Facile synthesis of liposome/Cu_{2-x}S-based nanocomposite for multimodal imaging and photothermal therapy

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A kind of multifunctional perfluoropentane (PFP) and ultrasmall Cu_{2-x}S nanodots (u-Cu_{2-x}S NDs) co-incorporated liposome (PFP@u-Cu_{2-x}S NDs@liposome) nanocomposite has been facilely and successfully synthesized for enhanced ultrasound/infrared thermal/photoacoustic multimodal imaging and photothermal therapy upon near infrared (NIR) laser irradiation. Such a liposome-based nanocomposite possesses a number of advantages, such as high dispersity and stability, excellent biocompatibility, small particle size (<100 nm), well-defined core/shell structure, strong NIR absorption and photo-triggered vaporization of PFP, etc. The detailed *in vitro* investigations demonstrate that the as-synthesized PFP@u-Cu_{2-x}S NDs@liposome nanocomposite is capable of enhancing the contrasts of ultrasound/infrared thermal/photoacoustic multimodal imaging, and substantially improving the photothermal therapeutic efficacy. This novel liposome-based theranostic nanoplatfrom shows great potentials in the future cancer diagnosis and therapy.

INTRODUCTION

Over the past decades, billions of people have suffered from various cancers worldwide [1]. Fortunately, diverse diagnostic imaging technologies [2–4] and powerful therapeutic methodologies [5–9] have emerged for cancer theranostics. As a minimally invasive and potentially effective therapeutic technique compared with conventional approaches, photothermal therapy (PTT) [10–13], which utilizes photothermal agents to convert near-infrared (NIR) energy into hyperthermia to kill cancer cells, has received extensive interest in recent years. Especially, imaging-guided PTT [14–17] shows great promise in improved efficiency and bio-safety.

Currently, ultrasound imaging [18–20] is believed to be one of the most widely used imaging tools in the clinic, owing to its considerably high resolution, deep tissue penetration, easy soft tissue recognition and low cost. However, the ultrasound waves are easily disrupted by air or gas and unable to penetrate bones [21]. Infrared thermal imaging [22–24] has recently attracted intensive interest thanks to

its high imaging speed, sensitivity and convenience, but it suffers from relatively low imaging resolution. Recently, the newly developed photoacoustic imaging [25–28] is emerging as a complementary imaging modality, which combines the high contrast of optical imaging and the high spatial resolution of ultrasound imaging. Therefore, the combination of ultrasound/infrared thermal/photoacoustic imaging techniques will bring unexpected potentials in offering more comprehensive and accurate information with the complementary features of these techniques. In addition, the ingenious strategy of the multimodal imaging-guided PTT will be more powerful and efficient in PTT pre-guidance, intra-monitoring and post-evaluation. Therefore, the exploration of such multifunctional theranostic nanoplatfroms, which combine both diagnostic and therapeutic functions to allow simultaneous multimodal imaging and therapy as well as the monitoring of tissue/organ's responses to the treatments, has been becoming a research hotspot in the current biomedicine filed.

The design of liposome-based nanocomposites [29–32] offered a great potential for the fabrication of such multifunctional theranostic nanoplatfroms. Perfluorocarbons (PFCs) incorporated liposome [33–36] has long been being a common contrast agent for the ultrasound imaging by providing contrast enhancement through an acoustic impedance mismatch or resonance. Recently, a laser-induced phase transformation [37,38] was proposed aiming at providing a safer and more controllable process of vaporization by using less energy than that of high-intensity ultrasound. Upon laser irradiation, a liquid-to-gas phase transformation of PFCs takes place due to the temperature elevation via the conversion of laser energy. The resulting PFCs bubbles are capable of enhancing contrast of ultrasound/photoacoustic imaging [39,40] and inducing cavitation for therapy [41–43] by the bubble formation and explosion. This kind of laser-induced phase transformation can be realized by the combination of PFCs droplets with various

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photosensitizers, such as indocyanine green (ICG)-loaded perfluorocarbon nanodroplets, gold nanoparticles and perfluorohexane liquid (PFH) co-loaded PLGA [42,43].

