REVIEW ARTICLE

Dual-function theranostic nanoparticles for drug delivery and medical imaging contrast: perspectives and challenges for use in lung diseases

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Abstract Theranostic nanoparticles with both therapeutic and imaging abilities have the promise to revolutionize diagnosis, therapy, and prognosis. Early and accurate detection along with swift treatment are the most important steps in the successful treatment of any disease. Over the last decade, a variety of nanotechnology-based platforms have been created in the hope of improving the treatment and diagnosis of a wide variety of diseases. However, significant hurdles still remain before theranostic nanoparticles can bring clinical solutions to the fight against chronic respiratory diseases. Some fundamental issues such as long-term toxicity, a precise understanding of the accumulation, degradation and clearance of these particles, and the correlation between basic physicochemical properties of these nanoparticles and their in vivo behavior have to be fully understood before they can be used clinically. To date, very little theranostic nanoparticle research has focused on the treatment and diagnosis of chronic respiratory illnesses. Nanomedicine approaches incorporating these theranostic nanoparticles could potentially be translated into clinical advances to improve diagnosis and treatment of these chronic respiratory diseases and enhance quality of life for the patients.

Keywords Chronic respiratory diseases · Theranostics · Nanoparticles

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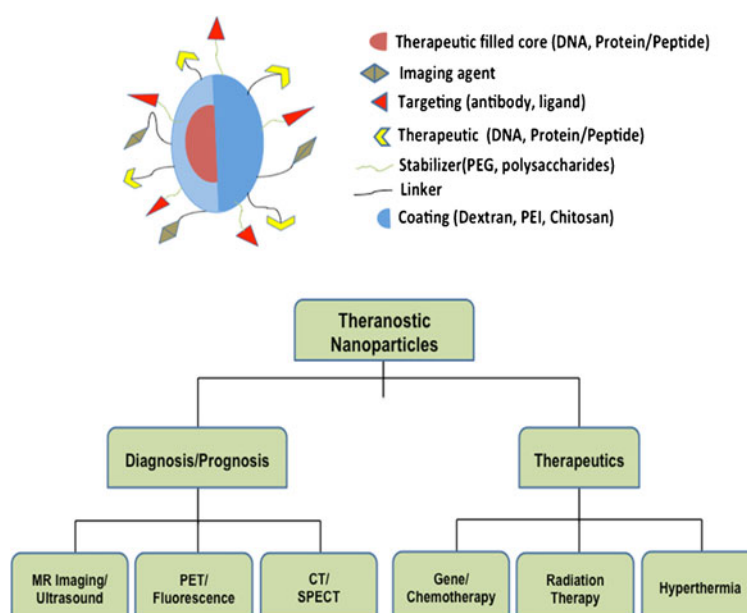12901 Bruce B Downs Blvd, MDC 7,
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Introduction

According to the World Health Organization, chronic respiratory diseases are one of major causes of death and disability for all age groups and regions in the world [1]. As of 2008, hundreds of millions of people suffer from chronic respiratory diseases and worldwide: 300 million have asthma, 210 million have COPD, and millions of others have other chronic respiratory diseases [2]. Thus, significant efforts need to be dedicated to their diagnosis, prevention, and treatment.

The current treatments for chronic respiratory diseases all have serious drawbacks, and improving their safety and therapeutic efficacy is one of the major challenges of this decade. The most common treatment for respiratory diseases that result in airway inflammation, like asthma and COPD, is corticosteroids; however, many systemic side effects can occur as a result of the chronic use of corticosteroids [3]. Treatment of TB is very complex and is becoming even more difficult with the evolution of multidrug-resistant TB. Current TB treatments involve daily administration of four or more antibiotics for a period of 6 months or longer [4]. The long duration of treatment and prevalence of side effects leads to noncompliance and poor therapeutic outcomes for current TB treatments [3, 4]. Viral respiratory infections, such as those caused by RSV, cannot be vaccinated against, and currently, there is no effective and safe antiviral compound [3]. Ribavirin has been used to treat a broad

spectrum of viral respiratory infections; however, clinical treatment with ribavirin requires high doses that can cause harmful side effects [3, 5]. Lung cancer diagnosis and therapies, along with many other cancers, are often limited by a multitude of biological barriers, which include nonspecific delivery and poor biodistribution of drugs or contrast agents, drug resistance of certain forms of cancer, lack of an effective modality for treatment monitoring, and toxicity to healthy tissues [6–8]. Clearly, there is a need to improve the treatment of these devastating chronic respiratory diseases.

Nanotechnology is the engineering and manufacturing of materials at the atomic and molecular scale [9]. Nanomedicine, the application of nanotechnology to medicine, has the potential to revolutionize healthcare, overcoming many current limitations in diagnosis, treatment, and management of human disease [10]. Nanoparticles have shown potential for in vivo imaging, thermo stimulation, phototherapy, site-specific targeting, and the capability to deliver a combination of drugs, peptides, nucleic acids, and imaging agents [6, 11–14]. Over the past decade, a variety of nanotechnology-based platforms have been created in the hope of improving the diagnosis and treatment of a wide variety of diseases, including lung diseases. However, significant hurdles still remain before nanoparticles can bring clinical solutions to the fight against both acute and chronic respiratory diseases. In this review, we intend to discuss both the anatomical and biological barriers to delivery of drugs and contrast agents and how medical researchers have sought to use nanoparticle technology to overcome these barriers to diagnosis and treatment of chronic lung diseases. In particular, this review focuses on the recent development of multifunctional theranostic nanoparticles (Fig. 1) and their applications with some relevant examples.

