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REVIEW ARTICLE / DERLEME MAKALE

NANOPARTICLES FOR DUAL IMAGING: PET AND FLUORESCENCE IMAGING

İKİLİ GÖRÜNTÜLEMEDE NANOPARÇACIKLAR: PET VE FLORESANS GÖRÜNTÜLEME

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ABSTRACT

Objective: *Molecular imaging methods are gaining popularity in clinical and preclinical fields. There are many different imaging methods such as computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), single photon emission computed tomography (SPECT) and Near-infrared fluorescence (NIRF), and each has different advantages and disadvantages. Multimodal imaging methods, a combination of two or more molecular imaging modalities, have been developed to overcome the disadvantages of these molecular imaging methods. However, these imaging methods are conjugated with different vectors to improve the multimodal imaging methods used. In this field, drug delivery systems, peptides, proteins, antibodies and aptamers have been widely used for conjugation of multimodal imaging modalities to overcome some of the disadvantages that come from imaging modalities. In this review, PET and NIRF combination imaging modalities were explained and more specifically PET and NIRF nanoparticle dual imaging modalities with their pros and cons were investigated.*
Result and Discussion: *Dual imaging modalities overcome to limitations of single imaging modalities and provide a better understanding of biological, anatomical, and physiological processes. Multimodal imaging modalities offer higher sensitivity, resolution, and specificity with lower cost and toxicity although have several disadvantages.*
Keywords: *Dual imaging, fluorescence imaging, nanoparticles, PET, quantum-dots*

ÖZ

Amaç: *Moleküler görüntüleme yöntemleri klinik ve preklinik alanlarda popülerlik kazanmaktadır. Bilgisayarlı tomografi (BT), pozitron emisyon tomografisi (PET), tek foton emisyon tomografisi (SPECT), manyetik rezonans (MRI) ve yakın kızılötesi floresans (NIRF) görüntüleme gibi birçok farklı görüntüleme yöntemi vardır ve her birinin farklı avantaj ve dezavantajları vardır. Bu moleküler görüntüleme yöntemlerinin dezavantajlarının üstesinden gelmek için iki veya daha fazla moleküler görüntüleme yönteminin bir kombinasyonu olan multimodal görüntüleme yöntemleri geliştirilmiştir. Bununla birlikte, bu görüntüleme yöntemleri, kullanılan multimodal görüntüleme yöntemlerini geliştirmek için farklı vektörlerle konjuge edilmiştir. Bu alanda görüntüleme yöntemlerinden kaynaklanan bazı dezavantajların üstesinden gelmek için multimodal görüntüleme yöntemlerinin konjugasyonunda ilaç taşıyıcı sistemler, peptitler, proteinler, antikorlar ve aptamerler yaygın olarak kullanılmaktadır. Bu derlemede PET ve NIRF kombinasyonlu görüntüleme modaliteleri anlatılmış ve daha spesifik olarak PET ve NIRF nanoparçacık ikili görüntüleme yöntemleri artıları ve eksileri ile incelenmiştir.*

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Sonuç ve Tartışma: *İkili görüntüleme yöntemleri, tek görüntüleme yöntemlerinin sınırlarını ortadan kaldırır ve biyolojik, anatomik ve fizyolojik süreçlerin daha iyi anlaşılmasını sağlar. Çoklu görüntüleme yöntemleri birkaç dezavantajı olmasına rağmen, düşük maliyet ve toksisite ile birlikte daha yüksek hassasiyet, çözünürlük ve özgüllük sunar.*
Anahtar Kelimeler: *Floresan görüntüleme, ikili görüntüleme, kuantum noktaları, nanopartikül, PET*

INTRODUCTION

Molecular imaging mainly provides the investigation of molecular abnormalities of diseases, not only the differences in the molecular stage. It was defined as an *in vivo* characterization and biological process measurements at a molecular level by Weissleder and Mahmood. Biomarkers, essential for molecular imaging, are used for targeting biological systems and provide high specificity and sensitivity [1,2]. Molecular imaging modalities such as CT, MRI, PET, SPECT, and optical imaging are showing increasing popularity in both clinical and preclinical areas [3]. Especially multimodal imaging, which combines two or three molecular imaging methods, has gained significant importance due to overcoming individual limitations [3,4]. Multimodal imaging techniques have been used to monitor structural, functional, and molecular changes, quantify, and identify biological processes at cellular and molecular levels in living organisms [4]. The limitation of each imaging modality can be overcome by combining different imaging techniques and providing better images in preclinic and clinic applications (Table 1).

Table 1. Molecular imaging modalities [5-7]

	PET	**SPECT**	**CT**	**MRI**	**NIRF**
Form of Energy	Annihilation photons	Gamma rays	X-rays	Radio frequency ways	Infrared light
Spatial Resolution (mm)	1-5	0.5-15	0.5-1	0.01-0.1	<1
Temporal Resolution	min	s-min	s-min	min-h	s-min
Penetration Depth	unlimited	unlimited	unlimited	unlimited	< 2 cm
Sensitivity	10^{-11}-10^{-12} M	10^{-10}-10^{-11} M	10^{-3} M	10^{-3}-10^{-15} M	10^{-9}-10^{-12} M
Cost	High	Medium-high	Medium	High	Low

CT is the technique that produces images depending on the different attenuation of X-rays by tissues. It is a common method used in clinical and shows high resolution, penetration, and fast acquisition time with low cost. However, high radiation doses and low quality of soft tissues are the main limitations of this imaging technique [4].

MRI is the common imaging method in radiology and provides good detection and characterization for soft tissue, unlike CT. This method shows high spatial resolution with high cost and low sensitivity [8,7]. MRI and CT imaging modalities provide better anatomic images and molecular changes [9].

Nuclear imaging techniques, PET and SPECT imaging, are the most common imaging modalities. SPECT is based on the detection of gamma rays decaying from gamma-emitting radionuclides. PET imaging method detects gamma rays from the two gamma photons (180°C direction) after the annihilation reaction between the electrons and positrons of PET radionuclides. These nuclear imaging methods show high sensitivity and quantification but also have a poor resolution [5]. PET and SPECT are also essential imaging modalities for personalized medicine and imaging models. They are the most common modalities for detecting diseases and monitoring treatments [4].

The optical imaging method is based on the detection of fluorophores, emitting the fluorescence after optical excitation by an optical microscope. This method shows high sensitivity and multiplexed imaging with low or medium-high costs, although the energies of fluorescence imaging are limited to

penetrate the tissues [7].

Nanotechnology, which is generally smaller than 100 nm, has been showing great importance for several decades. It is also one of the research subjects used as a medicine for diagnostic, therapeutic, and theranostic purposes. Nanotechnology applications in medicine provide the elimination of some deficiencies in conventional drug applications [10]. Organic and inorganic nanoparticles have been used in several imaging modalities [11].

In this paper, dual imaging modality (PET and fluorescence imaging combination) by using nano-size delivery systems and their advantages and disadvantages are reviewed.

