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Photothermal therapy of cancer cells mediated by blue hydrogel nanoparticles

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Abstract

Aim—The aim of this study was to investigate *in vitro* the utility of biologically compatible, nontoxic and cell-specific targetable hydrogel nanoparticles (NPs), which have Coomassie[®] Brilliant Blue G dye (Sigma-Aldrich, MO, USA) covalently linked into their polyacrylamide matrix, as candidates for photothermal therapy (PTT) of cancer cells.

Materials & methods—Hydrogel NPs with Coomassie Brilliant Blue G dye covalently linked into their polyacrylamide matrix were fabricated using a reverse micelle microemulsion polymerization method and were found to be 80–95 nm in diameter, with an absorbance value of 0.52. PTT-induced hyperthermia/thermolysis was achieved at 37°C using an inexpensive, portable, light-emitting diode array light source (590 nm, 25 mW/cm²).

Results & conclusion—Hydrogel NPs with Coomassie Brilliant Blue G dye linked into their polyacrylamide matrix are effective in causing PTT-induced thermolysis in immortalized human cervical cancer cell line (HeLa) cells for varying NP concentrations and treatment times. These multifunctional particles have previously been used in cancer studies to enable delineation, for glioma surgery and in photoacoustic imaging studies. The addition of the PTT function would enable a three-pronged theranostic approach to cancer medicine, such as guided tumor surgery with intra-operative photoacoustic imaging and intra-operative PTT.

Keywords

cancer cell; Coomassie[®] Brilliant Blue G dye; polyacrylamide nanoparticle; photothermal therapy

Cancer is still one of the leading causes of death in America; in 2010, 1,596,670 new cases and 571,950 cancer-related deaths were projected to occur [1]. To address this challenge, many advances have been made in the early detection and treatment of cancer, directly resulting in an increase in the treatment options available, including chemotherapy, radiation treatment and surgical removal. Despite these advances, cancer treatment has continued to be a hotbed of research because, in many instances, treatment plans must be robust to address the issues of probable damage to surrounding healthy tissues, tumor regrowth and a

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decrease in the quality of life for the patient. In recent years, many groups have devoted their efforts toward employing nanoparticle (NP) platforms for detecting and treating various types of cancer. NPs are advantageous because they are minimally invasive, have tunable characteristics (i.e., size, absorption and surface charge), and can be used as targeted, multifunctional or combined treatment platforms [2–4]. To date, NPs have been used as contrast agents for the detection and imaging of cancerous cells, both *in vitro* and *in vivo*, coupled with photosensitizing dyes for photodynamic therapy, or combined with chemotherapy drugs to facilitate cell-specific drug delivery, as well as coupled with external magnetic fields to induce cell death via hyperthermia [2–10].

Photothermal therapy (PTT) mediated by NPs has emerged as a promising cancer treatment modality that has been used to treat various cancerous cell lines. Gold, silver, iron oxide and magnetite are some of the materials that have been used in NP-mediated PTT [10–13]. Of these, the use of gold NPs has seemingly emerged as the frontrunner in PTT research, and various groups have created experimental protocols that yield a large variety in the gold NP structures, including nanorods, triangles, pyramids and shells [10,12]. Many groups utilize near-infrared radiation to excite the gold NPs and induce thermolysis in cells, taking advantage of the low absorption and scattering of the radiation by tissues at these wavelengths. Despite these advantages, there have been many studies conducted exploring the origins and levels of cytotoxicity of many types of gold nanostructures, with apparently conflicting conclusions regarding their use [8,14,15]. These concerns regarding cytotoxicity, along with concerns regarding bioelimination, motivated our development and assessment of the potential of another PTT modality aimed at widespread use in the treatment of various malignant cancers. Notably, polymeric NPs have been US FDA approved for clinical studies due to their apparent high therapeutic factor [16]. In particular, much research has been carried out on biocompatible, biodegradable and bioeliminable hydrogel NP platforms [3,4]. Incorporated in this study is the FDA-approved dye, Coomassie® Brilliant Blue G (CB; Sigma-Aldrich, MO, USA).

