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Theranostic Application of Mixed Gold and Superparamagnetic Iron Oxide Nanoparticle Micelles in Glioblastoma Multiforme

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Abstract

The treatment of glioblastoma multiforme, the most prevalent and lethal form of brain cancer in humans, has been limited in part by poor delivery of drugs through the blood-brain barrier and by unclear delineation of the extent of infiltrating tumor margins. Nanoparticles, which selectively accumulate in tumor tissue due to their leaky vasculature and the enhanced permeability and retention effect, have shown promise as both therapeutic and diagnostic agents for brain tumors. In particular, superparamagnetic iron oxide nanoparticles (SPIONs) have been leveraged as T2-weighted MRI contrast agents for tumor detection and imaging; and gold nanoparticles (AuNP) have been demonstrated as radiosensitizers capable of propagating electron and free radical-induced radiation damage to tumor cells. In this study, we investigated the potential applications of novel gold and SPION-loaded micelles (GSMs) coated by polyethylene glycol-polycaprolactone (PEG-PCL) polymer. By quantifying γ -H2AX DNA damage foci in glioblastoma cell lines, we tested the radiosensitizing efficacy of these GSMs, and found that GSM administration in conjunction with radiation therapy (RT) led to ~2-fold increase in density of double-stranded DNA breaks. For imaging, we used GSMs as a contrast agent for both computed tomography (CT) and magnetic resonance imaging (MRI) studies of stereotactically implanted GBM tumors in a mouse model, and found that MRI but not CT was sufficiently sensitive to detect and delineate tumor borders after administration and accumulation of GSMs. These results suggest that with further development and testing, GSMs may potentially be integrated into both imaging and treatment of brain tumors, serving a theranostic purpose as both an MRI-based contrast agent and a radiosensitizer.

Keywords

Gold Nanoparticle; Superparamagnetic Iron Nanoparticle; Glioblastoma Multiforme; Radiotherapy; Magnetic Resonance Imaging; Contrast Agent

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INTRODUCTION

Glioblastoma multiforme (GBM), the most prevalent form of brain cancer in humans, carries a poor prognosis with a median survival of just over one year^{1, 2} despite aggressive multimodal treatment involving surgical resection with adjuvant radiotherapy (RT) and chemotherapy. The blood-brain barrier (BBB), with its tight intracellular junctions and lack of fenestrations, limits the levels of chemotherapeutic drugs that can be delivered into the tumor while avoiding toxicity to normal tissue, often resulting in subtherapeutic drug levels at the tumor site.^{3, 4} Thus, RT has gained a central role in the management of GBM. In addition to its primary role in inducing free radical production and direct DNA damage, RT has also been demonstrated as a BBB permeabilizing agent that may allow greater penetration of anti-cancer drugs into brain tumor tissue.^{5–7} However, RT too is limited by the dose that can be administered to the tumor due to considerations of normal tissue radiation tolerances. Thus, much work has focused on development of radiation sensitizers, which by various mechanisms selectively enhance the radiation dose absorbed while limiting the dose administered, thus limiting collateral damage to normal tissues.

Magnetic resonance (MR) imaging for RT treatment planning has also become essential in designing RT-based GBM treatment regimens. Generally, clinical MR generates an image based on the decay of protons after magnetic pulses—characterized by T1 and T2 relaxation rates.⁸ Though presence of a tumor itself may cause tissue changes that alter T1 or T2 relaxation rates, these changes are often insufficient to detect or fully characterize the tumor volume with conventional MRI.⁸ Thus, a contrast agent, which can reliably produce measurable changes in relaxation rates, is often administered to better highlight tumors and distinguish them from surrounding vasogenic edema.⁹ However, traditional contrast agents such as gadolinium are limited by renal toxicity,¹⁰ rapid clearance, and need for repeated administration,^{11–13} as well as inaccuracies in delineating tumor margins due to heterogeneous BBB leakiness and interstitial diffusion patterns.¹⁴

