Development of Molecular Imaging Probe for Dual NIR/MR Imaging

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Imaging utilizing the near-infrared (NIR) light has attracted numerous attention due to the possibility in the deep tissue penetration as it can overcome the light scattering and absorption of tissue components. The ultraviolet (UV) unlikely penetrates the skin, while the visible (VIS) light can be scattered or absorbed by tissue components. This penetration likely improves as the wavelength shifts beyond 1000 nm region (also called NIR-II). Fat tissues are ascribed to the scattering of UV or VIS, while others such as water, melanin, hemoglobin are greatly attributed to absorbing light. Using NIR over 1000 nm (OTN-NIR) is currently considered as a critical approach for real-time dynamical visualization of the structure and functional features of tissues anatomically with refrained effects of fatty scattering and water absorption. However, the attempts to image anatomical structure by OTN-NIR is laborious and time-consuming. Then, for facile human applying, magnetic resonance imaging (MRI) is used as a guiding technique to localize the sites of interest. MRI is considered the most beneficial imaging technique without ionizing radiation which provides images with high resolution, preeminent tissue contrast. MRI also can visualize a large volume such as the human body to the few millimeter objects with great signal-to-noise ratio as well as contrast-to-noise ratio. This review highlights the design of imaging probe for multimodal NIR/MR imaging, including the potential applications.

Keywords: NIR, MRI, Multimodal imaging, Molecular probes

1. Introduction

In recent, using multimodal imaging is of great interest as it facilitates the accurate visualization of biological and dynamic processes, microstructure, and morphology in the anatomical aspects [1-4]. The multimodalities are constructed by integration of different imaging techniques simultaneously with design of favorable multimodal probes. The most widespread multimodalities are the combination of positron emission tomography (PET)/computed tomography (CT). It also can associate with single-photon emission computed tomography (SPECT) and/or CT [1-6]. However, the use of ionizing radiation to provide more functional and physiological information in the tissue has risen the concerns for patient safety [7,8]. Magnetic resonance imaging (MRI) is well-known for high spatial resolution and soft-tissue contrast that are good enough to discriminate morphological changes in a small structure [7-10]. The combination of MRI with optical imaging is then preferable because optical imaging can provide fast
and dynamic images with a high spatial-temporal resolution, and non-toxic effect. However, the optical imaging often uses VIS and UV light which are easily scattered and absorbed by tissue components, restricting the penetrating of light in deeper tissues [11-15]. The penetration depth of light is determined by the interaction between an electromagnetic wave and transmitted media according to Lorentz and Maxwell’s laws [16]. The tissue penetration of light with various wavelengths varied due to light frequencies and dielectric constant of media [17], whereas light absorption coefficient by hemoglobin greatly diminishes in low frequencies waves corresponding to NIR region. Although the tissue absorption contributed by water becomes significant above 950 nm [18]; there are clearer visions where tissue components have their lowest absorption [19,20]. The tissue-optical-transparent windows exit at 650-950, 1000-1350, and 1600-1870 nm, namely the NIR-I, NIR-II, and NIR-III biological window, respectively [11,12] especially in the region above 1000 nm. The near-infrared light over 1000 nm (OTN-NIR) is able to penetrate as deep as centimeter-level below the skin and is a potential approach for real-time dynamic visualization of the structure and functional features of tissues [18,19].

The merit of NIR/MRI registration is that a targeted image can be obtained with full dynamic, molecular as well as microstructure information.

Fig. 1. (A) Schematic figure of the dual NIR/MRI probe from Gd-ICG liposome. A bilayer of lipid molecules encapsulates an aqueous interior of Gd-chelate and ICG molecules. The encapsulated Gd-chelate is T1 contrast agent for MRI. The ICG is used in near-infrared imaging (NIR) (B). A coronal necropsy (C) and coronal open abdomen near infrared fluorescence images (D) of a nude mouse. Similar MR and NIR findings were noted with a second intraperitoneal tumor model, OVCAR-3. Arrow, tumor; I, intestine; K, kidney; L, Liver. Figure adapted from ref. 22 with permission.