Nanoparticles as drug/gene delivery vehicles for chronic respiratory diseases

Some therapeutic nanotechnology products have already been approved for clinical use, with drug-loaded liposomal or polymer nanoparticles being two major types [9, 15]. Inhalation delivery of corticosteroids is the treatment of choice for inflammatory airway diseases such as COPD and asthma, but obtaining reproducible delivery of the drugs with a sustained high dose and efficient distribution in the diseased area remains a challenge [16]. In the treatment of respiratory infections such as *Mycobacterium tuberculosis*, the drugs used tend to have poor bioavailability and thus must be used at relatively high doses with the accompanying risk of adverse effects [3, 4]. Nanoparticle delivery vehicles offer many benefits for patients with chronic respiratory diseases such as these, including improved bioavailability and pharmacokinetics, controllable sustained drug release, and a reduction in toxic side effects through encapsulation and targeting of therapeutics [9, 10, 17]. Nanoparticle drug carriers with targeting ligands such as antibodies or peptides that interact only with diseased tissue reduce damage to healthy cells and increase the therapeutic index [18].These nanocarriers are capable of delivering not only small-molecule drugs, but also therapeutic proteins, peptides and nucleic acids, paramagnetic metals for contrast imaging, and thermogenic compounds [16].

Cystic fibrosis (CF) is an inherited lung disease caused by a genetic defect in a transmembrane conductance regulator, CFTR. Delivery of a normal CFTR gene to the lungs of a CF patient is an attractive strategy to correct the disease [16], but getting nucleic acids to a specific site within the lung without degradation is no easy task. With molecular masses as high as 1 MDa and a strong negative charge, the physicochemical

Fig. 1 A schematic representation of theranostic nanoparticles and their applications

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properties of nucleic acids interfere with membrane passage into the cell and nucleus [19]. Nucleic acids can also be rapidly degraded by nucleases and cleared from the body [19]. These shortcomings can be overcome with the use of specific types of nanoparticles that allow nucleic acids to be successfully administered in vivo [19–21]. Nanoparticle constructs used for nucleic acid delivery can be categorized as condensing vector-based and non-condensing vector-based. Nanoparticles constructed from cationic polymers or lipids are able to bind to negatively charged nucleic acids, thus forming a condensed electrostatic complex [21]. Non-condensing nanoparticles possess either a neutral or net negative charge, with the nucleic acid payload encapsulated within the particles either by physical entrapment or through hydrogen bonding between the construct and nucleic acid bases [20].

Kumar et al. utilized chitosan nanospheres to encapsulate a cocktail of plasmid DNAs encoding all RSV proteins (except L) which proved to be an effective prophylactic intranasal gene transfer strategy for protecting mice against RSV infection [22]. After a single administration of the nanovaccine (25 μg/mouse), the mRNA and proteins of all antigens were expressed in the lungs of mice resulting in a several log reduction in viral titer and antigen load after acute RSV infection. The treatment also induced RSV-specific IgG antibodies, nasal IgA antibodies, cytotoxic T lymphocytes, and interferon-γ production in the lung and splenocytes. In another study, Vij et al. demonstrated the use of drug-loaded biodegradable nanoparticles (PLGA-PEGPS-341) to provide controlled and sustained drug delivery of the FDA-approved drug, bortezomib, which improves the inflammatory pathophysiology of CF cells [23]. This proteasomal drug is an extremely potent selective inhibitor of chymotryptic threonine protease activity; however, use of this drug may affect proteostasis and other consecutive processes. PLGA encapsulation and pulmonary delivery helps to mitigate this side effect. Intranasal administration of PLGA-PEGPS-341 in a mouse model of CF (Cftr−/−) resulted in a twofold decrease in lung proteasomal activity and reduction in *Pseudomonas aeruginosa* LPS induced inflammation, which demonstrates successful treatment of CF.

Nanoparticles as imaging contrast agents for chronic respiratory diseases

Medical imaging methods such as magnetic resonance imaging (MRI), X-ray computed tomography (CT), and positron emission tomography (PET) are used in the diagnosis and evaluation of many diseases. They are easily administered, minimally invasive, and capable of providing detailed images and information [24, 25]. In practice, PET scans are often read alongside MRI or CT scans because the combination gives both anatomic and metabolic information about a tumor [26]. Near infrared fluorescence optical imaging can be used for the

in vivo imaging of physiological, metabolic, and molecular function [27]. A variety of organic dyes, radioisotopes, and chelated metal ions conjugated to targeting ligands have been developed to provide contrast and enhance the quality of medical imaging [6, 26], but a multitude of new nanoparticles containing semiconductor quantum dots, carbon nanotubes and fullerenes, transition metal oxides, and noble metals has been receiving increased interest as contrast agents because of their advantages[6, 28]. Organic materials such as liposomes, micelles, and polymers are used in nanoparticles that encapsulate and deliver the new contrast agents [26].

