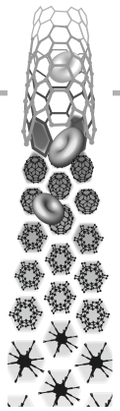

Nanoparticles as computed tomography contrast agents: current status and future perspectives

The importance of computed tomography (CT) as one of the leading radiology technologies applied in the field of biomedical imaging escalated the development of nanoparticles as the next generation CT contrast agents. Nanoparticles are expected to play a major role in the future of medical diagnostics due to their many advantages over the conventional contrast agents, such as prolonged blood circulation time, controlled biological clearance pathways and specific molecular targeting capabilities. This paper will describe the basic design principles of nanoparticle-based CT contrast agents and review the state-of-the-art developments and clinical applications of blood pool, passive and active targeting CT contrast agents.

KEYWORDS: active targeting ▪ atherosclerosis ▪ blood pool contrast agents ▪ cancer ▪ computed tomography ▪ *in vivo* imaging ▪ molecular imaging ▪ nanoparticles ▪ passive targeting

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Molecular imaging is an emerging field integrating molecular biology, chemistry and radiology in order to gain understanding about biological processes and to identify diseases based on molecular markers, which appear earlier than the diseases' clinical symptoms. (CT) is among the most convenient imaging/diagnostic tools used in hospitals today in terms of availability, efficiency, and cost. However, in contrast to other imaging modalities, such as PET, single-photon emission computed tomography and MRI, CT is not widely thought of as a molecular imaging modality. Recently, much research has focused on the development of nanoparticles to be utilized both as blood pool CT contrast agents and for specific molecular imaging applications. This paper will describe the basic design principles of nanoparticle-based CT contrast agents and review the *in vivo* studies, which can be divided into three main categories:

- Blood pool CT contrast agents, which remain confined to the intravascular space, highlighting blood vessels;
- Passive targeting agents that reach sites of disease via the reticulo-endothelial system (RES) or through the enhanced permeability and retention (EPR) effect;
- Active targeting agents, which selectively accumulate on specific cells and tissue by conjugation of antibodies, peptides, or other ligands onto the surface of nanoparticles.

The goal of the latter studies is to expand the role of CT beyond its present structural imaging capabilities, endowing it with functional- and molecular-based imaging capabilities as well.

Requirements for CT contrast agents

Currently, CT is one of the leading radiology technologies applied in the field of biomedical imaging. CT provides superior visualization of bone structures due to the inherent contrast between electron-dense bones and the more permeable surrounding soft tissues. CT, however, is limited in distinguishing between different soft tissues that have similar densities [1]. CT contrast agents were introduced in order to improve vascular contrast and to enable better delineation of soft tissue structures with similar or identical contrast properties.

The ability of the CT to distinguish between different tissues is based on the fact that different tissues provide different degrees of x-ray attenuation, according to **Equation 1**:

$$I = I_0 e^{-\mu x}$$

Where I_0 is the incident x-ray intensity, I is the transmitted x-ray intensity, x is the thickness of the absorber medium and μ is the mass attenuation coefficient. The most dominant factor impacting the mass attenuation coefficient is the photoelectric effect, which is proportional to the third power of the atomic number of the material (Z^3). Therefore, in order to provide good contrast in CT images, the key factor in the selection of CT contrast agents is high atomic number materials.

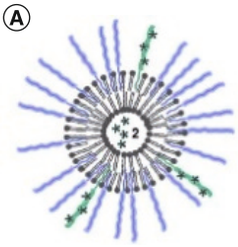
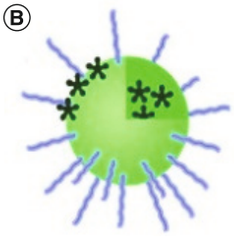
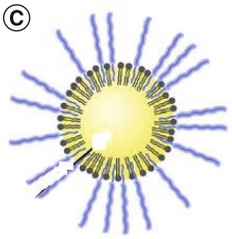
Future Medicine part of fsg

Another important parameter in the development of molecular CT – and indeed a major challenge in the implementation of high-Z contrast agents – lies in the relatively low sensitivity of the CT to the contrast agents' concentration. For example, in MRI contrast agents, proton relaxation must be altered, requiring agents that can perturb the local magnetic field around the proton. The perturbing field of a superparamagnetic particle is effective at up to 50-times its diameter, and therefore influences water protons in several cell layers around its location [2]. This phenomenon results in sensitivity of the MRI to micromolar contrast agents' concentration [3]. By contrast, CT contrast agents lack such amplification ability. CT is sensitive to millimolar contrast agent concentrations; therefore, in order to induce sufficient contrast in the desired organ, a much larger amount of high-Z molecules is needed, since the CT contrast is linearly proportional to the total amount of the high-Z molecules in a voxel.

The high Z nanoparticle contrast agents could also address the important issue of relatively high radiation exposure of CT. The new generation CT contrast agents that are based on high atomic number materials, such as gold and bismuth, have a great potential not only because of their ability to produce contrast higher than conventional iodine-based contrast agents, but even more importantly, because of the possibility to lower the overall radiation exposure to patients.

Low-to-middle x-ray photon energy (25–120 keV) is used for diagnostic radiology, producing significant contrast between bone and other tissues, resulting in high quality CT images. However, since most of this energy is being absorbed, it exposes the patient to a high dose of radiation. As the higher energy photons in the energy spectrum produced by the x-ray tubes will have a much lower interaction cross section for soft tissue than the nanoparticles, it is possible that by filtering the x-ray spectrum, yielding lower absorbed radiation dose to the patient, the uptake pattern of these particles can be visualized as distinct contrast relative to their soft tissue background [1]. Therefore, high-Z nanoparticles as contrast agents may permit CT imaging at lower patient doses, with better sensitivity and good specificity.

