Nanoprobes for hybrid SPECT/MR molecular imaging

Hybrid imaging techniques provide enhanced visualization of biological targets by synergistically combining multiple imaging modalities, thereby providing information on specific aspects of structure and function, which is difficult to obtain by a single imaging modality. Advances in the field of hybrid imaging have resulted in the recent approval of PET/magnetic resonance (MR) imaging by the US FDA for clinical use in the USA and Europe. Single-photon emission computed tomography (SPECT)/MR imaging is another evolving hybrid imaging modality with distinct advantages. Recently reported progress in the development of a SPECT/MR imaging hybrid scanner provides a cue towards the need for multimodal SPECT/MR imaging nanoprobes to take full advantage of a scanner's simultaneous imaging capability. In this review, we present some of the latest developments in the domain of SPECT/MR hybrid imaging, particularly focusing on multimodal nanoprobes.

KEYWORDS: gadolinium ▪ hybrid imaging ▪ magnetic resonance imaging ▪ multimodality ▪ nanoprobes ▪ single-photon emission computed tomography ▪ superparamagnetic iron oxide

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Hybrid imaging combines functional and anatomical information from different imaging modalities in a single scan to provide enhanced localization and molecular insight into structural abnormalities. By combining two or more detection techniques using multimodal probes, it is possible to combine the advantages of one imaging modality with another, and at the same time reduce the disadvantages of both. This synergistic combination of imaging modalities 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 [1]. PET/computed tomography (CT) and single-photon emission CT (SPECT)/CT are in widespread use worldwide in human patients with many thousands of scanners used in routine clinical practice, while PET/magnetic resonance (MR) imaging has also been recently approved for clinical use. The promise shown by these hybrid imaging modalities has encouraged advances in hybrid SPECT/MR technology. Hybrid imaging with PET/CT, SPECT/CT and PET/MR imaging has been widely reviewed in the past [2–6]. In this review, we present some of the latest developments in the domain of SPECT/MR hybrid imaging, particularly focusing on development of multimodal nanoprobes.

The major advantage of imaging with nuclear medicine techniques such as PET and SPECT is that a measurable signal can be obtained with only picomolar concentrations of radiotracers without interfering with the process under investigation. It is thus possible to detect and monitor a variety of molecular processes using tracer quantities of radiolabeled probes. Positron emitting radionuclides include short-lived isotopes of fluorine (^{18}F), carbon (^{11}C), nitrogen (^{13}N) and oxygen (^{15}O), and longer-lived isotopes of copper (^{64}Cu), gallium (^{68}Ga) and zirconium (^{89}Zr), all of which allow radiolabeling of biological compounds of interest [7], while SPECT relies more frequently on the use of radiolabeled analogs with radioisotopes such as indium (^{111}In) and technetium (^{99m}Tc) and radioactive iodine isotopes ^{123}I and ^{131}I. The relatively longer half-lives of SPECT tracers compared with PET tracers make them more suitable for imaging with probes that have slow kinetics. Also, in the clinic, a range of SPECT radiopharmaceuticals can often be produced on-site with instant kits, thus eliminating the need for an expensive on-site cyclotron/radiochemistry production facility typically required for PET tracers. Moreover, SPECT tracers are advantageous over PET tracers because of lower cost and easy availability. Both SPECT and PET imaging techniques provide functional information about molecular processes with exquisite sensitivity. However, missing anatomic information and relatively poor spatial resolution often make it difficult to delineate the precise location of the abnormalities. In the past, this often led to nuclear

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medicine being described by the typographical pun 'unclear medicine'. CT and MR imaging are two anatomic imaging techniques that have been used to complement SPECT and PET, thus providing a hybrid imaging tool for assessment of abnormalities. The advances in hybrid imaging techniques over the last decade have resulted in 'unclear medicine' now being described as the 'new-clear medicine' [8].

MR imaging has advantages over CT due to lack of ionizing radiation, high soft-tissue contrast and sensitivity to tissue alterations. In addition, MR imaging is capable for molecular imaging with the use of contrast agents such as those based on gadolinium and iron oxide, thus offering an excellent capability of examining soft tissues. MR imaging is several orders of magnitude less sensitive (millimolar vs picomolar) than PET or SPECT, due to the low quantum energy involved, and therefore requires signal amplification. However, MR imaging has higher spatial resolution than nuclear imaging methods (micrometer vs millimeter) (Figure 1) [9,10]. MR image contrast depends on fundamental parameters such as spin-lattice relaxation time (T_1) and spin–spin relaxation time (T_2), which are a function of the local chemical structure of the molecules being imaged. These parameters can be exploited to reflect the molecular content of the tissue being imaged, and therefore making MR particularly useful for molecular imaging [11]. More recent developments in the field of MR imaging have facilitated imaging of tissues, cells and molecules and include dynamic contrast-enhanced MR imaging, diffusion weighted imaging and (MR) spectroscopy. For example, the measurement of transendothelial transport of the MR contrast agent comprising gadolinium conjugated to albumin by dynamic contrast-enhanced MR imaging has been used as a marker for angiogenesis in tumors [12]. Cells labeled with MR contrast agents such as superparamagnetic iron oxide nanoparticles (SPIONs) have been developed [13] that can potentially be used for efficient *in vivo* tracking of stem cells, progenitor cells, or cell lines expressing transgenes, even at the single-cell level [14].

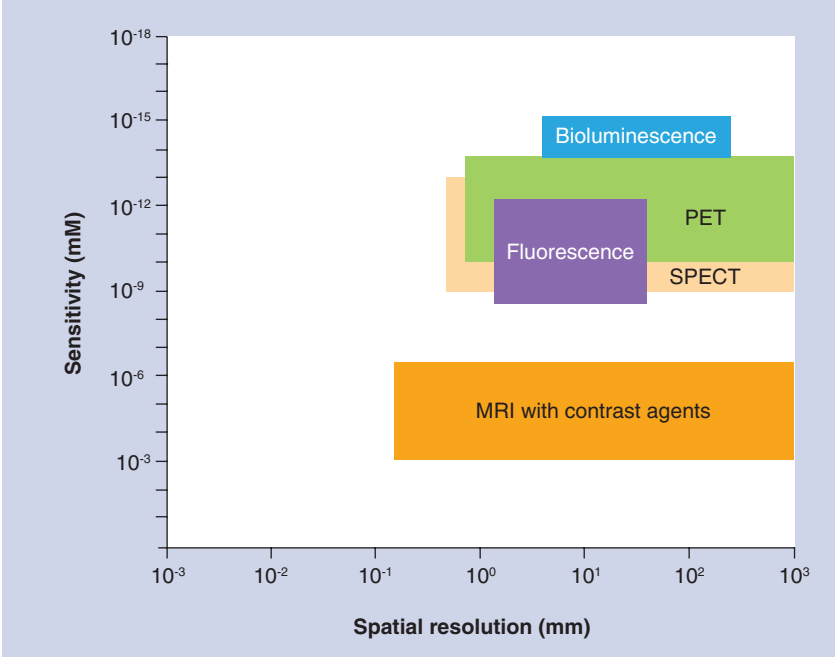

Figure 1. A comparison of imaging sensitivities and resolution of commonly used molecular imaging modalities.
SPECT: Single-photon emission computed tomography.

Hybrid imaging with SPECT and MR not only provides anatomical references in an image but also acts synergistically to combine the high spatial resolution associated with MR with the high sensitivity of SPECT [15]. Simultaneous SPECT/MR imaging can allow dynamic imaging with radiotracers and MR imaging contrast agents, which can facilitate noninvasive monitoring of treatment as well as study of pharmacokinetics and metabolism of drugs. Hybrid molecular imaging can also facilitate evaluation of specific parameters such as molecular abnormalities [16], growth kinetics [17], angiogenesis [18] and tumor cell markers [19], and provides potential for earlier detection, characterization of disease and evaluation of treatment. Currently, the assessment of tumor response to therapy is primarily based on unidimensional and bidimensional measurements of tumor size according to the Response Evaluation Criteria in Solid Tumors (RECIST) classification [20]. Imaging with hybrid techniques such as SPECT/MR imaging can offer the additional major advantage of assessing therapeutic effectiveness at a molecular level, long before phenotypic changes occur. Furthermore, simultaneous dual modality imaging with MR and SPECT can reduce the overall scan time, avoid multiple anesthesia sessions and the errors associated with co-registration.

