Copper sulfide nanoparticles for photothermal ablation of tumor cells

Aims: Copper sulfide (CuS) nanoparticles were developed as a new type of agent for photothermal ablation of cancer cells. Materials & methods: CuS nanoparticles were synthesized by wet chemistry and their application in photothermal ablation of tumor cells was tested by irradiation using a near-infrared (NIR) laser beam at 808 nm to elevate the temperature of aqueous solutions of CuS nanoparticles as a function of exposure time and nanoparticle concentration. CuS nanoparticle-mediated photothermal destruction was evaluated using human cervical cancer HeLa cells with respect to laser dose and nanoparticle concentration. Their toxicity was evaluated by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. Results: CuS nanoparticles have an optical absorption band in the NIR range with a maximum absorbance at 900 nm. Irradiation by a NIR laser beam at 808 nm resulted in an increase in the temperature of the CuS nanoparticle aqueous solution as a function of exposure time and nanoparticle concentration. CuS nanoparticle-induced photothermal destruction of HeLa cells occurred in a laser dose- and nanoparticle concentration-dependent manner, and displayed minimal cytotoxic effects with a profile similar to that of gold nanoparticles. Conclusion: Owing to their unique optical property, small size, low cost of production and low cytotoxicity, CuS nanoparticles are promising new nanomaterials for cancer photothermal ablation therapy.

KEYWORDS: cancer cells, cancer therapy, CuS nanoparticles, near-infrared irradiation

Nanotechnology refers to the interactions of cellular and molecular components and engineered materials with very small dimensions, between 1 and 100 nm. Nanometer-sized particles have novel optical, electronic and structural properties that are not present in either individual molecules or bulk solids [1]. One of the key aspects of the ‘nanophenomenon’ that has the potential to benefit biomedical research and nanomedicine is quantum size confinement, by which the properties of a semiconductor change dramatically as it approaches nanoscale dimensions. Quantum size confinement in nanoparticles can modify the physical and chemical properties of a semiconductor, changing the material’s energy structure and, thus, luminescence behavior and other optical properties [2]. These changes may lead to brighter luminescence and easier tunability of the emission color of semiconductor nanoparticles. Luminous nanoparticles have tremendous potential for use in cancer detection and imaging [2]. Furthermore, features, such as small size, water-solubility, good photostability, narrow emission bandwidth and enhanced surface functionalities, open up possibilities for in vivo applications where nanoparticles can be directed to specific disease sites, and even enter into different cellular compartments for selective imaging and/or therapy [3–7].

Hyperthermic therapy is the use of heat between 40 and 45°C to damage cancer cells while preventing the surrounding cells from being affected [8]. It is used to increase the effectiveness of other treatment modalities, such as chemotherapy or radiation therapy. At higher temperatures (>45°C), thermal energy alone may be used to directly ablate cancer cells. The heat for hyperthermic therapy and thermal ablation can be delivered by microwave, radiofrequency, magnetic field, focused ultrasound or laser stimulation. No matter what energy source is chosen, a proper amount of heat must be generated in a specific disease volume over a given period of time in order to activate efficient treatment. A significant obstacle to successful hyperthermic/thermal ablation therapy is that healthy tissues can also absorb electromagnetic and ultrasound energies. Therefore, the healthy tissues between the tumor and the external energy source can also heat up, resulting in a limited therapeutic window. The application of functional nanoparticles that interact with the external energy sources to mediate the thermal effect may overcome this limitation, because nanoparticles can be selectively directed to the cancer cells [3–6].

A variety of functional nanoparticles have been examined as potential thermal mediators. It has been demonstrated that magnetic
nanoparticles can improve hyperthermic cancer treatment [9–11]. Gold nanoparticles [12,13], nanoshells [14,15] and hollow nanospheres [6,16] have all been investigated for thermal ablation therapy induced by a near-infrared (NIR) light, hence the term photothermal ablation therapy (PTA). These gold nanostructures exhibit strong absorption of NIR light between a range of 700 and 1100 nm, in which light can penetrate deeply into the tissues [17]. The intense absorption is attributed to the surface plasmon phenomenon, and the absorption maximum is related to the particle size and shape, and to the dielectric constant of the surrounding matrix, such as the solvents and the core material in the core–shell gold nanostructures [18].