The development of a non-toxic contrast agent that improves the delineation of gross tumor volume would allow for improved tumor imaging and potentially more precise treatment planning. In addition, a long-lived contrast agent that persists in the tumor could assist in image-guided radiotherapy,¹⁵ as well as intraoperative assessment of tumor resection and monitoring of response early during the course of therapy to optimize individual management.^{16, 17} Finally, novel contrast agents have been shown to double as radiosensitizers, which, once delivered into the tumor, can be used in fractionated RT regimens to enhance the therapeutic index of RT.^{5, 15}

Nanotechnology provides a powerful direction for the development of novel agents that can be used in the diagnosis and treatment of cancer, by filling several of the roles proposed above. In particular, gold and iron oxide-based nanoparticles have attracted much attention for their promise as “theranostic” anti-cancer agents, showing exceptional performance in drug delivery, imaging, and therapy. We will focus on the potential of these nanoparticles to serve as both contrast agents for computed tomography (CT) and MR imaging and radiosensitizing agents to enhance the efficacy of RT.

Superparamagnetic iron oxide nanoparticles (SPIONs) have shown promise in a myriad of clinical applications such as separation techniques, lesion detection, magnetically assisted transfection of cells, and targeted drug delivery.^{18, 19} From a diagnostic perspective, SPIONs can act as superior contrast agents in T2-weighted MR imaging by virtue of their large magnetic moment and ability to enhance the proton relaxation rates, which produces hypointensity at their spatial location,^{18, 20} unlike traditional MRI-based contrast agents such as gadolinium which produce hyperintensity. They can also improve delineation of tumor margins compared to traditional contrast agents due to their low diffusivity and ability to be endocytosed by metabolically active cancer cells at sites of micrometastasis.¹⁴ Intravenously administered iron oxide nanoparticles have been shown to accumulate in tumor tissue and allow noninvasive tumor detection and monitoring by MRI in brain gliomas,²¹ squamous cell flank carcinomas,²² and choroidal melanomas.²³ Indeed, SPIONs are already used in MRI-based tumor detection in liver cancer,²⁴ and are currently in clinical trials to differentiate metastatic from inflammatory lymph nodes.¹⁸

Gold nanoparticles have also become the subject of recent studies as a potential anti-cancer agent due to their low toxicity and ability to potentiate tumor cells to radiation therapy by virtue of gold's high atomic number (Z).²⁵ Studies have shown that gold nanoparticles can enhance the effective radiation dose delivered to tumor cells by propagating electrons and free radicals induced by radiation.²⁶ These free radicals can directly damage DNA and indirectly induce cell apoptosis. In addition, prior work in our laboratories has shown that using gold nanoparticles as an adjuvant to radiation therapy improves survival of mice with brain tumors.⁵ In long-lived formulations, gold can also serve a diagnostic role as a CT blood pool contrast agent. Visualization of the intravascular space can serve purposes such as angiography, blood perfusion, and cardiac parameters; and can also aid in oncological imaging of angiogenesis by detecting blood flow abnormalities caused, for instance, by tumors with leaky vessels and dense neovasculature.^{27, 28}

Of note, the coating and surface functionalization of these nanoparticles is essential to their function. Naked nanoparticles administered intravenously suffer from rather short systemic circulation times due to rapid renal clearance, opsonization, and reticuloendothelial phagocytosis.²⁹ Thus, various natural and synthetic polymers, including dextran,³⁰ polyethylene glycol (PEG),³¹ and poly(vinylpyrrolidone) (PVP),³² have been employed as biocompatible coatings to prevent coagulation, promote particle monodispersion, and enhance the systemic circulation of the nanoparticles. In particular, polyethylene glycol-polycaprolactone (PEG-PCL) is an FDA-approved biodegradable amphiphilic diblock copolymer that can deter membrane opsonization and improve circulation times.^{25, 33}

In this paper, we combine the imaging, diagnostic, and therapeutic applications of iron and gold nanoparticles in a novel formulation of gold and SPIO-loaded micelles (GSMs) coated with PEG-PCL polymer. To investigate the radiosensitizing efficacy of GSMs, we used an *in vitro* model of GBM and subjected cell lines to radiation therapy in the presence or absence of GSMs. We then probed cells for γ H2ax, a marker of dsDNA breaks, and calculated the density of foci in different treatment groups to evaluate whether GSMs could effectively potentiate radiation-induced DNA damage.

