Multifunction in imaging

3.3.1. Magnetic resonance imaging

MRI uses hydrogen protons in tissue to form magnetic moments and excitation by a pulsed radiofrequency evacuation. Subsequently, the hydrogen proton will release the excitation energy received by radiofrequency coils and convert it to a grayscale image. MRI does not require injection of contrast agents, has no ionizing radiation, and has no adverse effects on the body, with excellent potential advantages for disease diagnosis. The multifunctional nanoparticles are introduced ferromagnetism or paramagnetic for enhancing tumor therapy with MRI-guiding. There are some MRI contrast agents that have been used in clinical or are undergoing clinical trials based on multifunctional backbone for treatment cancer. For example, SPIONS is injected to track the drugs for MRI cellular imaging of hepatic parenchyma (NCT04682847); Ferrotran® (a Fe_3O_4 preparation) is used to virtual histology of the bladder wall for bladder cancer staging (NCT04369560); and ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles are applied for measuring the activity of innate immune system within MS lesions (NCT05357833). Meanwhile, multifunctional nanoparticles containing SPIONs showed a typical darkening property under static magnetic fields because of the short transverse relaxation time (T_2) of protons. Thirunavukkarasu et al.prepared MIDEN with SPIONs (Fe_3O_4, SPIONs) and DOX for MRI-guided thermo-chemotherapy. In their design, SPIONs showed a typical darkening property under static magnetic fields due to the short transverse relaxation time (T_2) of protons and demonstrated that MIDEN could be used in MRI. After intratumoral injection of MIDEN in CT26 tumor-bearing mice, MRI signals were homogenously observed in the tumor region to achieve tumor imaging. Due to the reduced antiferromagnetic interaction, SPION multifunctional nanoparticles can be substituted by nonmagnetic atoms to enhance the MRI signal. For example, Gondan et al. prepared mZnSPION–polyIC–R837 based on PEGylated phospholipid-encapsulated $Zn_xFe_{3-x}O_4$ nanoparticles to deliver polyIC and the TLR7 agonist imiquimod (R837) for MRI and antitumor immunity effects. In their design, the overall magnetization and the spin–spin relaxation rate (R_2) of SPION could be optimized and significantly increased by zinc (II) doping, resulting in the improved MRI detection sensitivity of SPION. They illustrated that mZnSPION exhibited a strong MRI contrast effect with a transverse relaxivity (r_2) that was three times larger than mSPION. Due to the paramagnetic relaxed ability, multifunctional nanoparticles containing Mn^{2+} could be used for MRI with T_1 signals. Moreover, Chen et al.reported a biomimetic virus-like nanocomplex (SDN) composed of photosensitizer Ce6-loaded nanostructured lipid carrier and poly (allylamine hydrochloride)-functionalized MnO_2 nanoparticles. In their design, Mn^{2+} could be released quickly due to the presence of intracellular redox components. The SDN nanocomplex exhibited strong T_1 MRI signals, which were enhanced by Mn^{2+}.

3.3.2. **Fluorescence imaging**

FI uses a specific wavelength of laser light to irradiate fluorophores with fluorescent emission properties, absorb the light energy, and emit light for biological imaging. FI can be performed in vivo without puncturing the skin, and has the advantages of less influence on biological tissue, strong tissue penetration, and a high signal-to-noise ratio. Multifunctional nanoparticles with fluorescent emission properties can be used in FI with real-time tracking of drug delivery and release. In detail, multifunctional nanoparticles can be codelivered with bioactive agents containing luminescent groups to form nano prodrugs, such as