Table 1. A summary of the most pertinent iodine-based liposomes, polymers and micelles that were utilized as *in vivo* computed tomography contrast agents.

	Type	Size	Blood half-life time	Ref.
Ⓐ 	Liposome	>100 nm	Hours	[9–20]
Ⓑ 	Polymer	30–400 nm	Minutes	[21–24]
Ⓒ 	Micelle	~100 nm	Hours	[25,26]

Illustrations reproduced with permission from [14].

Beyond the aforementioned particular requirements for CT contrast agents, which are specifically derived from the physical principles of CT, additional general parameters exist as well, requiring consideration. These parameters, such as size, shape, pharmacokinetics and particle toxicity have been broadly examined in several recent reviews [4–8] and will be mentioned only briefly in this paper.

Iodine-based CT contrast agents

Present clinical CT contrast agents are predominantly based on the high atomic number iodine molecules (Z=53), which are effective in absorbing x-rays; however, the small size of iodine molecules allows very short imaging times due to rapid clearance by the kidneys. Larger sized iodine-based contrast agents, which provide longer blood circulation time, were developed in order to address this limitation. These 'soft' nanoparticles are based on liposomes [9–19], polymers [20–23] and micelles [24,25].

Liposome-based CT contrast agents

Liposome-based CT contrast agents are amphiphilic phospholipid vesicles with a bilayer membrane structure similar to that of biological membranes and an internal aqueous phase. Their amphiphilic nature allows them to transport both hydrophilic contrast agent molecules entrapped within their aqueous interior and hydrophobic molecules dissolved in their membrane. Several novel iodine-loaded liposomes have been developed and demonstrated long blood circulation time. Kweon *et al.* suggested an innovative approach to increase the iodine concentrations in the liposomes by coloading water-soluble iodinated compounds together with iodized oil [17]. Yet designing a liposome contrast agent requires special care given the relative instability of liposomes in biological media [14]. Table 1A summarizes recent *in vivo* studies with liposome-based CT contrast agents.

Polymeric CT contrast agents

Polymeric CT contrast agents are prepared by physical entrapment or covalent link of core molecules to polymeric chains [21–24]. The main challenge this kind of contrast agent presents is their stability in aqueous physiologic medium [22–24]. Therefore, polymeric nanoparticles must be carefully designed so as not to undergo decomposition and agglomeration after injection into the blood stream. Table 1B summarizes recent *in vivo* studies with polymeric CT contrast agents.

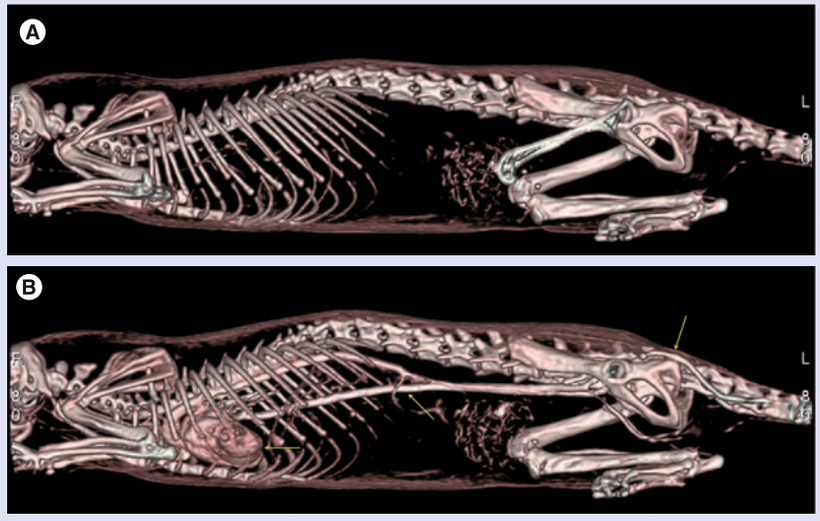


Figure 1. *In vivo* imaging before (A) and 10 s after (B) injection of tantalum oxide nanoparticles.
Reproduced with permission from [53].

Micelles

Since a low concentration of iodine in a contrast agent's total volume hampers its use in small animals due to restrictions on the injectable volume, particles with a higher iodine payload have been designed in micelles [25,26]. Polymeric micelles are spherical colloidal nanoparticles formed by the self-assembly of amphiphilic copolymers in aqueous media. This structure of a hydrophobic core stabilized by a hydrophilic corona enables the particles to deliver hydrophobic molecules within their interior and to link polar molecules to their surface. de Vries *et al.* developed radiopaque iodinated emulsions with high iodine payload (130 mg I/ml) that have been used as blood pool CT contrast agents [24]. They found that while the lipid-stabilized emulsions turned out to be unstable *in vivo*, polymer-stabilized emulsions remained stable. Table 1C summarizes recent *in vivo* studies with micelle-based CT contrast agents.

Metal nanoparticles as CT contrast agents

The use of metal nanoparticles has become a significant area of investigation. Metal nanoparticles have unique physical, chemical and biological properties, making them attractive candidates for CT contrast agents. As noted above, the higher the atomic number of the contrast agent, the better the resultant CT contrast (proportional to Z^3). The atomic number of the recently proposed metals tantalum, gold and bismuth (73, 79 and 83, respectively) is much higher than that of the currently used iodine (53) [27].

Table 2. A summary of blood pool *in vivo* CT contrast agents.