Herein, we propose a novel single NIR laser induced multifunctional theranostic nanoplatform for ultrasound/infrared thermal/photoacoustic multimodal imaging and photothermal therapy, based on the rational design and construction of perfluoropropane (PFP) and ultrasmall Cu_{2-x}S nanodots ($\text{u-Cu}_{2-x}\text{S}$ NDs) co-loaded liposome (abbreviated as $\text{PFP@u-Cu}_{2-x}\text{S}$ NDs@liposome). This multifunctional $\text{PFP@u-Cu}_{2-x}\text{S}$ NDs@liposome can be facily synthesized via a typical thin film method, and demonstrates a number of excellent properties, including high dispersity and stability in aqueous solution, excellent biocompatibility, small particle size (<100 nm), well-defined core/shell structure, and strong NIR absorption originating from the copper deficient $\text{u-Cu}_{2-x}\text{S}$ NDs, which is attributed to the localized surface plasmon resonances [44–46]. In addition, PFP bubbles can be detected upon NIR laser irradiation. Therefore, the $\text{PFP@u-Cu}_{2-x}\text{S}$ NDs@liposome is demonstrated to be capable of providing significant contrast enhancements for ultrasound/infrared thermal/photoacoustic multimodal imaging, and improving photothermal therapeutic efficacy for killing cancer cells *in vitro*. This novel NIR laser induced multifunctional theranostic nanoplatform based on $\text{PFP@u-Cu}_{2-x}\text{S}$ NDs@liposome

nanocomposite will attract extensive interest in future cancer diagnosis and therapy.

RESULTS AND DISCUSSIONS

The as-synthesized PFP and $\text{u-Cu}_{2-x}\text{S}$ NDs co-incorporated liposome ($\text{PFP@u-Cu}_{2-x}\text{S}$ NDs@liposome) for ultrasound/infrared thermal/photoacoustic multimodal imaging and photothermal therapy is illustrated in Scheme 1. The novel $\text{PFP@u-Cu}_{2-x}\text{S}$ NDs@liposome was facily and successfully synthesized by incorporating hydrophobic fluorocarbon compound PFP of low boiling point (29°C) in the core and $\text{u-Cu}_{2-x}\text{S}$ NDs in the shell.

Blank liposome was prepared via a typical thin film method [47]. The representative transmission electron microscopy (TEM) image (Fig. 1a) of the as-synthesized blank liposome shows that the liposome is quasi-spherical with a multilamellar structure and a mean diameter of 100 nm. This morphology offers high potential for the incorporation of PFP and $\text{u-Cu}_{2-x}\text{S}$ NDs with controllable bubbles release upon NIR laser irradiation. Moreover, the size of the liposome can be tailored in the synthetic process, ranging from several micrometers to dozens of nanometers, as shown in Fig. S1. The $\text{u-Cu}_{2-x}\text{S}$ NDs with a diameter of 2 nm (Fig. 1b) was synthesized as previously reported [48]. The typical scanning transmission electron microscopy (STEM) images (Figs 1c and inset) of $\text{PFP@u-Cu}_{2-x}\text{S}$ NDs@

Scheme 1 Schematic illustration of the as-synthesized $\text{PFP@u-Cu}_{2-x}\text{S}$ NDs@liposome for enhanced ultrasound/infrared thermal/photoacoustic multimodal imaging and photothermal therapy under NIR laser irradiation.

Figure 1 TEM images of blank liposome (a) and oleylamine-capped u-Cu_{2-x}S NDs (b). STEM (c) and HAADF-STEM image (d) of the PFP@u-Cu_{2-x}S NDs@liposome. (e) UV-Vis absorption spectra of PFP@u-Cu_{2-x}S NDs@liposome dispersions at different Cu²⁺ concentrations and (f) the corresponding linear fitting plots of absorbance vs. Cu²⁺ concentration at 1064 nm.