Next, we intravenously administered GSMs to mice implanted with human GBM tumors in either flank or brain, and assessed micelle accumulation within these tumors with the expectation that brain tumors would exhibit less micelle uptake than flank tumors due to the blood brain barrier (BBB). For selective delivery of GSMs to tumor sites, we relied upon the enhanced permeability and retention (EPR) effect, the intrinsic tendency of circulating agents to selectively accumulate in tumor tissue due to their leaky vasculature, disrupted endothelial cells, and poor lymphatic drainage.

Finally, we used both CT and MR to image mice with implanted tumors loaded with GSMs to assess the ability of GSMs to serve as contrast agents for potential imaging applications such as RT treatment planning. One aim of this study was to compare MRI to CT imaging, particularly in regards to the sensitivity of these modalities to low doses of iron oxide (MRI) versus gold (CT) for more effective tumor boundary distinction.

METHODS

Synthesis and Characterization of GSMs

Mixed gold and iron oxide micelles were synthesized as described previously.^{33–35} Laboratory stock chemicals, as well as iron and gold salts, were purchased from Sigma-Aldrich (St. Louis, MO, USA). In brief, dodecanethiol-capped AuNPs ($d = 1.9$ nm) were prepared by reduction of gold salts in a two-phase reaction as described by Brust et al.;³⁴ while oleic acid-stabilized SPIONs ($d = 15$ nm) were prepared by thermal decomposition and precipitation in acetone as described by Park et al.³⁵

A combined solution (200 μ L) of AuNPs (4 mg), SPIONs (1 mg), and PEG-PCL diblock copolymer (4 mg), was added directly to a glass vial containing 4 mL of Millipore water, and the mixture was emulsified for approximately 3 min in an ultrasonic bath (Branson 3510). The emulsions were then allowed to stand overnight in a desiccator to remove the toluene prior to their purification and characterization. The resulting dark brown solution was centrifuged at 400 RCF for 10 min to remove the largest micelles. The solution was then centrifuged twice at 3100 RCF for 30 min, after which the supernatant was removed, and the pellet was resuspended in phosphate-buffered saline (PBS). Different size fractions were collected using different centrifugal rates. Free polymer and smaller sized particles were removed by diafiltration using a MidGee hoop cross-flow cartridge with a 750 kDa molecular weight cutoff (GE Healthcare, Piscataway, NJ, USA) and were then filtered through a 0.2 μ m cellulose acetate membrane filter (Nalgene, Thermo Scientific) to remove any oversized particles. Finally, the nanoparticles were concentrated using 50 K MWCO centrifugal filter units (Millipore, Billerica, MA, USA).

A 4:1 AuNP:SPION nanoparticle ratio was chosen to achieve a relatively high concentration of AuNP to achieve radiosensitization and CT contrast enhancement, while maintaining sufficient SPION concentration to produce contrast enhancement using the more sensitive MR imaging modality. Gold and iron concentrations in each micelle sample were measured by inductively coupled plasma optical emission spectrometry (ICP-OES) (Spectro Analytical Instruments GMBH; Department of Earth and Environmental Sciences, University of Pennsylvania).

