Review

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Feature Review

Inorganic nanoparticle-based contrast agents for molecular imaging

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Inorganic nanoparticles (NPs) including semiconductor quantum dots (QDs), iron oxide NPs and gold NPs have been developed as contrast agents for diagnostics by molecular imaging. Compared with traditional contrast agents, NPs offer several advantages: their optical and magnetic properties can be tailored by engineering the composition, structure, size and shape; their surfaces can be modified with ligands to target specific biomarkers of disease; the contrast enhancement provided can be equivalent to millions of molecular counterparts; and they can be integrated with a combination of different functions for multimodal imaging. Here, we review recent advances in the development of contrast agents based on inorganic NPs for molecular imaging, and also touch on contrast enhancement, surface modification, tissue targeting, clearance and toxicity. As research efforts intensify, contrast agents based on inorganic NPs that are highly sensitive, target-specific and safe to use are expected to enter clinical applications in the near future.

NPs as molecular imaging agents

Molecular imaging is a new frontier of biomedical research for visualizing, characterizing and monitoring biological processes in cells, tissues and organisms using sensitive instrumentation and contrast mechanisms [1]. Molecular imaging interrogates biological processes to report on and reveal the molecular abnormalities that form the basis of diseases. As a result, molecular imaging provides a powerful tool for the diagnosis of diseases including cancer, cardiovascular syndrome and neurological disorders. It can also assist treatment planning by providing information on the physiological state of a tissue or stage of a disease. Molecular imaging differs from traditional imaging in that contrast agents are typically used to help identify particular biomarkers or pathways with high sensitivity and selectivity [2]. Ideally, the contrast agents would selectively accumulate at the site of interest; the accumulated agents then interact with the target physically, chemically and/or biochemically, and thereby alter the imaging contrast according to the ensuing changes. Recent advances in both the development of new imaging techniques and the synthesis of novel contrast agents offer a broad range of exciting opportunities, including early diagnosis and the effective treatment of disease.

Although small molecules such as organic dyes and radioisotopes conjugated to targeting ligands have been widely used as contrast agents in both research and clinical settings [2,3], inorganic NPs are receiving increasing attention as future contrast agents because of their superb properties [4–8]. For example, semiconductor QDs exhibit not only optical emission wavelengths similar to organic dyes (with peaks tunable in the visible and near-infrared regions) but also unique features such as superior brightness, extraordinary photostability and multicolor capability under single source excitation [4]. Inorganic NPs can also be readily designed and prepared to include an array of properties (e.g. magnetic and optical scattering, absorption or luminescence) for use with multiple imaging modalities [9–16]. In addition, the surfaces of inorganic NPs can be easily conjugated with different functional groups without changing their physical properties, making it feasible to selectively target the site of interest (e.g. cancerous tissue) for maximum contrast enhancement [17,18].

NPs made of organic materials (e.g. liposome, micelles and polymeric particles) have also been explored as contrast agents for molecular imaging [19,20]. However, most of them are simply used as carriers to encapsulate functional components such as inorganic NPs, coordination compounds and organic dyes. Part of the reason can be attributed to the fact that most organic materials that can be easily and conveniently processed as NPs do not exhibit the relevant magnetic and optical properties. Some organic materials such as conjugated polymers do exhibit interesting and tunable optical (fluorescence) properties, but they can be difficult to process and degrade in the body. As a result, inorganic NPs are beginning to receive more attention than their organic counterparts as contrast agents for molecular imaging.

Here, we highlight recent progress in the development of new contrast agents based on inorganic NPs for molecular imaging. Specifically, we first introduce imaging modalities that can benefit from contrasts agents based on inorganic NPs and we concentrate on some of the most extensively explored systems that are in various stages of preclinical and clinical development. We then discuss the mechanisms of targeting inorganic NPs by engineering the surface properties. We also touch on the clearance and toxicity issues associated with inorganic NPs. Finally, we elaborate on a set of recent applications enabled by contrast agents based on inorganic NPs.

Molecular imaging modalities

Inorganic NPs are an actively explored technology for the development of contrast agents for molecular imaging.

