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Photothermal Therapy Employing Gold Nanoparticle-Loaded Macrophages as Delivery Vehicles: Comparing the Efficiency of Nanoshells Versus Nanorods

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Abstract

Macrophages (Ma) loaded with gold-based nanoparticles, which convert near infrared light to heat, have been studied as targeted transport vectors for photothermal therapy (PTT) of tumors. The purpose of the experiments reported here was to compare the efficacy of gold-silica nanoshells (AuNS) and gold nanorods (AuNR) in macrophage-mediated PTT. Photothermal therapy efficacy was evaluated in hybrid glioma spheroids consisting of human glioma cells and either AuNS- or AuNR-loaded Ma, designated Ma^{NS} and Ma^{NR}, respectivly. Spheroids were irradiated for 10 minutes with light from an 810-nm diode laser at irradiances ranging from 0 to 28 W/cm^2. Photothermal therapy efficacy was determined from spheroid growth over a 14-day period. The uptake by Ma of pegylated AuNR (3.9 ± 0.9 %) was twice that of pegylated AuNS (7.9 ± 0.7%). Hybrid spheroids consisting of a 5:1 ratio of glioma cells to loaded Ma exhibited significant growth inhibition with Ma^{NS} when subjected to irradiances of 7 W/cm^2 or greater. In contrast, no significant growth inhibition was observed for the Ma^{NR} hybrid spheroids at this 5:1 ratio, even at the highest irradiance investigated (28 W/cm^2). Although AuNR were taken up by Ma in larger numbers than AuNS, Ma^{NS} were shown to have greater PTT efficacy compared to Ma^{NR} for equivalent numbers of loaded Ma.

Keywords

glioma; photothermal therapy; gold-silica nanoshells; gold nanorods; murine macrophages

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I. INTRODUCTION

The ability of gold nanoparticles to convert near-infrared (NIR) light to heat is highly efficient; thus, they are well suited for hyperthermia applications such as photothermal therapy (PTT).[1–3] The most studied nanoparticles for PTT applications are gold nanoshells (AuNS) and gold nanorods (AuNR). Both of these nanoparticles have strong absorption in the NIR, which makes them ideally suited for biological applications because NIR wavelengths have relatively deep penetration in tissues. The absorption peak of AuNS can be tuned by varying the ratio of gold shell to silica core thickness,[4] while peak absorption wavelengths for AuNR are dependent on their aspect ratio (length to width).[5,6]

A fundamental characteristic for the success of nanoparticle-mediated therapy is the ability of these constructs to penetrate the therapeutic site at sufficient concentrations while minimizing accumulation at undesired sites such as the reticulo-endothelial system. Nanoparticle delivery via passive approaches, such as those employing the enhanced permeability and retention (EPR) effect, is unlikely to achieve sufficient target specificity.[7] In contrast, macrophages (Ma) employed as nanoparticle delivery vehicles have the ability to leave the circulation and migrate to and infiltrate the tumor interstitium by active processes (e.g., by following gradients of chemokines, cytokines, and growth factors).[8–10]

AuNS-loaded macrophages for PTT have been found to be effective in a number of studies.[11–14] The overall objective of the this study was to compare the PTT efficacy of Ma loaded with either AuNS (designated Ma^{NS}) or Ma loaded with AuNR (designated Ma^{NR}). The Ma^{NS} or Ma^{NR} were incorporated into multicell hybrid spheroids formed by a mixture of loaded Ma and human tumor cells. The relative PTT efficacies of the two types of nanoparticles were evaluated based on inhibition of spheroid growth following exposure to NIR laser irradiation.

II. MATERIALS AND METHODS

A. Cell Lines

Murine macrophages (P388D1; ATCC# CCL-46) and human grade IV GBM cells (ACBT; G. Granger, University of California, Irvine) were used in this study.

