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Nanoparticulate X-ray CT contrast agents

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X-ray computed tomography (CT) has been widely used as a powerful diagnostic tool in clinics because it can provide high-resolution 3D tomography of the anatomic structure based on the distinctive X-ray absorptions between different tissues. Currently, CT contrast agents are mainly small iodinated molecules, which suffer from drawbacks such as short blood-retention time, nonspecific *in vivo* biodistribution, and renal toxicity. Utilization of nanoparticles as potential CT contrast agents to overcome the aforementioned issues has advanced rapidly. In this mini review, we introduce current research efforts in the development of nanoparticulate CT contrast agents and discuss the challenges for additional breakthroughs in this field.

X-ray computed tomography (CT), contrast agents, nanoparticles, iodine

1 Introduction

X-ray computed tomography (CT) is considered as one of the most useful diagnostic procedures in medicine [1]. When an X-ray beam traverses an object, the original beam is weakened due to the absorption and deflection of X-ray photons by the object. This process, referred to as X-ray attenuation, is closely related to mass attenuation coefficient. Different tissues have distinctive mass attenuation coefficients, hence, contrast occurs when the X-ray beam passes through a human body [2–4]. Under normal circumstances, this inherent contrast between bone and its surrounding tissues is large enough to distinguish them. However, it is difficult to differentiate soft tissues such as fat/muscle or normal organ/ tumors, owing to their similar mass attenuation coefficients [5]. To better delineate such tissues, seeking safe and efficient exogenous CT contrast agents has become a great challenge for researchers and physicians.

Currently, the CT contrast agents in clinical use are mainly small iodinated molecules and barium sulfate suspensions [6]. However, clinical practice indicates that these agents are not optimal. For example, barium sulfate suspensions are used only for gastrointestinal (GI)-tract imaging because Ba^{2+} ions can be very toxic to living organisms. Small iodinated molecules used for cardiovascular CT imaging also suffer from the following limitations: (1) short blood-circulation time, which makes them unsuitable for blood-pool imaging; (2) lack of applicability for patients who are hypersensitive to iodine; and (3) nonspecificity in *in vivo* biodistribution, which hinders their applications for target-imaging [7]. In addition, their low K-shell electron binding (K-edge) energy results in low contrast efficiency. Progress in nanotechnology has created new paradigm shifts for bioimaging. Owing to their unique properties such as controllable synthesis, facile surface modification, and special pharmacokinetics (EPR effect), nanomaterials, especially metal-containing inorganic nanoparticles, hold great promise to serve as CT contrast media [8]. In this mini review, we take a brief look at recent efforts in the field of nanoparticulate CT contrast agent research, with a specific focus on the design of metal-containing nanoparticulate contrast media.

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2 Iodine-based CT contrast agent

The use of iodine as CT contrast agents has a long and rich history. In 1923, Osborne and colleagues [9] found for the first time that sodium iodide solutions can be used for delineating the bladder. Since then, iodinated agents have undergone significant evolutions with numerous methods and materials approved to optimize their contrast properties. Most of the iodinated contrast agents in use are composed of 1,3,5-triiodobenzene and its derivatives, such as iohexol (trade name Omnipaque, GE Healthcare, USA), iopromide (trade name Ultravist, Bayer Healthcare, Germany), and iodixanol (trade name Visipaque, GE Healthcare) (Figure 1). Although these small molecule agents are nontoxic and cost-effective, extravascular leakage and rapid renal excretion occur when they are administered *in vivo*. To address these limitations, one strategy is to increase the particle size of the contrast agents with the purpose of avoiding renal filtration by the glomerular capillaries. Therefore, iodine-based nanoparticle systems such as nanoemulsions, liposomes, and polymeric nanoparticles have been developed [10].

2.1 Iodine-containing nanoemulsion

Emulsions are dispersions of one liquid phase in another immiscible liquid phase (e.g., oil in water). The way emulsions carry CT contrast agents is similar to the way soap micelles encapsulate oily dirt. Nanoemulsions allow for the months-long stable dispersion, in the form of droplets, of highly concentrated lipophilic iodinate agents in an aqueous phase. Furthermore, the formulation process can be very simple [11]. Nanoemulsion of iodinate poppy-seed oil, named Lipiodol, was among the earliest nanoparticulate contrast agents. In clinical settings, Lipiodol is commonly used as a contrast medium for lymphography and hysterosalpingography as well as for detecting liver tumors in certain cases [12]. Two other commercially available nanoemulsion contrast agents are Fenestra LC and Fenestra VC. These emulsions are manufactured by shear-induced rupturing with 10% iodinated triglycerides. Fenestra LC is indicated for hepatobiliary system and liver function imaging results in fast Apoliprotein E (ApoE) receptors mediated uptaken by hepatocytes. Because tumor cell surfaces hardly express ApoE receptors, Fenestra LC can also be used for image-guided tumor-seeking in livers. Fenestra VC, owing to the polyethylene glycol (PEG) coating which blocks fast recognition by hepatocytes via ApoE, is commonly used as a blood-pool contrast agent in vascular imaging. Given its toxicity issues, the use of Fenestra is still constrained to preclinical animal models. In contrast to small iodinated molecules, these nanoemulsions are eliminated by hepatocyte metabolism and thus reduce nephrotoxicity [13].

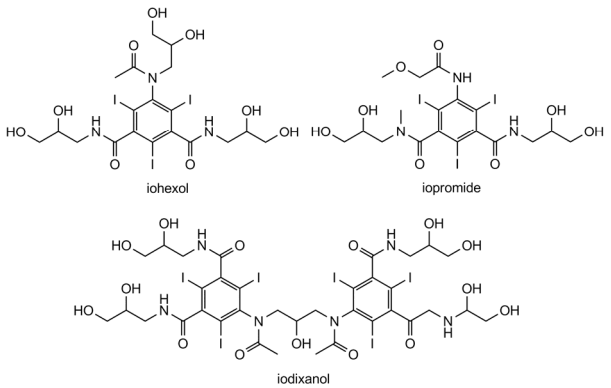

Figure 1 Structures of iodinated CT contrast agents.