Fluorescence Imaging

NIRF imaging, the most common optical imaging technique, is using for shallow lesion and superficial object imaging [12]. Fluorescent dyes emitting in the NIRF area are mainly divided into two regions: NIRF first window (NIRF-I: 700-900 nm) and NIRF second window (NIRF-II: 1000-1800 nm) [13,14]. The excitation photon travels and reaches the NIRF agents, and in the end, photon absorbance occurs depending on the absorbing components of tissues and organs [15]. The main absorbing components are water, lipids, oxy, and deoxy hemoglobins [16]. Also, the NIRF imaging method depends on many parameters such as dye properties, excitation light properties, biodistribution and pharmacokinetic properties of the imaging system, targeting tissue and cell properties, etc. [16]. The most common fluorophores are fluorescein isothiocyanate (FITC), cyanine and cyanine derivatives, pacific blue and alexa fluor. However, new dyes are investigated by researchers due to their limited photostability [17]. Rhodamines, fluorescein, boron-dipyrromethene, and cyanine dyes are commercial fluorescent dyes, and they are currently on the market [17].

NIRF imaging technique is getting more attention day by day due to their high sensitivity, high spatial and temporal resolution, low optical absorption, and scattering features [18]. Also, fluorescence imaging properties provide some functional features about the activity of molecules [19]. However deep tissue penetration is the main obstacle to clinical use [20]. The main problem of fluorescence imaging is the instability of the fluorophores [21]. Their stability problem affects the fluorescence signal in imaging techniques. Also, this technique faces some difficulty in imaging living organisms due to a lack of deep tissue penetration (Table 2) [11].

Table 2. Advantages and disadvantages of fluorescence imaging technique

Advantages	**Disadvantages**
Deep penetration in tissues and organs	Instability of fluorescent dyes
High sensitivity	Non-specific adsorption to proteins
High resolution	Rapid degradation

Drug delivery systems have been used for NIRF imaging to overcome these disadvantages. These systems could help to overcome the instability of the fluorescent dyes and the rapid degradation of these dyes. Another advantage of drug delivery systems in NIR imaging is higher loading capacity, providing better resolution [22].

Fluorescent dyes are generally placed in the core of nanoparticles which is protected by the shell from photobleaching. Quantum dots (QDs), carbon-based nanoparticles, silica nanoparticles, fluorescent dyes encapsulated nanoparticles, liposomes, and other drug delivery systems are mainly used for fluorescence imaging [23,24].

Pet Imaging

PET imaging is one of the nuclear medicine imaging modalities based on PET radionuclides, which are traditional radionuclides such as ^{18}F, ^{13}N, ^{15}O, and radiometals such as Gallium-68 (^{68}Ga), Zirconium-89 (^{89}Zr), Cupper-64 (^{64}Cu), etc. [25]. The positron, which is emitted from positron-emitting radionuclides, travels and is annihilated with electron [25]. After this annihilation reaction, two 511 keV gamma rays occurred in the opposite direction (180°C apart) and were detected by the PET scanner (Figure 1). [26]

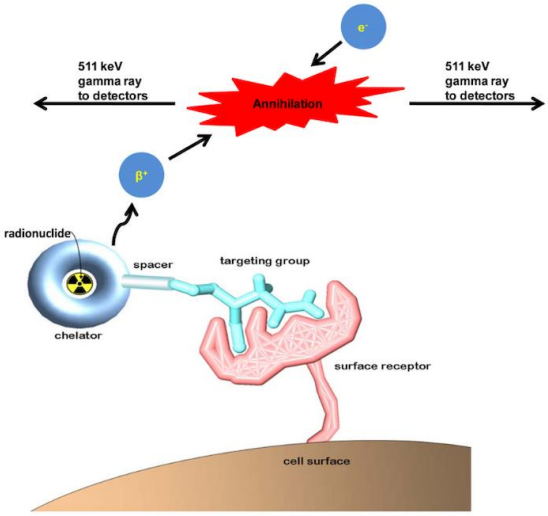

Figure 1. PET imaging principle [26]

PET imaging modality provides some advantages such as better resolution and high sensitivity; however, spatial resolution is the limitation due to the physical characteristics of the scanners (Table 3) [26,27]. Also, the sensitivity is highest among all imaging modalities without any depth limitation [27].

Table 3. Advantages and disadvantages of PET imaging technique

Advantages	**Disadvantages**
High sensitivity	Expensive method
High resolution	Low spatial resolution (about 5mm)
Three dimensional functional method	

The gold standard PET radiopharmaceutical, ^{18}F- fluorodeoxyglucose (FDG), a glucose analog, provides higher tumor uptake depending on the tumor tissue and overexpression on tumor area [9]. FDG PET scans all body with a single injection dose with 96% sensitivity and 77% specificity [28]. This imaging modality is used to detect dynamic changes in the body. It can also be used for basic physiological and molecular mechanisms [29]. Drug delivery systems can be radiolabelled with different radionuclides for many applications besides conventional PET imaging radiopharmaceuticals[30]. Nanoparticles can be radiolabelled with different methods (direct and indirect radiolabelling methods) depending on the various parameters such as half-lives of nanoparticles and radionuclides, energies of radionuclides, and properties of both nanoparticles and radionuclides [30].

Direct radiolabelling methods occurred attachment, incorporation, or encapsulation of radionuclides to nanoparticles. The interactions between nanoparticles and radionuclides can happen with physical interactions such as electrostatic interaction. This method is used mostly for nonmetallic PET radionuclides. Indirect radiolabelling methods, generally preferred ones, require chelators. These chelators act as a bridge between nanoparticles and radionuclides [30,31]. The chelators, also called bifunctional chelators, bind the radionuclides and nanoparticles; because of this, chelator choice is one of the most critical steps [30]. The most common bifunctional chelators for PET radionuclides are 2,2',2'',2'''-(1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetrayl)tetraacetic acid (DOTA), 1,4,7-triazacyclononane-N,N',N''-triacetic acid (NOTA), diethylenetriamine-N,N,N',N-pentaacetic acid (DTPA) and desferrioxamine (DFO) [30].

Combination of PET and NIRF Imaging

Dual imaging modalities, especially for diagnosing and monitoring diseases, provide many advantages due to taking advantage of each modality. NIRF imaging modalities have been frequently

used for dual imaging due to their complementary properties (Figure 2) [32]. NIRF's main principle is the selective and repeated activation of dyes with excitation light, while PET imaging is based on the detection of 511 keV gamma energies by the camera [21].

The combination of NIRF and PET imaging is improved the imaging quality and provides better sensitivity, specificity, and real-time visualization for preclinical and clinical applications [33]. These combination imaging agents can be used for surgical planning of whole-body imaging and molecular guiding for surgery, and also provide the correlation between these two imaging, which are for surgical planning and guiding [34].

PET-NIRF dual imaging agents can be synthesis different methods such as coupling and conjugation reactions, and several points should be considered before the synthesis studies:

1. Reaction times should be short if the short-lived radionuclides are used,
2. Reaction should result in as high a yield as possible due to expenses of materials and limited radioactivity,
3. Radiolabelling steps should be controlled because any reaction may occur that cause stability problem for fluorescent dyes.

The stability and optical properties of NIRF dyes can affect the quality and sensitivity of dual imaging, including PET radionuclides and NIRF dyes. Thus, researchers should pay attention to the radiolabelling steps and conditions; and the factors such as radiolabelling conditions, radiolysis, and interactions between the radionuclides and fluorescent dyes should be considered [35].