Despite the promise, combining MR imaging with nuclear medicine instrumentation has proven to be challenging, due to the technological challenges in operating a PET or SPECT scanner within an MR instrument since the radiofrequency radiating components of these scanners can potentially interfere with the MR imaging system. Moreover, PET and SPECT detectors based on scintillators coupled to photomultiplier

tubes and associated electronics are commonly highly sensitive to magnetic fields [21]. Due to the recent development of γ ray detectors based on avalanche photodiodes as well as the availability of fast scintillation materials such as lutetium oxyorthosilicate, it has become possible to incorporate fully magnetic-field-insensitive high-performance PET detectors within PET/MR imaging scanners [22]. An initial study with such a system has demonstrated the feasibility of structural, functional and molecular imaging of brain tumors in patients [23]. Moreover, silicon photomultiplier detectors are also being explored for use in PET/MR hybrid systems [24]. The recent approval of the world's first PET/MR imaging scanner (Siemens Healthcare, Erlangen, Germany) by the US FDA has paved the way for entry of simultaneous PET/MR imaging into the clinic [25]. Subsequently, another PET/MR hybrid imaging system (Philips Healthcare, MA, USA), capable of sequential PET and MR imaging acquisitions followed by automated co-registration of images, has gained FDA approval [101]. Using similar design principles as Siemens PET/MR imaging, small-animal SPECT/MR imaging scanners using semiconductor detectors (cadmium–zinc–telluride [CZT]) were shown to be insensitive to magnetic fields up to 7 T [26]. Using these CZT detectors, only recently a new prototype imaging system (Gamma Medica [NY, USA]) that combines the molecular imaging capability of SPECT with MR imaging has been developed [27]. We envision that with these developments SPECT/MR hybrid imaging will soon gain utility in the clinical setting.

Design considerations for a SPECT/MR hybrid imaging nanoprobe

A hybrid imaging nanoprobe for SPECT/MR imaging comprises an MR imaging contrast agent combined with a SPECT radioisotope into a single nano-sized construct. In this form, the nanoprobe exhibits a 'single pharmacological behavior', while combining advantages, such as the high sensitivity of SPECT with high resolution of MR imaging, thus providing a single image that combines information from both modalities to reflect the same biological process [28]. However, *in vivo* molecular imaging with hybrid probes is challenging as several criteria for development of molecular imaging bioprobes must be met. An imaging probe must clear from the blood pool as well as other irrelevant sites within the time frame compatible with the half-life of the radionuclide. The probes must be biocompatible and have an ability to overcome biological delivery barriers (vascular, interstitial, cell membrane) to bind specific receptors with high affinity [29].

SPECT radionuclides

Various selection criteria such as the physical half-life, γ energy range (100–300 keV) and stability of the daughter radionuclide must be considered when choosing a radionuclide for SPECT imaging [30]. ^{99m}Tc with a physical half-life of 6 h is widely recognized as the radionuclide of choice for most nuclear medicine studies, preventing excessive radiation dosage to the patient [31] and allowing for optimal imaging due to its major γ line at 140 keV [30]. Other advantages of ^{99m}Tc include easy availability and the fact that its decay product has a very long half-life, which does not deliver any additional dose to the patients and also does not obscure the image. In addition to ^{99m}Tc, ^{111}In is another isotope commonly used in nuclear medicine. ^{111}In has a half-life of 68 h and major γ energy lines at 171 and 245 keV, with no β-emission. ^{111}In is especially suitable for imaging with probes with slower kinetics, due to its sufficiently long half-life that allows imaging after a time period during which probes have cleared from the circulation, thus reducing the background. Moreover, ^{111}In provides more sensitivity (less scatter and internal shielding) than other longer half-life radionuclides (^{123}I and ^{125}I). ^{123}I and ^{125}I with half-lives of 13.2 h and 60.1 days and energies of 159 and 35.5 keV, respectively, can also be used for SPECT imaging; however, their clinical applications are limited due to the high cost, poor availability and relatively low *in vivo* stability [30,32]. Less used nowadays, but also useful as SPECT radionuclides, are thallium (^{201}TI) [33,34] and holmium (^{166}Ho) [35]. The useful lanthanide isotopes such as ytterbium (^{169}Yb), samarium (^{153}Sm), dysprosium (^{165}Dy) and ^{166}Ho, all also emit β-electrons and are thus mostly used for investigational brachytherapy applications where imaging of their γ component is additionally useful [36,37]. Similarly, the congener of technetium, rhenium in the form of both ^{188}Re and ^{186}Re, are also useful γ radioisotopes with a therapeutic β-component [38,39].

MR imaging contrast agents

There are essentially two types of agents used in MR imaging classified as T_1 and T_2 contrast agents. Although all contrast agents produce both T_1 (longitudinal) and T_2 (transverse) relaxation, the type of relaxation produced to a greater extent defines how we classify them. The ability of MR

contrast agents to produce relaxation, which is a multitude of processes by which nuclear magnetization prepared in a nonequilibrium state returns to the equilibrium distribution, is expressed in terms of relaxivity. The relaxivity value is a parameter that describes the enhancement of the water proton relaxation rate ($1/T_1$ or $1/T_2$) in solutions containing a 1 mM concentration of the magnetic solute. Due to low concentration of biological targets, contrast agents with high sensitivity are required for molecular MR imaging. This is a challenge, as most MR contrast agents are not very sensitive. Considerable research has thus focused on the design of metal complexes with high relaxivity values.

Hybrid SPECT/MR imaging T_2 agents

Transverse relaxation of contrast agents is characterized by change in relaxation rate of protons in the surrounding water molecules resulting in T_2 shortening, which produces a hypointense (dark) contrast [40]. SPIONs are able to efficiently shorten T_2, and particularly T_2* (contribution of both transverse relaxation and local magnetic field nonuniformities) of water protons and therefore have been utilized as MR imaging probes for molecular imaging experiments [41]. Recently, it has been shown that antibodies conjugated with SPIONs can noninvasively image HER-2-expressing cells or tissues both *in vitro* and *in vivo* by MR imaging [42].

In another study, ^{64}Cu-labeled SPION probes produced strong MR and PET signals and were stable in mouse serum for 24 h at 37°C making them attractive for the development of dual modality PET/MR imaging bioprobes [43]. Substituting ^{64}Cu (half-life of 12.7 h) with ^{67}Cu (half-life of 2.58 days) would easily result in a longer half-life nanoprobe capable of being imaged with SPECT and MR imaging. The good sensitivity and the ability to be efficiently taken up by cells, either by passive or active means, are specific advantages associated with SPIONs for MR imaging. However, the uptake of SPIONs by phagocytic cells make it necessary to use specific target approaches for their delivery into tumors or other cells of interest (e.g., cardiac myocytes, endothelial cells) [44–46]. In addition to iron oxide-based nanoparticles, other nanosystems utilizing bimetallic cores (e.g., iron cobalt [FeCo] or manganese ferrite [$MnFe_2O_4$]) have shown promise for developing T_2 contrast agents with higher relaxivity [47–49]. Such nanoparticles are still at the preclinical stage of development and their clinical translation will require a careful assessment of their toxicity.