Micro-computed tomography (micro-CT) is a new imaging modality for preclinical X-ray imaging. Compared with CT, the main advantage of micro-CT is high resolution, which allows for clear delineation of micro-sized tissues such as metastases. However, micro-CT is more time-consuming and possesses low efficiency [14]. The clinical blood-pool imaging agents with low iodine concentrations are thus not appropriate for micro-CT imaging. Recently, Li *et al.* [15] proposed a novel contrast agent (iodinated vitamin E nanoemulsions) with sufficient iodine content (around 106 mg/mL) for micro-CT. These agents, which were formulated in the form of nanoemulsion droplets by a spontaneous emulsification process, are surrounded by a PEG shell as stabilizer and confer stealth properties (Figure 2). Owing to the chemical nature of iodinated vitamin E, the contrast agents showed good biocompatibility and low toxicity, and could be internalized by hepatocytes. After intravenous administration into mice, the nanoemulsion exhibited long blood circulation time ($t_{1/2}$=9.0 h) and gradually accumulated in the liver, which provided strong contrast enhancement of this organ. Moreover, given its *in vivo* pharmacokinetics, it specifically accumulates in liver after long-term retention in the bloodstream. Thus these nanoemulsions show great potential for clinical use as well.

2.2 Iodine-based liposome

Liposomes are spherical nanoparticles composed of lipid bilayers that enclose an aqueous core. Compared with emulsion droplets, liposomes can transport both water-soluble iodinated molecules encapsulated in their aqueous core and water-insoluble iodine compounds dissolved in their lipid membrane. In addition, the toxicity of liposomes is reported to be lower than that of iodinated emulsions. Bare liposomes

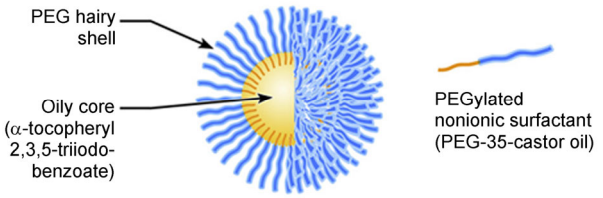

Figure 2 Schematic representation of a PEGylated nanoemulsion droplet [15].

can accumulate rapidly in the liver and spleen through the reticuloendothelial system (RES system) after intravenous injection; therefore, the initial motivation for using liposomes as CT contrast agents was to seek tumors in these organs [16]. The emerging of long-circulating liposomes benefits from the discovery of PEG. Annapragada's group [17] developed PEGylated liposomes loaded with the clinically used CT contrast agent iohexol for the purpose of achieving longer blood-residence time. The fabricated liposomes, 100 nm in diameter, provided sustained contrast enhancement of the blood pool and liver for more than 3 h after intravenously injection into rabbits. These liposomes were mainly excreted via the liver, and minimal early clearance through the kidney was also observed.

Sustained enhancement of blood-pool contrast allows for repeated imaging without the need for a second contrast injection, and thus offers the potential for online evaluation of the outcome of a therapy [10]. Burke *et al.* [18] used a PEGylated iohexol-containing liposomal agent to evaluate the diagnostic accuracy of pulmonary embolisms by monitoring the efficiency of a tissue plasminogen activator (t-PA, clinically an approved pharmaceutical for pulmonary embolism treatment) in rabbit modals. CT images taken over a time period of 4 h showed, a thrombus volume reduction in the range of 40%–60%.

In comparison to other nanoparticulate carriers, stability is the major drawback of liposomes [19]. Leakage of the encapsulated contrast agents within the inner core of liposomes often occurs in many liposomal formulations, mainly due to the differences in osmotic pressures as well as chemical potentials between the aqueous core of liposomes and the external bulk environment [20]. To address this limitation, Elrod and coworkers [21] created an iodinated lipid that could self-assemble into iodoliposomes. Because iodinated moieties of this formulation are contained within the vesicle's bilayer, leakage of iodinated molecules from the inner aqueous core is effectively avoided. Moreover, the unoccupied inner cores are available to encapsulate pharmaceutical agents to construct multifunctional nanoprobes or coload other contrast agents to further improve their contrast ability (Figure 3).

2.3 Iodine-based polymeric nanoparticles

Owing to their controllable synthesis and easy modification process, polymeric nanoparticles have recently emerged as promising contrast agents. Polymeric CT contrast agents are generally prepared by physical entrapment or covalent linkage of iodinated molecules to polymeric chains, which form nanospheres or nanocapsules [22,23]. Margel's group [24] demonstrated an iodinated radiopaque polymeric nanoparticles for body-organ imaging. By emulsion polymerization of 2-methacryloyloxyethyl(2,3,5-triiodobenzoate) (MAOETIB), polymeric nanoparticles composed of ca. 58% iodine (by weight) and with sizes ranging from 30 to 350 nm were obtained. These nanoparticles were administered intravenously into a dog, after which various organs including popliteal lymph nodes, liver, kidney, and spleen were visually observed in the obtained CT images 24 h postinjection. However, these nanoparticles could not allow blood-pool imaging because of their low particle concentration in aqueous solution (agglomeration occurred at higher concentration). Thereafter, copolymeric nanoparticles synthesized by emulsion copolymerization of MAOETIB and glycidyl methacrylate (GMA) were proposed by the same group for the achievement of blood-pool imaging. The obtained nanoparticles were small in size and had a more hydrophilic surface. Therefore, they are more stable against aggregation in physiological aqueous conditions. In a 5% dextrose solution, these nanoparticles can be condensed to 80 mg/mL without detectable agglomeration. Following intravenous injection into mice, significant enhancement of the blood pool was obtained at 2 min postinjection and lasted for 30 min. Moreover, these nanoparticles can be used to detect metastatic disease, owing to the strong uptake of the nanoparticles by the RES system. Cancerous tumor tissues in livers were clearly delineated 4 h postinjection in a tumor-bearing mouse from the *in vivo* CT image [25].