dioxetane. The dioxetane is unstable after being irradiated by NIR and forms two ketone structures located in the ground state and the excited state, respectively. When the excited ketone structure transitions back to the ground state, fluorescence is generated. For example, He et al.developed an organic afterglow protheranostic nanoassembly (APtN) for activating both pharmaceutical effects and diagnostic signals in the TME. The phenylboronic ester group of APtN could be cleaved by H_2O_2 to uncage the afterglow substrate and react with silicon 2,3-naphthalocyanine bis(trihexylsilyloxide) (NCBS) to form PEG–dioxetane. Due to the instability of PEG–dioxetane, fluorescence was generated under NIR. Their results showed that the afterglow of APtN in solution could be increased 820-fold after treatment with H_2O_2, causing permitting the real-time in vivo feedback for the status of prodrug activation. Additionally, multifunctional nanoparticles can be codelivered with persistent luminescence nanoprobes (such as rare-earth-doped nanoparticles), which can store photons in the traps by irradiating them with ultraviolet (UV) light and subsequently releasing the stored energy under thermal energy excitation. Kong et al.reported a persistent luminescence nanoprobe (TRZD) for the NIR imaging and therapy of glioma. Due to the Cr^{3+}/Sn^{4+} codoped $ZnGa_2O_4$, TRZD could cause persistent luminescence in NIR imaging under thermal energy excitation. Their results demonstrated that the luminescence of TRZD could last longer than the 7200 s and still maintain comparable intensity, and luminescence could also be renewed by UV light. Additionally, multifunctional nanoparticles with aromatic amino acids are intrinsically fluorescent under near-infrared light, such as tryptophan, tyrosine, and phenylalanine. Fan et al.prepared an NIR fluorescent peptide nanoparticle (RGD–f-P nanoparticles/EPI) with RGD and EPI for cell FI and target drug delivery. In their design, the cyclo [-($_D$-Ala-$_L$-Glu-$_D$-Ala-$_L$-Trp)$_2$-] peptide structure of f-P nanoparticles enabled it to emit visible and NIR fluorescence under 370 and 760 nm laser irradiation. Zinc coordination-limited energy dissipation during thermal relaxation pathways to obtain better quantum yield and fluorescence intensity. Their results showed that drug delivery to tumor sites could be monitored in vivo by the NIR fluorescence of RGD–f-P nanoparticles/EPI.

3.3.3. Photoacoustic imaging

PAI uses the photoacoustic effects of biological tissue to perform imaging. The imaging principle is that when biological tissue is irradiated by a pulsed laser, absorbed light energy, slight local heating and rapid thermal expansion results. This region of the biological tissue expands outward and generates acoustic waves to form a photoacoustic signal. The advantages of PAI include high resolution, strong contrast, and deep penetration, with excellent development potential in cancer therapy. Multifunctional nanoparticles are added with ultrasound for tracking drug release with PAI-guided cancer therapy. In detail, multifunctional nanoparticles with light absorption and high photothermal conversion efficiency could achieve PAI, such as inorganic materials (BP quantum dots). Black phosphorous quantum dots with unique layered structures could absorb laser light to convert heat energy and store the energy for PAI. For example, Li et al. prepared a multifunctional Fenton nanocatalyst (BCG) composed of Cu-doped BPQDs and GOx for cancer therapy with PAI guidance. Due to its unique layered structure and layer-dependent bandgap under 808-nm laser NIR, the BCG in BPQDs possessed strong absorption in the NIR region and allowed for the PAI. Their results suggested that PAI intensity was concentration-dependent and the PA signal was improved in the BCG NP solution with a concentration of 1 mg/ml. Additionally, multifunctional nanoparticles with photothermal conversion capabilities can also be used for PAI due to their ability to induce thermoelastic and ablative generation of ultrasound, such as PDA. Zhang et al. proposed the use of acorn-like Janus nanoparticles (PAA–mCaP/PDA–PEG J nanoparticles) based on mesoporous calcium phosphate for the

synergistic treatment of cancer with PAI-guided chemo-phototherapy. PDA possessed strong NIR absorption and high photothermal conversion efficiency under 808-nm laser illumination. Their results showed that the PA signal intensity increased gradually, reaching a peak after intravenous injection of these nanoparticles for 24 h in HepG-2 tumor-bearing mice. Moreover, multifunctional nanoparticles codelivered with metal materials can also induce PAI via photo-induced charge transfer, such as Mo (VI). Wang et al.reported a multifunctional nanoenzyme (*Ox*-POM@Cu) based on a polyoxometalate doping with Cu ions for NIR-II PAI-guided chemodynamical and PTT. The photo-induced charge transfer of Mo could realize PAI under 1064 nm laser illumination, leading to an increase in their absorbance in the NIR-II region. Their results showed that the PA signal was the strongest in tumor tissue after injection of these nanoparticles for 4 h in 4T1 tumors.