Semiconductor copper sulfide (CuS) nanostructures display interesting electrical, optical and catalytic properties, with potential applications in photodegradation of pollutants [19], biological labeling [20], laser light monitoring and eye protection [21], and DNA detection [22]. As a semiconductor, the infrared absorption in CuS nanoparticles is fundamentally different from that displayed by gold nanostructures. The former is derived from energy band–band transitions, while the absorption from gold nanostructures results from surface plasmons. In this study, we tested the hypothesis that the interaction of CuS nanoparticles with NIR light could generate heat, which could be harnessed for PTA of cancer cells. To the best of our knowledge, this is the first report on the use of semiconductor nanoparticles for PTA therapy.

Materials & methods

Materials

Thioglycolic acid (TGA), CuCl$_2$.2H$_2$O and thioacetamide were purchased from Sigma-Aldrich (MO, USA). Roswell Park Memorial Institute (RPMI)-1640 culture medium, calcein AM and EthD-1 LIVE/DEAD® viability kit were obtained from Invitrogen (Eugene, OR, USA). Gold nanoparticles (20 nm) were prepared by adding 5 ml of sodium citrate (25 mM) into a boiling aqueous solution of HAuCl$_4$ (0.25 mM). The mixture was stirred until the solution turned into a red wine color, indicating the completion of the reaction. Human cervix adenocarcinoma HeLa cells and human embryonic kidney 293 (HEK293) cells were obtained from American Type Culture Collection (VA, USA).

Nanoparticle synthesis & characterization

Thioglycolic acid-stabilized CuS nanoparticles were synthesized as follows. A total of 0.017048 g of CuCl$_2$.2H$_2$O (0.1 mmol) was dissolved in 100 ml of distilled water, 0.2 mmol of TGA (~14.2 µl) was added into the solution under constant stirring and the pH was adjusted to 9.0 by drop-wise addition of a 1 M solution of NaOH. The solution was placed into a three-necked flask fitted with a septum and valves, and was degassed by argon bubbling for 20 min. A solution of thioacetamide (8.0 mg, 0.1 mmol) in distilled water (20 ml) was added, and the whole solution was heated at 50°C for 2 h to promote nanoparticle growth.

The crystalline structure, size and shape of the nanoparticles were observed by x-ray diffraction (XRD) and high-resolution transmission electron microscopy (HRTEM). XRD was measured using a Siemens Kristalloflex 810 D-500 x-ray diffractometer (Karslruhe, Germany) under an operating mode of 40 kV and 30 mA, with $\lambda = 1.5406$ Å radiation. The nanoparticles in solution were placed onto holey carbon-covered copper grids for HRTEM observation. The HRTEM images of the particles were obtained with a JEOL JEM-2100 electron microscope (Tokyo, Japan) with accelerating voltage of 200 kV. The absorption spectra were recorded using a Shimadzu UV-2450 UV-vis spectrophotometer (Kyoto, Japan).

Photothermal effect in aqueous solution

The laser was a continuous wave GCSLX–05–1600 m$^{-1}$ fiber-coupled diode laser (China Daheng Group, Beijing, China) with a center...
wavelength of 808 ± 10 nm. A 5-m, 600-µm core BioTex LCM-001 optical fiber (Houston, TX, USA) was used to transfer laser light from the laser unit to the target. This fiber had a lens mounting at the output that allowed the laser spot size to be changed by changing the distance from the output to the target. The output power was independently calibrated using a hand-held optical power meter (Newport model 840-C, CA, USA) and was found to be 1.5 W for a spot diameter of 1.3 mm (~113 W/cm²) and a 2-Amp supply current. For measuring temperature change mediated by CuS nanoparticles, 808-nm NIR laser light was delivered through a quartz cuvette containing the nanoparticles (100 µl). A thermocouple was inserted into the solution perpendicular to the path of the laser light. The temperature was measured over a period of 15 min. Water was used as a control.

