Multimodal Molecular Imaging *In Vivo*

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Abstract: In medical research and practice, imaging is now playing a crucial role. With rapidly developing molecular imaging, the role of *in vivo* imaging is expected to expand much more. In molecular imaging, one of the recent trends is multimodal imaging, which theoretically combines all the strengths of each imaging modality and consequently provides synergism. For this, many multimodal imaging probes have been developed so far. However, multimodal probe imaging is different from simple fusion images using multimodal imaging instruments. To maximize the effectiveness of multimodal *in vivo* imaging, practical application methods and delicate design of imaging probes are essential. Here, imaging instruments and probes for multimodal *in vivo* imaging are briefly reviewed and practical application is discussed.

Keywords: Multimodal, *in vivo* molecular imaging, imaging probe, PET, MRI, optical imaging.

INTRODUCTION

In the recent standards of medical practice, imaging is a crucial part in the entire process of diagnosis and treatment, with which lesion detection, differential diagnosis, evaluation of disease severity and monitoring of therapeutic efficacy are performed. In many diseases, image finding itself is even regarded as a specific biomarker for a specific cell type or a specific biological process. Furthermore, the role of imaging is getting more important with development of molecular imaging. 'Molecular imaging' means the biomedical imaging methods which makes it possible to visualize molecular biomarkers or molecular processes in the living body. Therefore, pathophysiological changes at the molecular level can be assessed by molecular imaging, with high sensitivity, specificity, and most of all, non-invasiveness. ^{18}F-fluorodeoxyglucose (FDG) positron emission tomography (PET) is a good example of molecular imaging in which cellular status of glycolysis is visualized by PET with use of a glucose-analog probe, FDG.

At present, diverse imaging methods are available for molecular imaging. In addition to nuclear imaging modalities such as PET and single photon emission computed tomography (SPECT), conventional radiological modalities such as computed tomography (CT), magnetic resonance imaging (MRI), and ultrasonography (USG) have been used for molecular imaging. Optical imaging methods with fluorescent probes or bioluminescence have also been widely tried. Among them, CT and MRI can show normal structures of the body without any imaging probes, because CT and MRI are based on different radiation attenuation and magnetic relaxation time of tissues, respectively, and normal tissues have their own characteristics in those properties. In contrast, probe imaging methods that are used in most molecular imaging are limited in the localization of signals due to the lack of anatomical coordinates. For this reason, combination or fusion of CT and MRI with probe imaging has been tried since more than a decade ago. For example, co-registration of brain perfusion SPECT to MRI was tried as a trial of software-based image fusion between MRI and SPECT [1]. At present, hardware-based image fusion methods of PET/CT and SPECT/CT are prevalent through the world and PET/MRI is under active development [2, 3]. However, the use of these multimodal imaging approaches is still confined to anatomical localization of radioactivity signals, in most clinical practice.

In the field of molecular imaging, there has been much development in multimodal imaging, especially in imaging probes, during the last 3 or 4 years. Many novel imaging probes which can be imaged by multiple methods were developed and reported. Most of them are combining imaging probes for PET, MRI, and optical imaging. In addition to instruments for fusion imaging, these probes have suggested the potential of multimodal molecular imaging in living body. In this review, the present status of multimodal imaging techniques and the perspectives of them are discussed.

CHARACTERISTICS OF EACH IMAGING MODALITY

The advantage of multimodal imaging is that the strengths of each modality can be used in combination. The important properties of an imaging modality that should be considered in molecular imaging include sensitivity, spatial resolution, ability of signal penetration through tissues, and ability of lesion localization with anatomical reference images. These characteristics of each imaging modality are tabulated in Table **1**. In order to optimize multimodal *in vivo* imaging, it should be considered which properties would be effective when combined. CT and MRI may give synergistic effect to most probe-using molecular imaging methods, because CT and MRI can provide anatomical reference

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Table 1. Characteristics of Imaging Modalities

	Anatomical Localization with Structural Reference	Spatial Resolution	Signal Penetration Through Tissue	Sensitivity to Imaging Probe
CT	+++	+++ (0.05~0.5 mm)	+++	+
MRI	+++	+++ (0.05~0.5 mm)	+++	++
USG	++	++ (0.1~1 mm)	++	++
PET	+	+ (1~5 mm)	++	+++
Optical Probe Imaging	–	+++ (< 0.1 mm)	–	+++

images which is lacking in most probe imaging. This is also one of the reasons why instruments like PET/CT and SPECT/CT have prevailed so much. However, in case of probe imaging the need for multimodal imaging is somewhat different. For instance, a multimodal imaging probe that can be visualized on both MRI and PET may or may not be effective, according to the specific condition. An effective dual-modal probe for PET and MRI should give incremental information compared with a single PET probe imaged using PET/MRI. McCann *et al*. reported fusion imaging of fluorescent molecular tomographic image and MRI, by using a fluorescent-MRI dual-modal probe as a common landmark for image fusion (Fig. **1**) [4].