B. Nanoparticles

Polyethylene glycol (PEG)-coated AuNS (AuroShell™) and AuNR were purchased from Nanospectra Biosciences (Houston, TX, USA). AuNS were supplied at a concentration of 2.82×10^{11} particles/mL, and consisted of a 120-nm diameter silica core encased in a gold shell of 15-nm thickness, giving a total particle diameter of 150 nm. The peak absorption was found at a wavelength of 819 nm (optical density = 105). AuNR were supplied at a concentration of 2.0×10^{11} particles/mL and had a length of 45 nm and a width of 15 nm. The peak absorption occurred at a wavelength of 765 nm (optical density = 1.09). The absorption and electron micrographs for both types of nanoparticles (supplied by the manufacturer) are shown in Figure 1.

C. Nanoparticle Uptake

AuNS or AuNR uptake by Ma was studied using UV-Vis-NIR spectrophotometry. Ma (5.0×10^6) were incubated with either AuNS (4.29×10^9 per mL) or an equivalent concentration of AuNR for 24 h. Following incubation, Ma were centrifuged at 600 rpm for 7 min and then washed twice with phosphate-buffered saline (PBS) to remove excess particles, and the cells then resuspended. The absorbance of the resultant cellular suspension was measured with a Varian UV-Vis-NIR spectrophotometer (Cary 6000i, Varian, Palo Alto, CA). The percentage uptake of nanoparticles was calculated by applying the following formula:

$$A_{M+N}/A_N \times 100 \quad (1)$$

where A_{M+N} is the absorbance of endocytosed nano-shells at $\lambda = 819$ nm (or nanorods at 765 nm), and A_N is the absorbance of the reference nanoshell or nanorod solution at the same wavelength.

D. Hybrid Spheroid Generation

Hybrid tumor/Ma spheroids were formed as previously described.[12] Prior to spheroid formation, both nanoparticle-loaded and empty Ma were incubated for 1 hour in 20 μg/mL mitomycin C (Sigma-Aldrich, St. Louis, MO, USA) to inhibit cell division and their subsequent contribution to spheroid growth. Hybrid spheroids using either 5×10^3 ACBT cells and 1×10^3 or 2×10^3 loaded Ma (designated Ma^{NS} or Ma^{NR}) were generated. The cell combinations were alloquated into the wells of ultra-low attachment 96-well round-bottom plates (Corning, Corning, NY, USA) in 200 μL culture medium. The plates were centrifuged at 1,000 ×*g* for 10 min. The plates were incubated for 48 h to allow the spheroids to stabilize. Previous experiments employing two-photon microscopy have shown an even distribution of the Ma throughout the spheroids.[12]

E. External Hyperthermia Treatment

Forty-eight hours after initiation, hybrid spheroids containing empty Ma were incubated at temperatures ranging from 37 to 46°C for 45 minutes at each temperature. The spheroids were followed for 14 days, and their volume was calculated on the basis of of their diameters as determined from light microscopy measurements.

F. Photothermal Therapy of Spheroids

Individual spheroids in each well of a 96-well round-bottom plate were irradiated with 810 nm light from a laser diode (Intense, New Brunswick, NJ, USA) at irradiances ranging from 0 to 28 W/cm^2 with a beam diameter of approximately 3 mm and an irradiation time of 10 min. The maximum power output, corresponding to an irradiance of 28 W/cm^2, was 1.98 W. Treatment efficacy was monitored by spheroid growth following 14 days of incubation. Spheroid diameters were measured using an ordinary light microscope with a calibrated eyepiece and spheroid volumes were estimated assuming a perfect sphere.

G. Statistical Analysis

Microsoft Excel (Redmond, WA, USA) was used to determine the mean, standard deviation and standard error. Data were analyzed using one-way ANOVA at the significance level of $P < 0.05$ and presented as mean with standard error unless otherwise noted.