Nanoparticle core atom	Size (nm)	Coating	Blood circulation time	Ref.
Blood pool contrast agents				
Iodine	280	Liposome	Hours	[9–20]
Iodine	30–408	Polymer	Minutes	[21–24]
Iodine	82–112	Micelle	3 h	[25,26]
Gold	2–30	Various coatings	4–24 h	[55–59]
Tantalum	5–15	Various coatings	12 h	[53,54]
Bismuth	10–50	Polymer	140 min	[52]

Therefore, these proposed metals can induce stronger x-ray attenuation. In addition, the size of the metal nanoparticles can be precisely controlled, allowing optimization of the CT signal, which is directly correlated to the amount of contrast molecules in a voxel. For example, by increasing the radius of a particle by a factor of two, the amount of metal atoms will increase by a factor of eight (μr^3). For biological targeting, metal nanoparticles are readily amenable to bioconjugation and biomodification. Their surface has a strong binding affinity towards thiol, disulfide and amine groups, which allows, by simple chemistry, surface conjugation with various peptides, proteins, antibodies and other biomolecules [28–30].

Among the metal nanoparticles, gold nanoparticles (GNPs) have attracted special attention. Other than the high atomic number of gold, GNPs have unique optical properties that make them appealing both for optical imaging and for photothermal therapy. These wide applications of GNPs and their potential for clinical implementation have led to substantial research regarding their *in vivo* chemical stability [26,31,32], pharmacokinetics [31], biodistribution [32–37] and biotoxicity [31,33,38–41]. The well-known biosafety of gold since the 1950s [42,43], along with the high degree of flexibility in terms of particle size, shape and functional groups for coating and targeting, provides the GNPs with high potential to become the next generation of CT contrast agents.

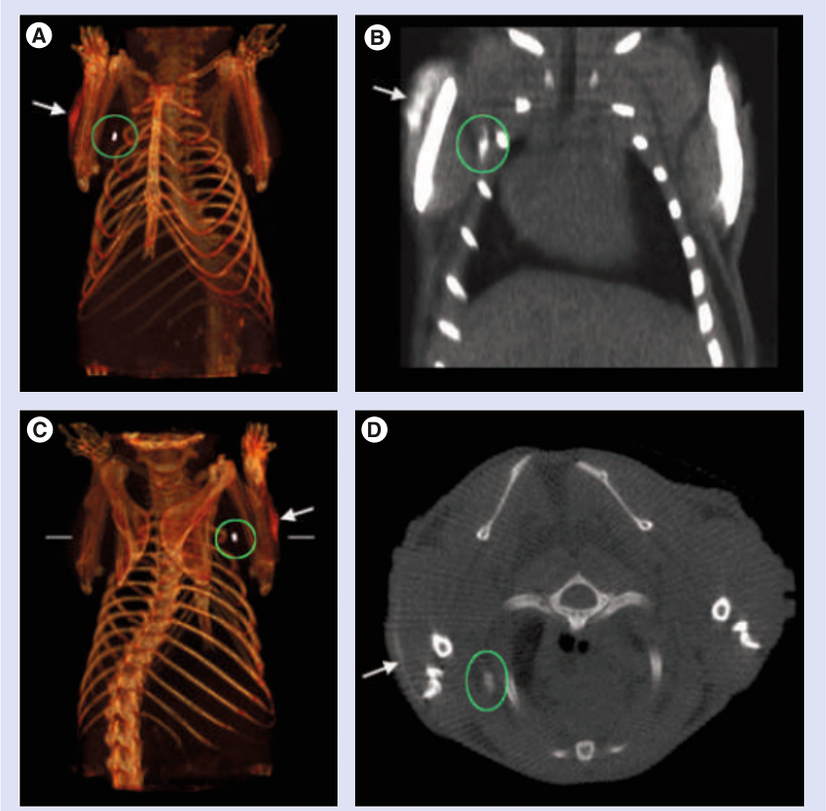


Figure 2. Lymph-node imaging: computed tomography imaging of a lymph node of a mouse with the BPNP imaging agent. (A & B) 3D volume renderings of the CT data set; **(C)** coronal slice; **(D)** transverse slice at the height indicated by the horizontal lines in **(B)**. The ovals indicate the position of the lymph node under the right shoulder, and the arrows show the injection site. Note the lack of contrast in the corresponding contralateral (left shoulder) lymph node.
Reproduced with permission from [52].

Imaging applications

Nanoparticle-based CT contrast agents have been suggested for several medical imaging applications depending on multiple parameters, such as particle size, coating material and targeting moiety. These parameters determine not only the efficacy of the contrast achieved in CT, but also the biodistribution and clearance mechanisms.

Blood pool imaging

The ideal blood pool CT contrast agents must be wisely designed so as to overcome biological barriers and to remain confined to the intravascular space for prolonged time. The role of these particles is to enable sharp blood vessel delineation, mainly for cardiovascular applications. A consensus exists regarding a few design principles, such as their size, shape and coating, which will endow the nanoparticles with minor macrophage uptake and prolonged blood circulation

time (from minutes for the conventional contrast agents to hours) [14,44–46]. Their optimal size (for spherical particles) should be larger than approximately 15 nm to avoid rapid clearance by the kidneys or uptake in the liver, and smaller than approximately 200 nm to avoid filtration in the spleen [14,46]. Anisotropic nanoparticles demonstrated extended blood circulation time (~17 h) [47]; however, anisotropic nanoparticles remain yet to be optimized and characterized. Chemical coating and surface charge of the nanoparticles plays a critical role in their blood half-life time. Various hydrophilic polymers have been studied [6,48] in order to create a nonfouling coating on the particle surface. Polyethylene glycol (PEG), however, has been the most widely used polymer, and proven to circumvent the activation of the immune system and to reduce nonspecific interactions [14,47,49,50]. Nevertheless, it is important to note that the size and the coating of the nanoparticles are not the only factors that influence the particles' half-life. MRI contrast studies have demonstrated that the blood half-lives are also dose dependent [51]. Increasing doses causes progressive saturation of macrophage uptake by the liver or other macrophage-rich organs, and consequentially increases particles' blood half-life time. This issue has not been fully elucidated for nanoparticle-based CT contrast agents.

