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What is new in nanoparticle-based photoacoustic imaging?

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Abstract

Photoacoustic imaging combines the high temporal and spatial resolution of ultrasound with the good contrast and spectral tuning of optical imaging. Contrast agents are used in photoacoustic imaging to further increase the contrast and specificity of imaging or to image a specific molecular process, e.g., cell-surface proteins or small molecule biomarkers. Nanoparticle-based contrast agents are important tools in photoacoustic imaging because they offer intense and stable signal and can be targeted to specific molecular processes. In this review, we describe some of the most interesting and recent advances in nanoparticle-based photoacoustic imaging including inorganic nanoparticles, organic/polymeric nanoparticles, nanoparticle coatings, multimodality imaging, as well as emerging topics in the field.

Introduction

In vivo molecular imaging is a powerful tool to study biology and practice medicine.¹ Ultrasound imaging is particularly useful because of its low cost, good depth of penetration, and high temporal and spatial resolution. One recent development in ultrasound is photoacoustic imaging, which uses the absorption of optical energy to generate acoustic waves. Photoacoustic imaging offers high contrast and good signal-to-noise ratios in large volumes of biological tissues without ionizing radiation or tissue damage. It combines the high temporal and spatial resolution of ultrasound with the good contrast and multiplexing capabilities of optical imaging. Different designs offer resolution from whole organs to organelles.²

Photoacoustic imaging is a “light in-sound out” effect versus the “sound in-sound out” in traditional ultrasound (Fig. 1A). The mechanism of contrast is absorption of photons and subsequent thermal expansion. First, a nanosecond-pulse laser beam irradiates the sample and is absorbed to raise the temperature (ΔT) and cause a pressure rise, p_0 , due to thermal expansion via $p_0 = \beta \cdot \Delta T / \kappa$. Here, β is the thermal expansion coefficient, and κ is the isothermal compressibility.² Most excitation sources used in photoacoustic imaging are between ~ 680 and ~ 1100 nm³. This is because near infrared (NIR) light has higher penetration depth than optical light due to lower hemoglobin absorption and tissue scattering.^{4, 5} A transducer then detects the pressure wave and software forms an image from the acoustic data.

Current challenges in photoacoustic imaging include light scatter and absorption, frequency/signal changes because of volume modifications, reconstruction inaccuracies, tissue background noise, attenuation of the excitation source, and poor penetration depth.⁶ Therefore, developing effective contrast agents to specifically increase signal at the site of interest is important in improving photoacoustic imaging for use in humans. Here, we discuss recent advances in nanoparticle contrast agents in photoacoustic imaging. We detail different materials and their properties as well as representative applications that demonstrate clear advantages. Finally, we discuss the challenges and broader impacts of photoacoustic imaging using nanoparticles. We close with some perspective on future developments in the field.

Contrast Agents in Photoacoustic Imaging

Imaging agents (or contrast agents) increase the specific contrast of a target organ or disease process such as cancer (Fig. 1B). Photoacoustic contrast agents should be effective at low doses, biocompatible, biodegradable, non-toxic, and complement the intrinsic ultrasound data.^{7, 8} There are both endogenous and exogenous photoacoustic contrast agents. Endogenous agents include hemoglobin and melanin—these naturally absorb in the infrared region.⁹ However, endogenous agents often have less contrast than exogenous agents such as nanoparticles or dyes because hemoglobin/melanin is also present in normal adjacent tissue.¹⁰ While this can be improved by using a reporter gene to specifically express a protein, e.g. tyrosinase,¹¹ at the site of interest, the use of reporter genes in humans is limited because of regulatory and safety challenges.

Small molecule dyes are a second class of photoacoustic contrast agents—many of these are already clinically approved. They also have good tissue penetration after intravenous injection and defined pharmacokinetics and pharmacodynamics. Examples include methylene blue, indocyanine green, and Prussian blue.¹² The disadvantages of small molecules include low photoacoustic signal, poor photostability, fast clearance, and small optical absorption cross sections.¹³

Nanoparticles are a third class of contrast agents. Nanoparticles are useful in photoacoustic imaging because they have high signal and stable signal. They can also be functionalized with many copies of targeting ligands to home the material to the site of interest. Nanoparticles can be made from a variety of responsive materials that produce signal only under the influence of a chemical cue. The rest of this manuscript describes some of the most interesting and recent advances in nanoparticles for photoacoustic imaging including inorganic nanoparticles, organic/polymeric nanoparticles, nanoparticle coatings, multimodality imaging, as well as emerging topics.