***In Vitro* Assays of GSM-Induced Radiosensitization by gh2ax Foci Immunofluorescence Imaging**

We investigated the ability of GSMs to sensitize U251 and U373 GBM cell lines to ionizing radiation. Quantification of RT-induced DNA damage was performed by immunofluorescent labeling of gh2ax, a well-established marker for unrepaired DNA double strand breaks (DSBs). U251 and U373 cells (ATCC) were cultured in Dulbecco's Modified Eagle Medium (Invitrogen) supplemented with 10% fetal calf serum/1% antibiotics, and kept in a 37°C humidified 5% CO₂ incubator. Cells were plated in chamberslides and incubated overnight with 100 ug/mL GSMs in culture medium. Cells in chamberslides were then irradiated with 4 Gy using a Small Animal Radiation Research Platform (SARRP) (150 kVp, 0.5 mA) administered through a wide-beam 15 cm collimator. After 24 hours, cells were fixed with 10% neutral buffered formalin (Sigma-Aldrich), nuclei were stained with Hoechst, slides were permeabilized with Triton-X, and cells were immunofluorescently stained for gh2ax as described previously.³⁶ Finally, fluorescence imaging on samples was performed using a Deltavision Deconvolution microscope (Applied Precision); and foci counting was accomplished using ImageJ as described previously.³⁶

Heterotopic and Orthotopic Mouse Models of Human GBM

6–8 week old female athymic nude mice from NCI were used for developing U251 xenograft and orthotopic brain tumor models.

Implantation—For flank tumor xenografts, anesthetized (140 mg/kg ketamine, 10 mg/kg xylazine) mice were subcutaneously injected with 2×10^6 U251 cells in 50 μ L DMEM in the lower rear flank. Orthotopic models of human GBM were performed as described previously.⁵ In brief, a drill mounted on a stereotactic rig (Stoelting Inc.) was used to create a 0.4 mm hole 2.0 mm posterior and 1.5 mm lateral to the bregma in the skull above the right cerebral hemisphere in anesthetized mice (140 mg/kg ketamine, 10 mg/kg xylazine). A 30 gauge needle attached to the rig was then used to inject 3×10^5 U251 cells in 6 μ L DMEM at a 0.5 μ L/min flow rate 3 mm deep to the skull. The hole was then sealed with bone wax (Ethicon) and the incision sealed with sterile veterinary tissue glue. Mice were sacrificed if they exhibited excessive weight loss (>20%), tumor metastasis, lethargy, or other signs of distress consistent with IACUC standards.

Bioluminescent Imaging—U251 cells used in this experiment were genetically engineered to express firefly luciferase, which enables bioluminescent imaging (BLI) of living tumor cells. As shown by our laboratory and by others, measured luminescence is proportional to cell proliferation, and consequently provides a reliable noninvasive indication of tumor size and progression (Fig. 3(c)).^{37, 38} Mice implanted with brain tumors were serially monitored with BLI every 2–3 days to assess tumor size and progression. Anesthetized mice (2% isoflurane in 100% oxygen) were injected subcutaneously with 100 μ L of 50 mg/mL solution of D-luciferin (GoldBio Inc.) in PBS. Continuous imaging was performed over 15–30 minutes using the IVIS Lumina II system (Xenogen) until the peak tumor radiance (photons/second/cm²/steradian) was reached (Fig. 3(d)).

GSM Administration and Accumulation in Brain and Flank Tumors

In order to ensure maturity and allow full development of abnormal vasculature characteristic of large GBM tumors, flank xenografts were allowed to grow to a diameter of approximately $0.7\text{ cm}^{-1}\text{ cm}$; and orthotopic brain tumors were allowed to grow to BLI $\sim 10^6\text{--}10^7\text{ photons/sec/cm}^2\text{/sr}$. Next, GSMs were intravenously administered via tail vein injection at a dose of 300 mg Au/kg body weight. Mice were monitored regularly after tail vein injection for signs of weight loss, abnormal behavior, or systemic toxicity.

ICP-MS (Inductively Coupled Plasma-Mass Spectroscopy) Analysis

After allowing 48 hours for full extravasation of GSMs, select mice were sacrificed by perfusion using PBS and Zinc. Muscle, flank tumors, normal brain, and brain tumor were harvested and sent for ICP-MS (inductively coupled plasma mass spectroscopy) analysis of gold and iron content (Pennsylvania Animal Diagnostic Laboratory System). Accumulation of GSMs was quantified as % injected dose/g tissue.

