REVIEW

Nanomaterials incorporated ultrasound contrast agents for cancer theranostics

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ABSTRACT

Nanotechnology provides various nanomaterials with tremendous functionalities for cancer diagnostics and therapeutics. Recently, theranostics has been developed as an alternative strategy for efficient cancer treatment through combination of imaging diagnosis and therapeutic interventions under the guidance of diagnostic results. Ultrasound (US) imaging shows unique advantages with excellent features of real-time imaging, low cost, high safety and portability, making US contrast agents (UCAs) an ideal platform for construction of cancer theranostic agents. This review focuses on the development of nanomaterials incorporated multifunctional UCAs serving as theranostic agents for cancer diagnostics and therapeutics, via conjugation of superparamagnetic iron oxide nanoparticles (SPIOs), CuS nanoparticles, DNA, siRNA, gold nanoparticles (GNPs), gold nanorods (GNRs), gold nanoshell (GNS), graphene oxides (GOs), polypyrrole (PPy) nanocapsules, Prussian blue (PB) nanoparticles and so on to different types of UCAs. The cancer treatment could be more effectively and accurately carried out under the guidance and monitoring with the help of the achieved theranostic agents. Furthermore, nanomaterials incorporated theranostic agents based on UCAs can be designed and constructed by demand for personalized and accurate treatment of cancer, demonstrating their great potential to address the challenges of cancer heterogeneity and adaptation, which can provide alternative strategies for cancer diagnosis and therapeutics.

KEYWORDS

Cancer; theranostics; ultrasound contrast agents; nanomaterials; nanomedicine

Introduction

Nanotechnology is a multidisciplinary research field involving physics, chemistry, biology, engineering, material science and clinical medicine, providing a variety of nanoscale materials with extraordinary features for diagnostics and therapeutics of cancer1-6. In the recent years, a unique cancer treatment strategy, theranostics, has been developed by combination of imaging diagnosis and imaging guided therapeutic interventions to enable accurate detection and personalized treatment of cancer with high efficacy7-12. Theranostics can deliver both diagnostic and imaging-guided therapeutic functions simultaneously, which can address the challenges of cancer heterogeneity and adaptation13,14. Therefore, nanomaterials with excellent nature of easy functionalization and significant biocompatibility as well as their optical, electronic, magnetic and structural properties, are ideal candidates as theranostic nanomedicine for cancer diagnosis and therapy. Furthermore, the combination of various imaging modalities could make it possible to carry out cancer therapy under the guidance of multi-modal contrast imaging, achieving visualized and personalized treatment of cancer15,16.

There are several medical imaging methods for clinical cancer diagnosis, including X-ray computed tomography (CT), ultrasound (US) imaging, magnetic resonance imaging (MRI), positron emission computed tomography (PET) and so on. Among all the diagnostic imaging techniques, US has its unique advantage due to its features of real-time, low cost, and high safety17, making it the ideal candidate for imaging guided cancer therapy. With the use of ultrasound contrast agents (UCAs), the resolution and sensitivity of clinical US imaging have made great improvements18-20. Typical UCAs are microbubbles (MBs) that composed of an inner gaseous core with a thin shell coating. The shell materials including proteins21, saccharides22, surfactants23, polymers24 and lipids25 are used to form a protective layer outside the inner gas cores like air, nitrogen, sulfur hexafluoride (SF6) and perfluorocarbons (PFCs)26-28.

Two or more biomedical imaging techniques are usually
applied together in clinic to reach more reliable diagnostic results, since each imaging modality has its specific advantages as well as limitations. Therefore, in the recent years, the idea of using multimodal imaging modalities in combination has attracted the attention of researchers. US could display real-time images, nevertheless, its spatial and anatomical resolution is relatively poor compared to other imaging methods. The disadvantages of US might be overcome by the integration of other imaging modalities with excellent spatial and anatomical resolution. Therefore, multimodal imaging agents composed of UCAs incorporated with other imaging probes could provide more detailed and accurate diagnostic results for better guidance of cancer treatment. Furthermore, by integration of proper nanomaterials with therapeutic functionalities, theranostic agents based on UCAs could be constructed for cancer treatment.