SPIONs typically consist of a crystal core of magnetite (Fe_3O_4) and/or maghemite (Fe_2O_3) coated with a suitable material with an overall diameter between approximately 60 and 250 nm [50,51]. Size characteristics play an important role in determining the pharmacological and magnetic properties of SPIONs. Particles of monodisperse crystal cores and a high degree of crystallinity are crucial to achieve the good relaxivity characteristics required to obtain imaging probes with high sensitivity. SPIONs in the size range of 20–50 nm are typically characterized by a higher r_2 (relaxivity due to transverse relaxation) value. For targeting to the intracellular space within a cell, a size of 5–10 nm is suggested, whereas for circulating nanoparticles in the bloodstream or nanoparticles targeting vascular and extravascular (tumors) structures, larger particles with a size of 30–100 nm seem to be useful [52].

For *in vivo* applications, the particles need to be coated with a suitable biocompatible material to prevent aggregation and sedimentation and to improve stability. In addition, the surface coating is also critical for defining the pharmacokinetic behavior of the particle, since the nanoparticles, when introduced into the body, are recognized as foreign and undergo phagocytic uptake into the macrophage-rich organs such as the spleen and liver. Coatings such as polyethylene glycol (PEG) reduce the ability of serum proteins to recognize the particle and thus prolong circulation times in the blood pool [43]. In addition to PEG, materials such as dextran, starch and silica are frequently utilized as coating materials for SPIONs [53–55]. SPIONs (Feridex IV®, Advanced Magnetics, MA, USA) have been in clinical use for many years, primarily for the detection of liver and lymph node lesions [56,57]; however, their manufacturing was ceased in 2008.

For hybrid imaging, Bligh *et al.* first tested the concept of combining radioactive imaging and MR imaging procedures [58]. They prepared hybrid imaging probes by conjugating Fe_3O_4 particles of 0.1–0.5 µm in diameter with either ^{99m}Tc or ^{111}In. Biodistribution and γ-camera imaging studies confirmed the main targets to be the liver and lung. Further exploring this concept, Lewin *et al.* developed a hybrid imaging probe capable of detection by the three modalities, MR imaging, fluorescence imaging and SPECT, by conjugating crosslinked iron oxide (CLIO) nanoparticles with an HIV-Tat peptide, followed by labeling with fluorescence (fluorescein isothiocyanate) and the radioisotope ^{111}In (**Figure 2**) [59]. The CLIO nanoparticles with a hydrodynamic diameter of 45 nm consisted of

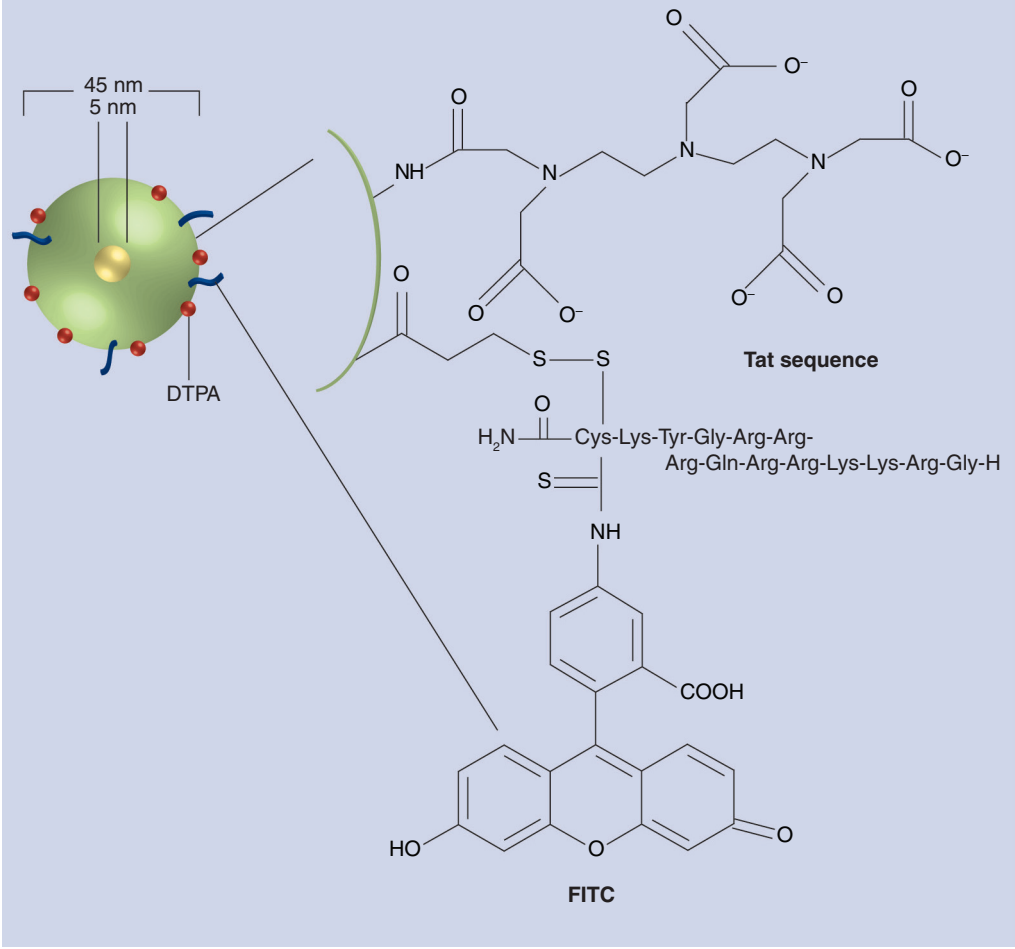

Figure 2. Triple-label crosslinked iron oxide-Tat. The developed magnetic particles consist of a central superparamagnetic iron oxide core (yellow), sterically shielded by crosslinked dextran (green). An average of four fluorescein isothiocyanate-derivatized Tat peptides (blue) was attached to the aminated dextran. The dextran surface was also modified with the chelator diethylenetriamine pentaacetic acid (red) for isotope labeling.
DTPA: Diethylenetriamine pentaacetic acid; FITC: Fluorescein isothiocyanate.
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a monocrystalline, superparamagnetic iron oxide core stabilized by crosslinked aminated dextran. The authors used cysteine and lysine residues at the C-terminus of the HIV-Tat peptide for bioconjugation to the magnetic particle and for fluorescein attachment, respectively. Radiolabeling with ^{111}In was performed by conjugating a diethylenetriamine pentaacetic acid (DTPA) chelator to CLIO nanoparticles via the dextran coating. The authors further demonstrated that triple-labeled CLIO–Tat probes (hybrid imaging probes) tagged to hematopoietic (CD34$^+$) and neural progenitor cells (C17.2) could be used to track the distribution and differentiation of progenitor and stem cells by high-resolution *in vivo* imaging techniques.

In a recent study, Torres *et al.* employed a novel strategy for the development of hybrid SPECT/MR imaging agents [28]. The authors developed a new dual-modality imaging agent by conjugating radiolabeled bisphosphonates (BPs) to SPIONs (5 nm Fe_3O_4 core and overall 106 ± 60 nm hydrodynamic radius including the dextran shell; Figure 3). Taking advantage of the high binding affinity of BPs to iron; the authors were able to directly label the iron oxide core of Endorem/Feridex®, a commercially available liver MR imaging contrast agent, with ^{99m}Tc-dipicolylamine-alendronate, a BP SPECT agent for bone imaging. The bimodal imaging capabilities of these nanoparticles were confirmed *in vivo* using MR imaging and nanoSPECT CT imaging, showing that ^{99m}Tc and Endorem colocalize in the liver and spleen (Figure 4). This design could potentially be used in the future to develop SPECT/MR hybrid nanoprobes utilizing BP as a platform, for incorporation of SPECT and MR motifs, as well as for conjugation of targeting ligands.