In this study, the utility of biologically compatible hydrogel NPs, which have CB dye covalently linked into their polyacrylamide (PAA) matrix (called here CB-PAA) as candidates for PTT of cancer cells *in vitro* is investigated. We have substantiated the capability of the CB-PAA NPs to serve as a platform for a multimodal therapeutic approach (photoacoustic imaging [17], visual surgical delineation [18–20] and now PTT). NPs of this type have previously been used in our laboratory for targeted *in vivo* photodynamic therapy (with photosensitizers such as methylene blue), combined with MRI and enhanced permeability and retention contrast enhancement agents, as well as for photoacoustic structural imaging and for the delineation of glioma boundaries (in the latter two cases with CB-PAA NPs), and have also been shown to be nontoxic, targetable, biodegradable and bioeliminable [17–20]. Thus, with the PTT effectiveness demonstrated here, the trifunctional CB-PAA NPs can be utilized simultaneously for three different aforementioned modes; furthermore, targetability can be easily added to the NP surface.

The simplicity of the method described here goes beyond the use of existing nanoplateforms. The excitation source employed in this study is an inexpensive, portable light-emitting diode (LED) array with a maximum emission at 590 nm. Based on its use, we find that CB-PAA-mediated PTT of immortalized human cervical cancer cell line (HeLa) cells, incubated with 1.2 mg/ml of NPs for 24 h, yields nearly complete cell death, within 1 h of treatment, after 40 min of illumination. Moreover, cells that were incubated for 24 h with only 0.6 mg/ml of the NPs and with only 20 min of illumination, yielded nearly complete cell death 3 h after treatment. On the other hand, under identical illumination, but without incorporation of the dye, there is little cell death. Likewise, with no illumination, but with the dye containing NPs, there is insignificant cell death. This promises, for *in vivo* use, an extremely high

selectivity of cell death, based on combined immuno and ‘illumo’ targeting, with minimal side effects. Overall, we find that CB-PAA-mediated PTT is characterized by high efficacy, with much promise for inclusion in a multipronged and safe approach for treating various types of cancers.

Materials & methods

Materials

CB, acrylamide, ammonium persulfate, *N,N,N,N*-tetramethylethylenediamine, sodium dioctylsulfosuccinate, Brij[®] 30, *N,N*-dimethylformamide, dimethyl sulfoxide (DMSO) and 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich. DMEM 1195 and 21063, Dulbecco's phosphate-buffered saline (DPBS), Roswell Park Memorial Institute medium (RPMI-1640), fetal bovine serum, penicillin–streptomycin glutamine, and a LIVE/DEAD[®] BacLight[™] Bacterial Viability Kit were all purchased from Invitrogen (NY, USA). *N*-(3-aminopropyl) methacrylamide hydrochloride (APMA) was purchased from Polysciences Inc. (IL, USA). Ethanol (95%) and hexane were purchased from Fisher Scientific (PA, USA). Phosphate-buffered saline solution (pH 7.4) was made with a phosphate-buffered saline tablet from Sigma-Aldrich. 100-mm, 35-mm and 96-well microplates were purchased from BD Biosciences (CA, USA). The water used throughout the experiment was deionized water, purified by a Milli-Q[®] system from Millipore Corp. (MA, USA).

Light source—The excitation source employed for the PTT experiments was a low-intensity LED array created with an emission peak of 590 nm. The array was fabricated in house as follows: an array of 256 wide-angle LEDs from Kingbright Corp. (CA, USA) were soldered to a fiberglass printed circuit board; wide-angle LEDs and mirrors were then attached around the perimeter of the array to increase illumination uniformity, and finally, an adjustable power supply was connected to the setup to enable control of the power output of the LED array. The intensity of the array was 25.4 mW/cm² and the deviation in irradiance at the face of the mirrors was found to be 6.2%.

Cell culture

HeLa 229 cells (American Type Culture Collection, VA, USA) were grown in DMEM 11995 supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin at 37°C and 5% CO₂ in a humidified incubator. Cells were plated and grown in 100-mm Petri dishes for the cellular uptake assay, in 96-well plates for the MTT assay and in 35-mm Petri dishes for the PTT tests.