In the molecular probe imaging *in vivo*, a basic need for imaging is sensitive detection of probes on the whole body images. For this purpose, PET (with instruments like PET/CT or PET/MRI) or MRI may be adopted as a basic imaging modality, with such characteristics as high sensitivity of PET or high resolution and soft tissue contrast of MRI. Meanwhile, optical imaging has the strengths of high sensitivity and direct detection of signals with simple optical devices, although it has poor penetration through soft tissues. Consequently, multimodal imaging probes for optical imaging and MRI or probes for optical imaging and PET will be effective and have synergism. For example, optical imaging may guide surgical procedure after lesion localization and surgery planning using PET or MRI.

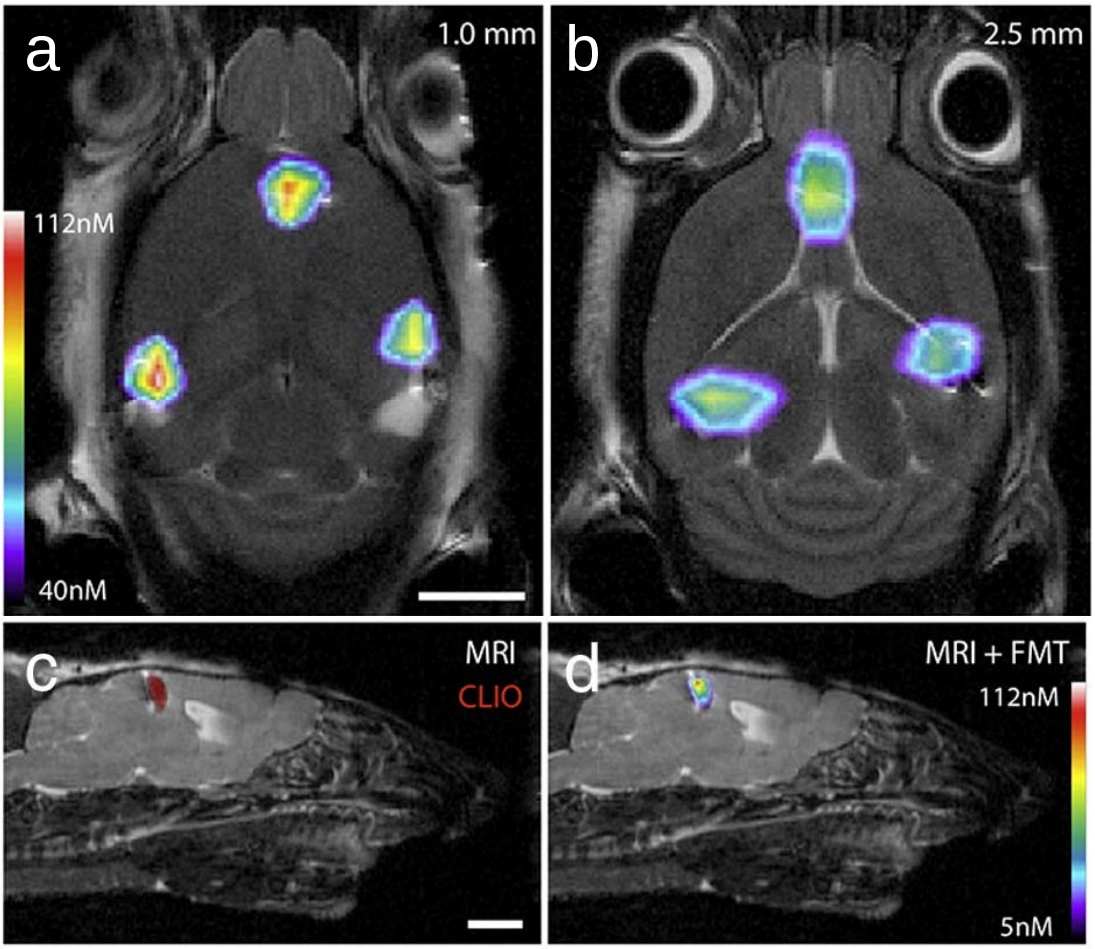

Fig. (1). Fusion image of fluorescent molecular tomography and MRI. A fluorescent-MRI imaging probe is used for common landmark for image fusion (Adapted with permission from ref. [4]).