Contrast agents used *in vivo* should be easily excreted after imaging to moderate any safety concern associated with their incomplete clearance. Cheng's group [26] demonstrated biocompatible poly(iohexol) nanoparticles for *in vivo* CT imaging. The nanoparticles were synthesized by cross-linking of iohexol and hexamethylene diisocyanate, followed by coprecipitation of the resulting polymers with mPEG-polylactide. Poly(iohexol) nanoparticles showed negligible toxicity and remarkable stability, and exhibited prolonged retention in the tumor bed compared to free iohexol. Furthermore, owing to the degradable domains (e.g., ester bond) in poly(iohexol), the nanoparticles could be degraded into small molecules *in vivo* at the end of the study, thus effectively avoiding unfavorable clearance profiles.

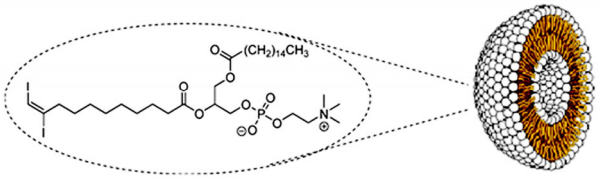

Figure 3 Liposomal CT contrast agent with iodine covalently incorporated into the lipid bilayer, which provides an empty cavity with potential coloading applications [8].

3 Metallic nanoparticulate CT contrast agents

The application of metallic nanoparticles to CT imaging has attracted great interest along with the blooming of nanotechnology [27]. In recent years, nanomaterials containing Au, Bi, Ta, Yb, etc. have been reported as novel CT contrast agents. Due to their high atomic numbers and K-edge values,

metallic nanoparticles can not only provide greater contrast than iodine but also lower the doses of radiation to which patients are exposed. In addition, facile surface modification with bioconjugates such as peptides, proteins, and antibodies make these nanoparticles attractive for target-specific imaging and molecular imaging.

3.1 Gold nanoparticles as CT contrast agents

Gold nanoparticles (GNPs) have long been studied for biomedical use because of their innate inertness and high biocompatibility. Owing to its high atomic number and high X-ray absorption coefficient, gold can provide about 2.7 times more contrast per unit weight than iodine [7]. In addition, GNPs show great affinity to thiol derivatives, thus allowing for facile surface modification. The use of GNPs as an *in vivo* CT contrast agent was first demonstrated by Hainfeld and coworkers [28]. When commercially available GNPs, 1.9 nm in diameter, were intravenously injected into a tumor-bearing mouse, detailed anatomic structures including blood vessels less than 100 μm in diameter and a 5 mm tumor were clearly visualized (from its increased vascularity) in CT images. Sharp delineation of the tumor could be obtained even at 24 h postinjection, which revealed that GNPs also hold great promise for contrast-enhanced tumour detection and therapy. These nanoparticles have low toxicity with a LD_{50} of 3.2 g Au/kg and are excreted through renal clearance due to their small size.

It was reported that bare nanoparticles easily aggregate *in vivo* due to surface absorbtion of plasma proteins and salts in blood; thus, the direct use of bare GNPs *in vivo* leads to rapid uptake by the RES system and the danger of plugged blood vessels [29,30]. To solve these problems, antibiofouling PEG-coated GNPs were developed as potential CT contrast agents for angiography and hepatoma detection. Using the citrate reduction method, they prepared them with a diameter of approximately 30 nm, followed by covalent conjugation of the PEG. Due to their larger size and the stealth property provided by PEG, the PEG-coated GNPs showed prolonged blood-circulation time (at least 4 h) and specifically accumulated in macrophage-rich organs such as the liver. After a 5 min intravenous injection of PEG-coated GNPs into a hepatoma-bearing rat, clear delineation between normal liver and hepatoma was observed. When the author performed an MTT assay to determine the toxicity of PEG-coated GNPs on a HepG2 hepatocyte cell line *in vitro*, results revealed that the nanoparticles did not induce any appreciable toxicity, even at a high concentration (1 mg/mL) [31].

The accurate detection of microcalcification, a common abnormality detected by mammography, is of vital importance for breast cancer diagnosis [32]. Cole and coworkers [33] developed bisphosphonate-functionalized gold nanoparticles (BP-AuNPs) for image-guided detection of breast microcalcifications based on the high binding affinity of bisphosphonates for hydroxyapatite (HA), the mineral component of breast microcalcifications. The synthesized monodisperse BP-AuNPs are spherical with a mean diameter of 12.8 nm and exhibit long-term stability in physiological media. The author utilized two breast microcalcification models comprising different concentrations of HA to evaluate the efficacy of BP-AuNPs for contrast-enhanced radiographic detection. Experimental results showed that the contrast enhancement of HA compositions bonded by BP-AuNPs was increased compared with unbonded compositions at the same concentrations of HA, even at a low concentration that was beyond the sensitivity of micro-CT.

Various GNP-based CT contrast media have been designed recently for laboratory and clinical research; some of them seem promising for commercial translation. However, unlike magnetic resonance (MR) or fluorescence imaging, CT is insensitive to low concentrations of contrast agents [8]. In order to induce sufficient contrast, large amount of contrast agents are needed. Considering their high price, GNPs are not an ideal choice. Therefore, researchers are seeking to develop metallic materials that are more economical and exhibit excellent contrast properties.

