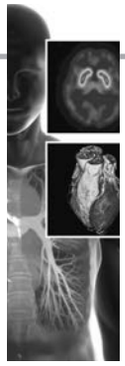

Nanoparticle contrast agents for CT: their potential and the challenges that lie ahead

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KEYWORDS: CT ■ gold ■ iodine ■ K-edge imaging ■ molecular imaging ■ multicolor CT ■ nanoparticles

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The number of reports of nanoparticle CT contrast agents has been growing swiftly over the past 5 years. These nanoparticles can be applied to produce long-lived CT contrast in the blood vessels and to perform targeted imaging, and can be used in conjunction with a new CT technique – multicolor CT. Such innovations offer the promise of new applications and diagnoses that can be performed with CT in fields such as image-guided surgery and evaluation of risk in atherosclerosis and cancer. However, there are a number of obstacles to be overcome in the field, such as the lack of standardized evaluation of new CT contrast agents, the cost of the agents and the need to prove biocompatibility.

Over the past 15 years, nanoparticles have become widely applied as contrast agents for MRI [1–3]. In comparison, there have been relatively few nanoparticle contrast-agent formulations reported for CT. Nevertheless, over the past 5 years, the number of publications on nanoparticle contrast agents for CT has been growing exponentially. There have been exciting recent reports of contrast agents that are long-circulating, that are targeted for molecular CT imaging and that allow multicolor CT imaging. There is great potential that these new formulations could allow major advances in preclinical and, eventually, clinical CT imaging. In this article, we will highlight some of the advances made and the challenges facing the field.

Long-circulating nanoparticle CT contrast agents

Long-circulating CT nanoparticle contrast agents can highlight the blood vessels for extended periods. Clinically approved CT contrast agents are small molecules and are therefore swiftly excreted via the kidneys. Nanoparticles, on the other hand, can be synthesized to be larger than the fenestra in the kidney and therefore remain in the bloodstream [4], as long as they possess a nonbiofouling coating and can thus avoid fast uptake in the liver. Long-circulating CT nanoparticle contrast agents could be very useful in image-guided surgeries, where the ability to visualize blood vessels over extended periods would be very helpful.

Torchilin and coworkers synthesized some of the first long-circulating nanoparticle CT contrast agents [5,6]. These formulations were micelles composed of iodinated amphiphiles whose headgroups contained polyethylene glycol. Gold nanoparticle formulations have been reported that produce sustained CT contrast in the blood vessels for as long as 12 h [7,8]. More recently, studies have been published where CT and MRI contrasts are produced by the same nanoparticle, by combinations of gold cores and gadolinium chelates or iron–platinum alloys [9,10]. Excitingly, a gold/gadolinium/Cy5.5 nanoparticle developed by van Schooneveld *et al.* was shown to induce contrast for three imaging modalities, that is, CT, MRI and fluorescence [11].

Other long-circulating platforms reported include a bismuth sulfide-based nanoparticle developed by Rabin *et al.* [12] and iodinated dendrimers developed by Fu *et al.* [13]. Furthermore, an iodinated emulsion known as Fenestra™ (Advanced Research Technologies) [101] and an alkaline earth-metal nanoparticulate called ExiTron™ nano (Miltenyi Biotec) are commercially available [102].

Targeted nanoparticle CT contrast agents

CT is relatively insensitive to contrast-generating materials compared with nuclear imaging techniques or even MRI. As a consequence, it was thought that targeted imaging would not

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be possible with CT [14]. Nevertheless, there have been several reports over the past 6 years on targeted imaging with nanoparticle CT contrast agents [15–20]. Usually, targeted imaging occurs via attachment of targeting ligands such as antibodies, peptides, proteins and small molecules, for example, to the nanoparticle surface. As a consequence, when these nanoparticles are applied to biological settings, the nanoparticles accumulate at their target site and contrast occurs in the images in the region of the target site.

> *"...there have been several reports over the past 6 years on targeted imaging with nanoparticle CT contrast agents."*

For example, Eck *et al.* used gold nanoparticles conjugated with anti-CD4 antibodies to specifically image lymph nodes [21]. Injections of these nanoparticles resulted in a 95-HU increase in intensity in lymph nodes, whereas only a 5-HU increase was observed for nontargeted nanoparticles. Further confirmation of the targeting effect of these nanoparticles was provided by examination of sections of the lymph nodes with transmission electron microscopy, where a large number of gold cores could be observed.