Table 2. Representative Multimodal Probes for Combining MRI and Optical Imaging

MRI Probe	Optical Imaging Probe	Platform	References
Cross-linked iron oxide	Various fluorophores (Cy5.5/Cy7/FITC/VT680)	-	[5-9]
Superparamagnetic iron oxide	Cy5.5	-	[10]
Cross-linked superparamagnetic iron oxide	Cy5.5	-	[11]
Iron oxide	Carbon nanotube (for Raman scattering signal)	Carbon nanotube	[15]
Gadolinium	Quantum dot	-	[16, 17]
	Quantum dot	Micelle	[18]
	Rhodamine	Liposome	[19]
	Cy5.5	Dendrimer	[20, 21]
	NBD-DPPE	HDL NP	[22]
	$[Ru(byp)_3]Cl_2$	Silica NP	[23]
	FITC/rhodamine	Gd_2O_3 NP	[24]
	Rhodamine	PLGA	[25]

NP; nanoparticle.

MULTIMODAL IMAGING PROBES

Probes for MRI and Optical Imaging

At present, most commonly reported multimodal probes are combining MRI and optical signals. Some of them are validated for *in vivo* imaging while others have shown just potential for multimodal imaging by *ex vivo* experiments yet. In these dual-modal imaging probes, signal for MRI is produced either by T1 or T2 contrast agents, which are attached to optical probes (Table **2**). Gadolinium for T1 contrast and iron oxide for T2 contrast have been selected in most dual-modal probes. Notably, various forms of iron nanoparticles (NPs) have been used for magnetic multimodal imaging probes. Cross-linked iron oxide (CLIO) [5-9], superparamagnetic iron oxide (SPIO) [10], and cross-linked superparamagnetic iron oxide (CL-SPIO) [11] were reported by many researchers. In addition to iron oxide-based NPs, other NPs have also been adopted as platforms to link MRI probes and optical probes. These NPs make the probes chemically flexible that a targeting moiety can also be attached to it. These multimodal imaging probes for MRI and optical imaging were thoroughly reviewed elsewhere [12].

For optical signals, most of the dual-modal imaging probes are using conventional fluorophores such as Cy5.5, rhodamine, and fluorescein isothiocyanate (FITC). Quantum dots were also used with its excellent optical properties. In addition to these conventional optical probes, optical signal from Surface-enhanced Raman scattering (SERS) is intriguing due to its multiplexing capacity. *In vivo* imaging with SERS signal was recently reported by several researchers (Fig. **2**) [13, 14], and a multimodal probe combining MRI and SERS probe was also reported [15].

Probes for Nuclear and Optical Imaging

As nuclear imaging has high sensitivity for detection of signal on the whole body scale, the combination of nuclear and optical imaging is expected to have considerable synergism (Fig. **3**). However, the limited half-life ($T_{1/2}$) and consequent limited time availability of imaging is an innate problem of nuclear imaging. As a result, radioisotopes with relatively long $T_{1/2}$ have been used for multimodal molecular imaging, such as ^{111}In ($T_{1/2}$ = 2.8 d), ^{64}Cu ($T_{1/2}$ = 12.7 h), and ^{177}Lu ($T_{1/2}$ = 6.7 d) (Table **3**). Although the optimal imaging time in molecular imaging is different according to the mechanism of targeting, used imaging probes, and the purpose of imaging, long imaging time may provide extended availability usually. Practically, however, increasing radiation exposure is a concern when radioisotopes with long $T_{1/2}$ are selected for labeling. For optical signals, several fluorophores and quantum dots were used like MRI-optical imaging probes.

In addition to multimodal imaging probes, another way of combining nuclear and optical molecular imaging is to use fusion reporter genes. Reporter gene imaging is a well-established molecular imaging method, in which an imaging probe itself is synthesized from a reporter gene or imaging probe is taken up by the product of a reporter gene. Multimodal reporter genes can be accomplished by linking reporter genes for different imaging methods. For instance, the genes for sodium iodide symporter and luciferase can be linked and produce multimodal imaging signals of radioactivity and bioluminescence. We have repeatedly reported multimodal imaging methods by combining reporter genes for both nuclear and optical imaging [26-28].