Image contrast agents were developed to enhance the amount and quality of information that can be obtained from MRI techniques. Most of these agents depend on metals to provide the contrast and some such as gadolinium can be highly toxic. Sequestering them inside nanoparticles can protect patients from harm, and nanoparticle-based contrast agents have become an extensively studied research area. Compared to commonly used contrast agents such as chelated metal ions, nanoparticles offer numerous advantages including the ability to control their imaging properties by altering their composition and structure, to modify their surfaces to allow targeting of specific cells, and to enhance the contrast they provide to much greater intensities [25, 26, 29]. The relatively weak MRI signal from the lungs is a major drawback in imaging lung disease and is a prime target for employment of nanotechnology. Metal-loaded nanoparticles with shortened relaxation times and entrapment of potentially toxic metal ions offer attractive possibilities in biomedicine as safe and effective MRI contrast agents [30, 31]. Branca et al. used SPIOs functionalized with luteinizing hormone-releasing hormone to specifically target and view pulmonary micro metastases with high-resolution hyperpolarized ^{3}He MRI [32]. Clinical X-ray CT contrast agents include barium and iodinated compounds, which have high densities causing them to appear radiopaque in CT images [30]. There are no clinically approved nanoparticle contrast agents for CT imaging; however, preclinical CT studies are investigating the use of gold, which has a high atomic number and density that provides a threefold improvement in contrast over conventional iodine contrast agents [30]. Recently, Wang et al. reported on folic acid-modified dendrimer-entrapped gold nanoparticles for use in targeted CT imaging of human lung adenocarcinoma [33]. Despite their useful properties and potential applicability, most nanoparticle contrast agents are still in primary development or preclinical phases [24, 26].

The role of inflammatory signaling and oxidative stress in COPD and CF has been established, but the lack of real-time diagnosis of inflammatory/oxidative states can result in improper treatment that can lead to chronic and fatal lung pathophysiology [17]. In one recent study, Cho et al. developed and tested chemiluminescent micelles capable of peroxalate reactions that allow detection of hydrogen peroxide (H_2O_2)

concentrations as low as 100nM and imaging of H_2O_2 generated in mice [34].With further research, nanoparticle contrast agents can be moved to the clinic to allow earlier diagnosis and real-time assessment of lung pathology in patients with chronic respiratory diseases.

Barriers to nanoparticle delivery to the lung

The major barriers to the delivery of nanoparticles for chronic respiratory diseases include the tight epithelial cell layer and mucus hypersecretion and the airway defense system [17], but will depend on the route of delivery, the disease site in the respiratory tract, and the progression of the disease [16]. Either intranasal, inhaled, or intravenous delivery can be used for treatment of pulmonary diseases; however, each poses unique problems. The preferred route will depend on the specific nanoparticle, the bioavailability requirement, and the airway target tissue [16]. Nanoparticles targeting the lung encounter not only the unique anatomy of the lung but also specific pulmonary, intravascular, and intracellular barriers.

Lung anatomy

From a drug delivery perspective, the respiratory tract consists of an upper part made up of the nasal cavity and the pharynx and a lower including the larynx, trachea, bronchi, and the alveolae. The trachea divides into two primary bronchi that divide into smaller bronchioles, which branch in the lungs forming passageways for air to travel to the terminal alveoli. Moving down the respiratory tract starting at the trachea, the tubes get smaller and divide into more and more tubes. There are estimated to be about 20 to 23 divisions, ending up at an alveolus. Even though the cross-sectional area of each bronchus or bronchiole is smaller, because there are so many, the total surface area is larger. This results in less resistance at the terminal bronchioles. Most resistance is around the three to four divisions from the trachea due to turbulence. Alveoli are very small but together they have a large total surface area for efficient gas exchange. The alveoli have only a single epithelial cell layer separating the air from the capillaries (~400 nm) [35]. Instead of posing an obstacle, this large surface area has a high capacity for drug absorption, and the avoidance of first-pass metabolism makes the pulmonary route very attractive for nanoparticle administration of drugs and theranostics [35–37].

Pulmonary barriers

There are several pulmonary barriers inbuilt for normal lungs and created in diseased lung for exogenous nanoparticles. First, the airway mucociliary system is the main defense mechanism to eliminate dust and microorganisms [35, 38, 39]. Goblet cells and submucosal glands produce mucus that covers the entire