In vitrod photothermal ablation of cancer cells with CuS nanoparticles

In this study, HeLa cells were used to evaluate the photothermal ablation with CuS nanoparticles. The cell line was derived from cervical cancer cells. The cells were chosen because NIR light can be potentially used colposcopically to illuminate cervical cancer and precancerous lesions. HeLa cells were seeded onto a 96-well plate at a density of 10,000 cells per well, 1 day before the experiment. The cells were washed three times with Hanks balanced salt solution (HBSS, Sigma-Aldrich), followed by incubation with CuS nanoparticles (0, 96, 192 or 384 µM equivalent CuS) at 37°C for 2 h. After incubation was completed, the culture media with nanoparticles was removed and the cells resupplied with fresh phenol red-free RPMI-1640 (Invitrogen, Carlsbad, CA, USA). The cells were irradiated with an NIR laser centered at 808 nm at an output power of 0, 24 or 40 W/cm² for 5 min or 64 W/cm² for 3 min (Diomed, Andover, MA, USA). The diode laser was coupled to 1-m long, 2 mm diameter core fiber, which delivered a circular laser beam of 2 mm in radius, covering the central area of the microplate well. Power calibration was carried out automatically. After laser irradiation, the cells were resupplied with RPMI-1640 containing 10% fetal bovine serum and incubated at 37°C for 24 h. The cells were then washed with HBSS and stained with calcein AM for visualization of live cells and with EthD-1 for visualization of dead cells, according to the manufacturer’s suggested protocol (Invitrogen). The cells were examined under a Zeiss Axio Observer.Z1 fluorescence microscope (Carl Zeiss MicroImaging GmbH, Göttingen, Germany). The fluorescence intensity of each well was measured using a Tecan microplate reader with Magellan software (Männedorf, Switzerland). The percentage of viable cells in each well was calculated according to the manufacturer’s protocol. Each experiment was performed in triplicate. Differences in viability between each treatment and the control (i.e., no laser, no nanoparticles) were analyzed using Student’s t-test, with p < 0.05 considered to be statistically significant.

Cytotoxicity

HEK293 cells were used to represent a normal cell line in order to evaluate the cytotoxicity of CuS nanoparticles. Cell viability was measured using the tetrazolium salt (WST-1) assay kit (Takara Bio, Inc., Shiga, Japan) after 48 h of continuous exposure to the CuS or 20-nm gold nanoparticles. WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate) works similarly to 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) by reacting with the mitochondrial succinate-tetrazolium reductase forming the formazan dye. The WST-1 reagent produces...
A water-soluble formazan rather than the water-insoluble product of the MTT assay. Exponentially growing HEK293 cells were dispensed into a 96-well flat bottom plate (10^3 cells/well, 100 µl). After allowing 24 h for cell attachment, each nanoparticle solution or aqueous solution of CuCl_2 was diluted appropriately in fresh media and added to the micro-wells (100 µl), three wells per concentration. Cell viability was determined by the addition of WST-1 solution (20 µl/well). The plate was incubated for an additional 2 h at 37°C and 5% CO_2, allowing viable cells to convert the WST-1 into a colored dye by using mitochondrial dehydrogenase enzymes. The soluble salt was then released into the media. Absorbance at 430 nm was measured against a background control as blank using a microtiter plate reader (Molecular Devices, Sunnyvale, CA, USA). Data were presented as mean absorbance ± standard deviation.

**Results & discussion**

Copper sulfide nanoparticles were readily synthesized in aqueous solution by reacting CuCl_2 and thioacetamide in the presence of TGA at pH 9. TGA served to stabilize the resulting CuS nanoparticles. Figure 1 shows the XRD pattern of CuS nanoparticle powder deposited from the aqueous solution, which is in agreement with that of the standard powder diffraction pattern of CuS with a hexagonal structure. The diffraction lines are indexed as labeled in Figure 1 for the hexagonal phase of CuS. The broadening of the diffraction peaks indicates the formation of nanoscale particles. No obvious impurity peaks were observed, indicating the acquisition of high-quality core-ellite CuS. Figure 2 shows the HRTEM images of CuS nanoparticles. The average size was approximately 3 nm with a uniform size distribution. Assuming a density of 4.6 g/cm^3 [23], each CuS nanoparticle is estimated to contain approximately 3260 CuS 'molecules'. The nanoparticles had a hexagonal structure and the crystal lattice fringes from the (102) and (103) lattice planes could be observed. The lattice spacing of the (102) plane measured from the images is approximately 0.30 nm and that of the (103) plane was approximately 0.28 nm. These results are very close to the lattice spacing of the (102) plane (0.305 nm) and of the (103) plane (0.282 nm) of previously reported hexagonal CuS nanostructures [24–26].

