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Tips and Tricks in Molecular Imaging: A Practical Approach

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Abstract

A variety of imaging modalities include X-ray-based Computed Tomography (CT) scan, Ultrasound (US), Magnetic Resonance Imaging (MRI), Nuclear Medical Imaging (NMI), and Optical Imaging (OI) are used to help diagnose and treat diseases through anatomical, physiological, and functional representation. With the advent of molecular imaging using nanoparticles, detailed information about properties is provided and facilitates early detection of malignancies. Now, novel approaches in nanoparticle designing, the development of hybrid imaging modalities, and improvements in the sensitivity of instruments have raised the level of disease diagnosis.

Regarding the fact that the molecular imaging fundamentals and basis of materials as contrast agents are different from each other, we have updated the brief synopsis of basic principles of imaging technique containing important points in detail with a practical approach.

Keywords: Computed Tomography; Ultrasound; Magnetic Resonance Imaging; Nuclear Medical Imaging; Optical Imaging; Contrast Agents.

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1. Introduction

Diseases manifest themselves by abnormalities in structure or function based on specific pathologic changes caused by various factors which may or may not be associated with certain signs and symptoms [1]. For starting the interventional procedures the medical imaging techniques have been widely used to diagnose diseases and highlight the physiological and pathological changes in organs [2, 3]. Currently, in the clinic, the algorithm for medical imaging is based on a step-by-step diagnosis. For example, clinicians recommend optical imaging (colonoscopy) or special radiography (Barium enema) as the main screening techniques for colon cancer and advise sophisticated imaging methods such as CT scan, MRI, or Positron Emission Tomography (PET) to determine the extent of cancer as the next step [4, 5].

Some imaging methods highlight detailed physiologic properties, others estimate the body's metabolism, and other ones demonstrate the body's structures [6, 7]. Regardless of the sensitivity level of the techniques, contrast agents are used to improve the aforementioned features by enhancing the sensitivity and selectivity [8, 9].

Most importantly, in the past few decades, Nanoparticles (NPs) have been developed to represent molecular changes, facilitate early detection of cancer, and have been used as treatment in conjunction with clinical or pre-clinical imaging techniques [10-12]. Besides, the variety of targeted NPs are used for the detection of disease by the use of different medical imaging techniques including X-ray-based CT, US, MRI, NMI, and OI [13-15]. Since the basic principle of each medical imaging technique is different, therefore there are several classes of NPs used as contrast agents for targeting of disease. Consequently, their respective basic imaging protocols and practical tips will be varied [16, 17].

Before commencing a discussion regarding molecular imaging, some general points should be considered. Each of the imaging modalities has its strengths and weaknesses that need to be considered; the relevant considerations include spatial/ contrast/ temporal resolution, depth of penetration, sensitivity, repeatability, and safety [18, 19]. MRI and PET are recommended to evaluate physiological and metabolic processes such as biochemical and molecular changes [20, 21]. Also, MRI or CT is preferred to obtain information about tissue structures and pathological changes. Combined imaging techniques such as PET/CT or PET/MRI can be utilized to obtain physiological and anatomical information together [22, 23]. For superficial and deep lesions, high-frequency US or OI in combination with MRI for soft tissues or CT for bone and lung are being used [24, 25]. Therefore, depending on the result of the goal-based evaluation, an appropriate imaging technique with corresponding contrast agents must be selected [26]. In some cases, the goal is to determine the functionality or to illustrate the structure of a target site, therefore, imaging will be limited to the specific region via targeted contrast agents. Conversely, whole-body imaging may be indicated in cases that require exploration of metastatic loci and assessment of bio-distribution [27, 28].

In terms of contrast agents, materials that have high safety and compatibility, low cost, easy preparation procedure, high affinity to the target site can be used in clinical application. Moreover, the most common types of contrast agents do not need to be stimulated for enhanced contrast, on the other hand, some of them such as fluorescent-based and liquid-based US contrast agents are excited by an external source and their light emission or transformation (from liquid to gas) produces an image [29-32].

Only spectral CT and OI modalities can detect multiple contrast agents simultaneously; therefore, these modalities can be recommended for different lesions in various tissues. The appropriate concentration and volume should be selected at the phantom level before injection into the body [33, 34].