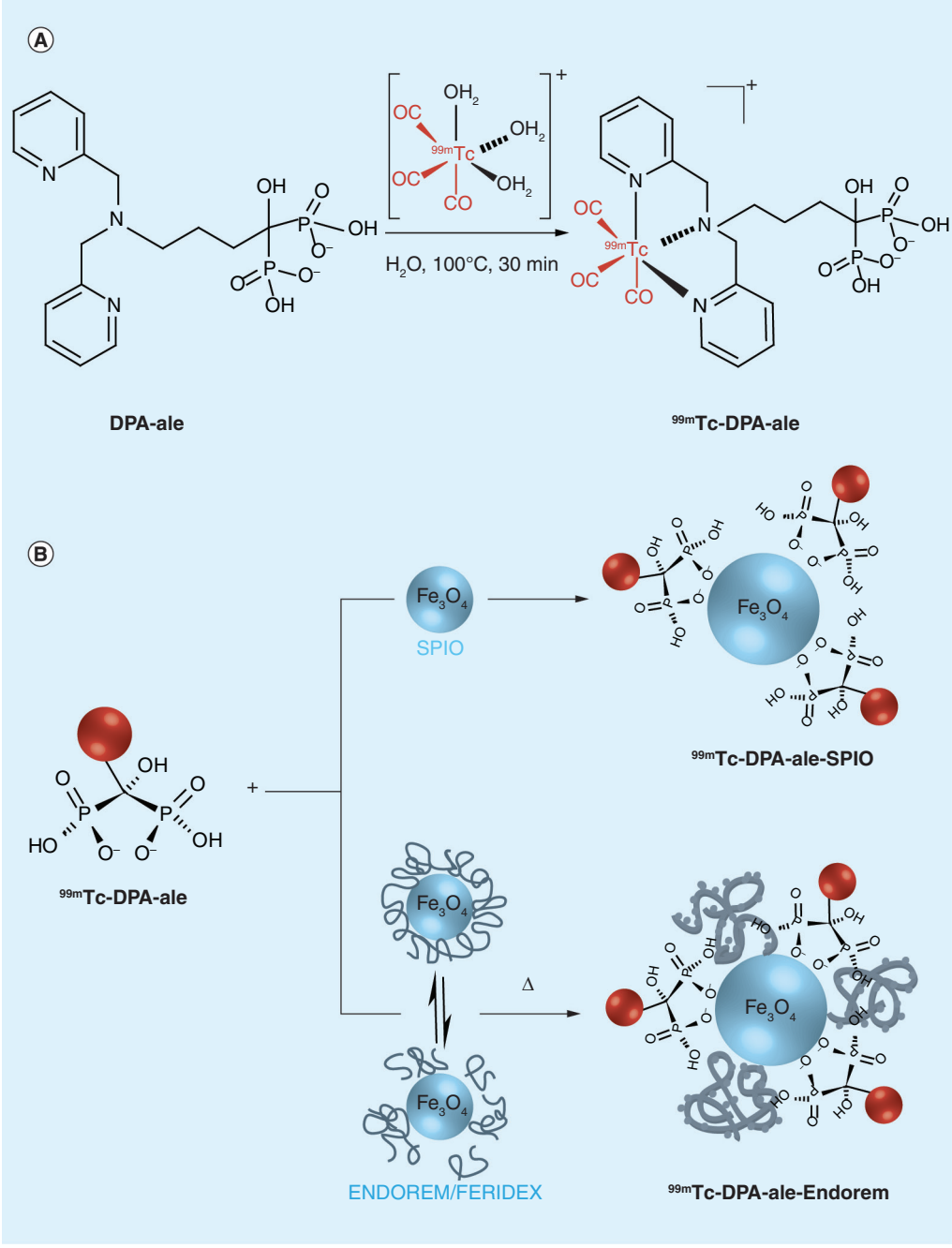

Figure 3. Radiolabeled bisphosphonate-superparamagnetic iron oxide hybrid imaging agent. (A) Synthesis of ^{99m}Tc-labeled dipicolylamine alendronate (^{99m}Tc-dipicolylamine-ale). **(B)** Schematic representation of the synthesis of radiolabeled superparamagnetic iron oxide nanoparticles ^{99m}Tc-dipicolylamine-ale-SPIO (top) and ^{99m}Tc-dipicolylamine-ale-Endorem (bottom). DPA: Dipicolylamine alendronate; SPIO: Superparamagnetic iron oxide.
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A hybrid SPECT/MR imaging agent for specifically targeting hepatocytes *in vivo* for evaluation of the hepatocytic function and the monitoring of disease progression was developed by Lee *et al.* [60]. The authors prepared a hepatocyte-specific dual modality nanoprobe by functionalizing 12 nm SPIONs with dopamine, which was then conjugated to lactobionic acid bearing a high affinity for the asialoglycoprotein receptor on the hepatocyte surface. Imaging hepatocytes with this hybrid nanoprobe could potentially be used in the clinic to provide critical diagnostic and prognostic information related to liver cancers and other liver diseases. Radiolabeling with ^{99m}Tc was performed by conjugating DTPA to unreacted functional groups of dopamine. The

authors confirmed the hepatocyte-specific liver accumulation of the ^{99m}Tc-labeled lactobionic acid SPIONs by microSPECT/CT and MR imaging, demonstrating the usefulness of the hybrid probes for evaluation of the hepatocytic function. In another study, Kaufner *et al.* developed SPIONs of Fe_2O_3 coated with poly(ethylene oxide)-block-poly(glutamic acid) (PEO–PGA) at a hydrodynamic diameter of 60 nm [61]. Using MR imaging and ^{111}In measurements, the authors demonstrated that PEO–PGA-coated Fe_2O_3 nanoparticles had a biodistribution in the liver comparable to carboxydextran coated SPIONs (Resovist®, Bayer HealthCare Pharmaceuticals, Berlin, Germany) used as the reference nanoscale MR imaging contrast medium.

Nanoparticles coupled with tumor-specific targeting ligands such as antibodies can be used to image tumors and detect peripheral metastases [62,63]. A hybrid imaging nanoprobe composed of Fe_3O_4 and conjugated to a radioisotope ^{125}I and antigastric cancer monoclonal antibody 3H11 (mAb 3H11), was designed by Liu *et al.* [64]. The authors prepared the ^{125}I-labeled antibody mAb 3H11 and then conjugated it to the PEG-coated Fe_3O_4. An alternative method of conjugating mAb 3H11 to the Fe_3O_4 nanocrystals through α,ω-dicarboxyl-terminated PEG chemically bonded on the particle surface, followed by labeling with ^{125}I by the Iodogen (Pierce Biotechnology, IL, USA) method, was also successful but required a purification step. A series of *in vivo* experiments demonstrated the ability of both probes to detect xenograft tumors in nude mice by MR imaging and γ-imaging techniques (FIGURE 5).

For the imaging of recurrent rectal carcinoma, Otsuji *et al.* radiolabeled the monoclonal antibody Mab A7 against human colonic carcinoma with ^{125}I using the chloramine-T method [65]. The radio-iodinated antibody was then conjugated to Fe_3O_4 nanoparticles ferromagnetic lignosite (mean diameter of 10 nm) with a conjugation efficiency of 40%. Based on *in vivo* biodistribution and MR imaging results, the authors concluded that ^{125}I-labeled A7-ferromagnetic lignosite to colorectal carcinoma may be a potentially useful imaging agent of recurrent rectal carcinoma. The incorporation of ^{125}I into the nano-construct also makes the probes capable of hybrid imaging with SPECT/MR.

Our own research work has focused on design of dual-modality SPECT/MR imaging nanoprobes, with the aim of improving imaging in a few of the most challenging cancers such as mesotheliomas, pancreatic and ovarian cancers.