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This one, however, is challenging because the processes of adapting and assembling data at all scale of resolution are complicated. Fortunately, all of these techniques employ exogenous probes to improve the signal-to-noise ratio; the development and use of multimodal probes are considered highly advantageous for obtaining images across the methods.

2. Multimodal imaging probes: critical points and design aspect

The advent in imaging techniques brings a flourished avenue for imaging probe development. The probes either organic molecules or inorganic nanoparticles are designed to localize a specific target within a biological specimen or to enable the target to be spectroscopically detectable as a response to a distinct stimulus. There are some criteria the probe must meet in the development in order to be applicable [21].

1. Responsibility to the multi-stimuli: the matter-energy interactions must be comparable with multimodal techniques. The signal’s characteristics must be less dependent on the nature of milieu.

2. Detectability: the probe characteristics must be accordant with the imaging modality. The emission or effect output of the probe must be sufficient to be recognized by the instrument in vastly complex biological system.

3. Stability: the probe must emit stable signals and sustain to the chemical environment without fading.

4. Enhancement and functionalization: tuning emission intensity must be addressed to image with multimodalities [22].

Functionalization of imaging probes includes peptide-targeted, multi-functional agents, that can carry and deliver both diagnostic agents and therapeutic drugs as in Ref. 22, the authors developed a tumour targeting theranostic combining of therapeutic and diagnostic agents that enables ligand-directed tumour targeting, multimodal imaging, and triggered drug release [22]. Another example of a multimodal imaging NIR/MRI application is shown in Fig. 1 where Gd-ICG liposome used as a dual NIR/MRI probe from ICG molecules-a NIR imaging probe, 780 nm for the excitation, 810-830 nm for emission and Gd\(^{3+}\) chelates were encapsulated by a bilayer of phospholipids. This probe was used to localize preclinically ovarian tumors by MR and plan for surgery. NIR imaging was used for resection during surgery. The results claimed that by a single injection dual-modal imaging probe (Gd-ICG) co-registration of ovarian tumors was obtained by MR and N IR in mice [23].

3. NIR molecular probes

There are numerous substances used as NIR/MRI imaging probes such as rare-earth-doped nanoparticles, the hybrid structure of metals nanoparticles, semiconductor, carbon, etc. [24-30] to metal-ion-derived complexes (Gd or Mn, etc.) [30] or iron oxide nanoparticles in order to be used in MRI [28]. However, there is a critical concern of toxicity for human using because the retention of the materials out of the body is a problem. These nanostructures are durable and undegradable in the body and may cross the blood-brain barrier [31-33]. These are a significant concern for clinical using of nanoparticles. While molecular dyes are well-defined architectures with rapid metabolism and low toxicity.

Many attempts in developing of molecular probes for NIR imaging have been made, especially, in the region over 1000 nm as the tissue transparency greatly augments. Although a large number of NIR organic molecules emitting in the NIR-I window are commercially available, the potential OTN probes are still under development. In which, polymethine dyes are a large class of compounds currently...
commercially available. This class of probes has a wide range of applications from lasers to diagnostic, as sensitizers for photodynamic therapy [34]. The molecular structure of these dyes is characterized by a conjugated (polymethine) chain terminated at each end by a heterocyclic moiety [35]. Polymethine dyes absorb and emit mostly visible light along with the chain length and of the terminating moieties. In general, as the chains are elongated, the dyes can absorb and emit longer wavelength, but it also causes a decrease in efficiency [36]. Among these groups, IR1061 dye is generally used for in-vivo imaging shown in Fig. 2a. Dai group reported IR-1061 incorporated to nanoparticles consisting of amphiphilic polymer poly (acrylic acid) (PAA) and polyethylene glycol-conjugated phospholipid (DSPEnPEG) [37]. IR-1061 is highly hydrophobic and quenched in water. Incorporation of IR1061 in nanoparticle increases the quantum yield to 1.8%. In other respect, Kamimura and co-authors reported IR1061 fluorescent copolymer micelles PLC-PEG as a NIR fluorophore and performed fluorescence imaging in the second NIR window [38]. In the effort of design of NIR-emitting fluorophores an aqueous solution, Dai, Cheng, and Hong reported a new type of benzobis(1,2,5-thiadiazole) NIR-emitting dyes with D-A-D charge structures shown in Fig. 2b. This type of NIR dye (CH1055-PEG) emits 1050 nm light with a quantum yield of 0.3% in an aqueous solution [39]. A modification of CH1055 sulfonated derivative (CH-4T) with the bovine serum to produce a 50-fold increase in NIR fluorescence. NIR imaging of tumors in mice using affibody functionalized CH1055 was also conducted [40]. More recently, our group and several others found that a commercially available dye, indocyanine green (ICG), the only NIR dyes that are approved by the Food and Drug Administration (FDA) for clinical use in humans, with an emission peak of 830 nm can be used to NIR fluorescence imaging in the second NIR window [41].