NP synthesis & characterization

Synthesis—The CB-PAA NPs used in this study were synthesized according to a protocol established in a previous study completed by our group and modified slightly using the CB derivative as the only cross-linker [18]. Briefly, the CB covalently linked PAA NPs were prepared by a reverse microemulsion polymerization method. A monomer solution was prepared by dissolving monomers, acrylamide (610 mg) and APMA (40 mg) in 1.2 ml of water. A dye solution was prepared by dissolving 165 mg of CB-linked APMA in 0.8 ml of *N,N*-dimethylformamide and then 0.4–1.0 ml of Brij 30 was added to the dye solution. The monomer and dye solutions were mixed and sonicated to make a homogeneous solution. The solution was then added to a deoxygenated hexane (60 ml) that contained two surfactants, sodium dioctylsulfosuccinate (4.8 g) and Brij 30 (total amount of 9.5 ml). After stirring the mixture under inert atmosphere for 20 min, a freshly prepared 66% (w/v) ammonium persulfate solution (400 µl) and tetramethylethylenediamine (200 µl) were added to the mixture solution to initiate polymerization. The solution was then stirred under inert

atmosphere at room temperature for 1.5 h. After completing the polymerization, hexane was removed with a Büchi RII rotary evaporator (Büchi Corp., DE, USA) and the residue was then suspended in ethanol. The suspension was then washed in an Amicon® filtration system (Millipore Corp.) with a 300,000 nominal molecular weight limit Millipore ultrafiltration membrane under 10–20 psi of pressure with ethanol ten times, followed by ten water washes to ensure all surfactants and unreacted molecules were removed. The NP solution was then filtered through a 0.22-µm Whatman syringe filter (Whatman, MO, USA). The resultant CB-PAA NPs were freeze dried and stored at room temperature in a ModulyoD® Freeze Dryer (Thermo Electron Corp., MA, USA) for later use.

Characterization—CB-PAA NPs were dissolved in DPBS, syringe filtered (0.2-µm pore size; Whatman) and diluted to 0.05 mg/ml, after which the size and zeta-potential of NPs were measured using a Delsa™ Nano dynamic light scattering particle sizer (Beckman Coulter, CA, USA). Representative sizing and zeta-potential data are shown in Figure 1. The percentage of CB dye in each batch of NPs was calculated, based on a standard calibration curve of the dye's absorption at 597 nm. Two batches of NPs were used; the NPs used in the MTT assays and PTT experiments had 8.5% CB dye loading, and the NPs used for cellular uptake experiments had 11% dye loading. Further characterization of the CB-PAA NPs was carried out to: measure the temperature change caused by the CB-PAA NPs alone (Supplementary Figure 1; see online at www.futuremedicine.com/doi/suppl/10.2217/NNM.12.190); investigate if CB dye degrades out of the nanoparticle matrix during illumination (Supplementary Figure 2); and to characterize singlet oxygen production during illumination (Supplementary Figure 3) [21].

CB-PAA NP solution calibration: A known amount of previously freeze-dried CB-PAA NPs were dissolved in fresh DPBS (14040) to make a stock solution. The stock solution was then used to make diluted concentrations of CB-PAA NP solutions between 0.01 and 0.35 mg/ml in DPBS with total volume of 1.5 ml each. The absorbance of dilute NP solutions was measured using a UV-1601 Spectrophotometer (Shimadzu, MD, USA); a representative plot is shown in Figure 2. A calibration curve relating CB-PAA NP concentration to absorbance at 600 nm was constructed and fit linearly. The CB-PAA stock solution was then filtered using a 0.2-µm Whatman syringe filter; a small sample was taken and diluted to a concentration between 0.01 and 0.35 mg/ml and the absorbance measured. The linear calibration curve was then used to extract the new concentration of the filtered NPs. Losses due to filtering ranged between 0 and 30%, depending on the amount of time the NPs had been stored.