3.2 Bismuth-based CT contrast agents

Like gold, bismuth (Z=83, K-edge value=90.5 keV) possesses good X-ray attenuation properties [34]. Bismuth, an abundant mineral, is highly cost-effective to use. In 2006, Rabin and coworkers [35] reported the use of polyvinyl pyrrolidone (PVP)-coated bismuth sulfide (Bi_2S_3) nanocrystals as an injectable CT contrast agent. The quasi-rectangular platelet-shaped Bi_2S_3 nanocrystals, 10−50 nm in width/length and about 4 nm in thickness, were synthesized through coprecipitation of bismuth citrate and sodium sulfide followed by surface modification with PVP. The nanoparticles exhibited long blood half-life (140±15 min) and were effectively used for *in vivo* imaging of the vasculatures, liver, and lymph nodes. Moreover, contrary to free bismuth ion (LD_{50} 8 mmol/L), bismuth in the form of Bi_2S_3 nanocrystal (LD_{50} 100 mmol/L) is less toxic to cells and shows a safety profile similar to clinically iodinated agents.

Pan *et al.* [36] demonstrated nanocolloids of bismuth (NanoK) to detect intra-arterial thrombi with spectral CT. NanoK was synthesized by encapsulating a commercially available hydrophobic bismuth complex (bismuth *n*-decanoates) in a phospholipid monolayer with the stabilization of surfactants. By surface modification of a monoclonal antibody (anti-fibrin mAb), along with the relatively large particle size (ca. 200 nm in diameter), antibody-labeled NanoK could be constrained to the vasculature; specifically, it could bind to the fibrin in the ruptured atherosclerotic plaque. After 30 min *in situ* incubation within the iliac artery of an atherosclerotic rabbit, an intra-arterial thrombus enhanced with fibrin-bound NanoK was clearly differentiated from the attenuation effects of the bone.

Bi_2S_3 nanoparticles labled with LyP-1 peptide have been reported for target imaging of breast cancer with CT. Peptide LyP-1 exhibits specific targeting to tumor lymphatic vessels as well as other cells in tumor tissues. The cellular receptor for LyP-1 is the p32 protein, which is overexpressed on breast cancer cells and their mitochondrial membranes. LyP-1 was bounded to PEGylated Bi_2S_3 nanoparticles through thioether formation between the N-terminal cysteine moiety on LyP-1 and the maleimide group on PEG_{5000}-DSPE. *In vitro* cell incubation experiments indicated that LyP-1-labled Bi_2S_3 nanoparticles could be effectively internalized by the p32-expressing 4T1 cells and did not affect cell growth. *In vivo* characterization of the LyP-1-labled Bi_2S_3 nanoparticles was carried out by intravenously injecting the nanoparticles into a tumor-bearing mouse. Micro-CT images were obtained over 1 d after administration; results showed that the LyP-1-labled Bi_2S_3 nanoparticles were more likely to accumulate in the tumor site than non-labled Bi_2S_3 nanoparticles. In addition, the contrast enhancement was still visible after 7 d. Although the nanoparticles were eliminated through a hepatobiliary/fecal route, agglomeration did occur during the *in vivo* circulation [37].

The feasibility of controlled synthesis and surface modification as well as large-scale production of these newly developed Bi_2S_3 nanoparticle preparations are the crucial determinants of their ultimate applications in clinics. Our group explored a facile strategy for large-scale fabrication of Bi_2S_3 nanodots [34] that were 2–3 nm in diameter with remarkable size uniformity and showed excellent monodispersity in organic solvents under the protection of oleic acid. After coating with PVP through a ligand exchange reaction, the obtained PVP-Bi_2S_3 could disperse well in water and remain stable for more than one month without detectable bismuth-ion leaching. The *in vivo* contrast efficacy of PVP-Bi_2S_3 nanodots was tested by intravenous administration of PVP-Bi_2S_3 suspension into mice followed by imaging with a CT scanner used in clinic (with peak voltage of 120 kV). Contrast enhancement was observed in the heart, kidney, liver, and spleen; the enhancement of the latter two lasted longer than 4 h. On the contrary, no enhancement of these organs was detected after the administration of iobitridol (a commercially available iodinated contrast agent). These nanodots exhibited long blood retention times of several hours and were eliminated from the mice within one month after injection with no acute damage to organ tissues.

3.3 Tantalum oxide nanoparticles as CT contrast agents

Water-soluble tantalum oxide nanoparticles are another alternative to GNPs. These nanoparticles are chemically inert, biocompatible, and have good radiopacity [38]. At equal molar concentrations, tantalum provides greater contrast enhancement than iodine throughout the diagnostic X-ray spectrum. Without surface modification, tantalum oxide nanoparticles are inappropriate for intravenous injection because of their hydrophobic property. In 2010, Bonitatibus and coworkers [39] demonstrated water-soluble tantalum oxide nanoparticles by conjugation with triethoxysilane for CT imaging. Following intravenous injection into mice, transient contrast enhancement was observed from the vena cava to the heart; however, rapid renal clearance also occurred. Hyeon's group [40] developed a multimodal tantalum oxide nanoparticle, PEG-RITC-TaO_x, that exhibited longer blood-circulation time; its preparation involved modification of PEG and fluorescent dye onto the surface of tantalum oxide nanoparticles via simple sol-gel reaction. The imaging probe not only exhibited prolonged (over 3 h) blood-vessel contrast enhancement but also can be used for bimodal (CT and fluorescence imaging) image-guided lymph-node mapping. When these researchers systematically examined the toxicity of these nanoparticles, experimental results revealed that PEG-RITC-TaO_x nanoparticles did not cause any significant adverse effect to organs.