4. MRI imaging probes

Currently, MR contrast agents are classified into two main groups which by reducing the T1 and T2 relaxation times in the target tissue [42]. They are described as either ‘T1 agents’ or ‘T2 agents’ depending on their effect is greater in T1 or T2. The contrast enhancement effect is obtained when one tissue has either a higher affinity for the contrast agents or higher vascularity than another one. T1-weighted image is also called positive image contrast, as the image signal intensity increase due to T1 shortening. T2-weighted images give negative contrast, due to the effect of T2 shortening. The positive contrast agents are mostly made from Gd$^{3+}$ paramagnetic complexes such as gadopentetate dimeglumine (Gd-DTPA, Magnevist), Gadobutrol (Gd-DOTA, Gadovist) are among the most widely used in MR molecular imaging. These agents have a low molecular weight, which helps them easily leak out the capillary into the intercellular space and be distributed nonspecifically. The agents then reach the equilibrium rapidly in normal tissues and lesion areas [42]. The negative contrast agent is superparamagnetic iron oxide nanoparticles (SPIONs), Fe$_3$O$_4$. This agent shows lower contrast medium toxicity as the nanoparticles can be degraded in the normal iron recycling pathways [42]. Moreover, SPION surface can be functionalized and combined with appropriate targeting ligands. The further agent made from non-lanthanide paramagnetic metal manganese that possesses good positive T1 enhancement effects, with five unpaired electrons in case of bivalent manganese. Manganese is an important element for cell biology; therefore, it also is minimally toxic in vivo of manganese chelates and allows using in large doses. Manganese-based contrast agents can be used in a variety of forms such as organic chelates [43], or oxide nanoparticles [44].

5. Design of NIR/MRI multimodal imaging

The combination of NIR and MRI encounters several challenges associated with the inherent difference in the sensitivity. Optical imaging is higher-order sensitive compared to MRI; therefore, higher MRI contrast ratios must be loaded into the probe relative to the number of fluorescence dyes to compensate for the low sensitivity of MRI. The second challenge is involved in the physical property mismatch between two agents. For example, iron oxide is an agent that causes quenching of fluorescence because of the contact of fluorescent molecules with the metal oxide particle surface, resulting in an energy transfer process [45]. Therefore, between iron oxide and the fluorophore must be separated by polymeric layers. Consider the fluorescence signal readout and the structural complexity of the probe is essential before adding targeting groups and biocompatible modifications. The other challenges are related to the nature of Gd ions as an MRI contrast agent as it is required to expose closely to water molecules in the primary or
secondary-sphere to give high T1-contrast enhancement signals [46]. The appropriate position for Gd chelates is at the hydrophilic outermost surface to achieve good signal contrast.

6. Application of multimodal molecular imaging
Generally, multimodal imaging is a combination of two kinds or more imaging techniques to form a new paradigm, which allows obtaining some further information in diagnosis, treatment, and monitoring. At present, multimodal molecular imaging has been widely investigated in medical researches and clinical applications. For instance, multimodal molecular imaging has been used for cardiovascular diseases [47], neuropsychiatric diseases [48], and other clinical diseases [49]. In addition, it can significantly increase the numbers of tumor images and effectively guide the surgical resection of the tumor [50].

7. Conclusion
In summary, multimodal NIR/MR imaging heralds a bright perspective. The advance in this field will bring a major breakthrough in medical imaging and molecular biology. Although NIR imaging remains at its early stage, there are still a great potential for further advancement.

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