Cytotoxicity of the CB-PAA NPs: Cytotoxicity of the CB-PAA NPs was tested with a standard MTT cell viability assay examining metabolic activity; cells that had not been incubated with any NPs were used as a control. Cells were plated on a 96-well plate with a cell density of 5000 cells/well and cultivated overnight. CB-PAA NPs and growth media were added into the wells so as to achieve for each sample an overall concentration ranging between 0.1 and 1.2 mg/ml, and then incubated with the cells for 24 h. The cells were then carefully washed, and 200 µl of clear growth media plus MTT reagent were added (20 µl of 5 mg/ml MTT reagent dissolved in 180 µl of media without fetal bovine serum). After 4 h, 200 µl of DMSO was added into each well, so as to solubilize the formazan crystals produced from the MTT in viable cells. The 96-well plates were covered with aluminum foil and placed on a rocking shaker (Reliable Scientific Inc., MS, USA) and allowed to gently mix overnight in the dark. Absorption at 550 nm was measured, for each well, with a SpectraMax® Plus 384 Absorbance Plate Reader (Molecular Devices, CA, USA). The results from 24 wells for each NP concentration were analyzed. CB dye-related toxicity was not investigated in this study, as no measurable toxicity was found in any of the previous

studies completed by our group [17–20], and CB has been successfully utilized in both laboratory and clinical studies at concentrations much higher (10- and 20-fold) than those used in the present study [22,23].

Cellular uptake assay

Cells were grown to at least 90% confluency, passaged and replated with half of the initial concentration of cells. The cells were then allowed to reattach and grow overnight. Varying amounts of CB-PAA NP stock solution were mixed with growth media to achieve concentrations between 0.1 and 1.2 mg/ml of particles in a total of 3 ml of solution. After 24 h, the cells were washed twice with DPBS and the particle solutions plus media were added to the cells. The cells were allowed to incubate with the NP plus media solution for 24 h, then again washed twice with DPBS to remove free NPs, trypsinized and centrifuged for 5 min at 900 r.p.m. Trypsin, media and DPBS were aspirated, then 1.5 ml DPBS was added to the cells. A total of 300 μ l of lysing reagent (M-PER[®] Mammalian Protein Extraction Reagent; Thermo Scientific, MI, USA) was then added to each. For reference, cells that had been allowed to grow for the same amount of time without CB-PAA NPs were counted and adjusted so that their concentrations matched those of the cells incubated with CB-PAA NPs. After 24 h, the absorbance at 600 nm was measured for the cells with CB-PAA NPs (i.e., the amount of absorbance from the CB-PAA in the cells was measured using UV-visible spectrometry with lysed reference cells). The spectra were then analyzed using a least squares method; the NP concentration, C_{NP} , was extracted by fitting the absorption spectrum to the linear combination in Equation 1:

$$A = R \cdot SPC_{ref} + C_{NP} \cdot SPC_{NP} + Const. \quad (1)$$

where A is the absorption spectrum, SPC_{ref} is the absorption spectrum of the lysed cells without NPs and SPC_{NP} is the absorption spectrum of NP solution in deionized water. R, C_{NP} and Const. are the fitting parameters. The absorbance of the CB-PAA NPs only was extracted from the analysis and converted to the corresponding concentration in mg/ml and then divided by the number of cells to get the average CB-PAA NP concentration per cell.

PTT & LIVE/DEAD[®] assay

PTT protocol LIVE/DEAD[®] assay—CB-PAA NPs dissolved in DPBS were mixed thoroughly with fresh growth media, added to cells and then allowed to incubate for 24 h. The cells were washed twice with DPBS to remove any NPs remaining outside of the cells and new clear growth media was added (DMEM 21063). A total of 5 μ l of 1.5 mM of propidium iodide dissolved in DMSO and 1 μ l of 2 mM of calcein acetoxymethyl ester dissolved in DMSO was added to the cells and allowed to incubate for at least 10 min. Baseline measurements for the PTT were performed on an Olympus IX71 inverted microscope (Olympus America Inc., NY, USA) using a 20 $\times$ objective. The cells were then placed under the light source for 10, 20 or 40 min, inside an incubator at 37°C and then immediately imaged following the treatment. The cells were then placed back in the incubator and only taken out for imaging at 30-min intervals for another 3 h. The images were analyzed using ImageJ[®] software (NIH, MD, USA) to determine the number of live or dead cells per image, with the presence of calcein acetoxymethyl ester fluorescence indicating a live cell, and propidium iodide fluorescence in the nucleus indicating a dead cell (Figure 3).