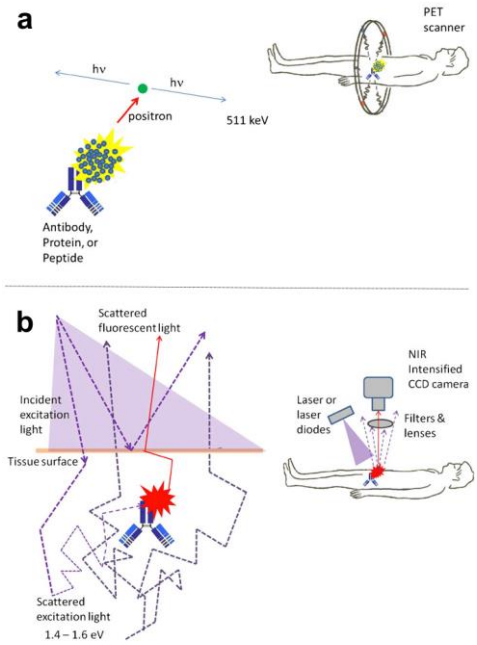

Figure 2. Schematic representation of PET imaging (a) and NIRF imaging (b) modalities [34] [Addepted from Azhdarinia et al. (2011)]

Nanoparticles with PET and NIRF Imaging

Dual imaging modalities with nanoparticles provide deep tissue detection of disease for image-guided surgery owing to PET imaging and also provide tissue resection owing to NIRF imaging [36]. Nanoparticles radiolabelled with PET radionuclides and conjugated or encapsulated fluorescent dyes can be used for tracking macrophages and detecting diseases [36].

Ariztia and co-workers explained the synthesis methods of PET and Fluorescence dual imaging agents with nanoparticles. Synthesis methods were divided into three main categories: 1. Dye approach; 2. Iterative approach; 3. Simultaneous approach (Figure 3) [27].

Dye Approach; This approach is not commonly used because radionuclides-bifunctional agent-fluorescent dyes synthesis is challenging. The radionuclides and fluorescent dye conjugation is in the first step and then followed by the conjugation of this dual imaging and nanoparticles. However, if radiometals will use for PET imaging, radiolabelling process is completed after fluorescent dyes-nanoparticles conjugation [27].

Iterative Approach; This approach is the most commonly used method for the dual imaging agent with radiometals. This strategy allows the radiolabelling in different steps. Thus, this convenience provides the prevention of chemical degradation and instability [27].

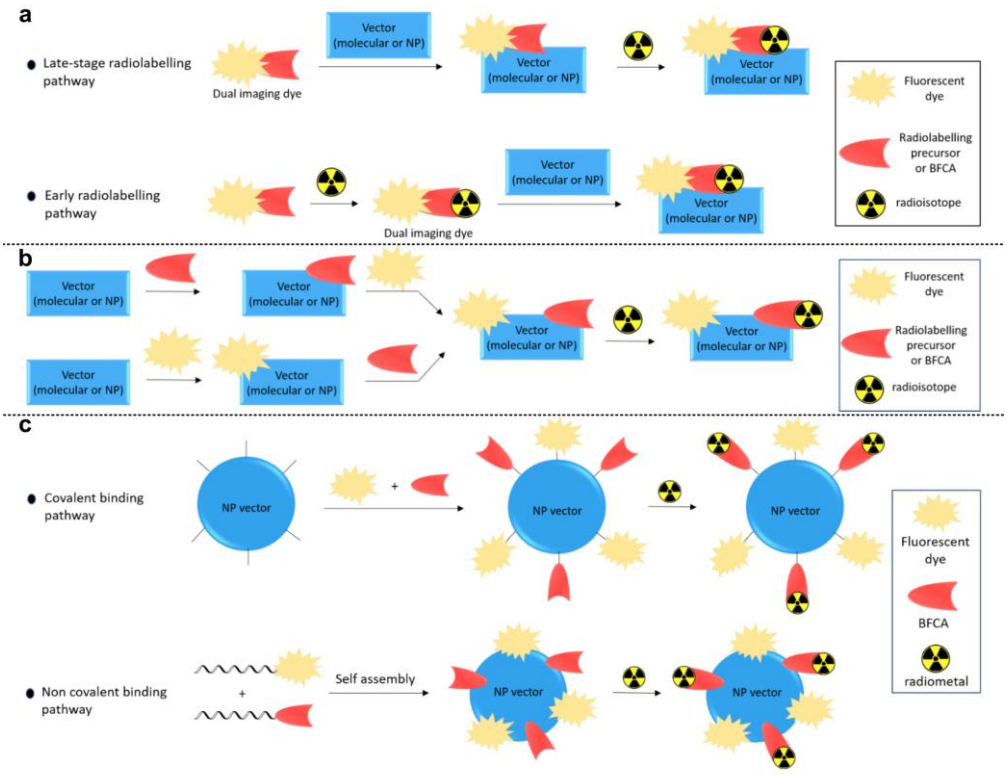

Figure 3. PET and NIRF dual imaging nanoparticle agents synthesis methods (a) dye approach (b) iterative approach (c) simultaneous approach [27] [Adapted with permission from Ariztia et al. (2022) American Chemical Society]

Simultaneous Approach; Covalent and non-covalent binding plays the primary role in this approach. Briefly, fluorescent dyes and bifunctional agents directly bind the surface of nanoparticles with covalent bind. This approach can only be used for radiometals and nanoparticles [27].

Imaging with contrast agents is essential in diagnosis, monitoring, and therapy for distinguishing physiological processes from anatomical processes. Although it has not yet had an Food and Drug Administration (FDA) approved agent, this imaging method is promising, and many preclinical studies have been reported in the literature [17]. PET/NIRF dual imaging combination in nano-size also has promising applications.

Recent studies show nanocrystals have a great potential for optical imaging [37]. QDs are fluorescent nanocrystals between 1-10 nm in size. They have optical and electrical properties and show better stability than other NIRF imaging nanoparticles. QDs consist of an inorganic core and inorganic shell structure [24,16]. They are not water-soluble due to their hydrophobic nature, and surface conjugation should be necessary to make them water-soluble probes[38]. Different types of molecules such as peptides[39], folates[40], dextrans[41,42], aptamer[43], antibodies[44], monoclonal antibodies (mAbs)[45] and commonly polyethylene glycol (PEG) can be conjugated [43,46].

Many studies have been conducted combining QDs with many different imaging methods [47]. NIRF with QDs and PET imaging combination could provide more information about the pharmacokinetics of NIRF QDs [33]. One of the main concerns of QDs imaging agents is the toxicity due to Cadmium (Cd) in QDs. Dual imaging could also solve this problem and decrease the toxicity of QDs [48]. ^{64}Cu radiolabelled PET/NIRF QDs showed significantly lower toxicity potential compared with the NIRF QDs. PET/NIRF imaging agents QDs require smaller amount for tumour imaging due to PET radionuclides, thus, the potential of toxicity decreases [33,49]. The toxicity levels of QDs depend on their size, charge, concentration, other bound groups or coats, and their stability [50,51]. Smaller QDs could be prepared with the combination of PET and NIRF imaging, which provide lower toxicity and reticuloendothelial system (RES) uptake herewith better imaging quality [52]. 21 nm (large) and 12 nm (small) size QDs biodistribution studies did not show any significant biodistribution differences between them. On the other hand, Cd, the reason for the toxicity of QDs, was not detected in *ex-vivo* studies of ^{18}F radiolabelled PEGylated QDs [53].