Evaluation of GSMs as Brain Tumor Contrast Agent for CT and MR Imaging

Both before and 48 hours after intravenous administration of GSMs to mice with sufficiently large GBM brain tumors, CT and MRI imaging were performed to visualize the tumor and assess whether enough GSMs had accumulated to serve as an effective contrast agent for both imaging modalities. The Small Animal Radiation Research Platform was used for CT imaging. Mice anesthetized with a mixture of ketamine and xylazine (140 and 10 mg/kg, respectively) were imaged using a 50 kVp potential (0.5 mA).

MRI was performed using the Small Animal Imaging Facility at the University of Pennsylvania, on a 9.4 T 31 cm horizontal bore MR Spectrometer equipped with a 21 cm ID gauss/cm and a 12 cm ID gauss/cm gradient tube and interfaced to a Varian DirectDrive console. T2 weighted imaging was conducted using a 25 mm coil, with TE and TR times of 2 s and 80 ms, respectively. Throughout the experiment, anesthesia was maintained on 100% oxygen and 1–2% isoflurane while monitoring respiratory rate and maintaining 37 °C body temperature.

RESULTS

Synthesis and Characterization of GSMs

GSMs were synthesized by self-assembled encapsulation of hydrophobic gold ($d = 1.9\text{ nm}$) and iron oxide ($d = 15\text{ nm}$) particles by PEG-PCL copolymer (Fig. 1(a)). TEM analysis of these GSMs showed spherical micelles with a mixture of iron oxide and gold nanoparticles (Fig. 1(b)), and ICP-OES analysis established that the gold:iron ratio of purified GSMs was approximately 3:1 by mass (Fig. 1(c)). The discrepancy in gold:iron mass ratio observed on ICP-OES compared to synthesis likely resulted from differential losses during the centrifugation and filtration steps of nanoparticle purification.

Dynamic light scattering (DLS) measurements (Fig. 1(d)) confirmed a low dispersity in the hydrodynamic diameter of GSMs, centered at approximately 100 nm. We observed a linear relationship between concentration of GSMs in suspension and T2 relaxation time produced

(Fig. 1(e)). GSMs exhibit a high relaxivity, with an R_2 value of $221.92 \text{ s}^{-1} \text{ mM}^{-1}$ at a field strength of 1.4 T at 25 °C (Fig. 1(e)). This behavior, due to the high magnetic susceptibility of the iron oxide, leads to remarkably hypointense contrast under T2-weighted imaging at relatively low concentrations.

***In Vitro* Radiosensitization of Glioma Cell Lines**

We used immunofluorescent labeling of gh2ax as a marker for DNA double strand breaks to investigate the radiosensitizing potential of GSMs. The upper panels in Figures 2(a) and (b) show representative fluorescent images of U251 and U373 glioblastoma cells treated with either vehicle or 100 $\mu\text{g/mL}$ GSMs, then given either mock irradiation or 4 Gy RT. In the absence of RT, we observed little difference in the density of gh2ax foci between cells in the control group and those incubated with GSMs for both U251 cells ($p = 0.78$) and U373 cells ($p = 0.82$), which suggests that GSMs themselves do not induce significant DNA DSBs in GBM cells. However, both U251 and U373 cells receiving 4 Gy RT following overnight GSM incubation showed 1.8-fold and 2.4-fold increase in foci density, respectively, as compared to cells receiving irradiation alone ($p < 0.01$). These results are summarized in the histograms shown in the bottom panels of Figures 2(a) and (b), which were obtained by calculating the density of gh2ax foci for >80 cells in each treatment group. This data suggests that GSMs can directly increase the induction of damage to cellular DNA when combined with RT, likely by virtue of mechanisms such as increased electron propagation and free radical generation.

Orthotopic Models of GBM

Figure 3(a) shows a schematic of the proposed theranostic applications of GSMs. Once administered intravenously, they circulate systemically and can accumulate in an orthotopically placed brain tumor. Upon administration of a magnetic field, GSMs can serve as an MRI contrast agent; and upon administration of ionizing radiation, they can serve as a radiosensitizer. A representative histological section of an orthotopically placed GBM tumor used in our experiments is shown in Figure 3(b); the cellular atypia and hyperchromaticity of the tumor tissue is evident in comparison with adjacent normal brain tissue. Orthotopic xenografts of U251 cells in mice have been shown in our laboratory and by others to recapitulate the relevant pathobiological features of human GBM;³⁹ thus, such preclinical models can provide valuable prognostic insight into behavior and response of these tumors to treatment. BLI imaging was successfully used to serially monitor tumor growth in orthotopic models (Figs. 3(c)–(d)).