Inorganic Nanoparticles

Metal nanoparticles were among the first materials to be used as photoacoustic contrast agents because of their high absorption cross section (intense photoacoustic signal) as well as established biocompatibility.^{14, 15} Gold nanoparticles are particularly popular because they have size- and shape-tunable absorption peaks and thus can facilitate multiplexing in

photoacoustic imaging (multiplexing is the measurement of multiple different signal types at the same time).¹⁶ Several configurations of gold nanoparticles have been studied including spheres, rods, shells, and liposomal formulations.¹⁷ While metal nanoparticles do not experience photobleaching, their morphology can change under irradiation to decrease signal.¹⁸ Most nanoparticles used in imaging are 20–150 nm. Particles that are smaller than ~10 nm are easily cleared from circulation via the kidneys and thus do not have adequate residence time to accumulate in target tissue. The signal strength of these smaller particles is usually low as well.

Recent advances in gold nanoparticles for photoacoustics have increased signal. For example, Emelianov and coworkers have improved contrast by using nonlinear photoacoustic effects. This was reported when plasmonic nanoparticles undergo aggregation and cellular endocytosis. This causes thermal coupling, localized temperature increases, and nonlinear effects for improved signal.¹⁹ In another example, Zharov created clusters of gold nanoparticles linked via photoswitchable proteins.²⁰ The distance between the nanoparticles changed upon activation of the protein, which in turn changed the photoacoustic signal.

Another challenge is poor biodegradation of gold nanoparticles. These materials accumulate in the liver and spleen with very long clearance times. More recently, < 5-nm gold particles were bound together into clusters with a biodegradable binder (Fig. 2).²¹ These larger particles have robust photoacoustic signal, and the cluster was sensitive to pH and acidic endosomes that could result in biodegradation and clearance of the smaller gold particles via the kidney.²² Similarly, 2–4 nm gold nanobeacons (GNBs) were placed inside phospholipid nanoparticle, and this contrast agent produced a photoacoustic signal nearly 10 times greater than blood.²³

Beyond gold, upconverting nanoparticles (UCNPs) also offer narrow excitation/emission profiles for photoacoustic imaging. These materials are based on phosphors like NaYF₄ doped with ytterbium (Yb³⁺), erbium (Er³⁺), and terbium (Tb³⁺). UCNPs convert NIR to visible light via donor (Yb)/acceptor (Er) relationships.²⁴ The heating capacity of NaYF₄:Yb³⁺, Er³⁺ UCNPs and luminescence quenching offers robust photoacoustic signal for small animal imaging at 980 nm.²⁴ These materials might be challenged by toxicity concerns related to injection of these exotic elements.

Copper and copper sulfide are also emerging materials. In one example, copper was encapsulated in a 80–90 nm phospholipid-entrapped nanoparticle (NanoCuN); it showed a 6-fold higher signal sensitivity than blood when used in a rodent model.²⁵ Copper sulfide (CuS) is also useful because it has a very tunable absorption peak.²⁶ In one example, CuS nanoparticles were used with a 1064 nm Nd:YAG laser to image human breast tissue up to 40 mm deep.²⁷ CuS nanoparticles were also used in a mouse model to visualize neural structures with clearance from the brain after 7 days.²⁷

Organic Nanoparticles

Organic polymeric nanoparticles offer good clearance and biodegradation. They often use materials with existing FDA approval.²⁸ Porphysomes were among the earliest organic

agents used for photoacoustic imaging and photodynamic therapy²⁹ and they have biocompatibility that has with very high translational potential.³⁰ An interesting approach to manufacturing of nanoparticles has been a “green” synthesis of nanoparticles using naturally occurring materials as the source of contrast. In one example, nanoparticles were derived from honey via a solvent-free rapid surface passivation of carbon nanoparticles with organic macromolecules.³¹ In another example, nanoparticles were made from the thermal and acidic degradation of cotton cellulose linters and later applied to ovarian cancer imaging. These cellulose nanoparticles were then shown to biodegrade into glucose in the presence of cellulase.³² Another example harvested melanin from melanocytes and created nanoparticles from this natural pigment for photoacoustic imaging.³³ We suspect that this trend will continue and might help in the clinical translation of these materials.