Figure 3 shows the optical absorption spectrum of CuS nanoparticles. The short-wavelength absorption edged at approximately 500 nm, which was a significant blue shift from the energy gap of bulk CuS, confirming the effect of quantum size confinement. The sample shows an increased absorption band in the NIR region, with maximum absorption at 900 nm. On the basis of the absorbance measurement, the absorption coefficient value (ε) was estimated to be approximately 2 × 10^7 M−1cm−1 at 900 nm. The peak absorption of our sample was assigned to the overlapping d–d transition of Cu^2+ in a trigonal environment [27], which was approximately a 20-nm blue shift, whereas the blue shift of other CuS nanoparticles reported in the literature was approximately 5 nm [28,29]. The observed blue shift in the absorption spectrum of the sample was most likely due to the weakening of the crystal field strength, since the nanoparticles were smaller in size than the previously studied CuS nanoparticles [28,29]. Owing to the smaller size of our nanoparticles, fewer ions were coordinated at sites near the surface than in the bulk CuS [30]. In addition, interaction with distant neighboring ions was much weaker or nonexistent in smaller CuS nanoparticles compared with that in bulk CuS [30]. Thus, it is expected that the crystal field interaction of these ions is weaker in smaller nanoparticles. As a result, the lowest excited state of the d–d transition of Cu^2+ is upshifted and the d–d transition is shifted to the blue region [30]. No shoulders were observed at 450 nm, which is a typical absorption peak of the CuS phase [28]. On the basis of these results, along with the XRD and HRTEM data, we conclude that we have made pure and high-quality CuS nanoparticles.
The intense absorption by CuS nanoparticles of the NIR should enable their use in PTA therapy. Figure 4 displays the temperature of an aqueous solution containing CuS nanoparticles as a function of exposure time to a laser beam at 808 nm. The temperature increased 12.7°C over a period of 5 min at an output power of 24 W/cm² and a concentration of 770 µM CuS ‘molecules’ (~1.42 × 10¹⁴ particles/ml). Under the same conditions, no change in temperature was observed with pure water (Figure 4). Thus, CuS nanoparticles can mediate photothermal effects at 808 nm in the NIR region, albeit to a lesser extent than gold core–shell nanostructures. Since the absorption of CuS nanoparticles peaks at 900 nm, it is anticipated that the photothermal effect mediated by CuS nanoparticles at the peak absorbance wavelength of 900 nm would be much stronger than that obtained at 808 nm.

Since in practice it is very difficult to obtain gold core–shell nanostructures with peak absorption of more than 850 nm [15], CuS nanoparticles that absorb light at greater than 850 nm may have interesting applications where photothermal response at a higher laser wavelength is needed.

To test the cell killing induced by the photothermal effects of CuS nanoparticles, HeLa cells were incubated with CuS nanoparticles for 2 h. The cell line was derived from cervical cancer cells. The cells were chosen because NIR light may be delivered colposcopically to illuminate cervical cancer and precancerous lesions in the cervix. The cells were then irradiated with a NIR laser centered at 808 nm. As shown in Figure 5, 24 h after laser treatment, cells treated with CuS nanoparticles plus a NIR laser experienced substantial cellular death. In fact, at a CuS concentration of 384 µM and an output power of 40 W/cm², cell death expanded beyond the zone of laser exposure, indicating the spread of heat outside the area of laser irradiation (Figure 5). No apparent cell death was observed in cells treated with CuS nanoparticles alone or with laser alone. Quantitative analysis of cell viability showed that at the laser power of 24 W/cm² for 5 min, the percentage of viable cells was 55.6 ± 5.8% when cells were pretreated with CuS nanoparticles at a concentration of 384 µM CuS. At the same nanoparticle concentration, the cell viability decreased to 21.2 ± 5.6% and 12.2 ± 3.7% when the laser power was increased to 40 W/cm² for 5 min and 64 W/cm² for 3 min, respectively. A similar trend was found when the nanoparticle concentration was increased and the laser power was maintained (Figure 6). These data indicate that the extent of cell death caused by the photothermal effect mediated by CuS nanoparticles is a function of the concentration of the nanoparticles and the output power of the laser used.