respiratory tract and increase the thickness of airway mucus (from 2–30 to >260 μm in disease).There is a periciliary liquid layer beneath that allows the coordinated, rhythmic beating of the cilia to constantly move mucus upwards toward the proximal airways, where it is either swallowed or expelled [35, 37–39]. Second, pulmonary surfactants reduce surface tension at the alveolar air–liquid interface and prevent collapse, but surfactant proteins SP-A and SP-D also play essential roles in pulmonary immune defense [35, 40, 41]. Both SP-A and SP-D play important roles in pulmonary immunity by binding and opsonizing invading microbes from the lung, ultimately causing their clearance by alveolar macrophages (AM) [35, 41, 42]. This aspect of these molecules also poses the most significant problem for nanoparticle administration as both SP-A and SP-D are able to bind to and cause clearance of nanoparticles from the lungs [43]. In a recent study, both molecules were able to enhance the AM uptake of nanoparticles, but for hydrophilic nanoparticles, this effect was strongest with SP-D, whereas for the hydrophobic nanoparticles, it was most pronounced with SP-A [43]. However, it must be noted that in this study both types of nanoparticles interacted to an approximately equivalent extent with AM in the presence of native surfactants, regardless of the different nanoparticle surfaces, suggesting that to fully understand this complex clearance mechanism of the lung, both SP-A and SP-D interaction with nanoparticles must be further studied [43]. Third, respiratory diseases such as cystic fibrosis, COPD, and asthma change the lung bifurcation angles and increase turbulent flow as well as obstruct the airways. This reduces deposition of particles to about 2 % in the obstructed areas that are most in need of treatment [37]. Using nanoparticles to overcome these problems will allow for great advantages when using the pulmonary route. The high level of vascularization, the large surface area of the respiratory tract, and the extremely thin barrier between the pulmonary lumen and the capillaries allows nanoparticles to easily enter the systemic circulation [37].

Intravascular barriers

For drugs or nanoparticles delivered by intravenous injection, following their entry into the systemic circulation, the blood with a high ionic strength may induce particle agglomeration and sequestration [8]. Plasma proteins such as opsonins may also adsorb onto the nanoparticles leading to their phagocytic recognition and elimination of the nanoparticles from the bloodstream within minutes of administration [8, 44, 45]. Size restriction by different endothelial layers throughout the body may pose yet another barrier as nanoparticles must extravasate from blood and lymphatic vessels to get to target tissues [45, 46]. Sometimes these intravascular barriers may promote targeted delivery, as in the case of the "leaky" tumor vasculature where nanoparticles have the ability to extravasate more readily in tumor

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tissues than healthy areas. The nanoparticles are also able to accumulate readily in the tumor interstitium due to poor lymphatic drainage, which along with the defective vasculature leads to an effect knows as enhanced permeation and retention [8, 47–49].

Intracellular barriers

Even after the nanoparticles have reached the diseased tissue, they must be able to penetrate the cell membranes to enter the cytoplasm, nucleus, or other organelles of the target cells to exert a therapeutic effect. The first barrier that intracellularly targeted nanoparticles encounter is the lipophilic cell membrane, which prevents large, charged molecules such as proteins, drugs, and DNA from entering except by endocytosis [50]. Clathrin-mediated endocytosis is a common mechanism for uptake of receptor-targeted nanoparticles, but a nanoparticle entering the cell through this pathway can become entrapped in late endosomes, which will fuse with lysosomes [50, 51]. Once inside the lysosome, enzymes can degrade the particles so only a small amount of the therapeutic agent or nanoparticle may make it into the cytoplasm [51].

Use of nanoparticles to overcome barriers to respiratory system delivery

Efforts to overcome the barriers imposed by lung anatomy and lung biology have led to the development of multifunctional nanoparticles with specific alterations in morphology, hydrodynamic size, and surface charge/hydrophobicity [8, 45]. The steric, electrostatic, and hydrophobic properties of inhaled nanoparticles must be optimized for evading the mucus barrier in order for the nanoparticles to reach the target site [16, 41]. Surfactants, lipids, proteins, inorganic molecules, polymers, dendrimers, and copolymers can be used to coat the nanoparticle's surface to improve stability [8, 52]. Polymer coatings such as dextran or polyethylene glycol confer biocompatibility on the nanoparticles and allow longer blood circulation times [35]. The most common functional groups on nanoparticle surfaces are amines and carboxyls, which allow attachment of peptides, antibodies, aptamers, fluorescent dyes, radionuclides, quantum dots, therapeutic agents, and other small molecules [53]. Peptides and antibodies can target the nanoparticles to a specific site or cell type, while fluorescent dyes and quantum dots allow imaging of the particles.

Multifunctional theranostic nanoparticles based on medical imaging contrast agents

Multifunctional nanoparticles are able to carry out two or more functions simultaneously, such as imaging and treatment of the target disease with theranostic nanoparticles. The combination of therapeutic molecules with safe and effective contrast agents in target-specific theranostic nanoparticles would allow physicians to diagnose disease at earlier stages, improve preoperative staging, provide site-specific delivery of drugs to limit side effects, and monitor treatment noninvasively [6, 11, 54]. Five classes of nanoparticle contrast agents that theranostic nanoparticles can be constructed with are currently under investigation. The types of nanoparticle contrast agents and some of the theranostic nanoparticles that contain these agents are summarized below and shown in Fig. 2.
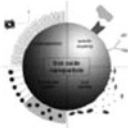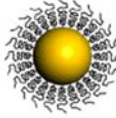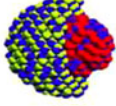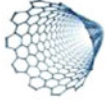
Quantum dot-based