Researchers are taking inspiration from materials used for light harvesting such as in photovoltaics, light emitting diodes, and energy transfer cascades. Stimuli-responsive nanoparticles have been designed to produce signal the presence of a specific temperature, enzyme, or small molecules. In one example, semiconducting polymer nanoparticles sensitive to reactive oxygen species were created via a benzothiadiazole scaffold. (Fig. 3).³⁴ These nanoparticles also have good structural flexibility, resistance to photodegradation, narrow photoacoustic spectral profile, and more photostability than gold nanorods.³⁴ In another example, light-harvesting porphyrins were used to synthesize a temperature-sensitive photoacoustic probe.³⁵ Organic nanoformulated naphthalocyanines have also been used in multispectral photoacoustic imaging to construct detailed maps of lymphatic drainage systems.³⁶ In another example, nanoparticles were made from perylene-diimide molecules. These materials had sufficient signal to image through intact skull and identify glioblastoma.³⁷ Additional work has described pyrrole-based nanoparticles¹³ and porphyrinoids.^{38, 39} In one example, porphyrinoid-based nanoparticle were sensitive to the presence of uranium and offered parts-per-billion detection limits *in vivo* (Fig. 4).³⁸

Other approaches have been inspired by traditional ultrasound. Microbubbles are used in diagnostic ultrasound because they have highly scattering acoustic properties and interact nonlinearly with incident ultrasound. However, they are generally to imaging vascular targets because of their size (>1 μm). One novel approach uses perfluorocarbon nanodroplets, which offer both ultrasound and photoacoustic contrast.⁴¹ These materials are constructed from bovine serum albumin-coated liquid dodecafluoropentane and gold nanoparticles. The gold nanoparticle acts as a fuse to vaporize the dodecafluoropentane and produce acoustic impedance and photoacoustic signal. Another approach utilized the conversion of microbubbles to nanostructures using an ultrasonic pulse. In this case, a bacteriochlorophyll–lipid shell was formed around a perfluoropropane gas and these microbubbles burst to form nanoparticles that retained the photoacoustic properties but with better tumor penetrating properties.⁴²

Nanoparticle Coatings and Targeting

The nanoparticle surface is where it interacts with the body. Thus, this layer has to be meticulously engineered to maintain low toxicity, high signal, and good targeting. While polyethylene glycol is often used as a coating agent on nanoparticles, silica also offers

important advantages. First, it can increase the signal stability and prevent optically induced nanoparticle deformation—especially with gold nanoparticles.⁴³ Second, the overall magnitude of signal is 3- to 4-fold higher with the silica coating.⁴⁴ Third, silica can increase cellular uptake of gold into cells, which is useful for photoacoustic imaging of stem cell therapy.⁴⁵

Other recent alternatives include non-cytotoxic PNIPAAmMA. This allows covalent attachment of targeting molecules like antibodies or magnetic nanoparticles and may also be used to improve target and anticancer therapies.⁴⁶ In another example, gold nanoparticles were coated with cinnamon-based phytochemicals (linalool, catechin, or epicatechin). These agents had uptake in PC-2 and MCF-7 cells and were internalized in cancer cells to report photoacoustic signal.⁴⁷

Cell surface receptor targeting is an area with interest, and Luke et. al showed that molecularly activated plasmonic nanosensors could detect metastases at low levels of approximately 30 cells by targeting to the epidermal growth factor receptor.⁴⁸ Protease activity has been visualized with the use of black hole quencher 3 conjugated to NIR-absorbing copper sulfide nanoparticles which exhibited two absorption peaks at 630 nm and 930 nm.⁴⁹ When in the presence of matrix metalloproteinases in the tumor, the black hole quencher 3 was released leaving the copper sulfide nanoparticles.

Another powerful approach to nanoparticle coating is the use of lysed cell membranes (Fig. 5). Although this has not yet been shown for photoacoustic imaging, Zhang et al. have shown that the membrane from lysed erythrocytes or cancer cells can be used to cloak nanoparticles similar to synthetic polymers.^{50, 51} These membranes retain all signaling components on the cell, and thus have significant utility in increasing circulation time and homing nanoparticles to target.