3.3.4. X-ray computerized tomography imaging

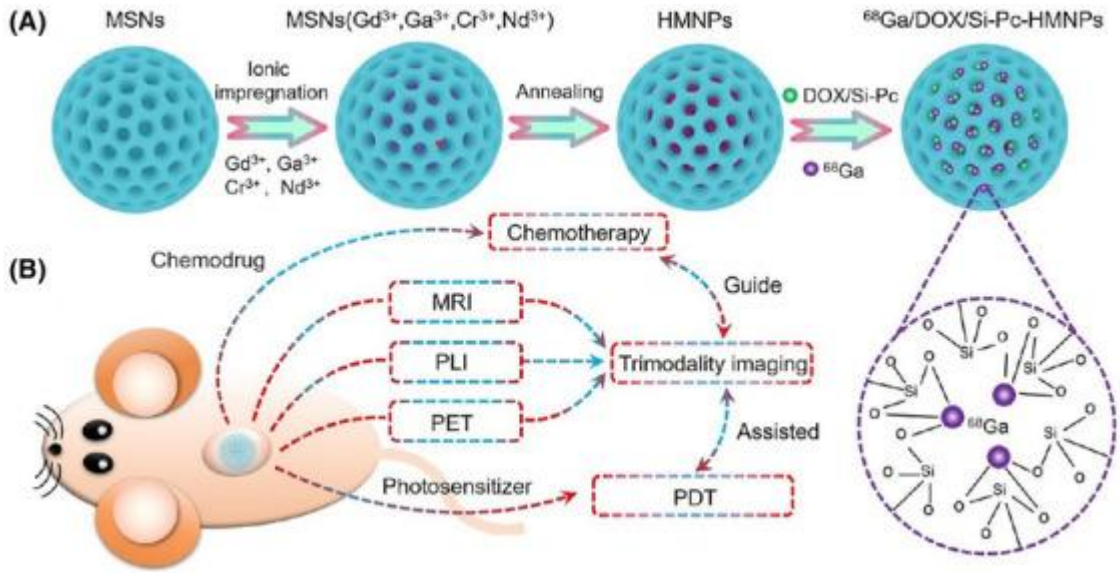
X-ray computerized tomography (CT) imaging is a medical imaging technique that uses the absorption properties of tissue under ray energy to realize tomographic imaging and visualize interior features of tissue. According to the structure and position of object, the intensity of X-ray energy could decrease and reflect as the grayscale images. CT imaging exhibits the great advantages of painless, noninvasive, and clear information. In addition, CT imaging is widely applied in cancer treatment and some iodinated contrast medium (ICM) are approved by US FDA, such as iohexol and iodixanol. Multifunctional nanoparticles modified with large X-ray attenuation coefficient could monitor drug effect and apply to CT imaging-guide cancer therapy. Due to the high electron density and atomic number, metallic nanoparticles show large X-ray attenuation coefficient used for CT contrast agents, such as Au, bismuth, barium, and gallium. For example, Wang et al.proposed a multifunctional nanosystem (Cu_3BiS_3–PEG–(Ce6–Gd^{3+})–FA NPs) using Cu_3BiS_3 NPs to anchor FA and chlorin e6 with Gd^{3+} for dual-modal CT and MR imaging guided cancer treatment. Bismuth element possessed excellent near infrared photothermal conversion performance and large X-ray attenuation coefficient, which made the prepared nanoparticles have excellent CT imaging performance. Their results indicated that these nanoparticles had a high X-ray absorption coefficient of 17.7 HU mmol Bi per L and the contrast of CT signal in the tumor was significantly enhanced after 4 h. Similarly, Zhao et al.also designed a Bi_2S_3-based multifunctional nanoparticle (FBPD NPs) for CT/PA dual-mode imaging-guided collaborative therapy of ovarian cancer. Moreover, iodides are small molecular with strong attenuation of X-ray absorption and have great compatibility in the body, which is usually used as contrast agent, such as iodinated nanoparticles. Fu et al. prepared LC@I-PANi nanoparticles through the combination of iodic acid and aniline monomers for CT imaging and PA imaging-guided PTT. In their design, iodine has a high atomic number, and the introduction of iodinated materials could produce image contrast due to different photoelectric absorption for enabling CT imaging. Their results showed that a strong CT signal was generated at the tumor site after intratumoral injection of LC@I-PANi in 4T1 tumor-bearing mice and LC@I-PANi possessed good biocompatibility in the body.