Theranostic nanoparticles incorporating semiconductor quantum dots (QDs) have been a booming research area for the past few decade [55]. QDs are nanometer-sized particles made from heavy metal compounds like CdSe, CdTe, InP, and InAs, which absorb light across a large spectral range, but have the unique ability to emit light of a well-defined wavelength regardless of the input wavelength [24, 26, 56, 57]. These absorption/emission properties are dependent on the size, shape, and chemical composition of the QDs, and tuning their size and composition allows for extremely fine control of the emission wavelength [24]. Compared with organic dyes and fluorescent proteins, QDs offer a range of emission spectra (400–2,000 nm) covering both the visible and near-IR, exhibit larger absorption coefficients, and have much greater photostability [24, 26]. Different QD-based nanoparticles that are under investigation are summarized in Table 1.

QDs are commercially available for use as contrast agents and in photoacoustic imaging [24, 26, 56, 57], but have not been investigated for lung diseases. One popular strategy is to have QDs and therapeutic agents co-encapsulated in polymeric micellar, or liposomal nanoparticles for theranostic applications [55, 58]. In a recent study, researchers encapsulated CdSe QDs and doxorubicin in polymer-lipid hybrid micelles allowing for simultaneous optical imaging and controlled drug delivery [59]. The encapsulation of QDs also reduces their toxicity in biological systems [60, 61]. RNA aptamers, QDs, and doxorubicin can be combined for cancer targeting and simultaneous imaging and drug delivery [60, 62]. These multifunctional theranostic nanoparticles can detect cancer at the single-cell level while simultaneously releasing a therapeutic agent in a reportable manner. With other disease-specific targeting molecules, they could be used for other conditions, and the integration into theranostic nanoparticles limits the toxicity of QDs [62].

Even though QDs provide high photostability and a fluorescence wavelength that can be easily manipulated, their utilization in biological systems is limited by their toxicity and non-biodegradability [56]. However, their

Fig. 2 Advantages and disadvantages of various contrast agents are shown

Contrast agent	Pros	Cons
Metal Oxide	➢ FDA approved for clinical use; examples: Combidex, Ferridex, Gadodiamide, LumenHance. ➢ SPIONs exhibit intrinsic magnetic properties. ➢ Flexible surface modification with different coatings, dyes and therapeutic molecules	➢ Toxicity and biocompatibility concerns. ➢ Responsible for inflammatory responses in the respiratory system.
Gold Nanoparticles	➢ Able to scatter and absorb light with strong excitation peaks in the visible and near-infrared region (wavelengths depend on nanoparticles size). ➢ Flexible surface modification with different coatings, ligands, dyes and therapeutic molecules.	➢ Toxicity concern.
Quantum Dots	➢ Narrow wavelength emission (absorption/emission properties are size dependent). ➢ High fluorescent efficiency and photostability with wide range of emission spectra (400 nm-2000nm) covering both the visible and near-infrared wavelengths.	➢ Toxicity and biocompatibility concerns specially heavy-metals containing QDs.
Carbon Nanotubes	➢ Intrinsic properties, enabling imaging modalities (NIR, Raman, and Photoacoustic imaging). ➢ Ultra-high surface area and hollow inside space available for efficient drug loading and bioconjugation.	➢ Toxicity and biocompatibility concerns.
Radioisotopes and Fluorescent Molecules	➢ Flexible incorporation into a variety of drug-delivering nanoparticles. ➢ Ability to perform as non-invasive real-time treatment monitoring and early diagnosis capabilities.	➢ Lack of targeting specificity, short half-life. ➢ Toxicity and biocompatibility concerns.

encapsulation into theranostic nanoparticles should help to limit the toxicity of QDs and improve their biocompatibility. QDs have played an important role in research and disease diagnostics as powerful imaging probes [55]. Their integration into intelligent, theranostic, non-toxic nanosystems could have the potential to revolutionize the treatment and diagnosis of chronic respiratory illnesses.

Table 1 Properties of various QDs

QD type	Optical properties	Biocompatibility
Heavy metal cadmium, lead, or arsenic	•High fluorescent efficiency and photostability •Wide band excitation with narrow light emission	•Potential toxicity, leakage of toxic heavy metal ions from QDs
Heavy metal free (CuInS2/ ZnS)	•Good quantum yield and photostability •Wide emission range from visible to infrared region	•Lower toxicity as compared to cadmium containing QDs
Silicon	•Strong photoluminescence and high quantum yield •Wide emission rang from visible to infrared region.	•Lower toxicity as compared to cadmium containing QDs •Degrade to silicic acid, which can be exerted through urine •Low water solubility, unstable in aqueous solutions
Carbon	•Strong photoluminescence and high photostability •Wide emission range from visible to infrared region	•No toxicity reported
Polymer	•High photostability and strong bright fluorescence •Suitable for fluorescence microscopy and laser excitation	•No toxicity reported

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Gold-based

Gold nanomaterials formulated as nanoparticles, nanoshells, nanorods, and nanocages have attracted considerable attention for their potential use in theranostic applications such as cancer diagnosis and testing [63–66]. They are highly biocompatible, undergo surface modification easily, and possess unique optical/electrical properties [67, 68]. Gold nanoparticles exhibit strong extinction peaks in the visible and near-infrared region [26, 69] and are able to scatter and absorb light, with the magnitude of this light being determined by the nanoparticles size and structure [26, 69, 70]. Gold nanoparticles are excellent CT contrast agents due to X-ray absorption that is two to five times greater than iodine, while gold nanoshells exhibit strong absorption in the near-infrared region (NIR) making them useful for NIR imaging [24, 26, 68, 71]. The hollow, porous structures of gold nanocages can be used to encapsulate therapeutic payloads or to act as effective photothermal transducers, capable of converting light into heat and causing local temperatures to rise substantially [64, 67, 72].