Multimodal Photoacoustic Imaging

Combining photoacoustic imaging with other modalities can utilize the advantages of both methods and recent advancements in image acquisition and reconstruction have allowed multimodal imaging to expand.⁵² Tantalum oxide-based polypyrrole (PPy) NPs were used for bimodal imaging to enhance X-ray CT and photoacoustic (PA) imaging.⁵³ MRI has excellent depth of penetration, but slower temporal resolution while photoacoustics has good temporal resolution and limited depth. In one example, a triple-modality nanoparticle combining magnetic resonance, photoacoustic, and Raman imaging (MPR) was used to resect glioma. Here, MRI signal is due to chelated gadolinium. The same probe can be used before (MRI) and during (photoacoustic) surgery for more accurate tumor resection.⁵⁴ These gold core/silica shell nanoparticles have an optical absorbance coefficient over 200 times higher than carbon nanotubes. Another interesting approach to MRI signal was shown via the copper component of copper sulfide nanoparticles.⁵⁵

A similar approach was used with an ovarian cancer model, but here the photoacoustic data was combined with Raman spectroscopy. Photoacoustics discriminated between tumor and

normal tissue, and Raman offered guidance during surgical resection. Nanorods offered a 10-fold improvement in SERS signal versus gold spheres, but with only 1% of the volume.⁵⁶

Challenges and Future Directions

The translation of NP-based photoacoustic imaging from laboratories to clinical settings is currently limited by toxicity concerns, poor signal, synthesis of NPs, and targeting of nanoparticles. More work is needed to increase the depth of penetration. Indeed, while photoacoustics can use diffuse photons and has better depth of penetration than optical imaging, its primary limitation is depth ($\sim <5$ cm). One approach is to use acoustic events triggered by stimuli other than light—stimuli that do not suffer absorption and scatter in tissue like optical photons. There are active research programs using radio frequency, microwave, x-rays to produce acoustic signal^{57–59}, i.e., thermoacoustic imaging. Magnetomotive ultrasound is another promising alternative that uses an external high-strength pulsed magnetic field to induce motion within magnetically labeled tissue; ultrasound is used to detect the induced internal tissue motion. In one example, this was used to discriminate between endocytosed iron oxide nanoparticles and extracellular nanoparticles via photoacoustic data as well as the concentration of each.⁶⁰ This approach also offers significant depth advantages. The challenge in all of these approaches is achieving sufficient signal and acoustic pressure differences to be detected with clinical transducers.

Another approach to overcoming depth limitation is via the development of novel transducers. That is, rather than trying to make the signal stronger, these approaches take the detector closer to the signal. There are active research programs developing small, implantable, and even wearable photoacoustic transducers suitable for endoscopic, transrectal,^{61, 62} transvaginal,⁶³ or vascular imaging. These devices will complement novel contrast agents and could be used for applications other than imaging such as wearable sensors sensitive to drug levels. A third approach to increasing signal moves into the second NIR window (NIR II). This region is generally from 1000–1500 nm and offers even lower absorption and scatter (except from water). Although this has thus far been primarily demonstrated for fluorescence imaging,⁶⁴ we suspect that photoacoustic imaging could take advantage of this region. It is likely that nanoparticles will play a key role in all three of these developments: sensitizers to the x-rays/RF, NIR II contrast agents, or for use with novel transducers.

Other advances will come from the design of the nanoparticles themselves. Above, we described recent advantages in new nanoparticle materials, coatings, and shapes. However, a paradigm-shifting innovation is underway in terms of nanoparticle construction. While top-down and bottom-up synthesis of nanoparticles has been established for many years, this new approach builds nanoparticles *inside* of living subjects based on chemical or biological cues (Fig. 6).⁶⁵ For example, work by Rao and colleagues has shown that these self-assembling materials can be used for imaging,⁶⁶ and Gianneschi and coworkers have similar designs using enzymatic-triggered activation of nanoparticle construction.⁶⁷ Although used primarily for MRI imaging, drug delivery, or as stem cell scaffolds, it seems likely that a

photoacoustic imaging probe will result from this technology. This could use fluorescence quenching (increased non-radiative relaxation) due to nanoparticle condensation.