In this review, we will focus on the latest development on nanomaterials incorporated multifunctional UCAs serving as theranostic agents for multimodal imaging and cancer therapy, via combination of different types of UCAs and nanomaterials including superparamagnetic iron oxide nanoparticles (SPIOs), CuS nanoparticles, DNA, gold nanoparticles (GNPs), gold nanorods (GNRs), gold nanoshell (GNS), Prussian blue (Pb) nanoparticles, and polypyrrole (PPy) nanocapsules, etc. (Figure 1). This review will provide a comprehensive design and fabrication of nanomaterials incorporated UCAs for cancer theranostics.

Theranostic agents based on protein UCAs

Feinstein et al. reported in 1984 that the albumin in the blood improved the stability of hand-made MB UCAs and sonication produced more stable MBs with controlled size. It resulted in the first commercial UCA, Albunex (Molecular Biosystems), which consisted of air-filled albumin MBs ranging from 1 to 15 μm with more than 95% being smaller than 10 μm. Albumin-based UCAs are widely used in clinical diagnosis and research field.

Gold nanomaterials exhibit good biocompatibility as well as magnificent optical and electronic properties, making them possible to be used in biological and medical applications. GNRs have well-defined shapes and their longitudinal plasmon resonances (LPRs) can be finely tuned as a function of aspect ratio to the near infrared (NIR) region, a spectral window which permits photons to penetrate biological tissues with relatively high transmittivity. Recently, Wang and co-workers developed GNRs loaded albumin microbubbles (AuMBs) for combined US and photoacoustic imaging and photothermal therapy. AuMBs were fabricated by sonication of the solution containing 2.67 nM GNRs, 2% human serum albumin, 0.04% (w/v) avidin and filling with PFC (C3F8) gas, then modified via biotin-avidin technique to result in antimouse VEGFR2 targeted AuMBs. In vivo ultrasonography revealed that the targeted AuMBs could identify the angiogenesis region in the tumor and extend the retention time for longer US imaging. The GNRs could induce photoacoustic imaging and thermal therapy under NIR irradiation, demonstrating the combined diagnostic and therapeutic properties of AuMBs.

Theranostic agents based on lipid/surfactant UCAs

Lipids and surfactants are extensively used as shell materials to fabricate MBs UCAs due to their capabilities to lower the surface tension and stabilize MBs via forming a coating layer. Amphiphilic molecules can self-assemble into a monolayer shell at the interface between the gas core and surrounding aqueous medium to form gas-filled MBs. When the MBs are exposed to US, they start to cavitate. The microstreams and shock waves generated during cavitation may lead to a local release of drugs loaded on the MBs. At the same time, temporary perforate cell membranes (sonoporation) may occur to result in the intracellular delivery of the released drugs. Furthermore, the microjets and shock waves could also permeabilize blood vessels, allowing the release of high molecular weight drugs and nanoparticles. Therefore, MBs with anticancer drugs or functional nanomaterials payload could serve as efficient US
triggered delivery system for imaging guided cancer treatment.

SPIOs are widely applied T2-weighted MRI contrast agents, which can provide a safe and strong negative contrast enhancement of the target lesion in MRI due to their high susceptibility and biocompatibility. Fan's group developed multi-functional phospholipid coated C\(_{3}\)F\(_{8}\) encapsulated MBs loaded with therapeutic agent (doxorubicin, DOX) and MRI contrast agents SPIOs via adapted thin-film hydration method. The blood-brain barrier (BBB) can be reversibly opened without damaging the neurons by US MB cavitation within the cerebral microvasculature for delivery of therapeutic compounds to the brain. Thus, the in vivo experiments proved that the DOX-SPIO-MBs could temporarily open the BBB and perform drug delivery to the brain due to cavitation upon the focused US exposure. Besides, they can carry out dual modal MRI/US contrast imaging diagnosis, and magnetic targeting to achieve enhanced drug delivery for imaging guided tumor treatment. Based on this study, they further fabricated a therapeutic SPIO-DOX (SD) complex that can be conjugated to MBs (SD-MB). The SD-MB could targeted release SD complexes through BBB under focused US exposure to allow dual modal brain imaging and drug delivery for chemotherapy. Moreover, their group also incorporated SPIOs into drug-embedded acoustic droplets to allow both magnetism-assisted targeting and MRI guided US-triggered acoustic droplet vaporization, which is a mechanical and chemical theranostic strategy for tumor treatment. The developed theranostic agents may provide a novel strategy for future imaging guided therapy of brain tumors.