Results & discussion

Cytotoxicity

Many hydrogels, including PAA, have been used extensively *in vivo*, with no major toxic effects [4,24]. Furthermore, they are very flexible in their molecular engineerability – that is, they can be modified for controlled, fast or slow biodegradation. NPs of this type can also be optimized towards fast or slow dye delivery. Often, the NPs are PEGylated for controlled circulation time and can be easily surface derivatized with molecular targeting moieties, for example peptides or aptamers, to achieve high specificity to certain cancer cells [4,25]. Radiolabeled, pharmacokinetic studies have been conducted on PAA NPs with and without the biodegradable crosslinker in the NP matrix. In the case of NPs without the biodegradable crosslinker, the NPs have “shown tissue distributions of the PAA NPs in the reticular endothelial systems that are consistent with other nanoparticle studies” [4]. No adverse effects were found when NPs with the biodegradable crosslinkers were utilized up to 90 days post-treatment [4,6,24,25].

As shown in Figure 4, there was no significant difference in cell viability among cells incubated for 24 h with CB-PAA NPs with concentrations ranging between 0.1 and 1.2 mg/ml and the control (no NPs), indicating the absence of any measurable dark toxicity associated with the NPs. It was noted that the NPs stay inside the cells and that the cancer cells used in the present study divide after approximately 24 h (the normal cell division being another indicator of the nontoxicity of these NPs in the dark). It was also noted that in potential medical applications, the effectivity of the NPs after long periods of time is irrelevant because the patient would not have to wait much longer than 24 h between administration of the NPs and the treatment. Furthermore, cytotoxicity due to dye leaching was not investigated in the present study because dye leaching of CB-PAA NPs was investigated and quantized in a previous study and published work completed by our group. The study was conducted under simulated physiological conditions and demonstrated that the “covalent linkage of the CB molecule to the NP matrix polymer backbone completely eliminates dye leaching” [20].

Cellular uptake

As shown in Figure 5, the average amount of CB-PAA NPs taken up by the cells increases with increasing NP concentration until 0.4 mg/ml, after which the uptake of NPs into the cells increases only moderately nearing saturation. These results are similar to findings from studies on the uptake of targeted gold and iron oxide hybrid NPs [11] and chitosan-coated silver NPs by two colorectal cancer cells [13]. PAA NPs can be targeted with the addition of targeting peptides, usually F3 or TAT, via simple wet-laboratory techniques. Multiple cell lines have been successfully targeted using PAA NPs [5,17,18,26]. Specifically, the addition of targeting moieties to the CB-PAA NPs has been shown to require shorter incubation times and result in preferential uptake in cancerous cells compared with nontargeted NPs [18,19]. Thus, the targetability of the CB-PAA NPs would enable further optimization of this mode of PTT through the likely decrease in the required NP concentration necessary for inducing thermolysis. The present study was a proof-of-principle study completed *in vitro*, so cell-specific targeting was unnecessary at this initial step.

Photothermal therapy

NP concentration effects—The survival rate (over time) of cells that had been incubated with CB-PAA NPs ranging from 0.2 to 1.2 mg/ml and illuminated for 20 min is depicted in Figure 6. Cells that had been incubated with CB-PAA NP concentrations of 0.2–0.8 mg/ml experienced little to no cell death during treatment; however, cells treated with the highest concentration of CB-PAA NPs exhibited a survival rate of only 70% when imaged

immediately after treatment. Furthermore, 1.5 h post-treatment, more than 90% cell death was observed for the cells incubated with 1.2 mg/ml of CB-PAA NPs. Near-complete cell death was also observed 3 h post-treatment for cells incubated with 0.6 and 0.8 mg/ml of CB-PAA NPs. Cells treated with 0.2 and 0.4 mg/ml experienced only 22 and 33% cell death, respectively, 3 h post-treatment. Interestingly, increasing the CB-PAA NP concentration from 0.4 to 0.6 mg/ml resulted in the observation of a particularly dramatic 14-fold increase in cell death after 3 h. As a control, cells were incubated with 1.2 mg/ml of blank polyacrylamide NPs without dye and illuminated for 40 min. The efficacy of the PTT increases with increasing NP concentration despite the notably smaller increase of uptake of CB-PAA NPs after 0.4 mg/ml. This somewhat surprising result may suggest a threshold beyond which changes in intracellular NP distribution cause a more efficient local thermolysis, although, on average, the overall NP uptake increases very slightly. This is under further study.