Studies showed that QDs can be radiolabelled with successful and high yield. Cai and co-workers (2007) radiolabelled the QD s with ^{64}Cu more than 90% yield. ^{64}Cu radiolabelled QDs were compared (in large and small sizes) with and without PEGylation. Both non-PEGylated QDs showed rapid uptake (2 mins) into the liver and spleen, unlike PEGylated QDs (6 mins). QDs size did not affect the biodistribution in their study, which is an unexpected result. However, size can be helpful in RES clearance, and smaller size QDs can affect the RES clearance and can help improve the NIRF image quality [33,38].

Peptide modified ^{18}F labelled QDs (^{18}F-Fluoropropionly (FP)-QD-arginine-glycine-aspartate acid (RGD)-bombesine (BBN)) were evaluated *in vitro* and *in vivo* for tumour detection/accumulation, imaging, biodistribution and compared with same QDs without ^{18}F-labelling (QD-RGD-BBN). ^{18}F-FP-QD-RGD-BBN showed higher uptake in kidney, liver and bladder unlike QDs due to metabolic stability of QD-RGD-BBN. ^{18}F-FP-QD-RGD-BBN dual imaging agent showed reduced toxicity and lower tissue penetration [49].

Radiolabelled QDs can also be used for *in vitro* and *in vivo* imaging. However, only a few *in vivo* imaging studies have been published. QDs show high photostability and brightness with changeable size and fluorescence wavelengths but for *in vivo* imaging, QDs should be more specific and effective to the targeted areas and organs [33]. The shortcomings in the acquired images have increased the search for dual imaging modalities for QDs. Also, tracking and quantification and, consequently, the biodistribution studies of the QDs *in vivo* by NIRF imaging are very limited due to their deep tissue penetration problems. However, due to the heavy metal toxicity of QDs, silica and carbon-based nanoparticles have been developed. Moreover, other nanoparticles may be more advantageous than QDs because their size can be adjusted easily, they can be formed from different materials, and they can be conjugated easily with different groups, which are helpful for desired circulation time [54].

NIRF dye encapsulated nanoparticles are also photostable systems that can overcome the stability problems of NIRF dyes. Moreover, prolonging the circulation half-life of NIRF dyes is the other primary advantage of this system [23]. Lee and co-workers developed the glycol chitosan-based nanoparticles for NIRF and PET dual imaging. Researchers indicated that the dual imaging agent developed by them provides biological features of the tumor as well as quantitative information on tumor targeting [19]. Moreover, dual imaging nanopartics provide better description for biodistribution for tumuors. PET images show better signal to noise ratio compared to NIRF images, however, NIRF provides *in vivo* and *ex vivo* visualization [55].

Silica-based nanoparticles show great potential for clinical applications in the future due to their biocompatibility. FDA also indicated silica-based nanoparticles as “Generally Recognized As Safe”. Silica nanoparticles, dense silica nanoparticles ($dSiO_2$), new generation $dSiO_2$ based Cornell prime dots (C’ dots), mesoporous silica nanoparticles (MSN), and hollow mesoporous silica nanoparticles (HMSN) are considered as a silica-based nanoparticles and are widely investigated for imaging properties. Several studies in radiolabelling silica-based nanoparticles with different radionuclides have been reported [56-59]. They can also be used for NIRF imaging by entrapment of fluorescence dyes [60]. This is an ideal drug delivery system for fluorescence dyes because it is photochemically inert and allows for the excitation and emission of light [23]. Other advantages of this drug delivery system are the water-

dispersible and microbial-resistant features. Their silica matrix allows the light to pass through and also protects the dyes from degradation [61]. Silica nanoparticles are biocompatibility, non-toxic characteristics, and easy modification with other molecules [22]. Different types of dual imaging with silica nanoparticles tagged/loaded with varying types of materials for targeting purposes are been investigated for primer or metastatic tumours. Many studies about aptamer, protein, and antibody conjugated silica nanoparticles for multimodal imaging using PET and NIRF systems have been reported for cancer imaging/therapy. One of them about aptamer-functionalized ^{64}Cu radiolabelled silica nanoparticles, was reported by Tang and co-workers (2012). Mono-disperse aptamer conjugated silica nanoparticles with 20 nm size for PET/NIRF imaging showed advantages for lymphatic imaging. Overcoming the depth insensitivity and low spatial resolution of each imaging modality with dual imaging showed potential for resection of metastatic lymph nodes [62].

MSNs have been widely investigated for drug and gene delivery systems, bioimaging, and cell markers due to their high drug-loading capacity, large surface area/high-surface modification, low toxicity and high stability [63]. MSNs are quite popular system for PET/NIRF imaging [64,65]. ^{64}Cu (PET radionuclide) and 800 CW (fluorescent dye) labelled targeted MSN were synthesized successfully and pharmacokinetics and targeting efficacy were evaluated *in vitro* and *in vivo* by using dual imaging modalities. Results showed that PET/NIRF MSN as a dual imaging agent could be a promising agent for imaging and also could be used for providing more information about accumulation, pharmacokinetics and targeting efficacy of agents [66].

MSN also have appropriate shell-thickness and size with protection for drugs. Hence, ^{64}Cu radiolabelled CuS@MSN nanoparticles tagged with TRC105 were synthesized for theranostic purposes. CuS nanoparticles were coated with MSN and after that, the anticancer drug was loaded into the CuS@MSN. It was followed by ^{64}Cu radiolabelling procedures for the purpose of the evaluation of their biodistribution and pharmacokinetics. Chen and co-workers (2015), mentioned that ^{64}Cu-CuS@MSN with a TRC105 tag was evaluated as a unique theranostic nanoparticle which provides *in vivo* active tumour targeting [67].

HMSN, which show a large drug loading capacity compared with mesoporous silica nanoparticles, were also used for dual imaging modalities [68]. Chen et al. (2014) reported the successfully prepared HMSN for PET and NIRF dual imaging purposes [68]. The chimeric antigen receptor (CAR) T cells tagged PET/NIRF silica nanoparticles were synthesized with success by Harmsen and co-workers (2021). ^{89}Zr radiolabelled NIRF silica (CF680R loaded) nanoparticles, were investigated for long-term whole-body CAR T cell tracking to provide a better understanding of CAR-T cell therapy and its limitations. CAR-T cell-tagged PET/NIRF nanoparticles provided whole-body tracking for 1 week. However, PET/NIRF nanoparticles were released from CAR-T cells after a week post-administration [69].

C'dots, inorganic silica nanoparticles, are Cy5 containing systems which can be used for NIRF images. One of the C'dots type which is ^{124}I-cyclic arginine-glycine-aspartic acid (cRGDY)-PEG-C'dots has been already approved by FDA Investigational New Drug for integrin-expressed cancer imaging This ultra small size nanoparticles evaluated as a bulk renal clearance, appropriate pharmacokinetics, excellent dual imaging modality without acute toxicity [70]. Following that, cRGDY-PEG-C'dots labelling studies with different radionuclides such as ^{89}Zr, ^{131}I etc. have been performed [70,57]. ^{124}I-cRGDY-PEG-C'dots were evaluated *in-vitro* and *in-vivo* by Benezra et al. (2011). In this study, Benezra and co-workers successfully obtained the binding affinity and levels of receptor expression, pharmacokinetic and clearance profiles, dosimetry and blood/tissue ratio of ^{124}I-cRGDY-PEG-C'dots. Results proved that ^{124}I-cRGDY-PEG-C'dots showed advantages in metastatic cancers [71]. ^{124}I-cRGDY-PEG-C'dots were also studied by Phillips and coworkers and this study were describes as a first-in-human trial of C'dots [72]. Phillips and co-workers investigated its safety profiles and also pharmacokinetics by using PET imaging. 124I-cRGDY-PEG-C'dots showed accumulation at tumour site with different pharmacokinetic with good clearance without RES uptake [72]. cRGDY-PEG-C'dots have already been labelled with 89Zr to evaluate as an agent for cancer detection and compare radiolabelling strategies by *in vitro* and *in vivo*. Results proved that ^{89}Zr-cRGDY-PEG-C'dots showed a great potential for clinical studies [57].