liposome show no significant morphology and size changes compared with blank liposome. The corresponding high angle annular dark field-STEM (HAADF-STEM) images (Figs 1d and inset) and elemental area mapping (Fig. S2) confirm the successful incorporation of u-Cu_{2-x}S NDs. The hydrodynamic diameter of the PFP@u-Cu_{2-x}S NDs@liposome was measured to be around 130 nm (Fig. S3a) and the Zeta potential was determined to be about −13 mV (Fig. S3b). In addition, there was no change in the hydrodynamic diameter and Zeta potential after being kept at 4°C

for 3 and 7 days, demonstrating the high stability of this multifunctional liposome nanoplatfrom. Moreover, it is worth noting that the as-synthesized PFP@u-Cu_{2-x}S NDs@liposome shows a broad and strong absorption from 800 to 1400 nm (Fig. 1e), with a peak locating in the second NIR window (1000–1350 nm). Additionally, the absorption intensity is linearly enhanced with increasing Cu²⁺ concentration (Fig. 1f), which is determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES).

In order to distinctly demonstrate the successful in-

Figure 2 Optical microscopic images of u-Cu_{2-x}S NDs@liposome (a, b) and PFP@u-Cu_{2-x}S NDs@liposome dispersion immersed in hot water (60°C) (c, d), and exposed to NIR laser irradiation (980 nm, 1.41 W cm⁻², 5 min) (e, f).

corporation of PFP in PFP@u-Cu_{2-x}S NDs@liposome, the bubble generation assays in both hot water (60°C) and by exposure to NIR laser irradiation were carried out. As shown in Figs 2a and b, the reference u-Cu_{2-x}S NDs@liposome sample without PFP shows no bubble generation both before and after being immersed in hot water. However, a great amount of smaller-sized phase-changed PFP micro-bubbles emerge in PFP@u-Cu_{2-x}S NDs@liposome dispersion after either being immersed in hot water (Figs 2c and d) or exposed to NIR laser irradiation (Figs 2e and f), which undoubtedly confirms that both PFP and u-Cu_{2-x}S NDs have been successfully incorporated into the liposome and great numbers of vaporized PFP nanoemulsions have been converted into gas bubbles. These small bubbles will further swell and merge into larger microbubbles, which is beneficial for enhanced ultrasound/photoacoustic imaging under NIR laser irradiation.

Encouraged by the strong NIR absorption of PFP@u-Cu_{2-x}S NDs@liposome and sustained generation of PFP gas bubbles under NIR laser irradiation, we further investigated its contrast enhancement capability for ultrasound/infrared thermal/photoacoustic multimodal imaging and photothermal effect *in vitro*. Two milliliter of PFP@liposome as a control and PFP@u-Cu_{2-x}S NDs@liposome dispersions (Cu²⁺ concentration: 50 ppm) were wrapped up by a plastic tube and immersed into the degassed water tank

for the following ultrasound imaging under NIR laser irradiation. The reference PFP@liposome dispersion shows no ultrasound imaging contrast enhancement after NIR laser irradiation (Figs 3a and b). Comparatively, a remarkable contrast enhancement (Figs 3c and d) is observed in plastic tube containing the PFP@u-Cu_{2-x}S NDs@liposome dispersion under exposure to NIR laser irradiation for 5 min (980 nm, power density: 1.41 W cm⁻²), which is attributed to the generation of large amount of PFP gas bubbles originated from the phase transformation of PFP. To further study the contrast enhancement capability of the PFP@u-Cu_{2-x}S NDs@liposome for infrared thermal imaging, infrared thermal images of reference water droplet and aqueous droplets containing the PFP@u-Cu_{2-x}S NDs@liposome (Cu²⁺ concentration, C1: 12.5 ppm, C2: 50 ppm) at different time intervals were recorded during NIR laser irradiation for comparison. As shown in Figs 3e–h, water droplet shows no contrast enhancement and negligible temperature rise. In contrast, significant contrast enhanced infrared thermal images and temperature elevation are detected in the aqueous droplets containing the PFP@u-Cu_{2-x}S NDs@liposome under the same experimental conditions, indicating that this multifunctional PFP@u-Cu_{2-x}S NDs@liposome is capable of enhancing the contrast of infrared thermal imaging. Furthermore, its potential of acting as a candidate contrast agent for photoacoustic imaging was also evaluated *in vitro*. Linear concentration-enhanced photoacoustic signals and images are obtained (Fig. 3i). In all, the PFP@u-Cu_{2-x}S NDs@liposome is demonstrated to be capable of substantially enhancing the contrasts of ultrasound/infrared thermal/photoacoustic multimodal imaging.