Analysis and Detection of GSMs Accumulation in Heterotopic and Orthotopic GBM Xenografts After Intravenous Administration

ICP-MS Analysis—After intravenously administering GSMs to mice with tumors, close monitoring revealed that mice tolerated the injection well, with no weight loss or signs of systemic toxicity. After waiting 48 hours post-injection to allow complete extravasation, we quantified accumulation of GSMs in brain and flank tumors using ICP-MS analysis. GSM uptake in flank tumors, which reached an average of 2.2% ID/g tissue, exceeded uptake in

muscle by a ratio of 11:1 (Fig. 4(a)). GSM uptake in brain tumors, which reached an average of 1.7% ID/g tissue, exceeded uptake in normal brain by a ratio of 97:1 (Fig. 5(a)).

CT and MR Imaging—We next sought to determine whether GSMs, after accumulation in heterotopic flank and orthotopic intracranial GBM xenografts, could be detected and used as a contrast agent with both CT and MR imaging. Pre- and post-injection CT scans show no discernible difference in tumor detection or outline for both flank tumors (Fig. 4(b)) and intracranial tumors (data not shown), likely because the relatively low concentration of GSMs injected (50 mg/ml Au) and amount of GSMs accumulated (~1–2% ID/g tissue) was below the sensitivity threshold needed for CT detection. However, even at these low concentrations, T2 weighted MRI shows striking hypointensity localized to both flank and brain tumor sites, as exhibited by the clear tumor outlines in Figures 4(c) (flank) and 5(b) (brain). At the concentrations injected and accumulated, only MRI, a more sensitive imaging modality, was capable of detecting the iron nanoparticles in the GSMs accumulated in flank and brain tumors. GSMs were detected using MRI even 5 days after initial injection (Fig. 5(b)), suggesting remarkable durability and persistence of GSMs at the tumor site.

Although CT imaging was insufficiently sensitive to detect GSM accumulation in brain and flank tumors, we did find that CT imaging successfully highlighted circulating GSMs in the vasculature, serving a blood pool contrast agent role. CT images taken of mice before and 5 minutes after intravenous GSM injection showed striking hyperintensity of the vasculature and heart post-injection (Fig. 6), suggesting that gold can be used as a blood pool agent for angiography-style studies.

DISCUSSION

In this paper, we have investigated several potential applications of novel gold and superparamagnetic iron oxide micelles as both therapeutic and diagnostic tools in glioblastoma multiforme. The gold nanoparticle component was used to sensitize tumor cells to ionizing radiation, while the iron oxide component was leveraged as a contrast agent for tumor imaging.

Toxicity of GSMs

The toxicity of nanoparticles is related to size and surface properties, which determines cellular uptake and cytotoxicity.^{18, 40} The biocompatibility of iron oxide nanoparticles has been proven for decades.⁴¹ Similarly, gold has been shown in multiple studies to be safe both *in vitro* and *in vivo*, causing very little inflammatory response and few side effects upon systemic administration.^{42–46} In this study, the lack of DSBs induced by GSM administration alone, along with the fact that mice intravenously injected with GSMs seemed to display no adverse side effects, suggest that GSMs in the formulation and dosages used in this work are relatively nontoxic and safe for use *in vivo*. Further formalized toxicity studies are needed to confirm their suitability for use in future clinical applications.