The synthesized contrast agents should also be validated in vitro with the appropriate protocol before clinical use. Due to the high sensitivity of NMI, there is a need for fewer contrast media volumes compared to MRI and CT. This balance is typically established to compare targeted versus non-targeted contrast agents. NM-based agents with the use of targeted materials have always been used to directly detect pathologic status [35-37].

Furthermore, the biological distribution should be investigated when using in vivo targeted imaging before identifying the target sites. Residual contrast material in other tissues may cause noise, signal loss, and decrease the Contrast to Noise Ratio (CNR). For this reason, delayed images are taken to wash the contrast media from other tissues [38, 39].

1.1. CT

CT is a non-invasive technique used to produce cross-sectional and 3-Dimensional (3D) images, based on different absorption levels of X-ray by the object. Therefore, contrast materials must have a high atomic number (Z) to enable strong absorption of the produced X-ray [40]. Contrast agents and NPs based on iodine, gold, bismuth, and tantalum have been developed to fulfill this requirement [41-45]. In CT, the amount of X-ray absorption is expressed in the Hounsfield Unit (HU), which is the difference between the linear coefficient of the material and water. In addition to demonstrating the CT images, the results should be presented as a CT number in terms of HU, HU/mM, a comparison to clinical iodine-based agents at similar concentrations, and also with the calculated CNR parameter [46, 47].

With the advent of the new generation of CT such as Multi-Detector (MDCT), Dual-Energy (DECT), and micro-CT, the possibility of fast imaging, quantification of compounds, and displaying more details of organs has been provided, respectively [48, 49]. It is suggested to employ DECT for the identification of several contrast agents simultaneously and utilize either micro-CT or the finest slice thickness by clinical MDCT for cellular level pathology [50, 51]. Also, MDCT is recommended for in-vivo small animal molecular imaging to display the vascular structures [49].

The new generation of micro-CT, using various model-based or hybrid reconstruction algorithm methods, allows the object to be imaged with the high spatial and temporal resolution facilitates volumetric imaging of the object or living tissues and small animals in a shorter time [51, 52]. The sensitivity of CT is lower than other modalities and requires a higher volume of contrast agents (especially when employing iodine-based contrast agents) in vascular and tumor studies [53]. For this reason, it has been suggested to use appropriate concentrations and targeted contrast agents as much as possible to minimize the related adverse reactions. Moreover, in the new approach for in vivo studies, the tendency to use less contrast medium volume using DECT has been raised [54].

Despite extensive efforts to optimize detectors and improvements in reconstruction algorithms, high levels of radiation are imposed on patients in vascular and multiphase studies [55-57]. Before the CT imaging, the scanner must be calibrated for accuracy and reproducibility of exposure rate output parameters [58]. Furthermore, it is necessary to design an appropriate protocol at the phantom level based on the calculation of the contrast-dose combination parameter (e.g. Figure Of Merit (FOM)) before implementation at pre-clinical or clinical trials [58, 59]. While selecting appropriate radiation parameters, the object should be placed in the center of the Scan Field-Of-View (SFOV), so that the lowest image noise is achieved at optimal delivered dose [60].

1.2. US

US images are acquired from acoustic impedance differences between interfaces of the subject [61]. It provides cross-sectional images from internal organs and assesses disease conditions using various modes. US molecular imaging technique has been proven promising to early-stage diagnoses and improves the image enhancement by contrast agents [62]. US contrast agents increase the impedance difference between the textures and enhancing the reflected echoes; these agents, including gas/liquid/solid based agents have been introduced to achieve echo enhancement [63]. Gas-based contrast materials have the highest echo but they are being replaced by nano-bubbles or solid and liquid-based materials due to their large size and short circulation half-lives [64]. Micron-sized particles are used for intravascular studies, while nano-sized particles are applied to display extravascular lesions (e.g. cancer research) [65]. The output US images are generally quantified by the Mean Pixel Intensity (MPI), and consequently, the CNR is calculated [66]. In addition, several parameters, including perfusion, distribution, Peak Enhancement (PE), vascular texture, Area Under the Curve (AUC), and volume can be evaluated by spatial, temporal, and machine learning analysis methods [62, 67].