Moreover, the strong NIR absorption of PFP@u-Cu_{2-x}S NDs@liposome motivated us to investigate its photothermal effect. The temperature elevations of the aqueous dispersions containing PFP@u-Cu_{2-x}S NDs@liposome at different Cu²⁺ concentrations and under NIR laser irradiation at different power densities were measured. Pure water as a control demonstrated a temperature rise of 5°C under NIR laser irradiation with a power density of 1.41 W cm⁻². Comparatively, the aqueous dispersions containing PFP@u-Cu_{2-x}S NDs@liposome showed a temperature increase of 10–20°C in 5 min under the same experimental condition (Fig. 3j), and the heating rate increased with the increasing power density (Fig. S4). Thus, it is believed that the PFP@u-Cu_{2-x}S NDs@liposome can efficiently and rapidly convert the 980 nm laser energy into hyperthermia, resulting from the strong absorption of Cu_{2-x}S NDs in NIR region. All these results demonstrate that this novel multifunctional PFP@u-Cu_{2-x}S NDs@liposome shows great potential in serving as an excellent contrast agent for ultrasound/infrared thermal/photoacoustic multimodal

Figure 3 Ultrasound images of PFP@liposome (a, b) and PFP@u-Cu_{2-x}S NDs@liposome dispersion (c, d) before and after NIR laser irradiation for 5 min. Infrared thermal images (e–h) of water droplet and aqueous droplets containing PFP@u-Cu_{2-x}S NDs@liposome (Cu²⁺ concentration, C1: 12.5 ppm, C2: 50 ppm) under NIR laser irradiation at different time intervals. (i) Linear plot of photoacoustic signal intensity vs. Cu²⁺ concentration, the inset shows the corresponding photoacoustic images of agar gel cylinders containing PFP@u-Cu_{2-x}S NDs@liposome at varied Cu²⁺ concentrations. (j) Temperature elevations of the PFP@u-Cu_{2-x}S NDs@liposome dispersion exposed to NIR laser irradiation (980 nm, 1.41 W cm⁻²) vs. Cu²⁺ concentration.

imaging and a highly efficient photothermal agent for photothermal therapy.

Furthermore, the cytotoxicity of the nanocomposite was evaluated by a standard 3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay on HeLa cells. As expected, the PFP@u-Cu_{2-x}S NDs@liposome appears to be basically non-toxic. The cell viabilities are all above 90% even at a high Cu²⁺ concentration up to 100 ppm, as shown in Fig. S5 (the black histogram). Afterwards, the photothermal effect on HeLa cells was investigated. As demonstrated in Fig. S5 (the red histogram), the PFP@u-Cu_{2-x}S NDs@liposome exhibits a concentration-dependent cell death feature under NIR laser irradiation. In addition, the cell death was visualized by trypan blue staining in optical microscope. The control groups including HeLa cells without treatment, treated with the PFP@u-Cu_{2-x}S NDs@liposome alone and NIR laser irradiation alone show no changes after trypan blue staining (Figs 4a–c). However, the cells