Several iodine-based 'soft' nanoparticles have been successfully applied *in vivo* as blood pool CT contrast agents [9–25]. Galperin *et al.* demonstrated the potential clinical use of iodinated radiopaque polymeric nanoparticles formulated into emulsions [22,23]. These polymeric nanoparticles showed significant CT contrast enhancement for 30 min post-injection. More recently, de Vries *et al.* demonstrated blood pool contrast enhancement of 220 Hounsfield units immediately after intravenous (iv.) injection of polymer-stabilized emulsions, with a half-life blood circulation time of 3 h and no noticeable *in vivo* toxicity observed [24]. Moreover, because of the high iodine payload per ml in these emulsions, it was possible to inject a small volume of contrast material, but still receive high-contrast enhancement. Yet although iodine-based nanomaterials seem to be a direct progression from the current clinical administration of iodine compounds, the development of large iodine payload nanomaterials with targeting properties that are both stable in physiological solution and nontoxic *in vivo* makes the chemical production challenging. Therefore, although theoretically iodine-based nanomaterials seem to be clinically attractive, it is far from being applicable.

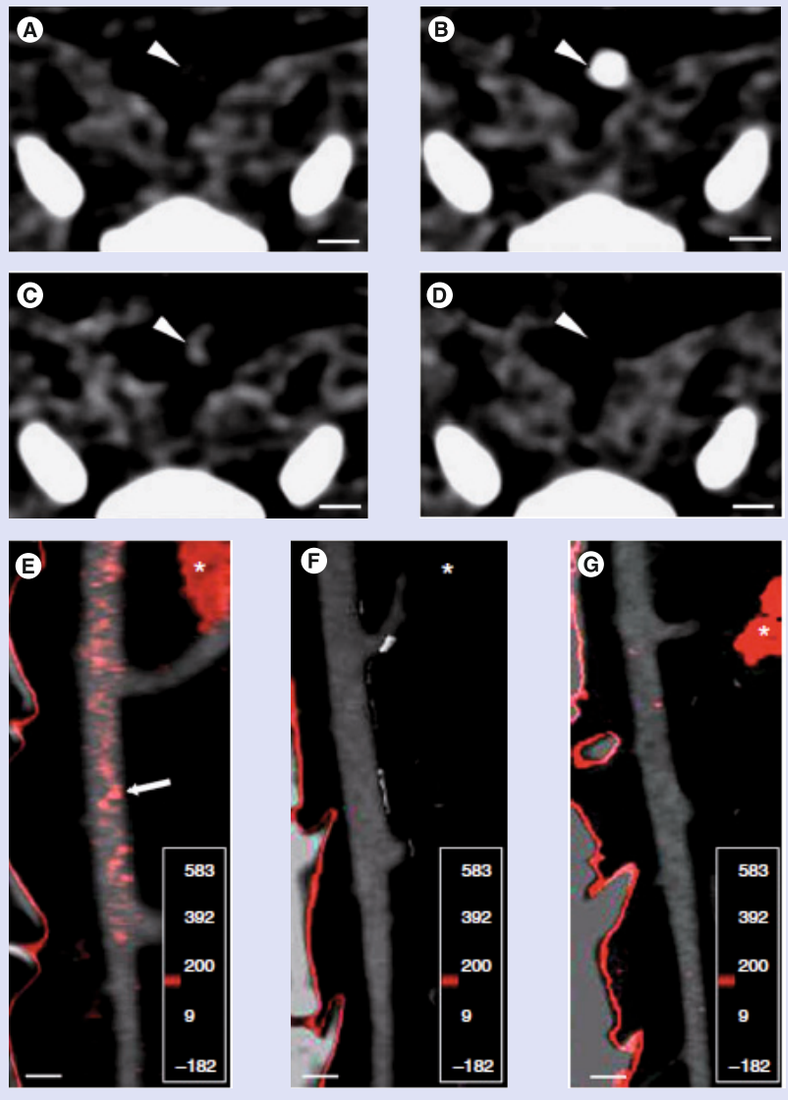


Figure 3. Imaging of macrophages in atherosclerotic plaques. (A–D) Axial views of the same atherosclerotic plaque (white arrowheads) in the aorta of a rabbit, obtained by CT before **(A)**, during **(B)** and 2 h after the injection of N1177 **(C)** or a conventional contrast agent **(D)**. These images were acquired in two separate imaging sessions. Before the injection of the contrast agent, the atherosclerotic plaque could not be differentiated from the surrounding tissues, whereas a strong enhancement was detected in the atherosclerotic plaque after the injection of N1177 (but not the conventional contrast agent). The same level and width windows were used for all views. Intense red spots were detected in atherosclerotic plaques after the injection of N1177 **(E)**, but not in atherosclerotic plaques after injection of a conventional contrast agent **(F)** or in the aortic wall of a control rabbit injected with N1177 **(G)**. The insert indicates the color scale of densities in Hounsfield units.
Reproduced with permission from [21].

Metal nanoparticles are attractive candidates as blood pool CT contrast agents. The main interest is attributed to gold; however, less expensive metal nanoparticles have been suggested as well, including bismuth sulphide (Bi_2S_3) and tantalum oxide. Rabin *et al.* proposed polymer-coated Bi_2S_3 nanoparticles [52].