Effective delivery of exogenous genes is a promising approach for the treatment of many diseases, especially those that are genetic in origin. Despite their high efficiencies as delivery vectors, viruses suffer from several limitations including high immunogenicity, the potential for positional mutagenesis, and high production costs [73]. Nonviral agents for gene delivery such as gold nanorods are attractive alternatives to viral vectors. Gold nanorods can also be used simultaneously for photothermal ablation, photothermally enhanced drug and gene delivery and biological imaging, thus making them a powerful theranostic platform [73].

Photoacoustic (PA) imaging is a powerful hybrid bioimaging modality that combines the strong optical absorption contrast associated with optical imaging with the high ultrasonic spatial resolution [64, 65, 74]. Nanoparticles with strong optical absorption have been investigated as contrast agents for PA imaging, especially those based on gold nanocages, which can be tuned to strongly absorb and scatter light throughout the NIR range from 700 to 900 nm [65]. Recently, Younan Xia et al. have reported on a new theranostic system with the capability to both enhance the contrast of PA imaging and control the release of a chemical or biological effector by high-intensity focused ultrasound [65]. The capabilities of this theranostic nanoparticle system can be further enhanced for in vivo molecular imaging, as well as chemo- and photothermal therapy [65].

Unlike their molecular equivalents, which often suffer from lack of specificity, nanocarriers offer both passive and active targeting to diseased tissues and cells. Active disease targeting can be achieved by conjugating specific targeting ligands such as peptides, antibodies, and aptamers that recognize and bind to specific receptors overexpressed on diseased cells [58, 66, 75]. In a recent study, Yuling Xiao et al. developed a multifunctional gold nanorod (GNR)-based platform for targeted DOX delivery and PET imaging of tumors [66]. The ligand cyclo (Arg-Gly-Asp-D-Phe-Cys) and the ^{64}Cu-chelator, 1,4,7-triazacyclononane-N,N',N''-triacetic acid, were conjugated onto the distal ends of the PEG arms to achieve active tumor targeting and PET imaging, respectively. This novel GNR theranostic nanoplatform combining targeting, chemotherapy, photothermal therapy, and imaging can potentially lead to improved therapeutic efficacy and cancer monitoring [66].

Thus, gold-based theranostics can have significant applications for diverse lung diseases including lung cancers. It is important, therefore, that these versatile compounds be tested for theranostic use in other conditions, such as chronic respiratory disease. They need to be thoroughly examined for biocompatibility, cytotoxicity, and environmental impact in order to be manufactured on a large scale for clinical usage in treating respiratory diseases [69, 76].

Carbon-based

Buckminsterfullerene (C_{60}) is a carbon allotrope and fullerenes such as carbon nanotubes (CNTs), graphene, nanodiamonds, and carbon dots have attracted a large amount of interest for biomedical applications, owing to their unique physical and chemical properties [48, 77]. Single-walled CNTs (SWNTs) exhibit an intrinsic NIR photoluminescence that has been utilized for biological imaging because biological tissues have low auto-fluorescence background at NIR wavelengths [78, 79]. Excitation of SWNTs by a laser with a frequency close to their electronic transition energies (E_{11} and E_{22}) induces Raman scattering and enables Raman imaging. In a recent study, SWNTs were utilized for Raman imaging and photothermal treatment of ovarian cancer [80]. Carbon nanotubes have high loading capacity and can be functionalized to carry therapeutic, diagnostic, and targeting agents simultaneously [81, 82]. Raman imaging of DNA-functionalized SWNTs incubated with 3T3 fibroblast and myoblast stem cells showed that the ultra-high photostability of SWNTs is orders of magnitudes better than NIR quantum dots and organic fluorescent dyes [83, 84]. RGD-conjugated SWNTs have also been utilized for photoacoustic imaging of U87MG tumors in a mouse tumor model [85].

Other imaging agents can be conjugated to CNTs. For example, the biodistribution of CNTs in mice was studied using CNTs labeled with the radioisotope iodine-125 [86]. Using iron as a catalyst to grow SWNTs produces CNTs with Fe_2O_3 nanoparticles attached to the ends that could be used for MR and NIR imaging in biological systems [87] or to carry drug molecules [88]. These theranostic nanoparticles are promising candidates for drug delivery and as MRI contrast agents because of their great drug-anchoring capability, high content of ferromagnetic iron, and low cytotoxicity. The safety

and biocompatibility of carbon nanostructures are still contro-versial [77, 84] with recent reports showing that carbon nano-structures may cause lung fibrosis and adverse immune reactions [81, 89, 90]. However, the many advantageous properties of CNTs dictate that more research be done to determine their potential for the treatment and diagnosis of chronic respiratory illnesses.