Before the ex-vivo cellular and in vivo experiments, the synthesized samples are placed in the human tissue-equivalent phantom or the samples are prepared in agar gel at different concentrations and compared to clinical US agents (or agar or deionized water). Depending upon the purpose, designate the image mode detection including B mode, Contrast Enhancement Ultrasound (CEUS), Doppler, and harmonic imaging. Adjust the experimental setup and imaging protocol parameters such as frequency, depth, dynamic range, and acoustic output depending on the procedure. A balance must be struck between penetration depth and frequency. Spatial resolution improves with increasing frequency at the expense of depth penetration. Concerning the low sensitivity

of the method as well as CT/MRI, it is better to use targeted NPs to show malignancy lesions [68, 69].

1.3. MRI

MRI images are obtained by measuring the signal generated from hydrogen nuclei in response to magnetic fields using many different sequences acquisition including T1-weighted, T2-weighted, Inversion Recovery (IR), and Gradient Echo (GE) [70]. New advanced techniques such as Diffusion Tensor Imaging (DTI), Susceptibility-Weighted Imaging (SWI), functional Magnetic Resonance Imaging (fMRI), and MR Spectroscopy (MRS) have emerged over the past decay for quantitative microstructural analysis, venous perfusion, brain activity, and evaluation of the chemical composition of textures, respectively [71].

Due to recent technological advances, next-generation sequencing, and also with the advent of novel theranostic contrast agents, molecular MRI imaging has become an early diagnostic tool and medicine against various diseases [72, 73]. Taking into account, MRI is a unique technique used to acquire anatomical images, functional information, and detailed illustrations of metabolic disorders by detecting small changes in magnetism [74]. The electron configuration of an element determines its magnetic behaviour and the elements are placed in paramagnetic, diamagnetic, or ferromagnetic classifications [15, 75]. Based on the specific properties, the various elements in micro or NPs have been developed in form of paramagnetic and super-paramagnetic MRI contrast agents. Like other imaging modalities, MRI contrast agents (either paramagnetic or super-paramagnetic) enhance the sensitivity of the study and can help to improve the accuracy of diagnosis. Both contrast agents shorten the T1 and T2 relation time. Also, paramagnetic-based agents increase the signal intensity on T1w images and super-paramagnetic agents decrease the signal intensity on T2 images that appear brighter and darker, respectively [73, 76, 77].

T1w sequences with different Repetition Times (TR) are applied for T1-based contrast agents, while T2 sequences with multi-Echo Times (multi-TE) are currently being used for T2-based NPs [78]. After the acquisition of the images, the correlation of mean signal intensity with TE or TR parameters at different contrast concentrations must be calculated. Additionally, in the phantom level study, the relaxation rates (R1 or R2) of contrast agents must be measured, correlated with concentration, and compared to standard samples. Depending on the particle-size distribution, the employment of Super-Paramagnetic Iron Oxide (SPIO) and Ultra-small Super-Paramagnetic Iron Oxide (USPIO) platforms may yield T1 or T2 images. Both T1 and T2 sequences must be taken, R1 and R2 as well as r1 and r2 measured, and r1/r2 ratios should be calculated [79, 80].

When using in vivo experiments and common T1w or T2w images, it is preferable to utilize complementary sequences including simple Gradient-Echo (GE), Fast Low Angle Shot (FLASH), Volumetric Interpolated Breath-hold Examination (VIBE), and True Fast Imaging with Steady-state Precession (True-FISP) [81-83]. Recent advances in molecular MRI include the synthesis of hybrid or three-modal NPs as MRI/CT, MRI/US, MRI/optical dual-contrast agents and CT/MRI/US multi-modal contrast medium to synergize the advantages of diagnosis methods [84-86].

1.4. NMI

NMI is typically comprised of gamma cameras, Single-Photon Emission Computed Tomography (SPECT), and PET scanners. In this method of imaging, the administered unsealed sources of radioactivity are identified, measured, and created 2D or 3D images by the gamma camera, SPECT and PET, respectively [87, 88].