Probes for Other Multimodal Imaging

Although combination of MRI or nuclear probes with optical imaging probes is most widely studied, other kinds of multimodal imaging probes have also been reported. For dual-modal imaging of PET and MRI, iron oxide NP is commonly used as platforms. Lee *et al*. developed polyaspartic acid-coated iron oxide NP labeled with ^{64}Cu with targeting integrin $\alpha_v\beta_3$ [41], and a dextran sulfate-coated SPIO NP labeled with ^{64}Cu was also developed [42]. In addition, Choi *et al*. reported a multimodal probe of Mn-doped magnetism-engineered iron oxide (MnMEIO) labeled with ^{124}I ($T_{1/2}$ = 4.2 d) [43]. Nevertheless, a concern related

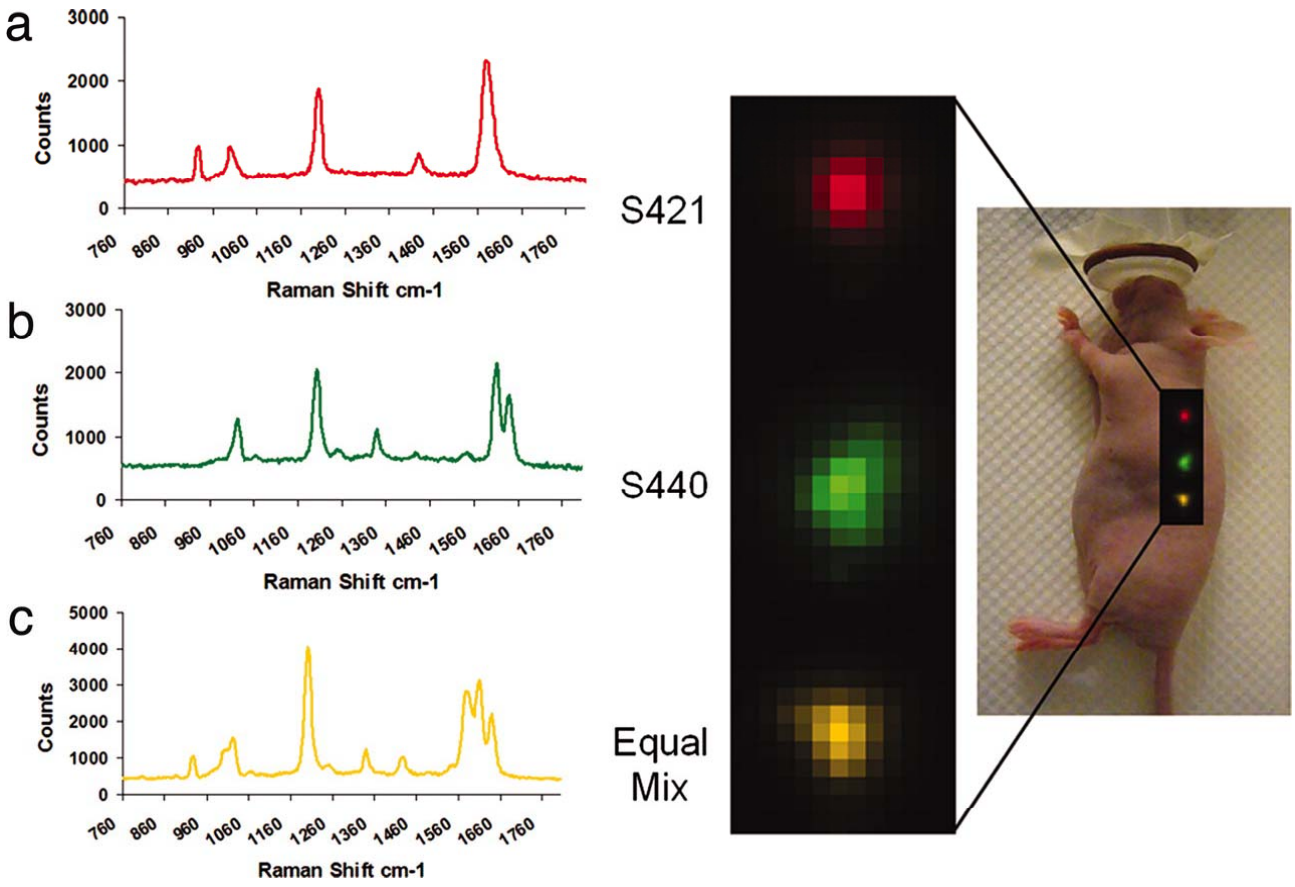

Fig. (2). *In vivo* Raman spectroscopy image. Probes for surface-enhanced Raman scattering were injected subcutaneously to a mouse and the Raman signals were imaged (Adapted with permission from ref. [13]).