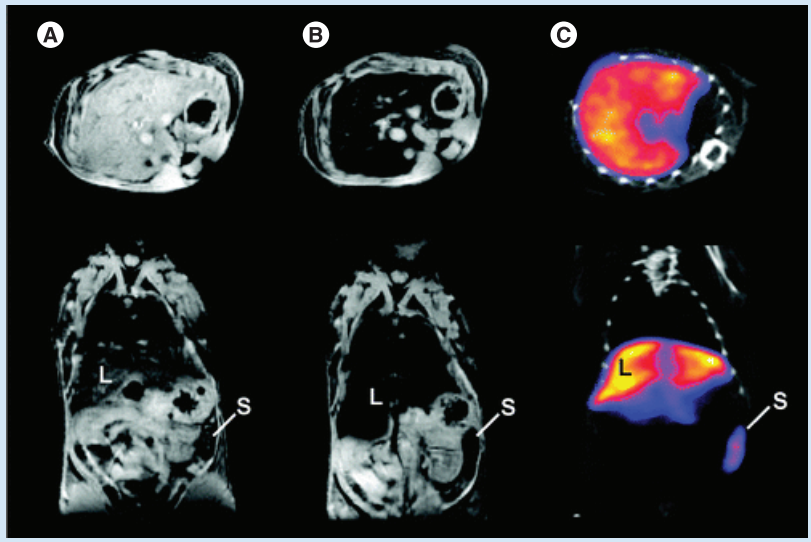

Figure 4. Dual-modality *in vivo* studies. Short-axis view (top) and coronal view (bottom) images: **(A)** T_2*-weighted MR images before injection of ^{99m}Tc-dipicolylamine-ale-Endorem, **(B)** T_2*-weighted magnetic resonance image 15 min postinjection and **(C)** nano-single-photon emission computed tomography/computed tomography image of the same animal in a similar view 45 min postinjection. Contrast in the L and S changes after injection is due to accumulation of ^{99m}Tc-dipicolylamine-ale-Endorem, in agreement with the nano-single-photon emission computed tomography/computed tomography image which shows almost exclusively liver and spleen accumulation of radioactivity. Magnetic resonance images were acquired with an Echo time of 2 ms.
L: Liver; S: Spleen.
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These cancers are characterized by low patient survival rates primarily due to late diagnosis. More established diagnostic methods have limited value in diagnosis of these cancers [66–69]. An imaging technique based on detection of molecular markers expressed in these cancers has the potential in identifying tumors early. This approach is also invaluable in tumor-node-metastasis staging by assisting oncologic surgeons to identify the resectability of the tumors prior to surgery [70]. Better imaging of the tumor sites will also permit more accurate, targeted drug or radiation delivery for subsequent management of the disease. Recently, we developed a tumor-specific molecular imaging bioprobe by conjugating radiolabeled antibodies to carboxymethyl dextran-coated SPIONs via carbodiimide coupling (FIGURE 6) [71]. The mAb MB that specifically targets a cell surface antigen known as 'mesothelin' overexpressed mainly by mesotheliomas, pancreatic and ovarian cancers, was radiolabeled with ^{111}In [72–74]. In tumor-bearing mice ^{111}In-labeled mAbMB antibody (^{111}In-mAbMB) showed specific uptake into mesothelin-expressing A431K5 tumors, when tested using SPECT imaging (FIGURE 7A) [75]. We then conjugated ^{111}In-mAbMB to SPIONs with

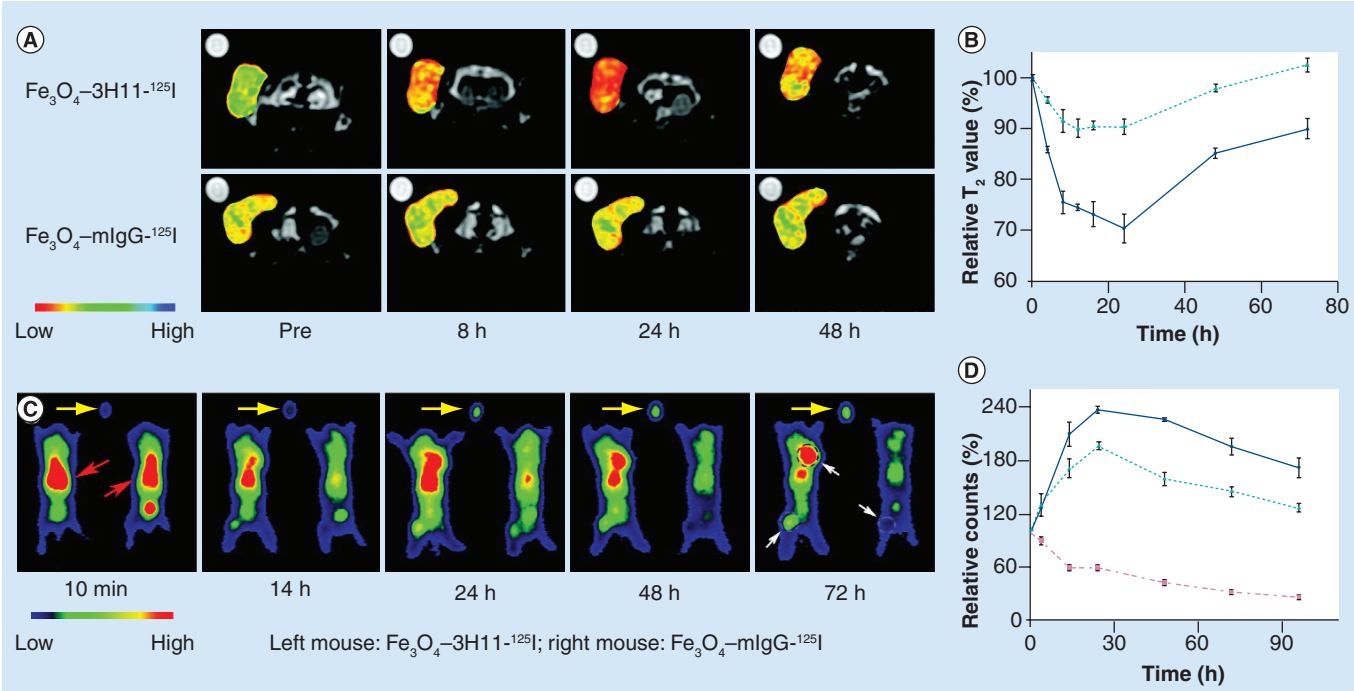

Figure 5. *In vivo* studies with single-photon emission computed tomography and magnetic resonance imaging. (A) T_2-weighted MR images of tumor-bearing nude mice acquired before and at different time points after intravenous injections of Fe_3O_4–3H11–^{125}I and Fe_3O_4–mIgG-^{125}I (superparamagnetic iron oxide nanoparticles with nonspecific antibody as control), respectively. **(B)** Variations of T_2 values of tumors after the injection of Fe_3O_4–3H11–^{125}I (solid line) and Fe_3O_4–mIgG-^{125}I (dotted line), respectively. **(C)** γ-images of tumor-bearing nude mice captured at different times postinjection. The Fe_3O_4-based probes quickly distributed in liver (red arrows) within the first 10 min postinjection and eventually at 72 h, the molecular probes distributed in both tumor and liver (white arrows). **(D)** The normalized γ-counts extracted after injection of Fe_3O_4–3H11–^{125}I from the whole body (dotted line), the tumor in the upper flank region (solid line), and the tumor in the proximal thigh region (dashed line) of the mouse at the left-hand side in each image, by using 1% injection dose (yellow arrows in **[C]**) as internal reference. Both magnetic resonance and γ-images are color-coded to more clearly show the tumors.
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an average diameter of 70 nm. The resulting ^{111}In-mAbMB-SPIONs showed specific uptake into mesothelin-expressing A431K5 tumors, retained their high relaxivity (469.57 $mM^{-1}s^{-1}$) and produced a change in the MR signal when tested in MR imaging experiments. The radioactivity associated with the hybrid nanoprobes allowed quantitative determination of their biodistribution and *in vivo* characteristics. MR imaging studies in mice demonstrated a change in MR signal in mesothelin-expressing tumors, which correlated well with biodistribution findings (Figure 7B). The high relaxivity and specific targeting of the bioprobes resulted in an enhanced MR contrast in mesothelin-expressing tumors. The intra-tumor distribution of the hybrid nanoprobe investigated using autoradiography correlated well with the regions of signal hyposensitivity observed in the tumors with MR imaging (Figure 7C). This novel hybrid molecular imaging probe would enable *in vivo* detection and characterization of mesothelin-expressing tumors based on both functional and anatomical information derived from MR imaging and SPECT. Successful clinical translation of such an agent would provide a powerful diagnostic tool for early diagnosis and monitoring of mesothelin-expressing cancers.