Morphologically, the untreated HeLa cells were polygonal, and few cells were stained red with ethidium homodimer-1 (EthD-1).

Figure 4. Temperature measured over a period of 15 min of exposure to 808-nm near-infrared light at an output power of 24 W/cm². The concentration of CuS NPs in water was 770 µM equivalent CuS. CuS: Copper sulfide; NP: Nanoparticle.
Figure 5. Cell viability after near-infrared irradiation. (A) HeLa cells were treated with different concentrations of CuS NPs and NIR light (808 nm) at 24 W/cm² for 5 min. After treatment with NPs at a concentration of 384 µM CuS plus NIR laser, most cells were dead in the zone of exposure (circled area). By contrast, after treatment with NIR laser alone, NPs alone or NPs at concentrations of 192 µM CuS followed by NIR laser, cells retained normal morphology and few dead cells were observed. (B) Following irradiation with the NIR laser beam at a higher power (40 W/cm² for 5 min), cell death was observed at a lower concentration of 192 µM CuS, and the cell death expanded beyond the zone of laser exposure when the NP concentration increased to 384 µM CuS. Viable cells were stained green with calcein, dead cells were stained red with EthD-1. Bar = 200 µm.

CuS: Copper sulfide; EthD-1: Ethidium homodimer-1; NIR: Near-infrared; NP: Nanoparticle.
However, after treatment with CuS nanoparticles (384 µM CuS) and the NIR laser, many cells that stained positive with calcein (green) became more rounded in shape (purple arrows, Figure 7), possibly as a result of the condensation of skeletal proteins. Some cells lost their viability, as indicated by calcein-negative staining (yellow arrows, Figure 7). The rest of the cells stained positive with EthD-1, which indicates loss of cellular membrane integrity (white arrows, Figure 7).

The cytotoxicity of CuS nanoparticles in HEK293 cells were compared with that of 20-nm gold nanoparticles, which are well accepted as biocompatible nanomaterials (Figure 8). The size of the gold nanoparticles was determined by transmission electron microscopy (TEM) and dynamic light scattering methods. Both CuS and gold nanoparticles (20 nm) had no cytotoxic effect on the cells at concentrations up to 100 µM after 48 h of incubation. At the highest concentration tested (1 mM), both nanoparticles caused a significant decrease in cell viability. The aqueous solution of CuCl₂, which was used for the preparation of CuS nanoparticles, was significantly more cytotoxic than its corresponding CuS nanoparticles at the same equivalent concentration of CuS, at greater than 100 µM. Almost all cells were dead after treatment with the aqueous solution of CuCl₂ at 1 mM. These data suggest that CuS nanoparticles have a cytotoxicity profile comparable to that of gold nanoparticles. Fisichella et al. found that mesoporous silica nanoparticles could enhance MTT formazan exocytosis in HeLa cells [31], giving an overestimation of the cytotoxicity of mesoporous silica nanoparticles.

No such phenomenon was observed with the WST-1 assay, which is similar to the MTT assay since in both assays tetrazolium salt reduction is driven by intracellular NADH contents. However, the formazan dye produced by enzymatic cleavage of the tetrazolium salt WST-1 is water-soluble and does not form crystals, as reduced MTT does. Casey et al. report interactions between single-walled carbon nanotubes and various dyes, including MTT and WST-1, used to assess cytotoxicity, resulting in false-positive toxicity [32]. In our studies, we did not observe a potential interaction between the dye molecules and CuS nanoparticles. Consistent with this notion, no morphological changes were observed at a CuS concentration of up to 384 µM (Figure 7).