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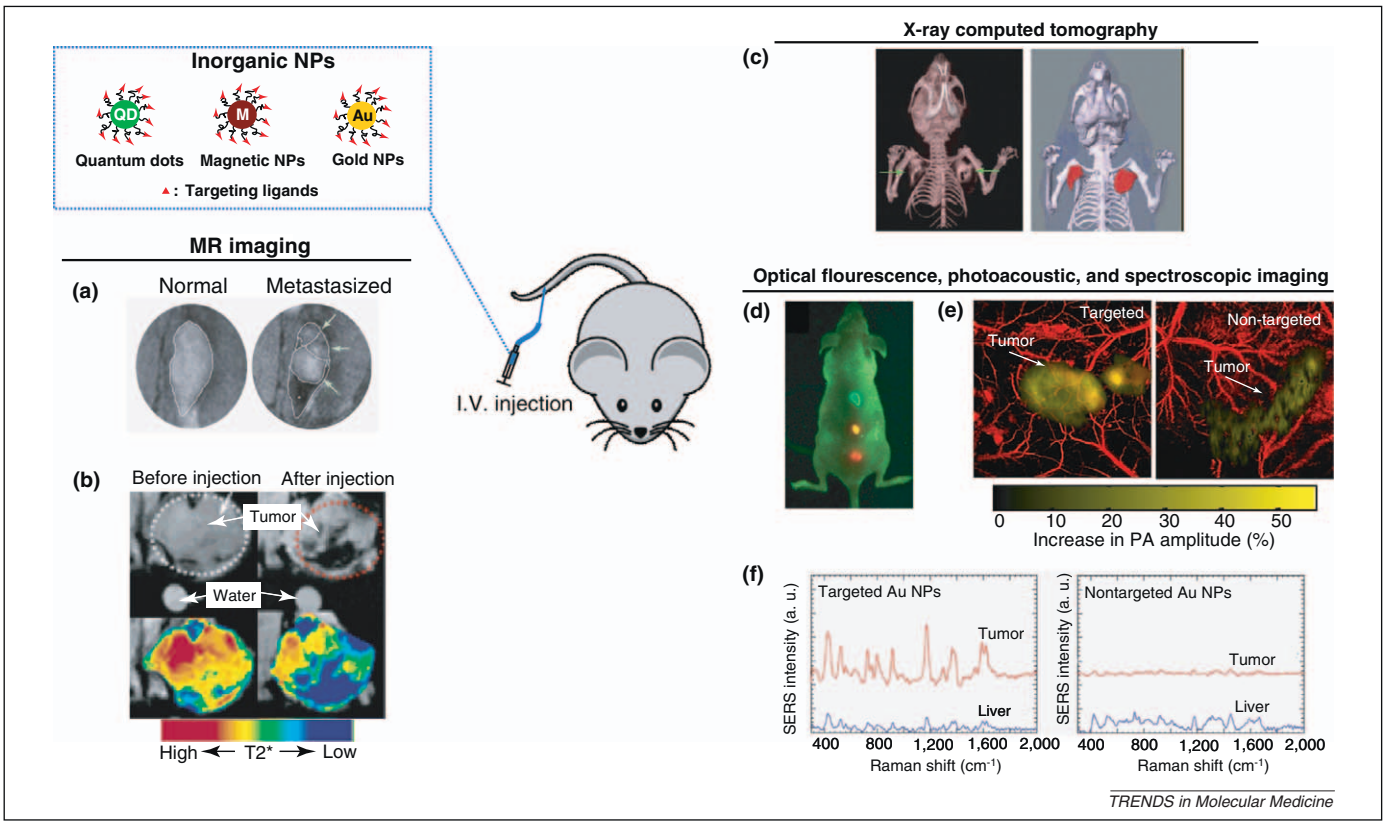

Figure 1. Typical examples of *in vivo* molecular imaging with inorganic NPs as contrast agents that are often modified with ligands to target tumors or other diseased lesions. **(a)** MR imaging of lymph nodes with iron oxide NPs for detecting the metastasis of prostate cancer. (Reproduced with the permission of [24].) **(b)** MR imaging with antibody-conjugated superparamagnetic iron oxide NPs for tumor targeting. (Reproduced with the permission of [25].) **(c)** X-ray CT of a mouse bearing tumors (indicated by arrows) as enhanced by PEGylated gold nanorods. (Reproduced with the permission of [31].) **(d)** Optical fluorescence imaging for detecting human prostate cancer with targeted QDs. (Reproduced with the permission of [21].) **(e)** Photoacoustic imaging with targeted gold nanocages for detecting human melanoma in a nude mouse. Reproduced with the permission of [27]. **(f)** SERS spectra with targeted gold NPs for detecting a human squamous cell carcinoma. (Reproduced with the permission of [15].)

Figure 1 and Table 1 list the types of inorganic NPs being developed as imaging agents, and Figure 1 also shows some typical examples of inorganic NP-based molecular imaging [6,9,13–15,21–33]. Before discussing the performance of these contrast agents, it will be helpful to provide a brief introduction to the imaging modalities whose success will greatly benefit from these contrast agents. We divide these modalities into two major groups depending on the penetration depth: deep tissue and shallow tissue modalities.

Deep-tissue modalities

Magnetic resonance (MR) imaging [34]. MR imaging is used clinically to visualize the structure and function throughout the body. MR imaging detects the different proton relaxation times (T) of water in response to a strong field in the radio frequency (RF) range. It offers great contrast between different soft tissues of the body. There are two types of MR imaging mechanisms: T1-weighted and T2-weighted.

T1, the "longitudinal" relaxation time required for a substance to regain longitudinal magnetization following an RF pulse, represents the correlation between the frequency of molecular motions and the Larmor frequency. The frequencies of small molecules (e.g. water) and large molecules (e.g. proteins) are significantly different from the Larmor frequency and thereby have long T1. By contrast, cholesterol, a medium-sized molecule, has natural frequencies close to those used for MR imaging, thereby it has a short T1. Thus, cholesterol-rich tissues appear bright, whereas water and proteins look dark on T1-weighted images. T1 can decrease from the interaction between

Table 1. Inorganic NPs that have been explored as contrast agents for molecular imaging.

NP type	Imaging modality	Stage of development[1]	Ref.
Semiconductor QDs	Fluorescence imaging	*in vivo*	[21–23]
Magnetic NPs	MR imaging	Clinical	[6,9,24,25]
Gold colloids	PAT	*in vivo*	[16,26,27]
	SERS	*in vivo*	[15,28]
	Multiphoton luminescence imaging	*in vivo*	[29,30]
	X-ray CT	*in vivo*	[13,14,31]
Rare-earth-doped NPs	Luminescence imaging	*in vivo*	[32]
	MR and near-infrared imaging	*in vivo*	[33]

[1]The stage of development is based on the data we could find in the literature when this review article was prepared. For example, magnetic NPs (especially iron oxide NPs) have been approved for clinical use so we denoted this agent ''clinical''. The references shown in the table are based on *in vivo* imaging with mice.