Hybrid SPECT/MR imaging T_1 agents

The paramagnetic complexes of metal ions with symmetric electronic ground states have an ability to catalyze the relaxation process of surrounding water protons, commonly expressed in terms of the longitudinal relaxivity (r_1), which results in T_1 contrast. Paramagnetic metals such as gadolinium Gd(III) and manganese Mn(II) therefore produce hyperintensity (bright contrast) in T_1-weighted MR images as a consequence of a predominantly longitudinal relaxation process.

Lijowski *et al.* designed and characterized dual-modality $\alpha_v\beta_3$-targeted nanoparticles that afford sensitive nuclear detection in conjunction with high-resolution MR for characterization of tumor angiogenesis [76]. The authors reported preparation of $\alpha_v\beta_3$-targeted perfluorooctylbromide

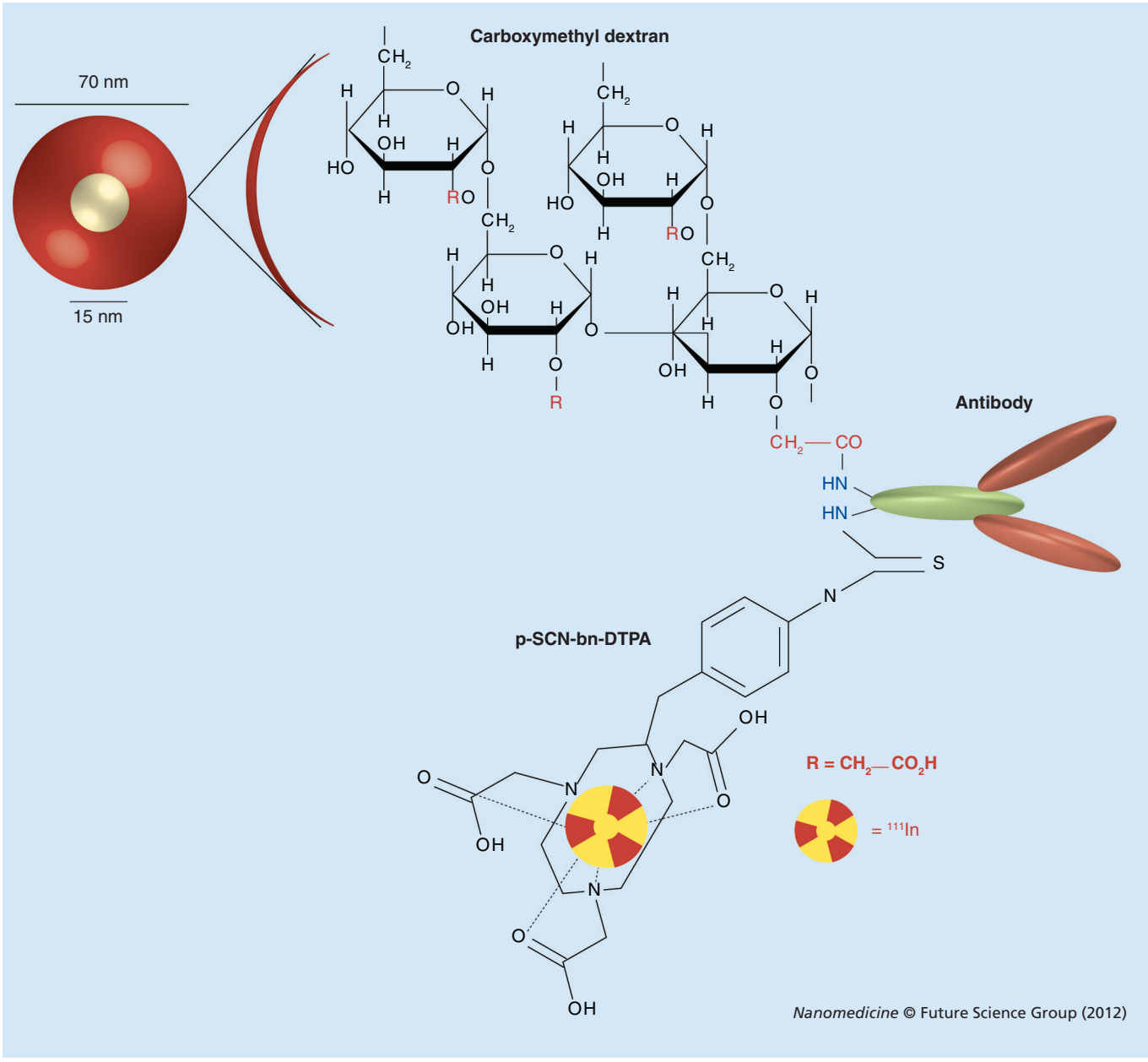

Figure 6. Schematic diagram of the hybrid single-photon emission computed tomography/magnetic resonance imaging nanoprobes (^{111}In-mAbMB-superparamagnetic iron oxide nanoparticles). The hybrid probes consist of a magnetic resonance motif in the form of superparamagnetic iron oxide nanoparticles coated with carboxymethyl dextran. The single-photon emission computed tomography motif used is diethylenetriamine pentaacetic acid-bound ^{111}In conjugated to the antibody.

nanoparticles, followed by ^{99m}Tc labeling of nanoparticles via a ^{99m}Tc-tricarbonyl precursor. Furthermore, they also incorporated gadolinium (T_1 contrast agent) into the outer phospholipid layer of the nanoparticles, thus developing a single nanoparticle construct capable of being imaged by both SPECT and MR imaging. In a VX2 rabbit tumor model using intravenous injections through the marginal ear vein, the authors first demonstrated an enhanced radiocontrast signal in the tumors at titrated doses from 11 to 44 MBq/kg by dynamic imaging. The tumor to muscle ratios were significantly higher than the nontargeted and competitively inhibited controls. Furthermore, by using clinical SPECT/CT imaging techniques, it was clearly possible to demonstrate the effectiveness of $\alpha_v\beta_3$-targeted ^{99m}Tc gadolinium nanoparticles for tumor neovascular imaging. The SPECT CT scan was followed by administration of an additional dose of the $\alpha_v\beta_3$-targeted gadolinium nanoparticles for MR imaging, which 2 h later highlighted the angiogenic regions in the tumor mass (FIGURE 8). The authors concluded that high-resolution MR molecular