Liposomes are unilamellar lipid bilayer drug delivery systems that can trap the hydrophilic and lipophilic molecules in the central core and lipid bilayer [73]. This system is commonly used and preferred because of its bio-compatible, non-toxic, and biodegradable features [18]. Also, their encapsulation ability, modification property, and transportation ability into the tumor are the other reasons for common use [74]. Due to these features, many liposomes have been approved by FDA, and many of them are also available in the market [75]. Liposomes are suitable systems for dual imaging due to their encapsulation and/or attachment capability of different types of molecules [18,76]. Perez-Medina and co-workers developed dual imaging agents, ^{89}Zr radiolabelled Cy5 dyes encapsulated liposomes. Mainly, two different radiolabelling chemistry were investigated, and after that, NIRF dyes were encapsulated into the lipid bilayer. Results proved that dual imaging agents could be prepared with high stability [74].

Table 4. PET/NIRF dual imaging nanoparticles mentioned in this article

	PET radionuclides	**NIRF dyes**	**Dual imaging agent**	**Reference**
QDs	^{64}Cu	-	^{64}Cu-labeled DOTA–QD	[33]
	^{64}Cu	-	^{64}Cu-DOTA-QD525 ^{64}Cu-DOTA-QD800 ^{64}Cu-DOTA-QD525PEG ^{64}Cu-DOTA-QD800PEG	[38]
	^{18}F	-	^{18}F-FP-QD-RGD-BBN	[49]
	^{18}F	-	^{18}F-QDs	[53]
Silica Nanoparticles	^{64}Cu	NC200 NC20	^{64}Cu-NC200 silica nanoparticle ^{64}Cu-NC20 silica nanoparticle	[62]
	^{64}Cu	800CW	^{64}Cu-800CW-MSN	[66]
	^{64}Cu	CuS	^{64}Cu-CuS@MSN-TRC105	[67]
	^{64}Cu	ZW800	^{64}Cu-HMSN-ZW800-TRC105	[68]
	^{89}Zr	CF680R	^{89}Zr-silica nanoparticle	[69]
Chitosan Nanoparticles	^{64}Cu	Cy5.5	^{64}Cu-DOTA-Lys-PEG$_4$-DBCO	[19]
C'dots	^{124}I 131I	Cy5	cRGDY-PEG-Cy5-C'dots	[70]
	^{89}Zr	Cy5	^{89}Zr-DFO- cRGDY-PEG-C'dots	[57]
	^{124}I	Cy5	^{124}I -cRGDY-PEG-Cy5-C'dots	[71,72]
Liposomes	^{89}Zr	DiIC@DFO-L (Cy5 analog 1,1-diododecyl-3,3,3,3-tetramethyl-indodicarbocyanine-5,5-disulfonic acid)	89Zr-liposomes	[74]
	^{64}Cu	IRDye800CW	Liposome-DOX- ^{64}Cu/800CW	[78]

PET and NIRF imaging systems can also be very attractive dual imaging modalities to evaluate in different ways the therapy of drug delivery systems. Lobatto and co-workers used dual imaging modalities to understand the atherosclerosis therapy success of liposomes. Biodistribution and vessel

wall targeting of liposomes were evaluated by PET/CT images, while vascular permeability was evaluated by NIRF imaging. In the clinics, PET/CT imaging systems are used to provide a better understanding of *in vivo* behavior of drug delivery systems [77]. Du and co-workers (2017) studied PD-1 specific doxorubicin loaded, NIRF dye, and ^{64}Cu labeled liposomes to evaluate the tumor detection sensitivity and also therapy approach. Researchers aim to monitor the pharmacokinetics of this agent and also to understand the antitumor activity of the PD-1 targeted doxorubicin-loaded liposomes. The reason for using PET and NIRF combination imaging is the advantages of using both imaging modalities to understand tumor therapy (Table 4) [78].

RESULT AND DISCUSSION

Multimodal imaging modalities have been widely investigated in different combinations such as PET/MRI, SPECT/MRI, PET/optical imaging, etc. The limitations of single imaging modalities can be overcome with these combinations and provide a better understanding of biological, anatomical, and physiological processes. Multimodal imaging modalities offer higher sensitivity, resolution, and specificity with lower cost and toxicity. However, some limitations still exist or still need to develop. Drug delivery systems in nanosize have important advantages in multimodal imaging due to their large surface area, high loading and modification capability, and extended circulation half-life properties. Also, some nanoparticles provide better stability results for NIRF imaging dyes. The combination of PET and NIRF imaging has shown important advantages compared to single imaging modalities and also could be promising properties for clinical application. Moreover, nanoparticles in PET/NIRF dual imaging provide many benefits in preclinical and clinical stages and overcome many of the disadvantages that come from PET/NIRF imaging modalities, such as better stability and blood circulation times, and better images.

Nanoparticles in PET/NIRF, dual imaging modalities, have been found as a promising research area and have an excellent potential for preclinical and clinical applications. However, more research should be done in this field, especially radiolabelled and fluorescent dyes encapsulated nanoparticles should be investigated due to their biocompatible features.

AUTHOR CONTRIBUTIONS

Concept: E.T.S.; Design: E.T.S.; Control: E.T.S.; Sources: E.T.S.; Materials: E.T.S.; Data Collection and/or Processing: E.T.S.; Analysis and/or Interpretation: E.T.S.; Literature Review: E.T.S.; Manuscript Writing: E.T.S.; Critical Review: E.T.S.; Other: -

CONFLICT OF INTEREST

The authors state that there are no actual, potential, or perceived conflicts of interest for this paper.

REFERENCES

1. Beckmann, N., Kneuer, R., Gremlich, H.U., Karmouty-Quintana, H., Blé, F-X., Müller M. (2007). *In vivo* mouse imaging and spectroscopy in drug discovery. NMR in Biomedicine, 20, 154-185. [CrossRef]
2. Weissleder, R., Mahmood, U. (2001). Molecular imaging. Radiology, 219, 316-333. [CrossRef]
3. Cui, X., Green, M.A., Blower, P.J., Zhou, D., Yan, Y., Zhang, W., Djanashvili, K., Mathe, D., Veres, D.S., Szigeti, K. (2015). $Al(OH)_3$ facilitated synthesis of water-soluble, magnetic, radiolabelled and fluorescent hydroxyapatite nanoparticles. Chemical Communications (Camb), 51, 9332-9335. [CrossRef]
4. Wu, M., Shu, J. (2018). Multimodal molecular imaging: Current status and future directions. Contrast Media & Molecular Imaging, 2018, 1382183. [CrossRef]
5. Ge, J., Zhang, Q., Zeng, J., Gu, Z., Gao, M. (2020). Radiolabeling nanomaterials for multimodality imaging: New insights into nuclear medicine and cancer diagnosis. Biomaterials, 228,119553. [CrossRef]
6. Smith, B.R., Gambhir, S.S. (2017). Nanomaterials for *in vivo* imaging. Chemical Reviews, 117, 901-986. [CrossRef]
7. Xing, Y., Zhao, J., Conti, P.S., Chen, K. (2014). Radiolabeled nanoparticles for multimodality tumor imaging. Theranostics, 4, 290-306. [CrossRef]
8. Blamire, A.M. (2008). The technology of MRI-the next 10 years? British Journal of Radiology, 81, 601-