More recently, nontoxic cationic tantalum oxide nanoparticles have been developed and used for target imaging of articular cartilage [41]. Core-shell tantalum oxide nanoparticles (Ta_2O_5 NPs) with different surface charges were prepared using phosphonate (neutral), ammonium (cationic), and carboxylate (anionic) ligands as end functional groups. *Ex vivo* murine modal experiments showed that cationic tantalum oxide nanoparticles could distribute into the entirety of the articular cartilage, thereby enabling clear visual differentiation of cartilage from both electron-dense bone and air, while the neutral and anionic nanoparticles accumulated only at the surface and did not produce sharp delineation of the cartilage-bone or cartilage-air interfaces. Because articular cartilage is negatively charged, this contrast-enhancement difference was mainly due to the favorable coulombic attraction between positively charged NPs and articular cartilage. The author next used cationic tantalum oxide nanoparticles to image a naturally occurring cadaveric osteoarthritic defect in a human cadaver distal metacarpophalangeal (MCP) joint. The obtained CT images revealed that the cationic nanoparticles penetrated into the cartilage such that the defect could be clearly observed (Figure 4). In general, positively charged nanoparticles are extremely cytotoxic to living systems. In order to demonstrate feasibility of use *in vivo*, cationic tantalum oxide nanoparticles were further modified with PEG to moderate their cytotoxicity. The obtained nanoparticles demonstrated an improved safety profile and were administered into the male rat knee through an intra-articular injection. The contrast-enhanced cartilage could be clearly visualized in both *in vivo* and *ex vivo* CT images. Moreover, the rat displayed no signs of distress and subsequent histological examination of the cartilage revealed no adverse effects.

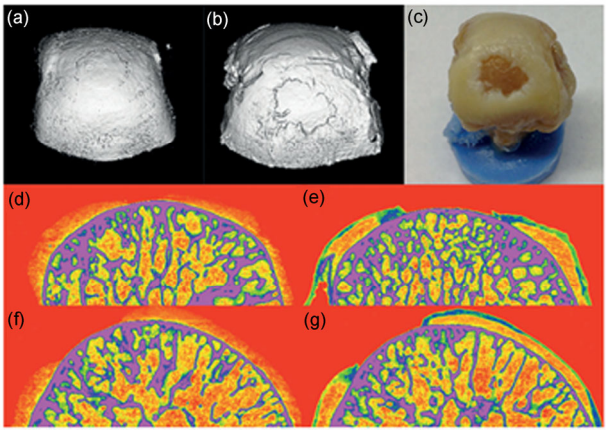

Figure 4 Cartilage defect in the proximal metacarpophalangeal (MCP) joint of a human index finger. The images were obtained before (a, d, f) and after (b, e, g) immersion in a cationic tantalum oxide nanoparticles (NPs) solution with a concentration of 40 mg/mL for 24 h. The 3D CT reconstruction in (b) shows the defect and is similar to that seen with the result of gross inspection (c). Coronal (d, e) and sagittal (f, g) cross-section 2D-CT reconstructions further document the necessity of using the NPs to visualize the cartilage and the defect in the MCP [41].

3.4 Ytterbium-based nanoparticulate contrast agents

Ytterbium (Yb) has a higher atomic number (Z=70) than Au, Bi, and Ta, as well as iodine. More importantly, Yb's K-edge is just located within the higher-energy region in the X-ray spectrum, which means that Yb can provide excellent contrast enhancement under normal operating conditions in clinical CT. Recently, research interests in Yb have mainly been focused on its use as a host material for NIR-Vis up-conversion luminescence (UCL). Our group, for the first time, explored the use of Yb-containing nanoparticles as an injectable CT imaging agent. Gd-doping $NaYbF_4$:Er nanoparticles (OA-UCNPs) with controllable shape and size were synthesized in an organic solvent containing oleic acid. Subsequently, PEGylation was implemented to yield water-soluble PEG-UCNPs. The obtained PEG-UCNPs showed excellent monodispersity in aqueous solution and negligible cytotoxicity at high concentrations, and could be uptaken by Hela cells *in vitro*. Compared to a clinically used iodinated contrast agent (iobitridol), PEG-UCNPs provided higher X-ray absorption and longer circulation time (more than 1 h) *in vivo*. Furthermore, at 120 kV, the obtained CT numbers (HU value) of PEG-UCNPs were higher than any other currently available metallic contrast agents (e.g., Au-, Bi-, Pt-, and Ta-based nanoparticles). After intravenously injection into a rat, clear delineations of the heart, great vessels, liver, and spleen were observed from the obtained CT images at different time intervals. Similar enhancement of these organs was obtained even when the injected dosage was lowered by half. Based on the long blood-circulation time and good contrast efficacy, we evaluated the feasibility of PEG-UCNPs to lymph-node imaging by subcutaneously injecting a solution of PEG-UCNPs into a rat's paw; contrast enhancement of the regional lymph node was obtained as anticipated. To assess the capability of PEG-UCNPs to serve as multimodal imaging (CT/UCL/MR) contrast agents, we next tested the *in vivo* contrast enhancement of fluorescence and magnetic resonance imaging, with good results [42].

After demonstrating the usefulness of Yb-containing CT contrast agents as above, we further explored their additional uses. First, we hybrid $BaYbF_5$@SiO_2@PEG nanoparticles to meet the clinical need for a universal contrast agent that can supply good contrast at different operating voltages. The presence of two contrast elements (Yb and Ba) enables better contrast because the operating voltage changes from 80 to 140 kV relative to clinical iodinated agents (e.g., iobitridol). After these nanoparticles were intravenously administered into a mouse, contrast enhancement of the vasculature was observed immediately and could last for more than 2 h; this long-term enhancement was also observed in heart. We next used a larger animal model, a rabbit, to clearly observe the blood pool by CT imaging; as anticipated, nice blood pool CT images were obtained (Figure 5). These results show that $BaYbF_5$@SiO_2@PEG nanoparticles hold great promise for applications in angiography imaging. When we detected the *in vivo* biodistribution of these nanoparticles by fluorescence imaging, we found that they are excreted via a hepatobiliary/fecal route. Other histological studies and cytotoxicity assessments have also indicated good safety profiles for these nanoparticles [43].