Compared with gold nanostructures, CuS nanostructures have several advantages. First, CuS is much less expensive than gold. The estimated costs of making 1.85 × 10²⁰ 3-nm CuS nanoparticles (equivalent to 1 mole of CuS ‘molecules’) and 7.25 × 10¹⁷ 40-nm hollow gold nanospheres (equivalent to 1 mole of Au atoms),
based on the costs of the starting materials, are US$330 and $52,200, respectively. Second, the NIR absorption in CuS originates from the d–d transition of Cu$^{2+}$ ions [27], whereas the NIR absorption in gold nanostructures is from the surface plasmon resonance [33–35]. The absorption in CuS nanoparticles is from d–d transition of Cu$^{2+}$, which is different from the free excitons in pure semiconductor quantum dots, such as CdSe, but is similar to trapped excitons in doped nanoparticles [36]. Therefore, the absorption wavelength is hardly changed by the particle size, shape or solvent, but its absorption intensity is highly dependent on the particle size as a result of quantum size confinement [18,35]. For small size and core–shell structures, the absorbance should be very large due to the strong confinement of the bound excitons [36]. We consider this as a significant advantage compared with the use of gold nanomaterials. First, the d–d transition peaks at 900 nm, which is in the NIR range for in vivo applications. Therefore, no complicated procedures are needed in order to obtain the NIR absorption in CuS nanoparticles, as there are in making special gold textures, such as hollow nanoshells, nanoshells or nanorods. Second, the surface plasmon absorption peak of gold nanostructures is dependent upon the dielectric constant of the surrounding matrix. Thus, when they are delivered to the cancer cells, the plasmon absorption maximum will shift compared with in vitro observations. This might complicate the treatment conditions. These issues do not exist when using CuS nanoparticles. Finally, the 3-nm CuS nanoparticles may have more favorable pharmacokinetic properties for targeted delivery after systemic administration than that of gold nanostructures displaying surface plasmon absorption in the NIR region. To date, the smallest gold nanostructure having NIR absorption was approximately 40 nm in diameter [6,16]. The much smaller CuS nanoparticles may have a better chance of reaching their targets and being cleared from the body through the renal system [6,37,38].

One of the limitations of CuS nanoparticles is their relatively low photothermal conversion efficiency. With the current formulation, both the concentration of CuS nanoparticles and the laser energy required to cause sufficient cell death in the in vitro monolayer setting are prohibitively high for in vivo applications. Therefore, further improvement of the physicochemical properties of CuS nanoparticles is needed. We believe there is scope to increase the absorbance of CuS nanoparticles. As highlighted, the d–d transition of Cu$^{2+}$ in CuS nanoparticles is similar to the transition of trapped excitons in doped nanoparticles, and its transition probability (absorbance) is determined by its oscillator strength [39]. According to the theory of trapped excitons, the oscillator strength of the trapped excitons is much higher than that of

Figure 7. Microphotographs of cells incubated with copper sulfide nanoparticles (384 µM copper sulfide) followed by near-infrared laser irradiation (24 W/cm$^2$, 5 min). Without laser treatment, the cells were viable and polygonal. By contrast, most cells treated with the NIR laser shrunk and had spherical morphology (purple arrows). Some cells lost viability, as evidenced by calcein-negative staining (yellow arrows). Others lost membrane integrity, as indicated by positive staining with EthD-1 (white arrows). Bar = 20 µm. DIC: Differential interference contrast; EthD-1: Ethidium homodimer-1; NIR: Near-infrared.
the free excitons [39,40] and, therefore, its absorbance should be much stronger. The concept of giant oscillator strength \( f_{BE} \) for bound excitons can be expressed as: \( f_{BE} = (a_{ex}/a)^3 f \), where \( f \) is the oscillator strength for free excitons, \( a \) is the crystal unit-cell length and \( a_{ex} \) is the excitonic Bohr radius [39]. Since the unit-cell length is almost an order of magnitude smaller than the Bohr radius, the oscillator strength of bound excitons is approximately three to five orders of magnitude larger than that of free excitons [39,40]. The increase in oscillator strength indicates the increase in the wave function overlap between the electron and the hole of the exciton because the oscillator strength is proportional to the overlap. Evidently, the increase in the wave function overlap will enhance the binding energy of the trapped excitons. Therefore, trapped excitons can have higher absorbance and are more stable than free excitons in II–VI quantum dots. In addition, coating CuS nanoparticles with zinc sulfide shells is another strategy to increase their absorbance because core–shell nanostructures can further confine the excitons in the core nanoparticles [41]. In the future we will prepare CuS/zinc sulfide core–shell structures, with the aim of increasing the absorbance as well as the heating efficiency. In addition, the absorbance at 900 nm is approximately 1.5-times stronger than that at 808 nm. The efficiency can be enhanced if a laser of 900 nm is used for the treatment.