These are among the pioneers of molecular imaging and provide the ability to evaluate physiological and biochemical processes at both the clinical and pre-clinical levels. The SPECT focuses on functionality, while the PET measures metabolic activities [89]. Contrast agents used in nuclear medicine are called radiopharmaceuticals or radiolabeled probes which are made up of basic and targeted parts. Overall, the radionuclides are conjugated with a targeting molecule and used for detection or therapy [90, 91]. For this purpose, the $\beta+$ or γ emitter radionuclides are applied for diagnosis while α and $\beta-$ emitter ones are used in therapy. The targeting agent leads the radiotracer to its specific receptors [92] (Figure 1).

The basic component is responsible for signaling and consists of materials such as ^{99m}Tc, ^{67}Ga, ^{123}I, ^{201}Tl, ^{133}Xe for SPECT imaging, and ^{18}F, ^{68}Ga, ^{89}Zr for PET imaging [94]. The targeted component determines the specified location of interest for the accumulation of radiopharmaceuticals [35, 95, 96]. Targeted parts can

be classified in a few ways depending on the type of disease and the target tissue containing small molecules such as peptides, antibodies, aptamer, folate, dopamine, Fluorodeoxyglucose (FDG), etc. Since several types of radiolabeled probes can be used simultaneously in SPECT, it is possible to evaluate multiple targets. This is in contrast to PET studies where only one radiopharmaceutical is used [97].

Another important point in nuclear medicine is the observation of all experiments involving the exposure of researchers to ionizing radiation and must comply with the requirements for radiation protection regulations [98]. Due to the excellent feature of high sensitivity in PET and SPECT, the amount of contrast used can be reduced to pico-mole or nano-mole levels. In this regard, the radioactivity of radiolabeled probes should be obsessively determined by a dose calibrator depending on the type of procedure [99].

New approaches in this field are the use of combined radiopharmaceuticals in the form of theranostic agents for the simultaneous diagnosis and effective therapy of various types of diseases (Figure 2). Another class of strategy for optimization the diagnosis of diseases is to combine several agents within a carrier as PET/SPECT hybrid radiolabeled probes [100]. Both PET and SPECT have poor spatial and contrast resolution. To reduce this limitation, it is recommended to use an animal PET or SPECT for preclinical studies or apply

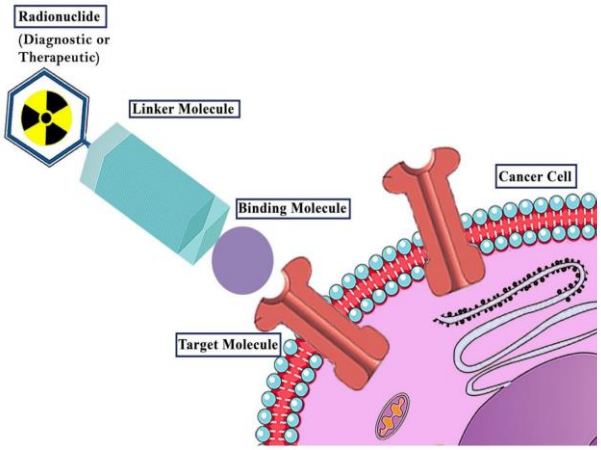

Figure 1. The schematic concept of radiopharmaceuticals; adapted with permission from ref [93]

hybrid SPECT/CT, PET/CT, and PET/MRI scanners to compensate for the shortcomings of one with another modality [101].

1.5. OI

OI which consists mainly of fluorescent, bioluminescence, and Optical Coherence Tomography (OCT) imaging is an ideal technique for evaluating biological processes with respect to visualization of cells, living tissues of the human body, and also the whole body of small animals in pre-clinical experiments. Owing to the low energy of the photons used, their penetration depth is limited to a few centimeters. To reduce this limitation, fluorescent-based nanomaterials such as dyes, indocyanine, Quantum