617. [CrossRef]
9. Karpuz, M., Silindir-Gunay, M., Ozer, A.Y. (2018). Current and future approaches for effective cancer imaging and treatment. Cancer Biotherapy and Radiopharmaceuticals, 33, 39-51. [CrossRef]
10. Sarcan, E.T., Silindir-Gunay, M., Ozer, A.Y. (2018). Theranostic polymeric nanoparticles for NIR imaging and photodynamic therapy. International Journal of Pharmaceutics, 551, 329-338. [CrossRef]
11. Ali, Z., Abbasi, A.Z., Zhang, F., Arosio, P., Lascialfari, A., Casula, M.F., Wenk, A., Kreyling, W., Plapper, R., Seidel, M., Niessner, R., Knoll, J., Seubert, A., Parak, W.J. (2011). Multifunctional nanoparticles for dual imaging. Analytical Chemistry, 83, 2877-2882. [CrossRef]
12. Bao, G., Mitragotri, S., Tong, S. (2013). Multifunctional nanoparticles for drug delivery and molecular imaging. Annual Review of Biomedical Engineering, 15, 253-282. [CrossRef]
13. Bhavane, R., Starosolski, Z., Stupin, I., Ghaghada, K.B., Annapragada, A. (2018). NIR-II fluorescence imaging using indocyanine green nanoparticles. Scientific Reports, 8, 14455. [CrossRef]
14. Gao, D., Hu, D., Liu, X., Zhang, X., Yuan, Z., Sheng, Z., Zheng, H. (2020). Recent advances in conjugated polymer nanoparticles for NIR-II imaging and therapy. ACS Applied Polymer Materials, 2, 4241-4257. [CrossRef]
15. Alfano, R.R., Demos, S.G., Gayen, S.K. (1997). Advances in optical imaging of biomedical media. Annals of the New York Academy of Sciences, 820, 248-270. [CrossRef]
16. Frangioni, J.V. (2003). *In vivo* near-infrared fluorescence imaging. Current Opinion in Chemical Biology, 7, 626-634. [CrossRef]
17. An, F.F., Chan, M., Kommidi, H., Ting, R. (2016). Dual PET and near-infrared fluorescence imaging probes as tools for imaging in oncology. American Journal of Roentgenology, 207, 266-273. [CrossRef]
18. Li, S., Goins, B., Zhang, L., Bao, A. (2012). Novel multifunctional theranostic liposome drug delivery system: Construction, characterization, and multimodality MR, near-infrared fluorescent, and nuclear imaging. Bioconjugate Chemistry, 23, 1322-1332. [CrossRef]
19. Lee, S., Kang, S.W., Ryu, J.H., Na, J.H., Lee, D.E., Han, S.J., Kang, C.M., Choe, Y.S., Lee, K.C., Leary, J.F., Choi, K., Lee, K.H., Kim, K. (2014). Tumor-homing glycol chitosan-based optical/PET dual imaging nanoprobe for cancer diagnosis. Bioconjugate Chemistry, 25, 601-610. [CrossRef]
20. Ballou, B., Ernst, L.A., Waggoner, A.S. (2005). Fluorescence imaging of tumors *in vivo*. Current Medicinal Chemistry, 12, 795-805.
21. Sevick-Muraca, E.M., Rasmussen, J.C. (2008). Molecular imaging with optics: Primer and case for near-infrared fluorescence techniques in personalized medicine. Journal of Biomedical Optics, 13, 041303. [CrossRef]
22. Santra, S., Dutta, D., Walter, G.A., Moudgil, B.M. (2005). Fluorescent nanoparticle probes for cancer imaging. Technology in Cancer Research & Treatment, 4, 593-602. [CrossRef]
23. Altinoglu, E.I., Adair, J.H. (2010). Near infrared imaging with nanoparticles. Wiley Interdiscip Rev Nanomed Nanobiotechnol, 2, 461-477. [CrossRef]
24. Rao, J., Dragulescu-Andrasi, A., Yao, H. (2007). Fluorescence imaging *in vivo*: Recent advances. Curr Opin Biotechnol, 18, 17-25. [CrossRef]
25. Cutler, C.S., Hennkens, H.M., Sisay, N., Huclier-Markai, S., Jurisson, S.S. (2013). Radiometals for combined imaging and therapy. Chemical Reviews, 113, 858-883. [CrossRef]
26. Wadas, T.J., Wong, E.H., Weisman, G.R., Anderson, C.J. (2010). Coordinating radiometals of copper, gallium, indium, yttrium, and zirconium for PET and SPECT imaging of disease. Chemical Reviews, 110, 2858-2902. [CrossRef]
27. Ariztia, J., Solmont, K., Moise, N.P., Specklin, S., Heck, M.P., Lamande-Langle, S., Kuhnast, B. (2022). PET/Fluorescence Imaging: An overview of the chemical strategies to build dual imaging tools. Bioconjugate Chemistry, 33, 24-52. [CrossRef]
28. Loudos, G., Kagadis, G.C., Psimadas, D. (2011). Current status and future perspectives of *in vivo* small animal imaging using radiolabeled nanoparticles. European Journal of Radiology, 78, 287-295. [CrossRef]
29. van Dongen, G.A., Visser, G.W., Lub-de Hooge, M.N., de Vries, E.G., Perk, L.R. (2007). Immuno-PET: A navigator in monoclonal antibody development and applications. Oncologist, 12, 1379-1389. [CrossRef]
30. Bentivoglio, V., Varani, M., Lauri, C., Ranieri, D., Signore, A. (2022). Methods for radiolabelling nanoparticles: PET Use (Part 2). Biomolecules, 12(10), 1517. [CrossRef]
31. Sarcan, E.T., Silindir-Gunay, M., Ozer, A.Y., Hartman, N. (2021). 89Zr as a promising radionuclide and it's applications for effective cancer imaging. Journal of Radioanalytical and Nuclear Chemistry, 330, 15-28. [CrossRef]
32. Same, S., Aghanejad, A., Akbari Nakhjavani, S., Barar, J., Omidi, Y. (2016). Radiolabeled theranostics: Magnetic and gold nanoparticles. Bioimpacts, 6, 169-181. [CrossRef]
33. Cai, W., Chen, K., Li, Z.B., Gambhir, S.S., Chen, X. (2007). Dual-function probe for PET and near-infrared