Illumination time effects—Cell viability as a function of exposure time of cells that had been incubated with 0.6 mg/ml CB-PAA NPs and illuminated for 10, 20 and 40 min is shown in Figure 7. A total of 10 min of PTT yielded a death rate of only approximately 20% of the cells at 3 h post-treatment; however, when the irradiation time was doubled, the efficacy of the PTT increased dramatically, yielding a 95% cell death at 3 h post-treatment. When the PTT treatment time was increased even further to 40 min, only 35% of the cells survived the treatment, and complete cell death is observed at 1 h post-treatment. Control experiments, where cells were incubated with 1.2 mg/ml of blank PAA NPs for 24 h and irradiated for 40 min, exhibited only 5% cell death 3 h post-treatment. This suggests that the major impetus of thermolysis-induced cell death is the intracellular heating due to the covalently linked CB-PAA NPs and not local or external heating due to the light source or any scattering effects.

Conclusion

The present work is a proof-of-principle study, which establishes the feasibility of a third modality, PTT, of the CB-PAA NPs. These NPs have been successfully used for both surgical delineation and photoacoustic imaging [17–20]. Although PAA gel is not FDA approved for use in NP-mediated PTT of cancer cells, it has been successfully utilized in *in vivo* and *in vitro* studies for many years. PAA has also been used successfully in implants in animal models [24,27]. The pharmacokinetics, biodistribution, bioelimination and toxicity of PAA gel NPs have been studied *in vivo*, and no acute toxicity has been associated with potentially therapeutic doses [4,27,28].

In the present study, the covalently linked CB-PAA NPs have been shown to be very effective in causing PTT-induced thermolysis in HeLa cells for varying NP concentrations and treatment times. This study can be used as demonstration that after the CB NPs are used to delineate a tumor and the majority of it is surgically excised, any remaining cancerous tissues can be located and destroyed by exploiting the photoacoustic imaging and PTT modalities without a need for another NP administration. It is worth noting that during or immediately after surgical debulking, any wavelength light source can be easily directed at the tumor residues. *In vivo* studies will follow. For optimal future use in *in vivo* studies, the nontoxic CB-PAA NPs used in this study can be further optimized, to be targeted and biodegradable, as was demonstrated before with PAA NPs [4].

Future perspective

The need for multimodal, multifunctional nanomedicine is becoming increasingly urgent in the face of the long-standing limitations of conventional diagnostic and treatment methods

of cancer. NPs that simultaneously provide intraoperative imaging and intraoperative therapy are of particular interest owing to their cellular-level selectivity, which is crucial in dealing with otherwise inoperable tumors and residual malignant tissues left after surgical resection. For example, through the use of a single, targeted, multimodal, multifunctional and safe nanoplatform, the neurosurgeon could be provided with continuous clear visual delineation of the tumor, as well as the capability of more refined imaging via photoacoustic imaging, followed by the utilization of PTT for the elimination of any residual boundary protrusions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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of interest

of considerable interest

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Executive summary

Nanomedicine preparation

- Biologically compatible hydrogel nanoparticles (NPs), which have medically safe Coomassie® Brilliant Blue G (CB; Sigma-Aldrich, MO, USA) dye covalently linked into their polyacrylamide (PAA) matrix, were prepared by a reverse microemulsion polymerization method.

Cytotoxicity

- No significant difference in cell viability was measured between cells incubated with CB-PAA NPs and the control, indicating the absence of any measurable dark toxicity associated with the nanoparticles.