MB UCAs have been developed as image-guided promising vehicles of genes for targeted delivery. Sonoporation is an effective method of promoting extravasation of large macromolecules, such as plasmid DNA, to improve delivery to tissue beyond the vasculature. Branched polyethyleneimine (PEI) was modified with polyethylene glycol (PEG) and thiol. The resulted PEI-PEG was then covalently attached to maleimide groups on lipid MB UCAs. Polyplex-MBs was prepared by loading DNA to achieve increased circulation in the bloodstream and decreased non specific adhesion. A luciferase bioluminescence reporter plasmid DNA was coupled to the PEI-MBs, and site-specific delivery was fulfilled using US applied over the tumor area following bolus injection of the DNA/PEI-MBs, showing over 10-fold higher bioluminescence from the tumor region compared to the untreated. It suggested that the PEI-MB UCAs would offer improved control of DNA loading for US-guided tissue transfection and gene therapy of cancer.

siRNA-loaded nanobubbles (siRNA-NBs) were also developed via hetero-assembling strategy by Shuai’s group using siRNA-complexed polymeric micelles and gas-cored lipid nanobubbles. Sufficient tumor accumulation of the nanoscale siRNA-NBs can be achieved via the EPR effect, allowing efficient delivery of siRNA micelles into cancer cells with the help of US exposure for imaging guided gene therapy. It indicates that US sensitive micro/nanobubble UCAs with DNA/siRNA payloads could act as potential theranostic platform for US-triggered and guided gene therapy.

CuS nanoparticles could penetrate into the tumor interstitium for efficient ablation of tumor cells due to their NIR absorption and small size. Furthermore, the NIR absorption of CuS nanoparticles is derived from d-d transition of Cu\(^{2+}\) ions, which is not affected by the solvent or the surrounding environment. Therefore, using surfactant MB ST68 as a template, CuS nanoparticles loaded MBs (CuS-ST68 MBs) was constructed by depositing photothermal conducting CuS nanoparticles onto the outer surface of gas-filled MBs, which consisted of inert C\(_{3}\)F\(_{8}\) gas and a monolayer shell of surfactant mixtures of Span 60 and Tween 80. In vitro and in vivo ultrasonography proved the contrast-enhancement capability of CuS-ST68 MBs. Besides, targeted CuS nanoparticles delivery using US-targeted MB destruction (UTMD) to destroy tumor cells by the photothermal effect could be fulfilled by CuS-ST68 MBs as well.

**Theranostic agents based on polymeric UCAs**

Polymeric microcapsules, typically composed of poly lactic acid (PLA) with outstanding biocompatibility and biodegradability, could serve as UCAs with good US contrast-enhanced capabilities and other advantages: they have good mechanical strength to be stable; they can load either hydrophilic or hydrophobic species or both during the double emulsion fabrication procedure to become functionalized; they are surface-charged and have functional groups on the surface so that they could be easily modified to acquire more utilities such as site-targeted capability.

By constructing echogenic PLA microcapsules employing the water-in-oil-in-water (W/O/W) double emulsion method, GNR-loaded PLA microcapsules have been developed for combined US contrast imaging and photothermal therapy via electrostatic adsorption of GNRs on the microcapsule surfaces under the help of polyelectrolytes. GNS was introduced to substitute GNRs to achieve higher payload of gold nanomaterials on...
microcapsules. GNS has a spherical dielectric core particle and a thin nanoscale gold shell around with highly tunable plasmon resonance, which determines the absorbing and scattering properties of the composite particles. By controlling the thickness of the gold shell and the diameter of the core, the plasmon resonance and the resulting optical absorption of GNSs can be tuned to the region of NIR, where the absorption of human tissues is minimal and penetration is optimal. The strong optical absorption of nanoshell can rapidly increase the local temperature under the NIR irradiation. Therefore, a novel multifunctional theranostic agent based on gold nanoshelled microcapsules (GNS-MCs) was designed and prepared by the electrostatic adsorption of GNPs on the PLA microcapsule surfaces as seeds, and the formation of GNS by surface seeding method (Figure 3 A-C). The microcapsules can cross pulmonary capillaries and show systemic enhancement. When irradiated with NIR radiation, GNS-MCs could raise the local temperature so that cancer cells were killed with surroundings remains unaffected.