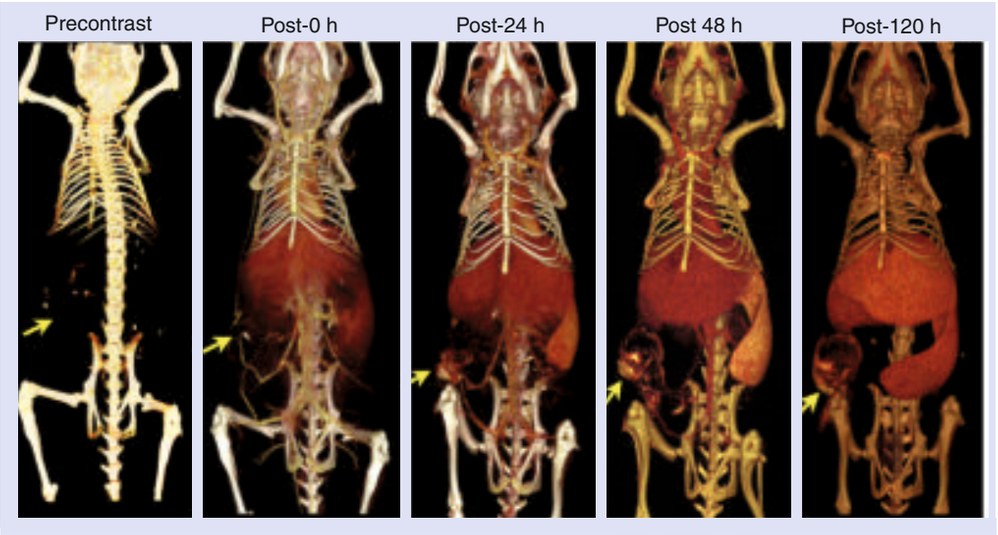


Figure 4. Dynamics of tumor signal enhancement. Coronal 3D volume-rendered images demonstrating the extravasation and accumulation of the nanoparticle contrast agent within the tumor (yellow arrow). Immediately after administering the nanoparticle contrast agent, the overall body and tumor vasculature is nicely demonstrated. Tumor accumulation of nanoparticle contrast agent was observed as early as 24 h postadministration.
Reproduced with permission from [13].

They demonstrated clear delineation and signal enhancement of 560 Hounsfield unit of the cardiac ventricles and all major arterial and venous structures, while providing a long circulation time (>2 h) and a safety profile that is comparable to or better than iodinated imaging agents. However, further studies and toxicity assessment with bismuth nanoparticles are lacking.

Bonitatibus *et al.* suggested tantalum oxide (Ta_2o_5) as a CT contrast agent [53]. Water-soluble, chemically inert and biocompatible nanoparticles (≤6 nm) showed clear delineation of a rats' vena cava and abdominal aorta (1–2 mm), as shown in Figure 1. These nanoparticles, which are smaller than 10 nm (within the size range of renal clearance [46]), were cleared from the blood within seconds following injection. More recent work [54] demonstrated a blood circulation time of 3 h after PEGylation for similar particles within the same size range (5–15 nm, before PEGylation).

Various gold-based nanoparticles have been suggested as blood pool contrast agents [55,56], including acetylation of dendrimer-entrapped GNPs [57], PEGylated dendrimer GNPs [58] and antibiofouling polymer-coated GNPs [59]. Indeed, all these studies demonstrated that GNPs extended the blood circulation time from several minutes (with the clinically used iodine compounds) up to 24 h [58]. Additionally, they showed stronger x-ray attenuation than the currently used iodine-based compounds (under the same clinically relevant conditions), thus allowing sharp blood vessel delineation, such as a 100 µm diameter blood vessel [55], the cardiac ventricles and great vessels [58], as well as the inferior vena cava and the pulmonary veins [57].

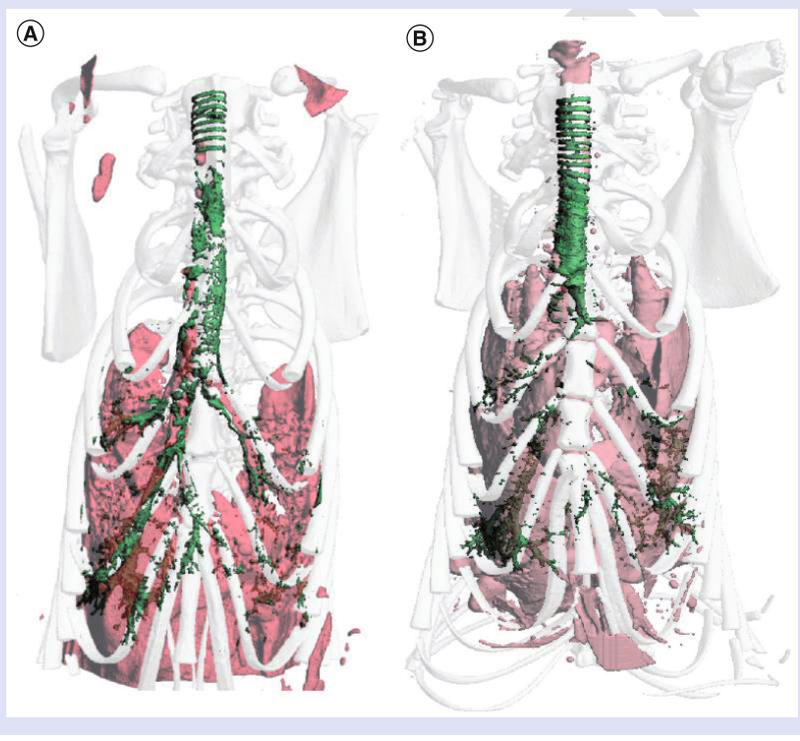


Figure 5. 3D reconstructed CT images of rat thoracic cavities after N1177 NanoCluster dry powder insufflations (A) directly after dose and (B) 2 h post dose. N1177 NanoCluster shown in green and soft tissue shown in pink.
Reproduced with permission from [67].