Multicolor CT imaging

Very recently there have been two reports on nanoparticles used in conjunction with a new form of CT known as multicolor CT [22,23]. This technique is based on novel cadmium telluride detectors that can determine the energy of absorbed x-rays [24]. Every material has a characteristic x-ray absorption profile and passing through any particular material will therefore produce a characteristic change in the x-ray spectrum. By analyzing the energy distribution of the x-rays transmitted through the subject, multicolor CT can identify different materials [25]. In particular, heavy elements that have a K-edge in the 25–100-keV range can be easily identified. Distribution maps of the materials in the field of view can be generated. These distribution maps are typically displayed in a different color for each material, thus leading to the name 'multicolor CT'. This type of technique is also known as K-edge imaging.

In a study published in *Radiology*, Cormode *et al.* injected atherosclerotic mice with macrophage-targeted gold nanoparticles [22]. The multicolor CT system used was able to distinguish the accumulations of gold nanoparticles in the arteries of these mice from iodine that was subsequently injected, calcified tissue and flesh. Pan *et al.* used a bismuth nanoparticle targeted to fibrin to detect thrombi induced in rabbits [23]. These nanoparticles allowed the clots, calcified material and tissue to be simultaneously visualized.

Problems in the field

Currently there is no existing standard protocol for testing the performance of novel contrast agents. In the field of MRI, the relaxivity of contrast agents is usually evaluated at 1.5 T and 37°C, either with a clinical MRI scanner [26] or with specially developed equipment that mimics the conditions of a clinical scanner [27]. Nanoparticle contrast agents have been tested using clinical scanners [28] and micro-CT systems [8], with x-ray tube voltages ranging from 50 to 210 kV [8,29]. Owing to the energy dependence of x-ray attenuation for a given substance, scanning at different voltages will probably result in differing estimations of contrast production, even with the use of iodine contrast-agent references. Consequently, to help the field move forward, there needs to be established a standard contrast evaluation protocol to allow new agents to be compared.

> *"For the field of CT nanoparticle contrast agents to show its worth, formulations must be translated to the clinic."*

Logically, such a standard protocol should use clinical scanners as this would predict the contrast generated when used in patients. In our experience, it is straightforward to gain access to clinical CT equipment to scan phantoms containing novel contrast agents and clinically approved iodine control agents. We suggest that novel agents be scanned in water to mimic scanning in patients and that a range of concentrations and tube voltages be used. Iodine contrast agents should be used as a comparison.

Future challenges

For the field of CT nanoparticle contrast agents to show its worth, formulations must be translated to the clinic. To achieve this, cheap and biocompatible formulations must be developed. Many of the new nanoparticles being reported for CT are based on gold and excellent results have been reported, however, gold is an expensive material. The reason for this, aside from the strong x-ray-attenuating properties of gold, is that the chemistry of gold nanoparticles is well established [30]. There have been a couple of reports of agents based on cheap elements such as bismuth or iodine, however, attaining high

densities of these elements in nanoparticle formulations is difficult. Therefore, new chemistries need to be developed to create high-density nanoparticle formulations of cheap, strongly x-ray-attenuating elements. Furthermore, agents must be developed that are either degradable or excretable. Studies must be performed to evaluate toxicity and excretion rates, thus providing confidence for investment in these agents.

The results from studies with multicolor CT are very exciting. However, the scan times of these devices are very long, due to the slow data-acquisition rates of the detectors used. This issue has to be overcome before the technique can be put into widespread use. A number of methods are being pursued to solve the problem, such as iterative reconstruction techniques and bow-tie-shaped filters [31].

Conclusion

Nanoparticle CT contrast agents hold great promise for new and advanced imaging applications. Long-circulating agents could be a boon for interventional radiology. Targeted agents could allow advanced diagnoses and evaluation of therapeutic efficacy. Multicolor CT will likely be used in clinical practice within 5 years, but new CT contrast agents could amplify its usefulness. For nanoparticle CT contrast agents to fulfill their promise, advances have to be made in the chemistry used to create them, common evaluation protocols must be agreed upon and biocompatibility must be proven.

Financial & competing interests disclosure

The authors received partial support from NIH grants R01 HL71021, R01 HL78667, R01 EB009638 (ZAF), K99 EB012165 (DPC) and NHLBI PEN Award, Contract #HHSN268201000045C. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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