We will also likely continue to see theranostic applications that combine photoacoustic imaging with drug delivery. This could be combined with stimuli-responsive nanoparticles for drug delivery due to vascularity, hemodynamics, local temperature⁶⁸, and pH.⁶⁹ In theranostics, the nanoparticle has both a therapeutic role (drug delivery, tumor ablation) and a diagnostic role (tumor margins, sub-typing). In one example, gold nanocages (AuNCs) encapsulated drugs and a phase-change material to load and release the drug in the presence of high intensity focused ultrasound—this allowed for site-specific delivery.⁷⁰ In another example, CuS nanoparticles were used for both photoacoustic imaging, enzyme-responsive drug therapy, and phototherapy—this combination therapy may be used as a multifunctional platform for cancer treatment.⁷¹ Future examples may combine photoacoustic imaging with photothermal therapy. This has already been shown with Fe₃C₂-based nanoparticles⁷² and is possible because of the different optical pulse sequences and wavelengths used in photoacoustic imaging versus photothermal therapy. Thus, this same nanoparticle could both image and ablate tumors. Although exciting, these combination approaches can actually be more challenging to clinically translate. Simpler materials are usually more easily approved by the FDA.

Conclusion

Photoacoustic imaging is a complementary acoustic imaging modality that increases the contrast of ultrasound data. The main limitation in the field remains poor penetration of light through tissue. Nanoparticle contrast agents help overcome this because they have high signal, stable signal, or activatable signal. Some of the most exciting developments in this field in the last five years have included nanoparticles that self-assemble *in situ*, nanoparticles that are reactive to a variety of small molecule and protein stimuli, and nanoparticles that are targeted to disease through novel strategies. New persons in this field would be wise to work on the NIR II window or other thermoacoustic excitation sources because they will offer better tissue penetration and thus more opportunities for clinical translation. Collaborations with persons developing novel photoacoustic imaging equipment is also helpful. We suspect that nanoparticle contrast agents will continue to play an important role in all of these approaches.

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Fig. 1. Basics of Photoacoustics

A) Ultrasound and photoacoustic imaging both use acoustic data (curved black lines) to create an image. In ultrasound, impedance mismatch creates contrast. In photoacoustics, incident light (red arrow) causes thermal expansion and hence a pressure difference (black lines). **B)** Contrast agents link biology and medicine via an imaging signal. Nanoparticles make excellent reporters because they have high signal intensity and stability.

Fig. 2. Biodegradable gold nanoparticles

A) Small gold spheres are bound together with a pH sensitive polymer. At endosomal pH values (~ 5), this polymer releases the small gold spheres. This causes a change in the absorbance spectrum (**B**). This is important because the larger gold cluster can be used for imaging, and the smaller spheres can then clear from the body via the kidney. Reproduced courtesy of American Chemical Society.²¹

Fig. 3. Imaging Reactive Oxygen Species with Acoustic Data and Semiconducting Nanoparticles Nanoparticles based on benzothiadiazole groups are responsive to reactive oxygen species and produce more blue-shifted acoustic emission in the presence of reactive oxygen species (+Zymosan; right) than control animals (–Zymosan; left). Ratiometric photoacoustic imaging (700 nm/820 nm) can then be used to monitor expression of these molecules. (Zymosan is a glucan that induces experimental sterile inflammation.) Reproduced courtesy of Nature Publishing Group.⁴⁰

Fig. 4. *In vivo* Imaging of uranyl cation

A) A porphyrinoid macrocycle produces photoacoustic signal when the uranyl cation is chelated because of increased aromaticity (heavy black line). **B)** Photoacoustic spectrum for free macrocycle (ligand), uranium-complexed porphyrin (complex), and the complex solubilized in PLGA nanoparticles (Complex-NP). **C)** TEM of nanoparticles at two different magnifications. The *in vivo* imaging of 100 μ L of 0.38 nM NPs with the uranium complex (**D**) or empty macrocycle (**E**) highlights the obvious signal difference. Note the scale and intensity bar in D and E apply to both panels. White dashed circle indicates the injection site. Reproduced with permission from The Royal Society of Chemistry.³⁸

Fig. 5. Homotypic Targeting

Nanoparticles can be coated with the cell membrane of lysed cancer cells. This membrane contains the entire repertoire of cell surface markers, which can help direct the nanoparticle to the tumor after injection into the systemic circulation. This is in contrast to traditional targeting approaches that use a single marker of interest, e.g. folate, epidermal growth factor receptor, integrins. Reproduced courtesy of American Chemical Society.⁵¹

Fig. 6. Strategy for Self-assembling Nanoparticles

In this strategy a small molecule contains an imaging agent (red), an enzymatic target (green), and activatable linkers (orange/yellow). When the enzyme cleaves the protecting group, the system self-cyclizes. This process can repeat to make nanoaggregates containing many copies of the imaging agent at the site of interest. This could potentially increase target accumulation more than systematically delivered nanoparticles.