with dual-modal probes for PET-MRI is the practical application of the probes rather than technological problems, which will be discussed below. It is notable that these PET-MRI imaging probes are also labeled with radioisotopes of relatively long $T_{1/2}$.

Combination of more than 2 imaging probes has also been tried by several researchers. We developed a quadruple-modal imaging probes with which PET, MRI, fluorescence and even bioluminescence imaging are possible, by attaching rhodamine, luciferase and ^{68}Ga to a cobalt ferrite NP (Fig. **4**) [44]. Stelter *et al*. also attached FITC and ^{68}Ga to aminosilane-coated SPIO NP to enable triple-modal probe imaging [45]. Reporter gene imaging as well as probe imaging was reported to be available for other multimodal molecular imaging. Kim *et al*. used tri-fusion gene for PET, fluorescence and bioluminescence imaging in order to track lymphocytes [46], and Waerzeggers *et al*. adopted a similar strategy to trace the fate of neural progenitor cells in rodents [47].

APPLICATION OF MULTIMODAL MOLECULAR IMAGING *IN VIVO*

As described above, there are many reported probes that make multimodal imaging available. However, their practical application should also be discussed for successful translation of the basic research hitherto, because most of them are just a proof of concept or development of materials. What will be the use of these multimodal imaging probes in molecular imaging *in vivo*, not only in preclinical research but also in clinical application?

One of the potential approaches using multimodal molecular imaging probes may be cross-site cross-modal imaging. For this purpose, optical imaging probes combined with PET or MRI probe is most useful. In case of cancer management, as an example, FDG PET is the most powerful imaging modality to detect primary or metastatic lesions, at present. However, it is not always easy at the operation room to find FDG-avid lesions that were preoperatively detected on PET, if they are so small or hard to be delineated from surrounding tissues. Consequently, there have been trials to use radioactive imaging probes as tumor indicators during surgery, by using portable gamma-ray or beta-ray detectors at the operation room. In those trials, radioactive probes such as ^{18}FDG, ^{123}I-MIBG and ^{99m}Tc-sestamibi have been used for intraoperative detection of several cancers, neuroblastoma, and parathyroid hyperplasia, and so on [48-50]. In cases like this, PET imaging before surgery and fluorescence-guided detection during the surgery would be available with optimal PET-optical dual imaging probes. As endoscopic surgery including robot-assisted surgery develops, the efficacy of optical probes at the operation field will get increased. Endoscopic diagnosis for hollow organs may be another potential application of multimodal probe imaging. After a lesion is detected on PET or MRI, endoscopic examination