Radiosensitization

GSMs were successfully used to radiosensitize glioma cell lines, effectively doubling the density of DSBs counted compared to radiation treatment alone for both U251 and U373 cell lines (Fig. 2). It is unclear whether increased DNA break formation or inhibition of subsequent DNA repair, both of which have been described as mechanisms of gold nanoparticle-induced radiosensitization,^{47, 48} is the primary mechanism driving this observed discrepancy between DSBs in the two treatment groups. Studies have shown that radiation-induced DSBs peak at 1 hour, then steadily decline over the next 24 hours due to DNA repair.⁴⁹ The 24 hour post-irradiation time used in the experiment shown in Figure 3 would allow time for both mechanisms to operate; however, identical supplementary experiments performed in the U251 cell line using a 30 minute post-radiation period showed similar results (data not shown). Since this 30 minute time period is too brief for significant DNA repair to occur, direct DNA damage due to radiosensitization is likely the primary mechanism driving the increased DSBs observed in cells treated with GSMs prior to irradiation.

These radiosensitization studies were exclusively performed *in vitro*; further studies are needed to confirm the radiosensitizing efficacy of GSMs *in vivo*. The U251 and U373 cell lines used in this study have both been widely used as models of malignant gliomas, and have been shown to recapitulate salient pathobiological features of clinical human GBM.^{50, 51} It is likely that when translated to *in vivo* models, radiosensitizing efficacy could also be demonstrated with GSMs. For instance, previous work in our lab has shown that similar gold-containing nanoparticles, when used as radiosensitizing agents in mice orthotopically implanted with U251-based gliomas, result in a doubling of mean survival time in mice treated with gold nanoparticles and radiation compared with radiation alone.⁵

It is also important to note that our radiosensitizing assays assessed only DNA damage as a mechanism of radiosensitization. Recent work on gold nanoparticles as radiosensitizers, particularly at clinically relevant MeV energy ranges, suggests that other biological mechanisms of radiosensitization and factors such as mitochondrial and cell wall damage, may also contribute to the full *in vivo* therapeutic benefit of gold nanoparticles.⁴⁸

Tumor Imaging

MRI is a superior modality for imaging and diagnosis of intracranial lesions, and possesses a high sensitivity to contrast agents such as GSMs even in low concentrations, as observed in Figures 4 and 5. This sensitivity is especially important for imaging of tumors of privileged organs such as the brain, in which the neuroprotective BBB can prevent effective extravasation of systemic agents into the tumor. CT imaging, for instance, was not sufficiently sensitive to detect the gold portion of GSMs in the concentrations accumulated in brain tumors.

Our results show that administration and accumulation of a small amount of GSM in brain tumors would be sufficient to serve as a contrast agent for MRI-based visualization. GSMs accumulated in both heterotopic flank and orthotopic brain GBM tumors, and provided reliable hypointense MRI contrast enhancement with good delineation of tumor borders as

well as minimal toxicity. In addition, the persistence of GSMs in tumor tissue for up to 5 days post-injection (Fig. 5(b)) suggests that this agent is durable enough to provide selective MRI contrast enhancement of tumor cells during the course of treatment, in applications such as image-guided radiation treatment planning, monitoring of surgical resection, monitoring of response to other targeted therapies, and long-term imaging of tumor through the course of treatment progression.^{15–17, 52}

We also propose that intravenously administered GSMs may be detected by CT imaging, and can thus serve as a blood pool contrast agent (Fig. 6). Although these results are preliminary and only sensitive enough to delineate the heart and major vessels, further refinement of this technique may enable more delicate imaging of tumor vessels or vascular pathology in general. In addition, GSMs leveraged as a vascular imaging agent may have utility in studying not only abnormal tumor vasculature but also vascular renormalization that may occur with various targeted chemosensitizers and radiosensitizers.^{53, 54}

CONCLUSION

In this study, we have demonstrated several potential applications of GSMs in the management of glioblastoma multiforme. By serving as both a safe and long-lasting MRI contrast agent as well as an effective radiosensitizer, GSMs may, with further development, be integrated into the standard paradigm of imaging and treatment of GBM.

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Figure 1.

Physical characterization of GSMs.