Metal oxide-based contrast agents

There are two types of MR imaging mechanisms: T1-weighted and T2-weighted. T1 refers to the longitudinal or spin–lattice relaxation time, which is the time required for a proton to regain longitudinal magnetization following a radio frequency pulse [26, 91]. Molecules with shorter T1 relaxation times will appear brighter on the MRI and longer T1 relaxation times will appear darker. T2 is the transverse or spin–spin relaxation time, which is the amount of time that a resonating proton can be coherent or rotated by a 90° radio frequency pulse [26, 91]. Molecules that are brighter in T1 images will appear darker in T2 images and vice versa. MRI contrast agents work by altering the T1, T2, or T1/T2 relaxation times of nearby protons. Super paramag-netic iron oxide nanoparticles, SPIONs, have shown success as T2 or negative contrast agents. For example, Combidex is a commercially available SPION T2 contrast agent for use in the differentiation of benign and metastatic lymph nodes [25]. To create a theranostic nanoparticle using SPIONs, the ability to simultaneously deliver a therapeutic compound must be incorporated into the nanoparticle.

Recently, we published a report on the development of dual-purpose chitosan and polyethyleneimine (PEI)-coated magnetic micelles (CP-mag-micelles) that can deliver nucleic acid-based therapeutic agents and also provide MRI [31]. These theranostic CP-mag-micelles are compo-sed of monodisperse hydrophobic SPIONs loaded into the cores of micelles that are self-assembled from a block copolymer of poly(D,L-lactide) and monomethoxy-polyethylene glycol. For efficient loading and protection of the nucleic acids, the micelles were coated with cationic polymers, chitosan, and PEI. These nanoparticles showed high MRI relaxivity and the ability to deliver genes with higher transfection efficiency and greater expression than Lipofectamine or PEI alone. These nanoparticles could be used for gene delivery and MRI monitoring in vivo.

Although SPION nanoparticles have been extensively studied for use in T2 contrast imaging in conjunction with a diverse array of nanotherapeutics [52, 92, 93], iron oxide is a relatively poor T2-type MRI contrast agent for the lung [94]. T1 contrast agents are better for lung imaging and diagnosis of chronic lung diseases. Currently, T1 MRI uti-lizes predominantly gadolinium (Gd)-based contrast agents with high spin states and long electronic relaxation times

[26]. Magnevist™, the first clinically approved Gd-based MRI contrast agent, appeared in 1981.The focus now is on using Gd-containing contrast agents in the design and syn-thesis of theranostic nanoparticles. Le Duc et al. reported ultrasmall Gd-based nanoparticles (GBNs) that produce pos-itive contrast for MRI and a radiosensitizing effect when activated by X-ray microbeams [95]. The combination of these GBNs and microbeam radiation therapy improved the survival of rats bearing aggressive brain tumors. These GBNs exhibited the properties required of a theranostic agent: contrast enhancement of medical imaging (MRI), therapeutic activity (dose enhancement of X-ray microbe-ams), and safety after intravenous injection (passive accu-mulation in the tumor, renal clearance) [95].

The high toxicity of Gd^{3+} requires that these contrast agents always be given in a chelated form. Several cases of nephro-genic systemic fibrosis have been reported in patients receiv-ing Gd-containing contrast agents [96, 97], highlighting the need for alternatives to Gd-containing T1 contrast agents. Manganese (Mn) is paramagnetic [98, 99], has five unpaired electrons in its bivalent state [98, 99], is a natural cellular constituent as a cofactor for enzymes and receptors [98, 99]; and thus, Mn may serve as a suitable T1 MRI contrast agent for lung tissues. Although Mn-containing contrast agents are FDA-approved for clinical use [98], Mn can be toxic at the high levels required to offset the short plasma half-life of ionic Mn [99, 100]. Sequestration of Mn within nanoparticles should reduce the risk of toxicity and overcome the problem of short plasma half-life.

We have synthesized "core–shell" cationic lipid mi-cellar nanoparticles (LMNs) in which the core contains hydrophobic drugs or imaging contrast molecules, and the shell is composed of phospholipids for loading DNA or peptides and attaching targeting moieties. The lipids, 3ß-[N-(N',N'-dimethylaminoethane)-carbamoyl] cholester-ol (DC-Chol) and dioleoylphosphatidyl-ethanolamine (DOPE), are used to facilitate endosomal escape and augment gene/drug delivery [101–105], while polyethyl-ene glycol-phosphatidylethanolamine (PEG-2000-PE) confers biocompatibility and longer blood circulation times [106, 107]. These LMNs can be loaded with MnO nanoparticles and the chemotherapeutic agent DOX in the core and with plasmid DNA on the surface to provide both MRI contrast and DNA/-drug delivery to target cells [108]. The synthesis of DM-LMNs is easily scalable and the combination of DOPE, DC-cholesterol, and PEG-2000-PE yields high gene transfection efficiency and drug uptake. When administered intranasally to mice, the DM-LMNs were found mostly in the lungs, in marked contrast to other polymers making them an ideal can-didate for lung cancer theranostics.