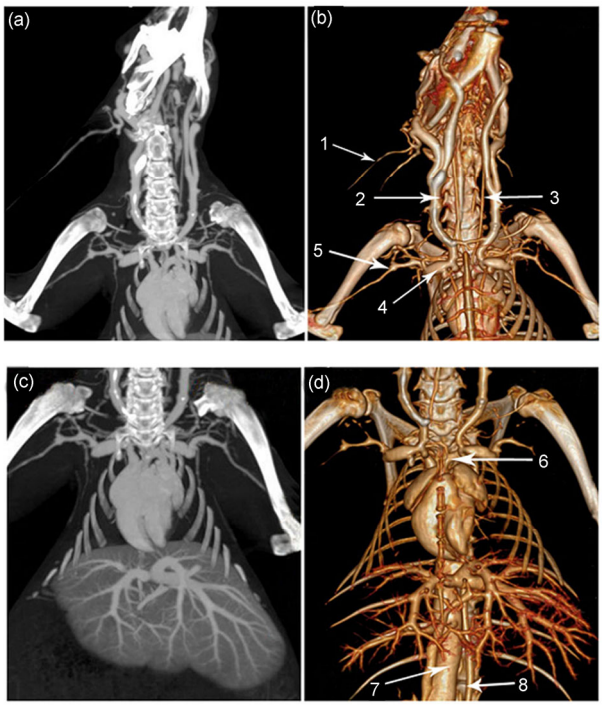

Figure 5 (a–d) Blood-poor imaging of a rabbit 10 min after intravenous administration of $BaYbF_5$@SiO_2@PEG solution. 1, Auricular vein; 2, jugular vein; 3, carotid artery; 4, subclavian vein; 5, axillary vein; 6, aortic arch; 7, inferior vena cava; 8, aorta [27].

3.5 Other metal-based contrast agents

Tungsten is an active site for enzymes in living organisms. Because it has higher atomic number (Z=74) and is more dense than iodine, tungsten is considered an appropriate candidate for CT imaging. Commonly, tungsten compounds such as tungsten oxide nanoparticles are so toxic that they can lead to mouse death within a few seconds after administration. This toxicity likely results from particle aggregation in blood vessels and capillaries, which causes embolisms and the subsequent plugging of blood vessels [44]. Jakhmola and coworkers [45] developed tungsten oxide (WO_3) nanoparticles modified with poly-ε-caprolactone that could be used as *in vivo* contrast agents for CT imaging. These nanoparticles show good stability in physiological conditions and do not induce detectable clinical signs of toxicity. They can provide good contrast at a low concentration (0.1 mol/L) and are cleared from the rat body within a few hours after administration.

Platinum, like gold, is another dense, highly unreactive noble metal with good radiopacity. FePt alloy nanoparticles were deveoped for dual modal CT/MR imaging [46]. After surface modification of monoclonal antibody, the obtained nanoparticles can be used for tumor-target imaging *in vivo*. The nanoparticles exhibit good biocompatibility and no noticeable cytotoxicity to mice.

Our group explored Gd-doping $NaYbF_4$:Er nanoparticles for CT/MR/UCL trimodel imaging. Similarly, Gd-doping water-soluble $NaLuF_4$:Yb/Er nanorods were demonstrated recently for synergistic X-ray/fluorescent dual-modal bioimaging. When both X-ray and fluorescence imaging were performed on a nude mouse that had been subcutaneously injected with the nanoprobes, results indicated that these nanoprobes were capable of synergistic imaging. They selectively accumulated in the liver and lungs, enabling clear delineation of these organs and even the blood vessels of the lungs in the X-ray image. Moreover, Gd-doping endows nanoprobes with magnetic properties to be used for magnetic resonance imaging. Toxicity issues were not mentioned by the author [47].

4 Conclusions

The importance of CT as a leading diagnostic technology in the field of biomedical imaging has promoted the development of nanoparticles as the next generation of CT contrast agents. In this mini review, we described the basic design principles and took a brief look at the state-of-art developments of nanoparticulate CT contrast agents based on iodine and metals. Research in this field has concentrated on the development of blood-pool, specific-targeting, and multifunctional contrast media that, allow clear blood-vessel visualization and early detection of certain diseases and abnormalities. More importantly, these newly developed nanoprobes have changed the concept of CT from diagnosis based on anatomical structures to diagnosis according to molecular markers [10]. Although these novel contrast agents are very attractive for clinical applications, their high costs (Au, Pt), complex syntheses, purification processes (liposome, emulsion), and long-term *in vivo* toxicity must be considered, particularly for metal elements (e.g., Bi, Ta, lanthanide elements) that don't naturally occur in the human body. In addition, toxicity observations to date have been constrained to *in vitro* experiments or preclinical studies; further investigations are needed of the overall toxicity and elimination of these exogenous agents [27].