Notes: (a) Schematic of GSM structure and composition, showing core of gold and iron nanoparticles coated by PEG-PCL polymer. (b) TEM image showing single micelle of ~75 nm diameter, with smaller Au nanoparticles interspersed with larger Fe nanoparticles. PEG-PCL polymer coating is not visualized on TEM. (c) Histogram of fraction by weight of gold and iron nanoparticles in individual micelles, as calculated by ICP-OES. Ratio of Au:Fe is approximately 3:1. (d) Dynamic light scattering plot showing homogeneously sized micelles with diameter of ~100 nm (Au-Fe core surrounded by PEG-PCL coating). (e) Linear relationship between concentration of GSM in suspension and T2 relaxation time produced ($R^2 = 0.3998$, R2 value = $221.92 \text{ s}^{-1} \text{ mM}^{-1}$).

Figure 2.

In vitro assay of radiosensitization: GBM cells incubated with GSMs show significantly increased gh2ax foci 24 hours after irradiation.

Notes: Representative immunofluorescent images of gh2ax foci in U251 (a) and U373 (b) cells in four treatment groups: Either untreated or treated with GSMs, and 24 hours after either mock irradiation or 4 Gy RT. Histograms (lower) show quantitative analysis of gh2ax foci density (# foci/100 μm^2) for $N > 80$ cells in each treatment group. Error bars represent 95% confidence intervals. Irradiation in the presence of GSMs led to a 1.8-fold and 2.4-fold increase in gh2ax density in U251 and U373 GBMs cells, respectively, compared to cells treated with RT alone. Of note, GSM treatment itself did not result in increased DNA damage compared to cells receiving no treatment, suggesting that GSMs are not directly cytotoxic.

Figure 3.

GSMs as theranostic agents for GBM.

Notes: (a) GSMs systemically administered to mice bearing orthotopic GBM xenografts can respond to an applied magnetic field (B_0) for MRI imaging and to X-rays for CT imaging and radiotherapy enhancement. (b) Hematoxylin and Eosin stain of normal brain boundary from a GBM mouse tumor. (c) Bioluminescent imaging (BLI) of a mouse orthotopic GBM. Tumor size was monitored via luciferase-expressing U251 cells reacting with injected luciferin, in units of radiance photons emitted. (d) Characterization of radiance emitted per number of luciferase-expressing U251 cells using BLI and Living Image software.

Figure 4.

CT and MRI imaging of GSM accumulation in flank tumors.

Notes: (a) ICP-MS analysis comparing muscle and flank tumor samples ($n = 4$), showing a ratio of flank tumor: muscle GSM uptake of 11:1. (b) Axial CT imaging of the same mouse before (upper) and 48 hours after (lower) GSM injection, showing no significant contrast enhancement. (c) T2-weighted MRI of the same mouse before (upper) and 48 hours after (lower) GSM injection. While the tumor in the pre-contrast image appears hyperintense on MRI due to edema, the post-contrast image shows a significant hypointensity in the flank tumor due to accumulation of GSMs (2.4% ID/g tissue in this mouse).

Figure 5.

GSMs as MRI-sensitive contrast agents for brain tumor imaging.

Notes: (a) ICP-MS analysis comparing GSM uptake in GBM tumor and normal brain ($n = 4$), showing a ratio of brain tumor:normal brain GSM uptake of 97:1. (b) Box-and-whisker plot and representative axial MRI images showing difference in MRI signal intensity between pre-contrast, 48 hour post-contrast, and 120 hour post-contrast brain tumors. Signal intensity in 48 hour post-contrast tumor was dramatically reduced compared to contralateral brain (average ratio 0.73), indicating hypointensity consistent with significant GSM uptake. Even 120 hours post-contrast, intensity ratio between tumor and normal brain remained decreased (average ratio 0.92), indicating persistence of GSMs in tumor tissue.

Figure 6.
GSMs as blood pool vascular contrast agents.

Notes: Vascular CT imaging of the same mouse before (left) and 5 minutes after (right) injection of GSMs, shown in axial (upper) and sagittal (lower) views. Significant hyperintensity can be seen in the major vessels after intravenous GSM administration, demonstrating the ability of GSMs to serve as blood pool contrast agents.