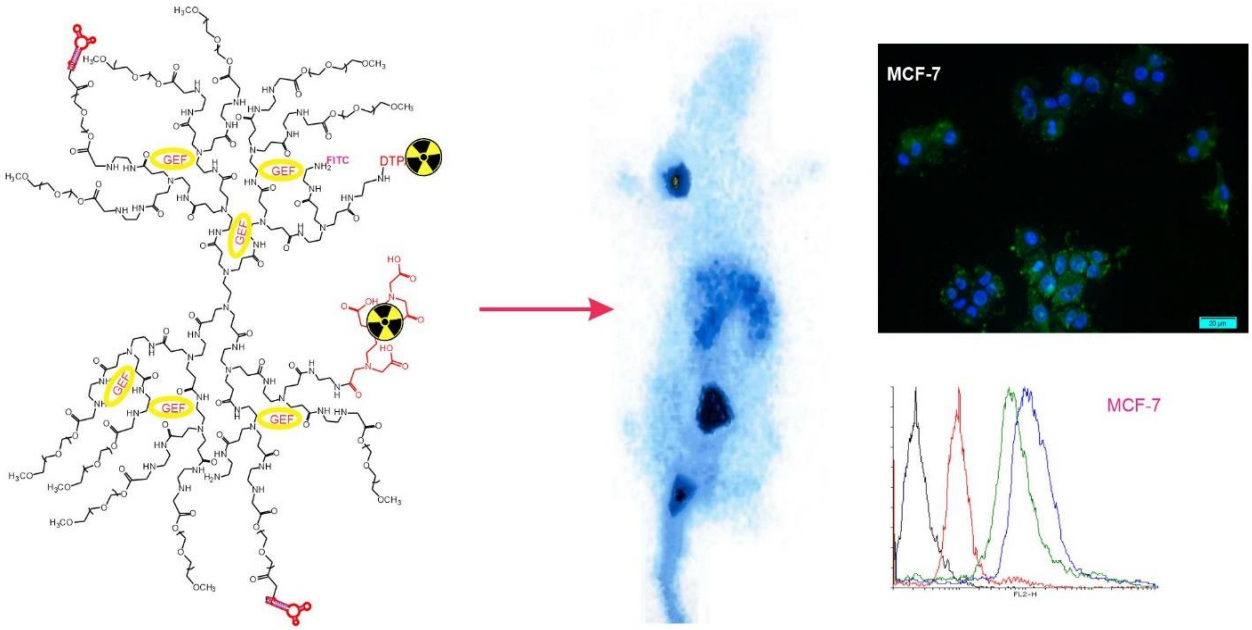

Figure 2. The ^{67}Ga labeled radiotracer as a theranostic agent for simultaneously drug delivery and SPECT imaging in MCF-7 tumor-bearing mice. Adapted with permission from ref [102]

Dots (Q-Dots), and fluorescent proteins in conjunction with Fluorescence Molecular Tomography (FMT) have emerged [25].

In fluorescent molecular imaging, stable targeted fluorescent-based agents with the appropriate emission wavelength must first be selected. Then an external light source corresponding to the agents must be selected to excite it (e.g. Q-Dots can be better excited by Ultra Violet (UV) light) and be fitted with a suitable filter proportional to the emitted light from the interaction of the laser and the agents [103].

Comparing to other optical imaging, the OCT technique provides cross-sectional and volumetric images with high-resolution in minimum time of tissue using a tiny volume of contrast agents or even without it [104]. Due to the high relative sensitivity of the OI, there is a need for a contrast agent at the level of ρ-mol. This reduces the tissue toxicity. Additionally, it is possible to attach several targeted ligands to identify multiple lesions simultaneously in the form of multiplex imaging [105]. After imaging, a quantitative image analysis is required. Quantifying optical images has its own set of complexities; in this context, the attenuation, adsorption, and dispersion rate as well as tissue correction factor related to depth and field size, must be calculated for each tissue and agent separately [106].

2. Conclusions and Future Prospects

The new approach tends to amplify the signal/intensity and improve contrast and mean pixel density. The combination of several elements leads to these properties [107]. Another approach is to reduce the limitations of current modalities by combining several elements with different properties into one carrier; each part of the platform gives a separate signal related to that modality (i.e. hybrid or three-modal nanoparticles) [108]. However, these types of combinations can compromise each other's properties to some extent. Additional trends for the simultaneous use of several contrast agents or radiopharmaceuticals to simultaneously evaluate the function of several tissues or diseases have been explored. It is highly predicted that improvements in the sensitivity of instruments and development of novel methods for the synthesis of contrast agents reduce the current limitations of imaging. In the clinical phase, more important roles should be assigned to these developments.

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