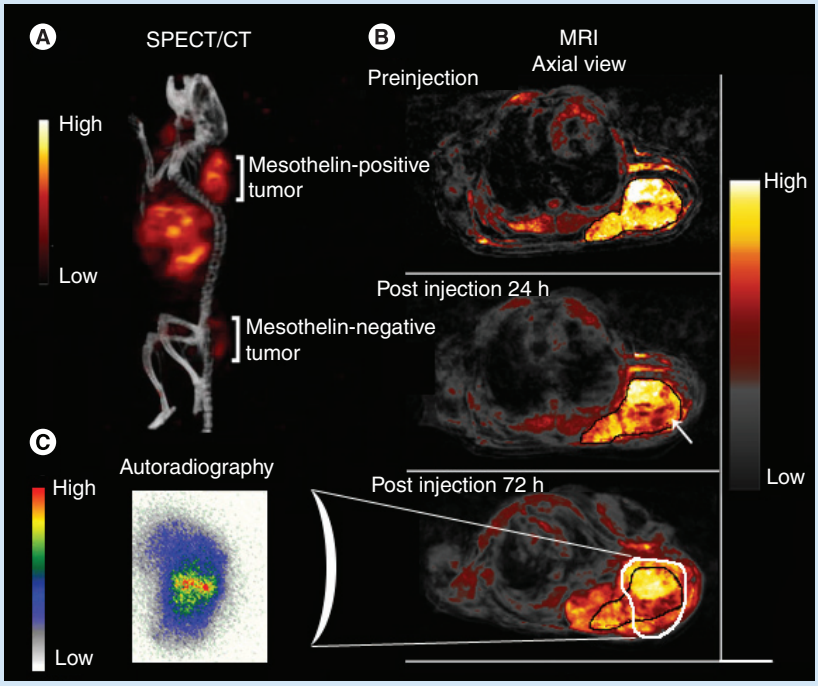

Figure 7. Single-photon emission and magnetic resonance imaging with hybrid nanoprobes. The SPECT/CT image **(A)** shows the preferential uptake of ^{111}In-mAbMB into A431K5 tumors (mesothelin-positive) compared with A431 (mesothelin-negative) tumors in SCID mice. **(B)** shows the T_2-weighted axial MR images at preinjection and 24 and 72 h postinjection time points for A431K5 xenograft tumors of SCID mice, injected intravenously with 15 mg/kg bodyweight iron equivalent of ^{111}In-mAbMB-superparamagnetic iron oxide nanoparticles (hybrid nanoprobes). **(C)** represents the autoradiographic image of a 20 µm tumor section obtained from the corresponding tumor.
CT: Computed tomography; SPECT: Single-photon emission computed tomography.
(A) Adapted from [71,75], © (2011), with permission from Elsevier.

imaging, combined with SPECT using $\alpha_v\beta_3$-targeted ^{99m}Tc-labeled paramagnetic nanoparticles could sensitively localize small tumors and also provided high-resolution MR characterization of the tumor neovasculature. Translated to the clinic, this technique could help identify the subset of patients with occult tumors who have a better chance of cure with a specific angiogenesis therapy.

Another T_1 contrast, based hybrid probe was reported by Kryza *et al.* [77]. They prepared gadolinium oxide nanoparticles with a mean hydrodynamic diameter of approximately 4 nm, embedded in a polysiloxane shell, functionalized with the fluorescent dye Cy5 for optical imaging and DTPA for conjugation with ^{111}In for SPECT imaging. The biodistribution and pharmacokinetics of the Gado-6Si-Np-^{111}In nanoparticles were evaluated in rats for up to 18 days. The findings demonstrated the ability of the nanoprobes to circulate in the blood pool and avoid uptake by the reticuloendothelial system into the liver and spleen. The clearance of the nanoprobes was through the kidneys with 95% of the nanoprobes being eliminated within 18 days. Both SPECT and MR imaging confirmed the pharmacokinetic behavior.

In another study, Zielhuis *et al.* designed liposomal nanodevices with a mean size of 130 nm capable of hybrid imaging as well as radiotherapy [78]. The liposomes were prepared by a thin film hydration technique and radiolabeled with ^{166}Ho (both a β- and γ-emitter and also highly paramagnetic) or ^{99m}Tc with high radiolabeling efficiency and radiochemical stability, by use of the amphiphilic molecule DTPA-lipid, which was incorporated into the liposomal bilayer [79]. The paramagnetic element gadolinium was co-loaded into the bilayer of the liposomes at concentrations up to 20 mol%. The authors further studied the effect of formulation variables on relaxivity values of the liposomal nanodevices. This study demonstrates the feasibility and potential of liposomal nanodevices as a matrix for the development of hybrid theranostic nanoprobes for SPECT/MR imaging and radiotherapy.

The uptake of low-molecular-weight contrast agents used in dynamic contrast-enhanced imaging provides data that can be interpreted as a combination of both tumor perfusion and extravasation [80,81]. A high-molecular-weight contrast agent that could be expected to remain intravascular even within leaky tumor vasculature would yield data that could be interpreted more specifically to represent the physiological parameters of permeability and perfusion. Recently, we reported a macromolecular platform as a probe for multimodal imaging where a unimolecular, nano-sized, long-circulating, biocompatible and nontoxic macromolecule (high-molecular-weight hyperbranched polyglycerols [HPG]) was equipped with MR, radioactive and fluorescence markers [82]. The HPG was modified with probes suitable for SPECT and MR imaging. The ligand (chelator) used (DOTA) was suitable to bind In^{3+} and Gd^{3+} metal ions. The addition of DOTA to HPG altered the molecule only minimally (e.g., in terms of changes to MW, chemistry, water solubility and charge), which resulted in no observable changes to *in vitro* behavior and biodistribution when compared to the original HPG molecule. Alexa 647 was also tagged to the same system for fluorescent imaging, resulting in a trimodal imaging agent. The fluorescent tag was to confirm the tumor distribution and extravasation by *ex vivo* imaging. The relaxivity of the HPG-Gd system was 1075 $mM^{-1}s^{-1}$, much larger than the commercially available gadolinium-labeled

albumin (Galbumin™ [BioPal, MA, USA], 82.85 $mM^{-1}s^{-1}$) as well as Gadovist (Bayer HealthCare Pharmaceuticals; 3.58 $mM^{-1}s^{-1}$), the clinically used small molecule contrast agent. An additional advantage for HPG-Gd was that it did not become highly viscous at high concentrations, a problem observed for Galbumin solutions ≥0.3 mM, making injections of that compound difficult. The fluorescent *ex vivo* imaging confirmed a heterogeneous distribution of HPG-Gd seen in the MR images and reflected the inhomogeneities of newly grown tumors in terms of not fully developed leaky vasculature and hypoxic tumor centers. Although there were no SPECT images taken in this study, the radioactive biodistribution showed clearly increased tumor uptake over time. At the 72 h time point postinjection, the activity in the tumor was 6% of the total activity injected, 2.4-times more than the activity measured there at the 24-h time point. Since HPG are long circulating macromolecules that are not processed rapidly by the reticuloendothelial system, such a high tumor uptake was likely related to the enhanced permeation and retention effect [83]. Overall, the three modalities gave similar and complementary information beneficial to gain quantitative data about the *in vivo* role of tumor vasculature.

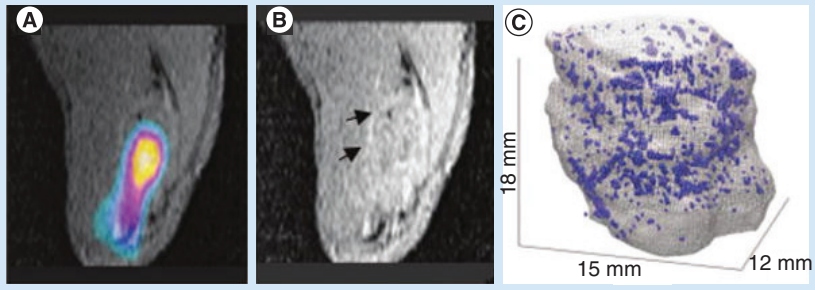

Figure 8. Dual modality imaging of tumor angiogenic regions. (A) Single-photon emission computed tomography-computed tomography image of nascent VX2 adenocarcinoma in rabbit popliteal fossa after injection of 11 MBq/kg of $\alpha_v\beta_3$-targeted ^{99m}Tc-gadolinium nanoparticles given via ear vein superimposed on the magnetic resonance image obtained at 2 h. **(B)** T1w, fat-suppressed, gradient echo image of the same section 2 h after administration of remaining MR dosage, revealing the asymmetric, peripheral neovascular signal enhancement previously reported for this model. **(C)** magnetic resonance 3D neovascular map of the same section illustrating heterogeneity of neovasculature in tumor periphery.
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Future perspective