It is known that Mn-based contrast agents have a lower longitudinal relaxivity than most Gd-based contrast agents. Yu Chen et al. recently reported on the fabrication of

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nontoxic MnO/mesoporous silica nanoparticles (MSNs) for use as T1 MRI contrast agents with comparable imaging performance to commercial Gd-based contrast agents and a sustained, pH-responsive release of anticancer agents [109]. The researchers expect that their present synthesis strategy can be used to produce various kinds of ordered, manganese-based mesoporous theranostics [109]. There is concern that metal oxide nanoparticles will have limited application in chronic airway diseases due to toxicity and the chronic inflammation they may cause [17, 110]. Encapsulating metal oxide nanoparticles within micelles, liposomes, or polymers as we have done should overcome the toxicity concerns, but it is important to explore other types of contrast agents for use in theranostic nanoparticles.

Radioisotope- and fluorescence-based contrast agents

Small molecules such as fluorescent dyes and radioisotopes have a long history of use as contrast agents in both research and clinical settings [24, 26]. PET scans have long utilized radioisotopes such as ^{11}C, ^{13}N, ^{5}O, ^{18}F, and ^{64}Cu conjugated onto certain biomolecules to measure the distribution and metabolism of these biomolecules throughout the body [24]. Fluorescent dyes incorporated into various macromolecules have been extensively used in biomedical research for confocal microscopy and flow cytometry to study the interaction of these macromolecules with cells and their biodistribution and fate [24]. However, both radioisotopes and fluorescent dyes suffer from drawbacks such as a lack of targeting specificity, short half-life, and toxicity [24, 26, 111]. It has been suggested that the integration of these contrast agents into theranostic nanoparticle systems can help to overcome the individual limitations and maximize the strengths of radioisotopes and fluorescent dyes.

Liposomes are vesicles composed of one or more concentric phospholipid bilayers and have been widely investigated as drug carriers. It is well known that the addition of a PEG coating prolongs the blood circulation times of liposomes by minimizing their removal by macrophages of the reticuloendothelial system. Marik and associated reported synthesis of radiolabeled diglyceride 3-[^{18}F] fluorodipalmitoyl-1, 2-glycerol (^{18}F-fluorodipalmitin, [^{18}F] FDP), and its potential as a reagent for radiolabeling long-circulating liposomes was investigated [112]. Radiolabeled long circulating PEG-coated liposomes were prepared using a mixture of DPPC, cholesterol, DSPE-PEG2000, and [^{18}F] FDP. PET imaging and biodistribution studies were performed, and the results showed that injected free [^{18}F] FDP was quickly taken up by the liver, spleen, and lungs; however, liposomal [^{18}F] FDP remained in circulation at near constant levels for at least 90 min. Incorporation of radiolabeled lipids inside the phospholipid bilayer allows radiolabeling of a wide variety of lipid-based drug delivery vehicles and gives these drug carriers' theranostic abilities.

Hydrogen peroxide (H_2O_2) is an essential oxygen metabolite of the antimicrobial respiratory burst of the human immune system, but it is also a major source of oxidative stress and has been implicated in inflammation and aging-associated diseases [34, 113]. Therefore, a nanoparticle capable of selective detection and scavenging of overproduced H_2O_2 could be used to treat a variety of inflammatory diseases [34]. Cho and colleagues reported the development of chemiluminescent micelles able to detect H_2O_2 and reduce generation of reactive oxygen species [34]. These micelles have great potential as a theranostic agent for H_2O_2-associated inflammatory diseases, such as COPD.

Integrating clinically approved, small molecule contrast agents, such as radioisotopes and fluorescent dyes, into theranostic nanoparticles can overcome many of the problems that plague the use of these small molecules when administered alone. Many contrast agents can be encapsulated or conjugated onto the surface of drug-delivering nanoparticles, which allows the nanoparticles to be used for noninvasive real-time treatment monitoring and early diagnosis. With further research, theranostic nanoparticle systems incorporating small molecule contrast agents could hold great potential for the treatment and diagnosis of a range of chronic respiratory illnesses.

Conclusions

Considerable research has been directed toward development of theranostic nanoparticles for targeted cancer imaging and therapy [6, 28], but these same efforts have not been applied to other chronic lung diseases. A majority of the theranostic nanoparticles reviewed here were designed and tested for cancers. A significant advantage of many theranostic systems is their versatility, as encapsulated drugs with similar properties can often be swapped for one another and targeting ligands are usually interchangeable. This versatility may make it possible to use the same theranostic nanoparticles for the treatment of a variety of lung diseases. Theranostic nanoparticles for the treatment of acute and chronic lung diseases will have to overcome additional hurdles due to the complexity of the airway, lung anatomy and biology, but this should not stop researchers from developing and testing theranostic systems for the acute and chronic lung diseases that are such serious global health problems.

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