Concentration effects

- Near-complete cell death was observed 3 h post-treatment for cells incubated with 0.6 and 0.8 mg/ml of CB-PAA NPs and illuminated for 20 min; more than 90% cell death was observed for the cells incubated with 1.2 mg/ml CB-PAA NPs 1.5 h post-treatment.

Time effects

- Cells that had been incubated with 0.6 mg/ml CB-PAA NPs and treated with 20 and 40 min of photothermal therapy yielded nearly complete cell death 3 h and 1 h post-treatment, respectively.

Conclusion

- This study establishes a third treatment mode, photothermal therapy, using the same CB-PAA NPs, which can be combined with the previously established modes of photoacoustic imaging and visual delineation (for surgery) of malignant cancerous cell lines.
- For optimal future use in *in vivo* studies, the nontoxic CB-PAA NPs can be made selectively targetable to tumor cells and with tailor-made biodegradability.

Figure 1. Size and zeta-potential characterization of the Coomassie[®] Brilliant Blue G (Sigma-Aldrich, MO, USA) polyacrylamide nanoparticles was carried out using a Delsa[™] Nano dynamic light scattering particle sizer (Beckman Coulter, CA, USA) Coomassie Brilliant Blue G polyacrylamide nanoparticles are 80–95 nm in diameter and have a zeta-potential of 25–27 mV.

Figure 2. Nanoparticle concentration-dependent absorbance spectra of Coomassie® Brilliant Blue G dye and resulting calibration curve

(A) Absorbance of Coomassie Brilliant Blue G (Sigma-Aldrich, MO, USA) polyacrylamide nanoparticles dissolved in Dulbecco's phosphate-buffered saline at concentrations between 0.1 and 0.35 mg/ml. (B) Coomassie Brilliant Blue G polyacrylamide nanoparticle concentration versus peak absorbance value at 600 nm used to calculate the concentration of filtered Coomassie Brilliant Blue G polyacrylamide nanoparticles.

Figure 3. Fluorescent images of cells that have been previously incubated with Coomassie[®] Brilliant Blue G (Sigma-Aldrich, MO, USA) polyacrylamide nanoparticles and then exposed to photothermal therapy

The images were taken before, immediately after and every 30 min post-treatment. Green fluorescence indicates viable cells while red indicates dead cells.

For color images see online at www.futuremedicine.com/doi/full/10.2217/NNM.12.190

Figure 4. Cell viability of immortalized human cervical cancer cell line (HeLa) cells after 24 h of incubation with increasing concentrations of Coomassie[®] Brilliant Blue G (Sigma-Aldrich, MO, USA) polyacrylamide nanoparticle solutions as determined by 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide assay

The results indicate no significant change in toxicity with increasing nanoparticle concentration. Error bars represent standard error in absorbance at 550 nm. The samples were not exposed to any illumination, so that any dark toxicity due to the Coomassie Brilliant Blue G polyacrylamide nanoparticles could be observed.

Figure 5. Coomassie® Brilliant Blue G (Sigma-Aldrich, MO, USA) polyacrylamide nanoparticle uptake into cells after 24 h of incubation analyzed by nanoparticle absorbance at 600 nm
Error bars denote standard error (n = 2).
CB-PAA NP: Coomassie Brilliant Blue G polyacrylamide nanoparticle.

Figure 6. Survival rates of the cells over a period of 3 h after 20 min of photothermal therapy with Coomassie® Brilliant Blue G (Sigma-Aldrich, MO, USA) polyacrylamide nanoparticle solution concentrations of 0.2–1.2 mg/ml

Error bars denote standard error in average number of live cells per time point; experiments were carried out in triplicates, except for 0.8 mg/ml, which was carried out in duplicate. The shaded area represents the illumination time.

Figure 7. Survival rates over time of cells treated with 0.6 mg/ml of Coomassie[®] Brilliant Blue G (Sigma-Aldrich, MO, USA) polyacrylamide nanoparticles in growth media for 24 h with 10, 20 and 40 min of illumination time (photothermal therapy) and the control measurement (blank polyacrylamide nanoparticle, 40-min illumination time)

Error bars represent the standard error in the average number of live cells at each time point; experiments were carried out in triplicates. All illuminations started at time 0.