fluorescence imaging of tumor vasculature. Journal of Nuclear Medicine, 48, 1862-1870. [CrossRef]
34. Azhdarinia, A., Ghosh, P., Ghosh, S., Wilganowski, N., Sevick-Muraca, E.M. (2012). Dual-labeling strategies for nuclear and fluorescence molecular imaging: A review and analysis. Molecula Imaging and Biology, 14, 261-276. [CrossRef]
35. Lee, S., Chen, X. (2009). Dual-modality probes for *in vivo* molecular imaging. Molecular Imaging, 8(2), 87-100. [CrossRef]
36. Abou, D.S., Pickett, J.E., Thorek, D.L. (2015). Nuclear molecular imaging with nanoparticles: Radiochemistry, applications and translation. The British Journal of Radiology, 88, 20150185. [CrossRef]
37. Padmanabhan, P., Kumar, A., Kumar, S., Chaudhary, R.K., Gulyas, B. (2016). Nanoparticles in practice for molecular-imaging applications: An overview. Acta Biomaterialia, 41, 1-16. [CrossRef]
38. Schipper, M.L., Cheng, Z., Lee, S.W., Bentolila, L.A., Iyer, G., Rao, J., Chen, X., Wu, A.M., Weiss, S., Gambhir, S.S. (2007). MicroPET-based biodistribution of quantum dots in living mice. Journal of Nuclear Medicine, 48, 1511-1518. [CrossRef]
39. Cai, W., Chen, X. (2008). Preparation of peptide-conjugated quantum dots for tumor vasculature-targeted imaging. Nature Protocols, 3, 89-96. [CrossRef]
40. Pandey, S., Choudhary, P., Gajbhiye, V., Jadhav, S., Bodas, D. (2023). *In vivo* imaging of prostate tumor-targeted folic acid conjugated quantum dots. Cancer Nanotechnology, 14, 30. [CrossRef]
41. Rees, K., Massey, M., Tran, M.V., Algar, W.R. (2020). Dextran-Functionalized Quantum Dot Immunoconjugates For Cellular Imaging. In: Fontes A, Santos BS (eds) Quantum Dots: Applications in Biology. Springer US, New York, NY, pp 143-168. [CrossRef]
42. Rees, K., Tran, M.V., Massey, M., Kim, H., Krause, K.D., Algar, W.R. (2020). Dextran-functionalized semiconductor quantum dot bioconjugates for bioanalysis and imaging. Bioconjugate Chemistry, 31, 861-874. [CrossRef]
43. Chu, T.C., Shieh, F., Lavery, L.A., Levy, M., Richards-Kortum, R., Korgel, B.A., Ellington, A.D. (2006). Labeling tumor cells with fluorescent nanocrystal-aptamer bioconjugates. Biosensors and Bioelectronics, 21, 1859-1866. [CrossRef]
44. Fatima, I., Rahdar, A., Sargazi, S., Barani, M., Hassanisaadi, M., Thakur, V.K. (2021). Quantum dots: Synthesis, antibody conjugation, and HER2-Receptor targeting for breast cancer therapy. Journal of Functional Biomaterials, 12(4), 75. [CrossRef]
45. Yemets, A., Plokhovska, S., Pushkarova, N., Blume, Y. (2022). Quantum dot-antibody conjugates for immunofluorescence studies of biomolecules and subcellular structures. Journal of Fluorescence, 32, 1713-1723. [CrossRef]
46. Stroh, M., Zimmer, J.P., Duda, D.G., Levchenko, T.S., Cohen, K.S., Brown, E.B., Scadden, D.T., Torchilin, V.P., Bawendi, M.G., Fukumura, D., Jain, R.K. (2005). Quantum dots spectrally distinguish multiple species within the tumor milieu *in vivo*. Nature Medicine, 11, 678-682. [CrossRef]
47. Mulder, W.J., Koole, R., Brandwijk, R.J., Storm, G., Chin, P.T., Strijkers, G.J., de Mello Donegá, C., Nicolay, K., Griffioen, A.W. (2006). Quantum dots with a paramagnetic coating as a bimodal molecular imaging probe. Nano Letters, 6, 1-6. [CrossRef]
48. Kirchner, C., Liedl, T., Kudera, S., Pellegrino, T., Muñoz Javier, A., Gaub, H.E., Stölzle, S., Fertig, N., Parak, W.J. (2005). Cytotoxicity of colloidal CdSe and CdSe/ZnS nanoparticles. Nano Letters, 5, 331-338. [CrossRef]
49. Hu, K., Wang, H., Tang, G., Huang, T., Tang, X., Liang, X., Yao, S., Nie, D. (2015). *In vivo* cancer dual-targeting and dual-modality imaging with functionalized quantum dots. Journal of Nuclear Medicine, 56, 1278-1284. [CrossRef]
50. Cai, W., Hsu, A.R., Li, Z.B., Chen, X. (2007). Are quantum dots ready for *in vivo* imaging in human subjects?. Nanoscale Research Letters, 2, 265-281. [CrossRef]
51. Derfus, A.M., Chan, W.C.W., Bhatia, S.N. (2004). Probing the cytotoxicity of semiconductor quantum dots. Nano Letters, 4, 11-18. [CrossRef]
52. Zimmer, J.P., Kim, S.W., Ohnishi, S., Tanaka, E., Frangioni, J.V., Bawendi, M.G. (2006). Size series of small indium arsenide-zinc selenide core-shell nanocrystals and their application to *in vivo* imaging. Journal of American Chemical Society, 128, 2526-2527. [CrossRef]
53. Ducongé, F., Pons, T., Pestourie, C., Hérin, L., Thézé, B., Gombert, K., Mahler, B., Hinnen, F., Kühnast, B., Dollé, F., Dubertret, B., Tavitian, B. (2008). Fluorine-18-labeled phospholipid quantum dot micelles for *in vivo* multimodal imaging from whole body to cellular scales. Bioconjugate Chemistry, 19, 1921-1926. [CrossRef]
54. Stockhofe, K., Postema, J.M., Schieferstein, H., Ross, T.L. (2014). Radiolabeling of nanoparticles and polymers for PET imaging. Pharmaceuticals (Basel), 7, 392-418. [CrossRef]
55. Xie, J., Chen, K., Huang, J., Lee, S., Wang, J., Gao, J., Li, X., Chen, X. (2010). PET/NIRF/MRI triple