Using BT474 breast cancer xenografted models, GNS-MCs were able to serve as an US enhancer to guide the intratumoral injection and ensure their uniform distribution. In vivo studies showed the intratumoral temperature could be heated up to nearly 70 °C for 8 min when treated with GNS-MCs injection with NIR laser irradiation, resulting in the gradual tumor volume decrease with 6 out of 7 mice cured at 17 days after treatment. It demonstrated that US imaging-guided photothermal therapy with theranostic GNS-MCs would be a promising technique for in situ treatment of breast cancer (Figure 3 D and E). Although US imaging could display the real-time images with higher contrast under the help of UCAs, the resolution of the US images is relatively poor compared to other imaging methods, and the imaging process can be easily interfered by bone- and gas-filled structures like the brain.
and stomach. The drawbacks of US happen to be the merits of CT with excellent spatial resolution based on density. Therefore, it might be a great combination of US and CT for better diagnostics to guide and monitor the cancer therapy. GNP s with large atomic number (z=79), high X-ray absorption coefficient (5.16 cm$^2$/g at 100 keV), and especially the great biocompatibility and non-toxicity, could be the ideal candidates for contrast-enhanced CT imaging. On the other hand, GOs exhibit superior optical absorption in the NIR region and photothermal conversion, large surface area and lower cost compared with noble metal nanoparticles, making GOs an appealing candidate for photothermal ablation of cancer. Theranostic microcapsules were successfully fabricated by introducing GNP s into PLA microcapsules with depositing GO onto the surface via electrostatic layer-by-layer self-assembly technique. The obtained microcapsules could serve as a contrast agent to simultaneously enhance US and CT imaging greatly both in vitro and in vivo. As an effective photothermal enhancer, the NIR laser light ablated the tumor completely within 9 days in the presence of the microcapsules and the tumor growth inhibition rate was 83.8% (Figure 4).

SPIO s could also be integrated into polymer UCAs for MRI contrast enhancement. Using PLA/PLGA or Pluronic F127 based UCAs as an encapsulation vehicle, SPIO s were loaded by emulsion methods, with other functional
materials like DOX\textsuperscript{80}, Carmustine (bis-chloroethyl-nitrosourea, BCNU)\textsuperscript{81} and GOs\textsuperscript{82}. Huang et al.\textsuperscript{81} designed and prepared SPIO-stabilized and BCNU-loaded nanobubbles based on the thermosensitive polymer F127 and polyacrylic acid (PAA). Both US and MRI contrast enhanced imaging were achieved with the help of the multifunctional contrast agents. Moreover, under the external magnetic targeting, higher US and MR signals could be displayed compared to the same site without magnetic targeting. The tumor growth profile of different treatment revealed that, the tumors exhibited half the growth rate of the control group with injection of BCNU loaded nanobubbles and high intensity focused ultrasound (HIFU) exposure, showing the significant tumor inhibition. The multifunctional system for dual-modal US/MRI imaging guided HIFU therapy and US triggered drug delivery would provide a potential alternative solution for efficient and imaging-guided cancer treatment\textsuperscript{82}.

PPy materials have received great attention in bioelectronics and biomedical application due to their inherent features, including high conductivity, outstanding stability and good biocompatibility\textsuperscript{83-85}. PPy NPs have been demonstrated as an attractive and photothermal agent with high photothermal conversion efficiency for tumor ablation owing to the strong NIR absorption spectrum\textsuperscript{86}. Therefore, a photothermal UCA of PPy hollow microspheres (PPyHMs) was first time constructed from the PPy polymer via a facile oil-in-water (O/W) microemulsion method. PPyHMs could generate US contrast consistently with the echo signals for no less than 5 min, and completely ablate the tumor in the presence of NIR laser light\textsuperscript{87}.