3.3.5. Multimodal imaging

Multimodal imaging is the organic superposition of multiple modal image information, which is better used in clinical research and treatment. Multimodal imaging has high sensitivity and resolution, which solves the limitations of a single imaging method and expands tumor imaging methods. In this method, multifunctional nanoparticles are added with multiple imaging capabilities to track the process of cancer therapy by multimodal imaging. In detail,

multifunctional nanoparticles with ferromagnetism or paramagnetic for MRI can be combined with photosensitizers for FI and PAI, such as Fe_3O_4 codelivery with MnO_2 and Ce6. The Fe^{3+} and Mn^{2+} ions have the short transverse relaxation time (T_2) of protons for MRI, and Ce6, with a tetrapyrrole ring structure, can absorb the NIR light for FI. Fan et al.developed a multifunctional nanoplatform (Fe_3O_4@MnO_2–CSL/Ce6) composed of Fe_3O_4 nanoparticles with MnO_2 coated on the surface, with celastrol and Ce6 for multimodal imaging-guided chemo-PDT. Due to the Fe^{3+}/Mn^{2+} ions and Ce6, these nanoparticles can significantly improve the magnetic resonance signal and have been used in FI of Bel-7402 tumor-bearing mice. The LSPR of Fe^{3+} and Mn^{2+} enabled Fe_3O_4@MnO_2–CSL/Ce6 for PAI under 850 nm wavelengths. Additionally, multifunctional nanoparticles could be codelivered with PFP, which could expand them from nanoscale nanobubbles to microbubbles to achieve the magnetic droplet vaporization (MDV) effect under alternating magnetic field (AMF) irradiation, resulting in further enhancement of USI. Wang et al.reported magnetic nanodroplets (Fe/Art-Lip@PFP) based on Fe_3O_4 and PFP encapsulated-liposomes and artesunate (Art) for enhancing the antitumor efficacy of ferroptosis with the guidance of multimodal imaging. The LSPR of Fe_3O_4 was useful for PA imaging under 690 nm wavelengths, and the results showed that the PA signal increased linearly with increasing concentrations of MNDs. MNDs filled with PFP could achieve a MDV effect under AMF irradiation to enhance USI. Moreover, multifunctional nanoparticles grafted with radionuclide that have paramagnetic properties and a photothermal conversion ability can be used for MRI/PET/FI. The Gd^{3+} radionuclides emitted positrons during its decay and could be used in PET imaging. Zou et al.developed multifunctional mesoporous nanoparticles (^{68}Ga/DOX/Si-Pc-HMNPs) composed of Ga_2O_3 (Cr^{3+}, Nd^{3+}), Gd_2O_3, and ^{68}Ga for multimodal imaging-guided PDT and chemotherapy (Figure 7). Their results showed that the longitudinal relaxivity (r_1) of HM nanoparticles was estimated to be 8.908 mM^{-1} s^{-1}, higher than that of a commercial positive contrast agent, indicating that HM nanoparticles are effective MRI-positive contrast agents. After intravenous injection of HM nanoparticles with UV lamp excitation, NIR-PL signals were detected in the whole LNCaP tumor-bearing mouse within 5 min. The ^{68}Ga of HM nanoparticles emitted positrons during their decay and could therefore be used in PET imaging.

FIGURE 7.



(A) The preparation of of ^{68}Ga/DOX/Si-Pc-HMNPs based on multifunctional mesoporous nanoparticles. (B) ^{68}Ga/DOX/Si-Pc-HMNPs could act as an “all-in-one” nanotheranostic tool for multimodal imaging-guided PDT and chemotherapy. Reprinted with permission from Ref. [500], Copyright 2021 American Chemical Society.