functional iron oxide nanoparticles. Biomaterials, 31, 3016-3022. [CrossRef]
56. Ni, D., Jiang, D., Ehlerding, E.B., Huang, P., Cai, W. (2018). Radiolabeling silica-based nanoparticles via coordination chemistry: Basic principles, strategies, and applications. Accounts of Chemical Research, 51, 778-788. [CrossRef]
57. Chen, F., Ma, K., Zhang, L., Madajewski, B., Zanzonico, P., Sequeira, S., Gonen, M., Wiesner, U., Bradbury, M.S. (2017). Target-or-Clear Zirconium-89 labeled silica nanoparticles for enhanced cancer-directed uptake in melanoma: A comparison of radiolabeling strategies. Chemistry of Materials, 29, 8269-8281. [CrossRef]
58. Juthani, R., Madajewski, B., Yoo, B., Zhang, L., Chen, P.M., Chen, F., Turker, M.Z., Ma, K., Overholtzer, M., Longo, V.A., Carlin, S., Aragon-Sanabria, V., Huse, J., Gonen, M., Zanzonico, P., Rudin, C.M., Wiesner, U., Bradbury, M.S., Brennan, C.W. (2020). Ultrasmall core-shell silica nanoparticles for precision drug delivery in a high-grade malignant brain tumor model. Clinical Cancer Research, 26, 147-158. [CrossRef]
59. Shi, S., Chen, F., Goel, S., Graves, S.A., Luo, H., Theuer, C.P., Engle, J.W., Cai, W. (2018). *In vivo* tumor-targeted dual-modality PET/Optical imaging with a yolk/shell-structured silica nanosystem. Nanomicro Letters, 10, 65. [CrossRef]
60. Smith, B.R., Gambhir, S.S. (2017). Nanomaterials for *in vivo* imaging. Chemical Reviews, 117, 901-986. [CrossRef]
61. Rampazzo, E., Genovese, D., Palomba, F., Prodi, L., Zaccheroni, N. (2018). NIR-fluorescent dye doped silica nanoparticles for *in vivo* imaging, sensing and theranostic. Methods and Applications in Fluorescence, 6, 022002. [CrossRef]
62. Tang, L., Yang, X., Dobrucki, L.W., Chaudhury, I., Yin, Q., Yao, C., Lezmi, S., Helferich, W.G., Fan, T.M., Cheng, J. (2012). Aptamer-functionalized, ultra-small, monodisperse silica nanoconjugates for targeted dual-modal imaging of lymph nodes with metastatic tumors. Angewandte Chemie, 124, 12893-12898. [CrossRef]
63. Rosenholm, J.M., Mamaeva, V., Sahlgren, C., Lindén, M. (2012). Nanoparticles in targeted cancer therapy: Mesoporous silica nanoparticles entering preclinical development stage. Nanomedicine, 7, 111-120. [CrossRef]
64. Caltagirone, C., Bettoschi, A., Garau, A., Montis, R. (2015). Silica-based nanoparticles: A versatile tool for the development of efficient imaging agents. Chemical Society Reviews, 44, 4645-4671. [CrossRef]
65. Zheng, X., Zeng, S., Hu, J., Wu, L., Hou, X. (2018). Applications of silica-based nanoparticles for multimodal bioimaging. Applied Spectroscopy Reviews, 53, 377-394. [CrossRef]
66. Chen, F., Nayak, T.R., Goel, S., Valdovinos, H.F., Hong, H., Theuer, C.P., Barnhart, T.E., Cai, W. (2014). *In vivo* tumor vasculature targeted PET/NIRF imaging with TRC105(Fab)-conjugated, dual-labeled mesoporous silica nanoparticles. Molecular Pharmacology, 11, 4007-4014. [CrossRef]
67. Chen, F., Hong, H., Goel, S., Graves, S.A., Orbay, H., Ehlerding, E.B., Shi, S., Theuer, C.P., Nickles, R.J., Cai, W. (2015). *In vivo* tumor vasculature targeting of CuS@MSN based theranostic nanomedicine. ACS Nano, 9, 3926-3934. [CrossRef]
68. Chen, F., Hong, H., Shi, S., Goel, S., Valdovinos, H.F., Hernandez, R., Theuer, C.P., Barnhart, T.E., Cai, W. (2014). Engineering of hollow mesoporous silica nanoparticles for remarkably enhanced tumor active targeting efficacy. Scientific Reports, 4, 5080. [CrossRef]
69. Harmsen, S., Medine, E.I., Moroz, M., Nurili, F., Lobo, J., Dong, Y., Turkekul, M., Pillarsetty, N.V.K., Ting, R., Ponomarev, V., Akin, O., Aras, O. (2021). A dual-modal PET/near infrared fluorescent nanotag for long-term immune cell tracking. Biomaterials, 269, 120630. [CrossRef]
70. Chen, F., Ma, K., Benezra, M., Zhang, L., Cheal, S.M., Phillips, E., Yoo, B., Pauliah, M., Overholtzer, M., Zanzonico, P., Sequeira, S., Gonen, M., Quinn, T., Wiesner, U., Bradbury, M.S. (2017). Cancer-targeting ultrasmall silica nanoparticles for clinical translation: Physicochemical structure and biological property correlations. Chemistry of Materials, 29, 8766-8779. [CrossRef]
71. Benezra, M., Penate-Medina, O., Zanzonico, P.B., Schaer, D., Ow, H., Burns, A., DeStanchina, E., Longo, V., Herz, E., Iyer, S., Wolchok, J., Larson, S.M., Wiesner, U., Bradbury, M.S. (2011). Multimodal silica nanoparticles are effective cancer-targeted probes in a model of human melanoma. Journal of Clinical Investigation, 121, 2768-2780. [CrossRef]
72. Phillips, E., Penate-Medina, O., Zanzonico, P.B., Carvajal, R.D., Mohan, P., Ye, Y., Humm, J., Gonen, M., Kalaigian, H., Schoder, H., Strauss, H.W., Larson, S.M., Wiesner, U., Bradbury, M.S. (2014). Clinical translation of an ultrasmall inorganic optical-PET imaging nanoparticle probe. Science Translational Medicine, 6, 260ra149. [CrossRef]
73. Mitchell, N., Kalber, T.L., Cooper, M.S., Sunassee, K., Chalker, S.L., Shaw, K.P., Ordidge, K.L., Badar, A., Janes, S.M., Blower, P.J., Lythgoe, M.F., Hailes, H.C., Tabor, A.B. (2013). Incorporation of

paramagnetic, fluorescent and PET/SPECT contrast agents into liposomes for multimodal imaging. Biomaterials, 34, 1179-1192. [CrossRef]

74. Perez-Medina, C., Abdel-Atti, D., Zhang, Y., Longo, V.A., Irwin, C.P., Binderup, T., Ruiz-Cabello, J., Fayad, Z.A., Lewis, J.S., Mulder, W.J., Reiner, T. (2014). A modular labeling strategy for *in vivo* PET and near-infrared fluorescence imaging of nanoparticle tumor targeting. Journal of Nuclear Medicine, 55, 1706-1711. [CrossRef]
75. Puri, A., Loomis, K., Smith, B., Lee, J.H., Yavlovich, A., Heldman, E., Blumenthal, R. (2009). Lipid-based nanoparticles as pharmaceutical drug carriers: From concepts to clinic. Critical Reviews in Therapeutic Drug Carrier Systems, 26, 523-580. [CrossRef]
76. Ghazanfari Hashemi, M., Gholami, M., Alaei, M., Ghazanfari Hashemi, M., Miratashi Yazdi, S.N., Talebi, V., Helali, H. (2023). The most common nanostructures as a contrast agent in medical imaging. Nanomedicine Research Journal, 8, 127-140. [CrossRef]
77. Lobatto, M.E., Binderup, T., Robson, P.M., Giesen, L.F.P., Calcagno, C., Witjes, J., Fay, F., Baxter, S., Wessel, C.H., Eldib, M., Bini, J., Carlin, S.D., Stroes, E.S.G., Storm, G., Kjaer, A., Lewis, J.S., Reiner, T., Fayad, Z.A., Mulder, W.J.M., Perez-Medina, C. (2020). Multimodal positron emission tomography imaging to quantify uptake of (89)Zr-labeled liposomes in the atherosclerotic vessel wall. Bioconjugate Chemistry, 31, 360-368. [CrossRef]
78. Du, Y., Liang, X., Li, Y., Sun, T., Jin, Z., Xue, H., Tian, J. (2017). Nuclear and fluorescent labeled PD-1-liposome-DOX-(64)Cu/IRDye800CW allows improved breast tumor targeted imaging and therapy. Molecular Pharmaceutics, 14, 3978-3986. [CrossRef]