Therefore, nanoparticle-based blood pool contrast agents may have a major role in future angiography and potentially can substitute invasive procedures. TABLE 2 summarizes recent studies regarding *in vivo* blood pool CT contrast agents.

Passive targeting

Nanoparticles that exhibit localization to specific organs or to sites of disease via biological mechanisms, such as the RES or the EPR effect, are known as 'passive targeting agents'.

Liver, spleen & lymph nodes

Nanoparticles that are taken up by macrophages can be used for CT signal enhancement of macrophage-rich organs, such as healthy liver, spleen and lymph nodes, or for pathologies such as atherosclerotic plaques. The RES is a diffuse system consisting of phagocytic cells that are associated with the connective tissue framework of the liver, spleen and lymph nodes. In most contrast agent studies, specific attention is dedicated to protecting the nanoparticles against RES uptake, since fast removal of the contrast agents from the blood circulation decreases their ability to reach their target organ. However, a few studies took advantage of the RES mechanism for mapping the liver [22,23,52,55,58,59], spleen [23,54,55,60–62] and lymph nodes [23,52,54]. The mapping of the lymph node is very important for cancer staging and for precise determination of the tumor metastasis, which could prevent unnecessary dissection surgery. For example, the aforementioned bismuth sulphide nanoparticles [52] distributed to organs containing phagocytic cells (liver, spleen and lymph nodes) 12–24 h post-iv. administration. Regional lymph nodes were clearly contrasted, as shown in FIGURE 2. Liver signal intensity was also highly increased, reflecting uptake of bismuth sulphide nanoparticles into macrophages (Kupffer cells) and hepatocytes, and therefore, by exact delineation of the tumor region this method might be developed to improve detection of hepatic metastases by CT.

Different routes of contrast agent delivery have also been demonstrated in lymph node imaging [54]. PEGylated tantalum oxide nanoparticles were injected intradermally into rats' paws, and the location of the lymph nodes were determined by the CT. Dissection of the lymph nodes specified the presence of the nanoparticles by fluorescence imaging.

Atherosclerosis & plaques

Atherosclerosis is the leading cause of heart attacks, cerebrovascular events and peripheral vascular disease. A critical factor in the determining the risk of acute ischemic events is the composition and stage of atherosclerotic plaques. Rupture-prone plaques are composed from smooth muscle cells, lipids, calcium and high-macrophage densities [63,64]. In unstable rupture-prone plaques, the fibrous cap can suddenly rupture and expose the content of the plaque to the blood. This exposure would lead to the formation of a thrombus, which can occlude the artery lumen and cause myocardial ischemia. This high-macrophage density turns the macrophages into a leading marker in the evaluation of unstable atherosclerotic plaques. CT can provide valuable information about the plaque composition because lipid-rich tissue (as presented in high-risk plaques) attenuates x-rays less than fibrous tissue [21,63,64]. Yet contrast agents that can accumulate specifically on these plaques can enhance the detection ability of atherosclerosis utilizing CT. In an experimental model of atherosclerotic plaques generated in the aorta of hypercholesterolemic rabbits, iodinated nanoparticles contrast agents (N1177) were iv. injected and demonstrated rapid accumulation on macrophage-rich tissues [21]. N1177 is a suspension composed of crystalline iodinated particles, with an average diameter of 257 nm, and surfactant, which is responsible for spreading the particles. These particles induced a significant enhancement of the plaques, whereas only low contrast was obtained utilizing iopamidol,

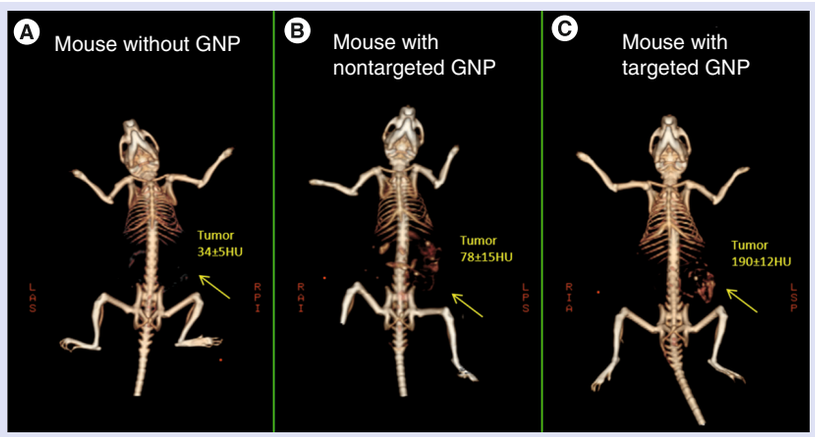


Figure 6. *In vivo* x-ray computed tomography volume-rendered images of (A) a mouse before GNP injection, (B) a mouse 6 h postinjection of nonspecific IgG GNP as a passive targeting experiment, and (C) a mouse 6 h postinjection of anti-EGFR coated GNP that are specifically targeted the SCC head and neck tumor. The anti-EGFR targeted GNP show clear contrast enhancement of the tumor (**(C)**, yellow arrow), which was undetectable without the GNP contrast agents (**(A)**, yellow arrow). Computed tomography (CT) numbers represent the average Hounsfield unit of the whole tumor area. All scans were performed using a clinical CT at 80 kVp, 500 mAs, collimation 0.625 x 64 mm and 0.521 pitch size (A 64-detector CT scanner, LightSpeed VCT, GE Medical Systems). Submitted to the *International Journal of Nanomedicine* [71].