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1 Kalender WA. X-ray computed tomography. *Phys Med Biol*, 2006, 51: R29–R43
2 Ahn S, Jung SY, Lee JP, Lee SJ. Properties of iopamidol-incorporated poly(vinyl alcohol) microparticle as an X-ray imaging flow tracer. *J Phy Chem B*, 2011, 115: 889–901
3 Aillon KL, El-Gendy N, Dennis C, Norenberg JP, McDonald J, Berkland C. Iodinated nano clusters as an inhaled computed tomography contrast agent for lung visualization. *Mol Pharmaceut*, 2010, 7: 1274–1282
4 Alric C, Taleb J, Le DG, Mandon C, Billotey C, Le Meur-Herland A, Brochard T, Vocanson F, Janier M, Perriat P, Roux S, Tillement O. Gadolinium chelate coated gold nanoparticles as contrast agents for both X-ray computed tomography and magnetic resonance imaging. *J Am Chem Soc*, 2008, 130: 5908–5915
5 Yu SB, Watson AD. Metal-based X-ray contrast media. *Chem Rev*, 1999, 99: 2353–2377
6 Lee N, Choi SH, Hyeon T. Nano-sized CT contrast agents. *Adv Mater*, 2013, 25: 2641–2660
7 Liu YL, Ai KL, Lu LH. Nanoparticulate X-ray computed tomography contrast agents: from design validation to *in vivo* applications. *Acc Chem Res*, 2012, 45: 1817–1827
8 Lusic H, Grinstaff MW. X-ray-computed tomography contrast agents. *Chem Rev*, 2013, 113: 1641–1666
9 Osborne ED, Sutherland CG, Scholl AJJr, Rowntree LG. Roentgenography of urinary tract during excretion of sodium iodide. *J Am Med Assoc*, 1923, 80: 368–373
10 Shilo M, Reuveni T, Motiei M, Popovtzer R. Nanoparticles as computed tomography contrast agents: current status and future perspectives. *Nanomedicine*, 2012, 7: 257–269
11 Hallouard F, Anton N, Choquet P, Constantinesco A, Vandamme T. Iodinated blood pool contrast media for preclinical X-ray imaging applications: a review. *Biomaterials*, 2010, 31: 6249–6268
12 Chung YE, Hyung WJ, Kweon S, Lim SJ, Choi J, Lee MH, Kim H, Myoung S, Lim JS. Feasibility of interstitial CT lymphography using optimized iodized oil emulsion in rats. *Invest Radiol*, 2010, 45: 142–148
13 Ashokan A, Menon D, Nair S, Koyakutty M. A molecular receptor targeted, hydroxyapatite nanocrystal based multi-modal contrast agent. *Biomaterials*, 2010, 31: 2606–2616
14 Montet X, Pastor CM, Vallee JP, Becker CD, Geissbuhler A, Morel DR, Meda P. Improved visualization of vessels and hepatic tumors by micro-computed tomography (CT) using iodinated liposomes. *Invest Radiol*, 2007, 42: 652–658
15 Li X, Anton N, Zuber G, Zhao MJ, Messaddeq N, Hallouard F, Fessi H, Vandamme TF. Iodinated α-tocopherol nano-emulsions as non-