**Theranostic agents based on liquid PFC UCAs**

PFCs with unique properties such as high oxygen solubility, low surface tension, hydrophobicity and lipophobicity, inertness and absence of metabolism, safety and biocompatibility, have been developed for various medical purposes\textsuperscript{88,89}. Because of their high difference of density with air and their poor solubility in water, liquid PFCs such as perfluorooctyl bromide (PFOB) have been encapsulated in the biodegradable and biocompatible polymeric shells to prepare nano-scale UCAs with great stability and echogenicity\textsuperscript{90,91}. Unlike blood-pool MB based UCAs, nano-sized UCAs are advantageous for tumor imaging mainly due to their unique size range that promotes a high tissue extravasation rate, resulting in an increased number of agents passing through the vessels feeding the tumor to achieve satisfactory imaging\textsuperscript{92,93}.

As mentioned above, gold nanomaterials could serve as CT contrast agent due to their high X-ray absorption coefficient. GNS is widely used as “light-activated nanoscopic heater” to...
significantly enhance the efficiency of photothermal therapy\textsuperscript{94,95}. Therefore, gold nanoshelled PFOB nanocapsule UCAs were developed for US/CT imaging guided photothermal ablation of tumors. It’s the first report of GNS serving as the dual-functional nanomaterials for both CT contrast imaging and photothermal tumor ablation\textsuperscript{96}. Gold nanoshelled PFOB nanocapsules (PGsP NCs) were constructed by the same gold seeding method\textsuperscript{69} on the PFOB nanocapsules generated via an adapted oil-in-water emulsion solvent evaporation process\textsuperscript{90}, with surface PEG modification to increase anti-fouling capabilities for long circulation time. In vivo US/CT contrast imaging was successfully carried out through intravenous injection of PGsP NCs. Using human glioblastoma tumor-bearing nude mice as xenograft models, the local temperature of tumors could be heated up to about 60 °C with the irradiation of 808 nm NIR laser, and the temperature lasted more than 7 min during the whole 10 min exposure, ensuring the effective thermal ablation of tumors. On 16th day after treatment, the tumor shrunk 67.6% in tumor size with 5 out of 8 mice cured. Compared with control group, the tumor growth was inhibited by 96.7%, suggesting great treatment effect of PGsP NCs induced photothermal tumor ablation\textsuperscript{96}.

By incorporation of SPIOs into the gold nanoshelled PFOB nanocapsules, a novel theranostic nano-UCAs, PGs-SP NCs were obtained for dual-modal US/MRI contrast imaging guided photothermal therapy\textsuperscript{97}. PGs-SP NCs could be not only used for the guidance under US contrast imaging, but also applicable via intravenous administration to targetly accumulate in the tumor sites for MRI guidance due to the EPR effect. The abnormalities in the tumor tissue lead to higher permeability of tumor vessels to the nanocapsules compared with normal vessels, and the impaired clearance of the nanocapsules from the interstitial space of the tumor could result in targeted accumulation on tumor sites (due to longer retention enhanced by PEGylation)\textsuperscript{6}. The targeted accumulated nanocapsules can display the tumor areas to guide the NIR laser irradiation for photothermal ablation of tumors without damaging the surrounding healthy tissues. The tumor growth was inhibited by 82.2% by two times of intravenous injection of PGs-SP NCs with NIR laser irradiation, suggesting that sufficient accumulation of PGs-SP NCs could trigger the great photothermal effect locally for effective photothermal tumor ablation (Figure 5).

Niu et al.\textsuperscript{88} developed SPIOs and PFOB co-loaded organic/inorganic hybrid vesicles via facile self-assembly/sol-gel approach for dual-modality US/MR imaging and guided HIFU ablation. With the help of the theranostic agent, the focused US can be precisely located on the VX2 tumor tissue in the liver of rabbits under MRI guidance for enhanced HIFU therapeutic efficacy. Using PPy as loading materials as well as photothermal absorbers, PFOB encapsulated PPy nanocapsules were obtained. Owing to the encapsulated liquid PFOB and strong NIR absorption of PPy polymeric shell, the resulted PPy nanocapsules showed great promise in US imaging guided photothermal ablation of xenografted tumors without inducing any significant side effect\textsuperscript{99}.