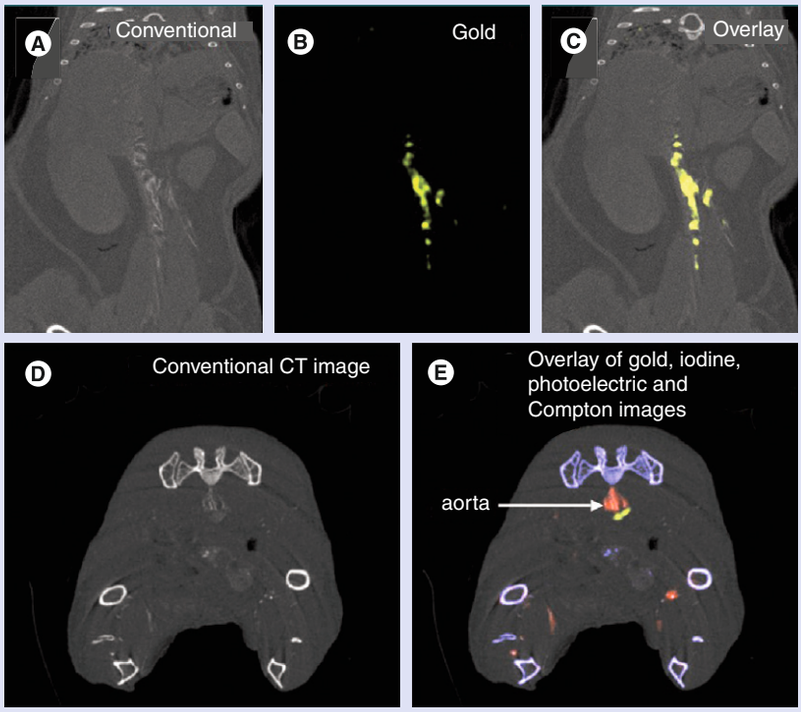


Figure 7. TITLE. (A–C) Spectral computed tomography images of thorax and abdomen in apoE–KO mouse injected 24 h earlier with Au-HDL. **(D & E)** Spectral CT images near bifurcation of aorta in apoE–KO mouse injected with Au-HDL and an iodinated emulsion contrast agent (Fenestra VC) for vascular imaging. Reproduced with permission from [72].

a clinical iodinated contrast agent. Moreover, the results showed a direct correlation between increasing plaque densities (data obtained from histological sections) and the intensity of macrophage infiltration that were obtained from the CT images (Figure 3). A disadvantage of this approach, however, is the necessity of a baseline scan, subjecting the patient to a double dose of radiation and requiring pre- and post-scan image comparison [65]. This pioneer work demonstrated the possibility of performing CT molecular imaging of atherosclerosis. This ability to image the macrophages' infiltration *in vivo* and thereby to classify the plaque type and stage in a noninvasive way may become an important adjunct to the clinical evaluation of coronary arteries with CT.

Cancer

One of the principle applications of nanoparticle-based CT contrast agents is in cancer detection. Nanoparticles of a certain size range, with minor macrophage uptake and prolonged blood half-life time can passively accumulate on tumors, taking advantage of the progressive permeation through trans-endothelial pores on tumor blood vessels (the EPR effect). This nonselective 'passively targeting' approach was demonstrated for breast tumor detection in a recent study [13]. This study showed significant CT contrast enhancement caused by accumulation of nanoparticle contrast agent both within the tumor and in areas surrounding it (Figure 4).

Whereas most of the CT imaging studies were performed by iv. injection, a few other routes of administration have been suggested. Acetylated dendrimer-entrapped GNPs (Au DENPs) have been injected both intratumorally and intraperitoneally in order to detect human lung cancer in a mouse model [66]. As expected, a significant enhancement was achieved after intratumoral injection of the Au DENPs. But more interestingly, the intraperitoneal administration indicated that nanoparticles could be delivered through the capillary network in the peritoneum and reach the tumor site via the EPR effect, which caused significant CT enhancement of the tumor. For lung cancer detection with CT, Aillon *et al.* suggested insufflations or inhalation of the nanoparticle contrast agents [67]. Insufflation of iodinated (N1177) NanoCluster powders provided enhanced CT images of the trachea, the bronchi, the respiratory bronchioles and some alveolar structure in the lung periphery. These results indicated that the NanoClusters reached the deep lung (Figure 5). The contrast agent was cleared from central airways 2 h postinhalation [67].

Active targeting

Conjugation of antibodies, peptides, or other ligands onto the nanoparticle surface produces active targeting agents, which can selectively accumulate on specific cells or tissues. This innovative imaging approach expands the role of CT beyond its present structural imaging capabilities, endowing it with functional and molecular-based imaging capabilities as well.

Cancer

Molecularly targeted nanoparticles reach tumor tissues through the EPR effect (as in passive targeting). However, the active targeting has additive values; the nanoparticles home selectively onto specific tumors and remain at the tumor site for an extended time, thereby increasing the local accumulation of the nanoparticles in sites of interest.

Specific targeting could be achieved through the conjugation of nanoparticles to a variety of ligands, including antibodies, peptides, aptamers or small molecules that possess high affinity toward unique molecular signatures found in diseases such as cancer. Hainfeld

et al. demonstrated molecular imaging of cancer with actively targeted CT contrast agents [68]. They showed that GNPs can enhance the visibility of mm-sized human breast tumors in mice, and that active tumor targeting (with anti-Her2 antibodies) is 1.6-fold more efficient than passive targeting. They also showed that the specific uptake of the targeted GNPs in the tumor's periphery was 22-fold higher than in surrounding muscle. In another study, Chanda *et al.* reported enhanced CT attenuation of bombesin functionalized GNPs that selectively targeted cancer receptor sites that are overexpressed in prostate, breast and small-cell lung carcinoma [69]. In our own research, we recently demonstrated, *in vitro* [66] and *in vivo* [67], that the CT number of molecularly targeted head and neck cancer is over five-times higher than the corresponding CT number of an identical but untargeted tumor, and that active tumor targeting is more efficient and specific than passive targeting (**Figure 6**) [70,71]. This specific interaction between antigen and antibody or receptor and its ligand was shown to be an effective strategy to improve the amount and residence time of contrast agents in tumors, as well as to provide specific molecular knowledge regarding the findings.