toxic contrast agents for preclinical X-ray imaging. *Biomaterials*, 2013, 34: 481–491
16 Desser TS, Rubin DL, Muller H, Mclntire GL, Bacon ER, Toner JL. Blood pool and liver enhancement in CT with liposomal iodixanol. *Acad Radiol*, 1999, 6: 176–183
17 Kao CY, Hoffman EA, Beck KC, Bellamkonda RV, Annapragada AV. Long-residence-time nano-scale liposomal lohexol for X-ray-based blood pool imaging. *Acad Radiol*, 2003, 10: 475–483
18 Burke SJ, Annapragada A, Hoffman EA, Chen E, Ghaghada KB, Sieren J, van Beek EJR. Imaging of pulmonary embolism and t-PA therapy effects using MDCT and liposomal lohexol blood pool agent: preliminary results in a rabbit model. *Acad Radiol*, 2007, 14: 355–362
19 Zingel C, Sachse A, Rössling GL, Müller RH. Lyophilization and rehydration of iopromide-carrying liposomes. *Int J Pharm*, 1996, 140: 13–24
20 Schneider T, Sachse A, Rossling G, Brandl M. Generation of contrast-carrying liposomes of defined size with a new continuous high pressure extrusion method. *Int J Pharm*, 1995, 117: 1–12
21 Elrod DB, Partha R, Danila D, Casscells SW, Conyers JL. An iodinated liposomal computed tomographic contrast agent prepared from a diiodophosphatidylcholine lipid. *Nanomedicine: NBM*, 2009, 5: 42–45
22 Ho KW, Jae LW, Yun CZ, Hyun BK, Gwan PT, Hoon KJ. Nanoparticulate carrier containing water-insoluble iodinated oil as a multifunctional contrast agent for computed tomography imaging. *Biomaterials*, 2007, 28: 5555–5561
23 Rogers WJ, Basu P. Factors regulating macrophage endocytosis of nanoparticles: implications for targeted magnetic resonance plaque imaging. *Atherosclerosis*, 2005, 178: 67–73
24 Galperin A, Margel D, Baniel J, Dank G, Biton H, Margel S. Radiopaque iodinated polymeric nanoparticles for X-ray imaging applications. *Biomaterials*, 2007, 28: 4461–4468
25 Aviv H, Bartling S, Kieslling F, Margel S. Radiopaque iodinated copolymeric nanoparticles for X-ray imaging applications. *Biomaterials*, 2009, 30: 5610–5616
26 Yin Q, Yap FY, Yin LC, Ma L, Zhou Q, Dobrucki LW, Fan TM, Gaba RC, Cheng JJ. Poly(iohexol) nanoparticles as contrast agents for *in vivo* X-ray computed tomography imaging. *J Am Chem Soc*, 2013, 135: 13620–13623
27 Liu YL, Liu JH, Ai KL, Yuan QH, Lu LH. Recent advances in ytterbium-based contrast agents for *in vivo* X-ray computed tomography imaging: promises and prospects. *Contrast Media Mol Imaging*, 2014, 9: 26–36
28 Hainfeld JF, Slatkin DN, Focella TM, Smilowitz HM. Gold nanoparticles: a new X-ray contrast agent. *Br J Radiol*, 2006, 79: 248–253
29 Rogers WJ, Basu P. Factors regulating macrophage endocytosis of nanoparticles: implications for targeted magnetic resonance plaque imaging. *Atherosclerosis*, 2005, 178: 67–73
30 Raynal I, Prigent P, Peyramaure S, Najid A, Rebuzzi C, Corot C. Macrophage endocytosis of superparamagnetic iron oxide nanoparticles-mechanisms and comparison of ferumoxides and ferumoxtran-10. *Invest Radiol*, 2004, 39: 56–63
31 Kim D, Park S, Lee JH, Jeong YY, Jon S. Antibiofouling polymer-coated gold nanoparticles as a contrast agent for *in vivo* X-ray computed tomography imaging. *J Am Chem Soc*, 2007, 129: 7661–7665
32 Gulsun M, Demirkazık FB, Arıyürek M. Evaluation of breast microcalcifications according to breast imaging reporting and data system criteria and Le Gal's classification. *Eur J Radiol*, 2003, 47: 227–231
33 Cole LE, Vargo-Gogola T, Roeder RK. Bisphosphonate-functionalized gold nanoparticles for contrast-enhanced X-ray detection of breast microcalcifications. *Biomaterials*, 2014, 35: 2312–2321
34 Ai KL, Liu YL, Liu JH, Yuan QH, He YY, Lu LH. Large-scale synthesis of Bi_2S_3 nanodots as a contrast agent for *in vivo* X-ray computed tomography imaging. *Adv Mater*, 2011, 23: 4886–4891
35 Rabin O, Perez JM, Grimm J, Wojtkiewicz G, Weissleder R. An X-ray computed tomography imaging agent based on long-circulating bismuth sulphide nanoparticles. *Nat Mater*, 2006, 5: 118–122
36 Pan D, Roessl E, Schlomka JP, Caruthers SD, Senpan A, Scott MJ, Allen JS, Zhang H, Hu G, Gaffney PJ, Choi ET, Rasche V, Wickline SA, Proksa R, Lanza GM. Computed tomography in color: nanoK-enhanced spectral CT molecular imaging. *Angew Chem Int Ed*, 2010, 49: 9635–9639
37 Kinsella JM, Jimenez RE, Karmali PP, Rush AM, Kotamraju VR, Gianneschi NC, Ruoslahti E, Stupack D, Sailor MJ. X-ray computed tomography imaging of breast cancer by using targeted peptide-labeled bismuth sulfide nanoparticles. *Angew Chem Int Ed*, 2011, 50: 12308–12311
38 Xiao Q, Bu W, Ren Q, Zhang S, Xing H, Chen F, Li M, Zheng X, Huab Y, Zhou L, Peng W, Qu H, Wang Z, Zhao K, Shi J. Radiopaque fluorescence-transparent TaO_x decorated upconversion nanophosphors for *in vivo* CT/MR/UCL trimodal imaging. *Biomaterials*, 2012, 33: 7530–7539
39 Bonitatibus PJ Jr, Torres AS, Goddard GD, FitzGerald PF, Kulkarni AM. Synthesis, characterization, and computed tomography imaging of a tantalum oxide nanoparticle imaging agent. *Chem Commun*, 2010, 46: 8956–8958
40 Oh MH, Lee N, Kim H, Park SP, Piao Y, Lee J, Jun SW, Moon WK, Choi SH, Hyeon T. Large-scale synthesis of bioinert tantalum oxide nanoparticles for X-ray computed tomography imaging and bimodal image-guided sentinel lymph node mapping. *J Am Chem Soc*, 2011, 133: 5508–5515
41 Freedman JD, Lusic H, Snyder BD, Grinstaff MW. Tantalum oxide nanoparticles for the imaging of articular cartilage using X-ray computed tomography: visualization of *ex vivo*/*in vivo* murine tibia and *ex vivo* human index finger cartilage. *Angew Chem Int Ed*, 2014, 53: 8406–8410
42 Liu YL, Ai KL, Liu JH, Yuan QH, He YY, Lu LH. A high-performance ytterbium-based nanoparticulate contrast agent for *in vivo* X-ray computed tomography imaging. *Angew Chem Int Ed*, 2012, 51: 1437–1442
43 Liu YL, Ai KL, Liu JH, Yuan QH, He YY, Lu LH. Hybrid $BaYbF_5$ nanoparticles: novel binary contrast agent for high-resolution *in vivo* X-ray computed tomography angiography. *Adv Healthcare Mater*, 2012, 1: 461–466
44 Dong K, Liu Z, Liu JH, Huang S, Li ZH, Yuan QH, Ren JS, Qu XG. Biocompatible and high-performance amino acids-capped $MnWO_4$ nanocasting as a novel non-lanthanide contrast agent for X-ray computed tomography and T_1-weighted magnetic resonance imaging. *Nanoscale*, 2014, 6: 2211–2217
45 Jakhmola A, Anton N, Anton H, Messaddeq N, Hallouard F, Klymchenko A, Mely Y, Vandamme TF. Poly-epsilon-caprolactone tungsten oxide nanoparticles as a contrast agent for X-ray computed tomography. *Biomaterials*, 2014, 35: 2981–2986
46 Chou SW, Shau YH, Wu PC, Yang YS, Shieh DB, Chen CC. *In vitro* and *in vivo* studies of FePt nanoparticles for dual modal CT/MRI molecular imaging. *J Am Chem Soc*, 2010, 132: 13270–13278
47 Zeng SJ, Wang HB, Lu W, Yi ZG, Rao L, Liu HR, Hao JH. Dual-modal upconversion fluorescent/X-ray imaging using ligand-free hexagonal phase $NaLuF_4$:Gd/Yb/Er nanorods for blood vessel visualization. *Biomaterials*, 2014, 35: 2934–2941