Apart from various organic polymers as protective shells outside the liquid PFC cores, inorganic nanomaterials could also be utilized to form hollow nanostructures with PFC in the inner cavities. Shi’s group constructed a nanometer-sized inorganic enhancement agent (MSNC-PFH) for HIFU treatment of cancer, which consists of mesoporous silica nanocapsules (MSNCs) as the shell and encapsulated temperature-sensitive perfluorohexane (PFH) as bubble generator\textsuperscript{100}. With exposure to HIFU, PFH bubbles could be generated and released through the mesopore channels of MSNCs for contrast enhanced US imaging, owing to the HIFU-induced local temperature rise. Moreover, HIFU therapeutic efficacy can be significantly enhanced by the generated PFH bubble, which can offer a unique superiority in localized tumor necrosis and drug delivery.

PB is a conventional dye used for hundreds of years. PB nanoparticles have been developed recently for catalysts, contrast agents, drug carriers, photacoustic tomography and photothermal therapy\textsuperscript{101}. Jia et al.\textsuperscript{101} fabricated phase transition hydrophobic liquid PFC perfluoropentane (PFP) encapsulated hollow PB nanocubes (HPB-PFP) for cancer theranostics. The PB shell could induce excellent photothermal effect under NIR laser irradiation, resulting in not only superior photothermal therapeutics but also PFP gasification and bubble formation for enhanced US contrast imaging. In vivo tumor imaging demonstrated significant signal enhancement could be observed with HPB-PFP injection and laser exposure. The enhanced US images could be utilized for better diagnosis and guidance of photothermal tumor ablation, making HPB-PFP a potential theranostic nanomedicine for cancer treatment\textsuperscript{101}.

**Summary and perspectives**

Numerous functional nanomaterials including SPIOs, CuS nanoparticles, GNPs/GNRs/GNS, DNA, siRNA, GO, PPy nanocapsules and PB nanoparticles have been incorporated to different types of UCAs to obtain additional functionalities for cancer diagnosis and therapeutics. The differences among UCAs for preparation of theranostic agents are shown in Table 1. Protein-based UCAs were the first commercial...
products available in the market due to the great biocompatibility. However, the relatively poor stability limits their functional modification, with rare reports in the construction of theranostic agents. Lipid/Surfactant-based UCAs are extensively studied for contrast enhanced imaging and UTMD drug delivery due to the excellent echogenicity and their similarity to conventional drug carriers such as liposomes or micelles. Nevertheless, the nanomaterials could not be easily incorporated into lipid/surfactant UCAs owing to the instability of their soft shells. On the contrary, polymeric UCAs with tremendous stability and easy functional decoration capability were able to load various nanomaterials to prepare multifunctional theranostic agents.

Although exhibiting mediocre echogenicity, polymeric UCAs could facilitate HIFU therapy, providing potential alternative functionalities for theranostic agents. Unlike the other three types of blood-pool MB UCAs, liquid PFC encapsulated UCAs with nanomaterial payloads could be generated in nano-scale sizes, leading to enhanced vessel permeability to improve tumor targeting and retention for efficient tumor imaging and therapy. With proper optimization and rational design, the well-constructed nanomaterials incorporated nano-UCAs based on liquid PFC might have great potential for clinical application of cancer theranostics.

The cancer treatment could be more effectively and accurately carried out under the guidance and monitoring via...
multimodal imaging with the achieved multifunctional UCAs. In the cancer theranostic procedure, multimodal imaging guidance could display dynamic complementary information about the tumors for planning, targeting, monitoring, controlling and assessing treatment response for the therapeutic treatment, which helps alter the treatment strategy accordingly with personalization and high efficacy. Notably, all the procedures in the whole cancer treatment based on theranostic UCAs could be noninvasive or minimally invasive to avoid unnecessary risks. Moreover, the nanomaterial-loaded UCAs could be designed and developed by demand for personalized treatment of cancer, which makes nanomaterials incorporated multifunctional UCAs involved treatment strategy a potential alternative methodology for noninvasive cancer theranostics.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

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