treated with PFP@u-Cu_{2-x}S NDs@liposome and NIR laser irradiation are stained in blue, indicating that severe cell death (Fig. 4d) took place. Moreover, the cellular uptakes of the PFP@u-Cu_{2-x}S NDs@liposome at varied duration were studied prior to confirming the cell death by confocal laser scanning microscopy (CLSM). It can be clearly found that most of the PFP@u-Cu_{2-x}S NDs@liposome particles are in the cytoplasm of HeLa cells in 1 h (Fig. S6a) and 4 h (Fig. S6b) of co-incubation, demonstrating that the PFP@u-Cu_{2-x}S NDs@liposome can be rapidly endocytosed by HeLa cells. The severe cell death was further verified by co-staining with calcein-AM and prodium iodide (PI) by CLSM. Similarly, the control cell groups mentioned above are stained in green after calcein-AM and PI co-staining, corresponding to live cells (Figs 4e–g). In contrast, the cells treated with the PFP@u-Cu_{2-x}S NDs@liposome and NIR laser irradiation are stained in red, assigning to dead cells (Fig. 4h). These data are consistent with the results of MTT

Figure 4 Optical microscopic images (a–d) and CLSM images (e–h) of HeLa cells stained with trypan blue, calcein-AM and prodium iodide (PI) after different treatments: (a, e) without treatment as a control; (b, f) treated with PFP@u-Cu_{2-x}S NDs@liposome alone; (c, g) treated with NIR laser irradiation alone; (d, h) treated with the PFP@u-Cu_{2-x}S NDs@liposome and NIR laser irradiation (980 nm, 1.41 W cm⁻²) together. All the scale bars in (a–h) are 50 μ m.

assay and optical microscopic imaging. Therefore, it is believed that the PFP@u-Cu_{2-x}S NDs@liposome is a highly effective photothermal agent to kill cancer cells *in vitro*, which is mainly attributed to the strong NIR absorption property, and therefore shows great potential in photothermal therapy *in vivo*.

CONCLUSION

In summary, a novel NIR laser induced multifunctional theranostic nanoplatfrom based on PFP@u-Cu_{2-x}S NDs@liposome has been facily and successfully fabricated. This kind of PFP@u-Cu_{2-x}S NDs@liposome nanocomposite demonstrates high dispersity and stability in aqueous solution, excellent biocompatibility; small particle size (<100 nm) and well-defined core/shell structure with PFP in the core and u-Cu_{2-x}S NDs in the shell. Specially, this nanocomposite exhibits strong absorption in the NIR region originating from unusual defect structure of u-Cu_{2-x}S NDs. Therefore, this nanocomposite is capable of converting laser energy to hyperthermia to induce the phase transformation of PFP, resulting in sustained generation of gas bubbles. Under the same NIR laser irradiation, the PFP@u-Cu_{2-x}S NDs@liposome is capable of generating significant contrast enhancement for ultrasound/infrared thermal/photoacoustic multimodal imaging and serving as an efficient photothermal agent for killing cancer cells. Thus, the as-synthesized PFP@u-Cu_{2-x}S NDs@liposome will be a promising agent in multimodal imaging-guided photothermal therapy, which, as we believe, will also provide an ingenious strategy for the intensive exploration of liposome-based multifunctional nanomaterials in future *in vivo* cancer diagnosis and therapy.

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Author contributions Shi J and Chen H initiated and guided the work. Mou J designed and conducted the experiments. Chen Y, Ma M, Zhang K gave useful suggestions. Wei C set up the experimental devices.

Conflict of interest The authors declare that they have no conflict of interest.

Supplementary information Experimental details are available in the online version of the paper.

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中文摘要 包裹氟碳化合物的脂质体已被广泛用作超声成像造影剂；而硫化铜由于具有表面等离子体共振效应在近红外区域具有强光学吸收特性，可以同时作为一种性能优异的近红外热/光声成像造影剂和光热治疗剂。本文以有机脂质体为载体，同时包裹氟碳化合物和硫化铜，在近红外激光照射下，硫化铜吸收光能将其转化为热能，达到氟碳化合物的相变温度时使氟碳化合物由液态变为气态，产生的气泡可增强超声成像造影能力，从而构建了单一波长激光诱导的超声/近红外热/光声多模式成像引导下的光热治疗，极大提高了癌症治疗的安全性和治疗效率。体外实验结果表明该有机无机复合物不仅能克服单纯有机脂质体的不稳定性等缺点，而且具有显著增强的多模式成像造影能力和优异的光热治疗效果，具有重要的临床应用前景。