**Conclusion**

In summary, our data suggest that CuS nanoparticles with strong absorption in the NIR region are promising new nanomaterials for PTA treatment of cancer. Following irradiation by a NIR laser beam at 808 nm, the temperature in CuS nanoparticle aqueous solution is increased as a function of exposure time and nanoparticle concentration. CuS nanoparticles induced photothermal destruction of HeLa cells in a laser dose- and nanoparticle concentration-dependent manner, and displayed minimal cytotoxic effects with a profile similar to that of gold nanoparticles. The unique optical property, small size, low cost of production and low cytotoxicity make CuS nanoparticles a promising and new type of nanomaterials for cancer PTA. Further studies on the in vivo pharmacokinetics, biodistribution, photothermal ablation efficiency and systemic toxicity are warranted.

**Future perspective**

As a new type of agent for photothermal treatment of cancer, CuS nanoparticles have many advantages, as reported in this research. The most favorable features are the low costs, simple and easy preparation, and small size for targeting. In addition, the intrinsic d–d transition of Cu\(^{2+}\) determines its merits for practical application. The only challenging issue is their weak absorbance, as a high powered laser is required for activation. This is particularly true when

![Figure 8. Cytotoxicity of copper sulfide nanoparticles in HEK293 cells.](image-url)

*Figure 8. Cytotoxicity of copper sulfide nanoparticles in HEK293 cells.* The cells were incubated in culture medium containing NPs at concentrations ranging from 1 nM to 1 mM for 48 h. CuCl\(_2\) solution and 20-nm Au NPs were included in the study. Cell viability is expressed as the absorbance at 430 nm. Control: untreated cells. Data represent mean ± standard deviation.

*p < 0.05 compared with untreated control.

**p < 0.05 compared with CuS NPs.

Au: Gold; CuS: Copper sulfide; NP: Nanoparticle.
the therapy is for in vivo applications. Once this problem is solved, their application is vast and may change the direction for photothermal treatment of cancer. The selection of proper lasers at approximately 900 nm to fit the absorption peak and the improvement of the nanoparticle qualities (surface passivation and size distribution), particularly the development of core–shell nanostructures to enhance the oscillator strength, are the possible solutions for enhancement. Numerous studies have shown that in vivo delivery of laser light at the NIR region is not a problem \[6,16\]. In fact, NIR can penetrate intact skull and skin in mice \[12\]. Therefore, the treatment based on CuS nanoparticles is promising and will be the focus of our future studies.

Financial & competing interests disclosure
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Ethical conduct of research
The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

- Copper sulfide (CuS) nanoparticles have an optical absorption peak at 900 nm, which is good for deep cancer treatment.
- Procedures for making CuS nanoparticles are easy and simple.
- CuS nanoparticles are inexpensive to make.
- The absorption position of d–d transition of Cu\(^{2+}\) in CuS nanoparticles is not varied with the particle size and shape, but its absorption intensity can be enhanced by quantum size confinement.
- CuS nanoparticles have low toxicity.
- Owing to their unique optical property, small size, low cost of production and low cytotoxicity, CuS nanoparticles are promising new nanomaterials for cancer photothermal ablation therapy.

Bibliography
Papers of special note have been highlighted as:

** of interest


** New application of nanotechnology in cancer treatment.


** Brand new concept for using nanotechnology in deep cancer treatment.


** Important for drug delivery and targeting.


** Combination of important therapies for cancer treatment through nanotechnology.


** Progress for using gold nanostructures for photothermal ablation of cancers.


Copper sulfide nanoparticles for photothermal ablation of tumor cells

**Interesting and important for copper sulfide nanoparticle synthesis.**


29 Interesting and important for copper sulfide optical properties.


41 Important physics to understand transition properties of trapped excitons.


43 Important to understand how to use core–shell structures to improve nanoparticles absorbance and emissions.