Hybrid imaging can provide early disease detection through improved imaging and screening protocols, as well as through patient-specific treatment selection based on understanding of the biochemical changes and the effect of therapeutic drugs *in vivo* with enhanced resolution and sensitivity. While there is lots of activity in the hybrid PET/MR imaging area (e.g., the first commercial PET/MR imaging hybrid scanner was unveiled by Siemens Healthcare in 2010; PET/MR imaging systems were then approved for use in human patients in USA and Europe in 2011), only one hybrid SPECT/MR imaging system is currently under development for preclinical use [15]. Gamma Medica is developing this system using CZT detectors, which allows them to accumulate SPECT images in the high magnetic fields necessary for MR imaging (FIGURE 9) [27]. The system is currently being evaluated in detail with experimental phantoms and small animal imaging studies. The same

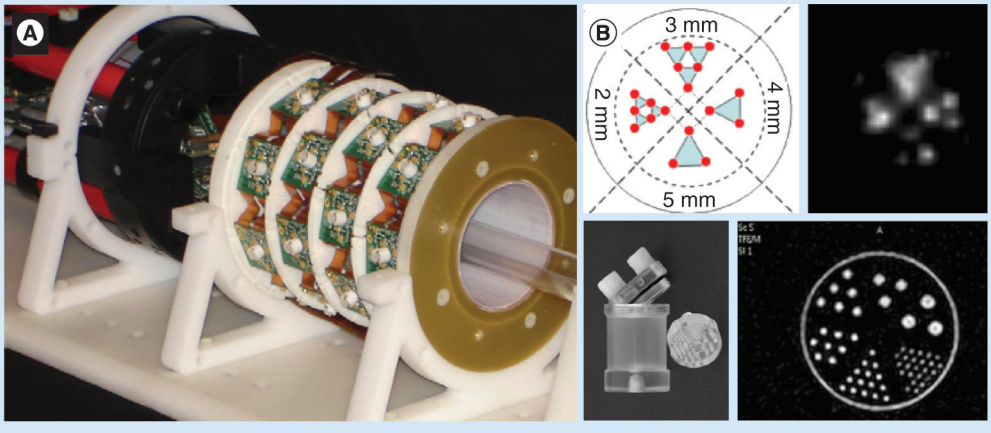

Figure 9. Development of a hybrid single-photon emission computed tomography-magnetic resonance scanner. (A) First magnetic resonance-compatible single-photon emission computed tomography camera from Gamma Medica (CA, USA). **(B)** Top: drawing of the distribution of γ-radiation point sources (left), and slices of the reconstructed image from the radiation phantom (right). Bottom: photograph (left) and axial MR image (right) of an ultra-microphantom. The smallest white circles have a 0.75 mm diameter.
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technology developed in this project may in the future be used for development of a clinical (human) SPECT/MR imaging system [27]. While it is understood that there are many potential clinical applications for both PET/MR imaging and SPECT/MR imaging, their long-term added benefit and cost–effectiveness are still to be completely evaluated.

PET/MR hybrid imaging has already been commissioned in the clinic, while SPECT/MR hybrid imaging has lagged behind. However, as we appreciate the advantages of SPECT/MR imaging, research in SPECT/MR hybrid imaging will gain further momentum. While PET/MR imaging may be as efficient as SPECT/MR imaging, the advantage associated with use of generator-produced SPECT radionuclides is difficult to ignore. The use of PET-based molecular probes is generally an expensive proposition due to the need for a cyclotron for producing PET radionuclides. Even a small table-top cyclotron would cost US$2 million, not including the overhead costs of operation [84]. Furthermore, most of the PET isotopes produced have a shorter half-life thus limiting their ability to be shipped from off-site locations. The short half-life of PET isotopes may also limit their suitability for incorporation into hybrid probes requiring longer time for conjugation chemistry. Also, a radioisotope with half-life compatible with the half-life of the hybrid nanoprobes (slower kinetics) would be required to study a molecular process over time. A longer half-life SPECT radioisotope such as ^{111}In would, therefore, be more suitable, especially for imaging extravascular sites (tumors), since such probes are expected to have longer blood circulation times. Another major advantage of SPECT isotopes over PET isotopes is their ability to simultaneously image different radioisotopes, thus making it possible to image different biomarkers at the same time [85].

Executive summary

Hybrid imaging

- The synergistic combination of imaging modalities ensures enhanced visualization of biological targets thereby providing information on specific aspects of both structure and function, which is difficult to obtain by a single imaging modality.

Why single-photon emission computed tomography/MR imaging?

- Investigations with single-photon emission computed tomography (SPECT) can be performed with only picomolar concentrations of tracers. In addition, SPECT radioisotopes are easier and cheaper to obtain than PET radioisotopes for the development of hybrid nanoprobes.
- MR imaging has a higher spatial resolution than nuclear imaging methods and can be used to reflect the molecular content of the tissue being imaged. Furthermore, no radiation dose is added.

Design considerations for a SPECT/MR hybrid imaging nanoprobe

- A SPECT/MR hybrid nanoprobe comprises an MR imaging contrast agent, combined with a SPECT radioisotope into a single nano-sized construct.
- An imaging probe must exhibit a single pharmacokinetic behavior, which is matched with the half-life of the radionuclide.
- The probes must be biocompatible and have an ability to overcome biological delivery barriers to bind specific receptors with high affinity.

SPECT radionuclides

- Selection of a radionuclide for design of multimodal nanoprobes is based on factors such as half-life and γ energy range, minimal emission of secondary radiation, stable daughter product, specific activity, availability and cost.

MR imaging contrast agents

- Due to low concentration of biological targets, contrast agents with high sensitivity are required for molecular MR imaging. Considerable research has thus focused on the design of metal complexes with high relaxivity values.

Hybrid SPECT/MR imaging T_2 contrast agents

- Novel SPECT/MR imaging agents capable of producing T_2 contrast are comprised of a radionuclide and iron oxide in a single matrix.
- Although iron oxide itself can serve as the matrix, various novel materials such as polymers and bisphosphonates can be used to confer unique properties to the nanoprobes. Nanoparticle coating materials play an important role.

Hybrid SPECT/MR imaging T_1 contrast agents

- Paramagnetic metal complexes such as gadolinium, Gd(III), and manganese, Mn(II), produce hyperintensity (bright contrast) in T_1-weighted MR images, and therefore can be combined with radionuclides to develop hybrid nanoprobes for SPECT/MR imaging.

Future perspective

- In an era of personalized medicine and with the recent technological advances in the development of hybrid SPECT/MR imaging scanners, the need for accelerated development of hybrid SPECT/MR imaging nanoprobes cannot be understated.

Some of the advantages of SPECT radiotracers are offset by PET radiotracers such as ^{18}F (110 min half-life), which is routinely shipped from off-site locations, although this often increases the complexity around shipping, patient scheduling, PET suite staffing logistics and scanning protocols [86]. In addition, generator-produced PET isotopes such as gallium (^{68}Ga), copper (^{64}Cu) or rubidium (^{82}Ru) can be used to offset the cost associated with cyclotron-produced isotopes. However, due to unreliable availability, their clinical use is very limited [87,88].

The identification of new molecular targets together with advances in SPECT/MR imaging instrumentation presents an unprecedented opportunity for hybrid imaging, which can directly impact the diagnostic and therapeutic management of a disease. However, to take full advantage of this synergy, there is a need to accelerate development of hybrid SPECT/MR imaging probes. Needless to say, nanotechnology has an increasingly important role to play in the design of hybrid SPECT/MR molecular imaging bioprobes. Modifications of iron oxide nanoparticles provide a common means of developing SPECT/MR imaging agents by introduction of radionuclides capable of SPECT imaging. In addition, novel nanomaterial platforms need to be developed that can allow rational conjugation of MR imaging and SPECT signal motifs, as well as the targeting ligands to the same matrix without affecting the effectiveness of individual motifs. For successful translation into clinical applications, numerous challenges need to be met. Most importantly, their biodistribution and biocompatibility, as well as the long-term effects on biological systems, need to be studied. Finally, we envision that with availability of a SPECT/MR imaging hybrid animal scanner to researchers, more research efforts will focus on development and validation of a variety of hybrid probes capable of SPECT/MR imaging.

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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