Atherosclerosis

Rupture-prone plaques, which are rich in macrophages, have been specifically targeted by gold high-density lipoprotein nanoparticles (Au-HDL) [72,73]. The targeted particles induced CT contrast enhancement specifically in macrophage-rich, rupture-prone plaques, while no significant enhancement was observed for stable plaques that are not rich in macrophages. In addition, the same group proposed a new concept of multicolor spectral CT, in which incident x-rays are divided into six different energy bins that can be used for multicolor imaging. This imaging method enabled differentiation of Au-HDL, iodine-based contrast material, and calcium phosphate in the phantoms and in a mouse model. Accumulations of Au-HDL were detected in the aortas of the mice, while the iodine-based contrast agent and the calcium-rich tissue could also be detected and thus facilitated visualization of the vasculature and bones (skeleton), respectively, during a single scanning examination (**Figure 7**) [72,73]. In another study multicolor spectral CT imaging was applied to visualize intravascular pathologic epitopes with fibrin-targeted bismuth nanoparticles in rabbit models of atherosclerosis [74]. Performing multicolor CT could enable a simultaneous tracking of two differently labeled cell populations and a study of their interactions or the interaction of cells with specific receptors, while each retains its own unique label.

These studies demonstrated that molecularly targeted particles yield the potential to significantly improve CT contrast and specificity through increasing the local accumulation of nanoparticles in sites of interest. However, since the active targeting approach is based on the existence and the degree of overexpression of specific tissue biomarkers, it is applicable only under particular biological conditions. **Table 3** summarizes recent studies regarding passive and active *in vivo* CT contrast agents.

Table 3. A summary of passive and active *in vivo* computed tomography contrast agents.

Targeted organ	Nanoparticles type	Size (nm)	Coating	Ref.
Passive targeting				
Plaque (macrophages)	Iodine	259	Biocompatible surfactants	[21]
Liver tumor	Gold	30	PEG	[59]
Breast tumor	Iodine–liposome	100		[13]
Lung tumor	Dendrimer-entrapped GNPs	2.6		[66]
Lung	Iodine	300		[67]
Lymph nodes	Bismuth sulphide	30	Polymer (PVP)	[52]
Lymph nodes	Tantalum	19	PEG	[54]
Lymph nodes	Gold	28/38	PEG with anti-mouse CD4 monoclonal antibodies	[75]

GNP: Gold nanoparticle; PEG: Polyethylene glycol; PVP:.

Conclusion & future perspective

High Z nanoparticles as contrast agents may permit CT imaging at lower radiation doses and with better sensitivity and good specificity. The main focus of the research in this field has concentrated on the development of blood pool CT contrast agents. The reviewed studies demonstrated extended blood circulation time and enhanced CT contrast, which allowed sharp blood vessel delineation and early detection of structural and functional vascular abnormalities. The new generation of molecularly targeted CT contrast agents has changed the concept of CT from diagnosis based on anatomical structures to diagnosis according to molecular markers.

Although these studies seem very promising and feasible for clinical applications, it is difficult to draw definite design principles owing to the differences in materials, size and shape of the nanoparticles, as well as variability between animal models. Further research is essential in order to establish basic design principles linking the size, shape, chemical coating and targeting agents with *in vivo* biodistribution and pharmacokinetics. There is still uncertainty regarding the toxicity of these nanoparticle contrast agents; although most of the reviewed studies have stated that no toxicity has been observed, a comprehensive prospective toxicity study has yet to be performed. Once the toxicity issue is clarified, clinical trials could be initiated.

Recent advances in the field of genomics, proteomics and molecular biology have prompted a paradigm shift in medicine from 'one-size-fits-all' to a personalized medicine concept. Molecular imaging with specifically targeted nanoparticle-based contrast agents is expected to follow this pathway and to enable personalized imaging according to the patients' disease characterizations. In addition, the possibility to combine imaging with therapy has led to the exiting approach of theranostic applications. Concurrent encapsulation of metal nanoparticles (diagnosis) and drug molecules (therapy) into carriers, such as emulsions or liposomes, is highly advantageous as it offers *in vivo* imaging and tracking of drug efficacy.

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Executive summary

High Z nanoparticles as computed tomography contrast agents

- Computed tomography (CT) contrast agents are introduced in order to improve vascular contrast and to enable better delineation of soft tissue structures with similar or identical contrast properties.
- Metal nanoparticles have unique physical, chemical and biological properties, making them attractive candidates for CT contrast agents. The higher the atomic number of the material, the better the resultant CT contrast (proportional to Z^3).
- High Z nanoparticles as contrast agents may permit CT imaging at lower radiation doses and with better sensitivity and good specificity.

Blood pool CT contrast agents

- The optimal nanoparticle should be larger than approximately 15 nm to avoid rapid clearance by the kidneys or uptake in the liver, and smaller than approximately 200 nm to avoid filtration in the spleen.

CT molecular imaging with specifically targeted contrast agents

- Conjugation of antibodies, peptides or other ligands onto the nanoparticle surface produces active targeting agents, which can selectively accumulate on specific cells or tissues.
- Molecularly targeted particles yield the potential to significantly improve CT contrast and specificity through increasing the local accumulation of nanoparticles in sites of interest.
- The utilization of molecularly targeted CT contrast agents expands the role of CT beyond its present structural imaging capabilities, endowing it with functional- and molecular-based imaging capabilities as well.
- However, since the active targeting approach is based on the existence and the degree of overexpression of specific tissue biomarkers, it is applicable only under particular biological conditions.

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