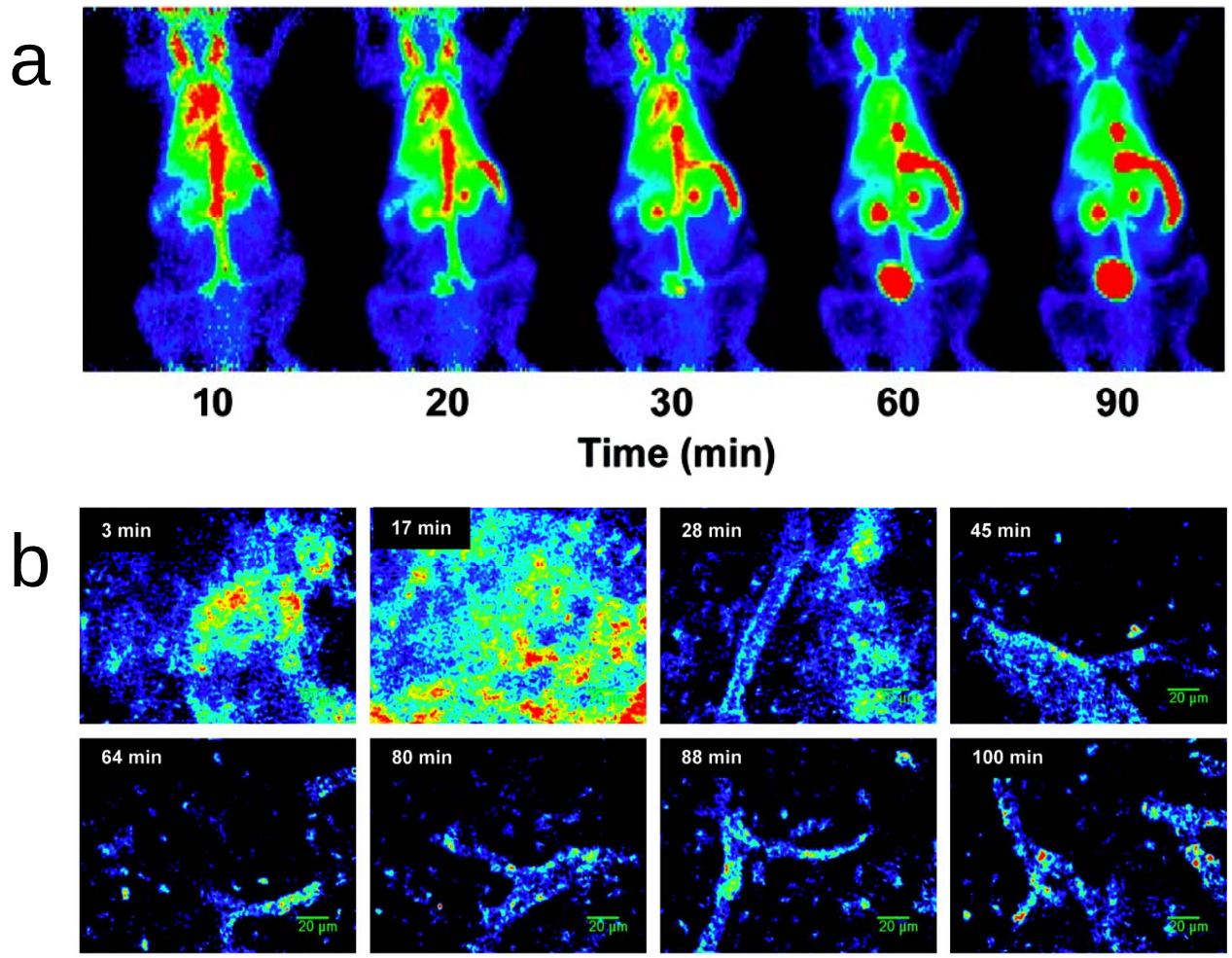

Fig. (3). Images using ^{18}F-labeled quantum dots. *In vivo* PET image (**a**) and *in vivo* optical image of spleen using the fibered confocal microscope (**b**) (Adapted with permission from ref. [29]).

Table 3. Representative Multimodal Probes for Combining Nuclear and Optical Imaging

Radioisotope	Optical Imaging Probe	Target	References
^{18}F	Quantum dot	-	[29]
^{111}In	Cypate	-	[30]
	IRDye800	-	[31]
	IR-783-S-Ph-COOH	Interleukin-11 receptor	[32]
	IRDye800CW	HER2	[33]
	Cy5/Alexa family	Lymphatics	[34]
^{64}Cu/^{177}Lu	Cypate	Somatostatin receptor	[35]
^{64}Cu	Quantum dot	Integrin $\alpha_v\beta_3$/EGFR/etc.	[36-38]
^{64}Cu/^{99m}Tc	Cy5.5	VEGFR	[39]
^{99m}Tc	IRDye800CW	Hydroxyapatite	[40]

and biopsy can be guided by optical signals. It may also be applied to intravascular lesions such as vulnerable plaque, like lesions in hollow organs, because it can be accessed by intravascular catheters or endoscopy. Some prototype instruments have been reported as intravascular optical signal detectors which can be inserted through catheters [51, 52].

