast agent (tumor imaging), because of typically lower accumulation of the contrast agent. For such applications, SPIONs are especially suitable because they possess high transverse relaxivity due to each particle being composed of many thousands of iron atoms. Additionally, coupling with specific molecular targeted ligands (like antibodies and peptides) provides them an ability to accumulate in extravascular tissue (tumors) at appropriate concentrations to significantly change local magnetic field and produce an optimum MR effect. Moreover, the MR effect of SPIONs extends beyond their immediate proximity, thus making it more effective in detecting even very small amounts of the agent. Several groups have successfully developed high-relaxivity contrast agents with \(r_2\) values ranging between 281 mM\(^{-1}\)s\(^{-1}\) to 453 mM\(^{-1}\)s\(^{-1}\). In our in vitro relaxivity studies, we observed a signal hypointensity gradient in proportion to the increasing iron concentrations for \(^{111}\)In-mAbMB-SPIONs. Qualitatively, the signal hypointensity of unconjugated SPIONs and \(^{111}\)In-mAbMB-SPIONs was equivalent, indicating that the conjugation did not affect the relaxation properties of the SPIONs. The \(r_2\) relaxivity values of SPIONs (397.33 mM\(^{-1}\)s\(^{-1}\)) as well as \(^{111}\)In-mAbMB-SPIONs (469.57 mM\(^{-1}\)s\(^{-1}\)) were higher than the relaxivity value for Feridex I.V. (224 mM\(^{-1}\)s\(^{-1}\); from our own data).

The use of radiolabeled MR probes adds a quantitative component and allows for the determination of the biodistribution...
and in vivo characteristics of the imaging bioprobes, which is difficult to achieve solely by MRI. The main purpose of our biodistribution study was to evaluate specific tumor uptake of 111In-mAbMB-SPIONs in A431K5 tumors and compare it with nonspecific uptake in A431 tumors. The observed activities in A431K5 tumors were significantly higher ($P < 0.05$) than in A431 tumors, which points to specific uptake of 111In-mAbMB-SPIONs in A431K5 tumors, a finding also confirmed by Prussian blue staining (Figure 5). Although blood levels of 111In-mAbMB-SPIONs decreased between 24 and 72 hours, the corresponding activity levels in A431K5 tumors increased slightly from about 4% ID/g at 24 hours to about 4.8% ID/g at 72 hours. To achieve high tumor penetration of molecular probes, it is necessary to achieve longer circulation times in the blood, but the rapid sequestration of intravenously injected colloidal particles from the blood by the reticuloendothelial system (liver and spleen) poses a major barrier. Previous studies have shown that the spleen localization of nontargeted SPIONs can range between 10%–20% ID/g.22,39,40 In our studies 111In-mAbMB-SPIONs showed high localization in spleen, similar to that reported previously for 111In-mAbMB.14 This spleen uptake (Figure 4) can be attributed to the binding of 111In-mAbMB-SPIONs to shed mesothelin antigen from A431K5 tumors, forming immune complexes that are sequestered by the spleen.14,41 Spleen, being composed of lymphatic tissue, is highly radiosensitive: therefore accumulation of 111In-mAbMB-SPIONs in spleen can be harmful due to the emission of auger electron radiation from 111In as reported previously.42-44 In comparison with spleen, the liver uptake was much lower at 24 hour, but increase in activity accumulation was observed by 72 hours, indicating that blood clearance mediated by hepatic midzonal and periportal Kupffer cells increased over time.38 The SPIONs taken up by the liver and spleen are metabolized and transferred to the body’s iron stores and incorporated into erythrocyte hemoglobin.45

MRI correlated well with biodistribution findings. The MR signal change was clearly visible in A431K5 tumors between pre- and post-injection time points in the axial MR images, indicating the presence of iron oxide particles in the tumors (Figure 6). Furthermore, the regions of signal hyposensitivity correlated well with the intratumor distribution of the probe, as evident from autoradiography (Figure 6). MR signal intensity in A431K5 tumors was reduced, especially apparent at the 24-hour time point (Figure 6, D). At 72 hours, signal hypointensity remains greater than at pre-injection, but was reduced in comparison with the 24-hour time point. This reduction can be partly attributed to tumor growth and the decline in blood-pool reservoir of 111In-mAbMB-SPIONs during the 24-hour and 72-hour periods, thus resulting in reduced incremental uptake as indicated by the biodistribution studies.

Despite high expression of mesothelin by A431K5 tumors, the shed mesothelin creates a major impediment to mesothelin-directed delivery of the 111In-mAbMB-SPIONs. Although we observed this phenomenon in a previous study with 111In-mAbMB,14 the unavailability of a relevant mesothelin-expressing tumor model that does not shed mesothelin limited our choice to A431K5 cells for these studies. Other commonly used cell lines, like NCI-H226 cells express about seven times...
lower amounts of mesothelin,14 much less than observed in mesothelioma patients,47 and are therefore not a useful model. We demonstrated the potential of 111In-mAbMB-SPIONs for simultaneous MR- and γ-imaging by following MRI with terminal biodistribution and autoradiographic studies (Figure 4). Further validation by SPECT imaging should be obtained in the future.

Receptor-directed agents such as monocrystalline iron oxide NPs, sized less than 40 nm are most capable of passing through capillary fenestra and interendothelial junctions to reach the extravascular space. This passage is a prerequisite for targeted imaging and would help to achieve higher tumor concentrations, but no such agents are yet clinically approved.28 The use of surface-modified SPIONs with larger size is therefore a reasonable choice as they possess greater magnetic susceptibility, thus making them capable of shortening spin-spin relaxation of surrounding water molecules.48 The T2-enhancing effects observed in MRI as well as the higher A431K5 tumor uptake provide evidence of the ability of 111In-mAbMB-SPIONs to specifically target mesothelin expressed by tumors. In addition, 111In-mAbMB-SPIONs have relaxation rates as well as biocompatibility characteristics suitable for MRI. With the progress made recently in development of combined PET/MRI and SPECT/MRI scanners, dual-modality imaging agents such as the one developed in the current study have enormous potential to be used in the clinic in the near future.3,49,50

In conclusion, we successfully developed and tested a novel dual-modality molecular imaging probe for in vivo detection of mesothelin-expressing tumors by both MRI and SPECT. This imaging bioprobe demonstrated better size distribution and relaxivity properties in comparison with the previously developed MRI and SPECT/MRI contrast agents. The high relaxivity and specific targeting of the bioprobe resulted in an enhanced MR contrast in mesothelin-positive tumors. Covalently bound 111In acted as a SPECT detection motif and was used to provide proof of principle by autoradiography and biodistribution studies. It is anticipated that combining SPECT and MRI modalities will enable characterization of tumors based on both functional and anatomical information and will provide a powerful diagnostic tool for early diagnosis and monitoring of mesothelin-expressing cancers.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.nano.2011.10.013.

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