In case of multimodal imaging probes for PET and MRI, it is still somewhat unclear what will be the efficient

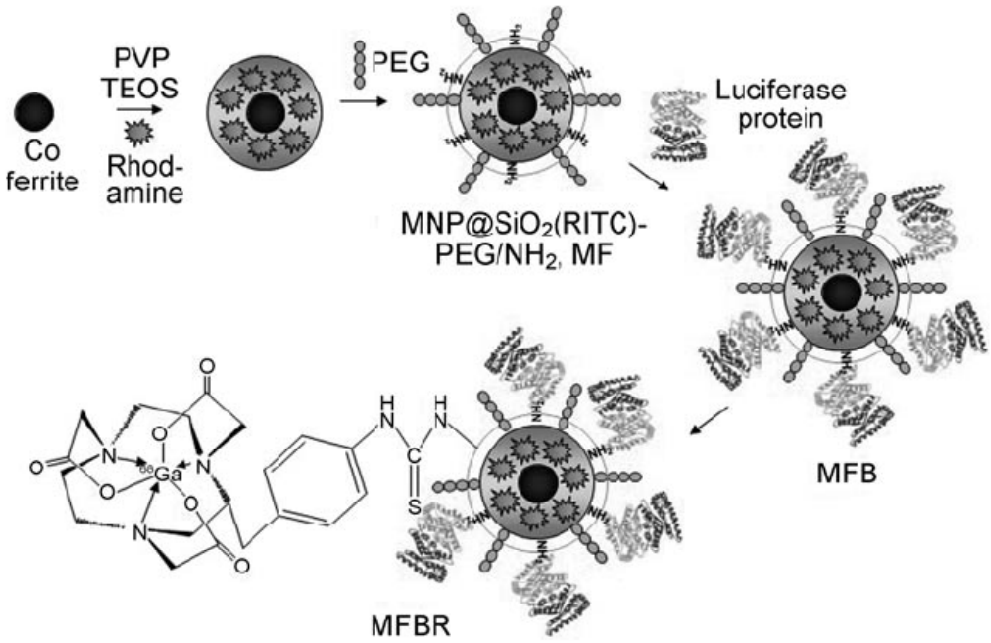

Fig. (4). Schematic diagram of the synthesis of magnetic-fluorescent-bioluminescent-radioactive particle (Adapted with permission from ref. [44]).

application of these probes. As mentioned above, an efficient multimodal PET-MRI probe imaging should have incremental value compared with single PET probe imaged with a fusion instrument of PET/MRI. If PET and MRI have different functions from those of each other, there will be synergism. However, usual function is similar between PET and MRI in that imaging probes are detected and localized on the scale of whole body with each of the methods. Furthermore, because PET is definitely more sensitive than MRI in detection of probes there might be little incremental value by just adding MRI, if it is only for the detection of probes. Therefore, it is required to find specific need and application when a multimodal PET-MRI probe is designed.

As a general principle, one imaging should be complementary to the weakness of the other even when PET and MRI probes are combined. One of the weak points of PET that can be complemented by MRI is spatial resolution. Although PET has better sensitivity in detection of signal than MRI, it is significantly affected by partial volume effect due to low spatial resolution. This partial volume effect compromises sensitivity of PET, in case of small, cystic, and heterogeneous lesions. Combined MRI images for the same imaging probe would be complementary for PET. In addition, some diseases that require more specified localization of imaging probe will be a good indication of multimodal PET-MRI probe imaging. In such diseases, PET would be used for detection of probes, and MRI, for specific localization with high spatial resolution. In case of myocardial infarct, as a putative example, transmurality of lesion is an important information, which cannot be revealed with the resolution of PET but with that of MRI.

Another effective application of multimodal imaging probe is to support the development of an imaging probe itself. All drug candidates including imaging drugs require pharmacokinetic data including absorption, distribution, metabolism, and excretion, to be introduced into clinical fields. These data can be acquired directly from *in vivo* imaging study with the concept of 'microdosing', which means administration of non-pharmacological minimal dose of a drug into human body [53]. With this approach, considerable amount of time and money can be saved during development of new drugs including imaging agents. Pharmacokinetic studies for some molecular imaging probes based on quantum dots [38] or microbubbles [54] have been performed using animal PET studies. However, some imaging probes such as quantum dots have such a high toxicity that it is hard to be considered for human application. Some problems such as toxicity, and non-biodegradability of some imaging probes should be resolved for clinical application in future.

CONCLUSION

With the development of many multimodal imaging probes, *in vivo* multimodal molecular imaging is getting more and more available. However, more delicate design of probes and consideration of application method are required in order that they are practically adopted with effectiveness. In discreetly selected indications, *in vivo* multimodal molecular imaging is expected to play a crucial role by combining strength of each combined modality, and expand the role of molecular imaging in both research and clinical field of medicine.

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