H. Experimental Protocol

The basic experimental protocol used in this study is shown in Figure 2. As seen in the figure, Ma loaded with either AuNS or AuNR were used to form hybrid spheroids consisting of ACBT glioma cells and a variable number of Ma^{NS} or Ma^{NR}. The experimental groups were subjected to NIR laser light at irradiances of 2, 7, 14, or 28 W/cm^2, while control groups received no irradiation. Spheroid growth in both groups were monitored for 14 days.

III. RESULTS AND DISCUSSION

A. Nanoparticle Uptake by Macrophages

Equal concentrations of gold nanoshells and nanorods were incubated with macrophages. Following 24 hours of coincubation, spectrophotometric analysis revealed uptakes of 3.9 ± 0.9 and 7.9 ± 0.7% for AuNS and AuNR, respectively. Based on the initial nanoparticle concentrations, these uptakes corresponded to 337 AuNS and 680 AuNR per macrophage, respectively. The difference in uptake was statistically significant ($P < 0.05$). Macrophage phagocytosis is influenced by size, shape, and surface chemistry of the nanoparticle.[15–17] The increased ability of Ma to take up more AuNR compared to AuNS might be due to the smaller size of the AuNR and their rod-like shape, which is similar to many pathogens.

B. Toxic Effects of Hyperthermia

The direct effect of hyperthermia on hybrid spheroid growth was examined in the wells of 96-well plates. Forty-eight hours after spheroid generation, the plates were incubated at 37, 40, 44 or 46°C for 45 minutes at each temperature. Following hyperyhermia, the plates were incubated at 37°C and monitored for growth for an additional 14 days. A temperature of 46°C for 45 minutes resulted in total spheroid death, while the majority of spheroids subjected to 44°C or below for the same interval, survived (Fig. 3). The toxic threshold for hyperthermia was estimated to be between 44 and 46°C and could therefore be used to infer the temperature obtained during PTT.

C. Photothermal Therapy of Nanoparticle-Loaded Hybrid Spheroids

Survival of ACBT/loaded Ma hybrid spheroids exposed to a range of laser irradiances is illustrated in Figure 4. Control spheroids consisting of ACBT cells and “empty” macrophages (Ma^E) were irradiated over a range of 0–28 W/cm^2. Minimal growth inhibition at all irradiances compared to nonirradiated controls was demonstrated.

Ma^{NS} mediated PTT significantly inhibited spheroid growth at irradiances of 7 W/cm^2 and higher ($P < 0.05$). Spheroid survival was approximately 40%, 30%, and 18% of controls at irradiances of 7, 14 and 28 W/cm^2, respectively. In contrast, Ma^{NR} hybrid spheroids showed no significant growth inhibition compared to Ma^E controls at any of the irradiances investigated. Increasing the number of Ma^{NR} in each spheroid to 2×10^3 resulted in a

modest inhibition of spheroid growth but only at an irradiance of 28 W/cm^2. A 30% reduction of spheroid volume compared to controls was achieved (data not shown). Although Ma ingested approximately twice as many AuNR compared to AuNS, AuNS have a much larger cross-sectional area compared to AuNR, resulting in a larger photothermal transduction cross-section.[18] Calculations show that the absorption efficiency of AuNS similar to the ones used in this study is approximately ten times greater than that of the AuNR used in this work.[19] The significantly increased conversion efficiency of NIR light to heat demonstrated by Ma^{NS} compared to Ma^{NR} is the likely explanation for the results shown in Figure 4.

IV. CONCLUSIONS

Although AuNR were taken up by Ma in larger numbers than AuNS, Ma^{NS} were shown to have greater PTT efficacy than Ma^{NR} for equivalent numbers of loaded Ma in the tumor cell spheroids. This finding was most probably due to the considerably larger cross-sectional area of the AuNS compared to the AuNR used in this study, resulting in a larger photothermal transduction cross-section. Theoretical calculations suggest the ratio of nanorods to nanoshells required, for equivalent PTT effect, is approximately 10:1

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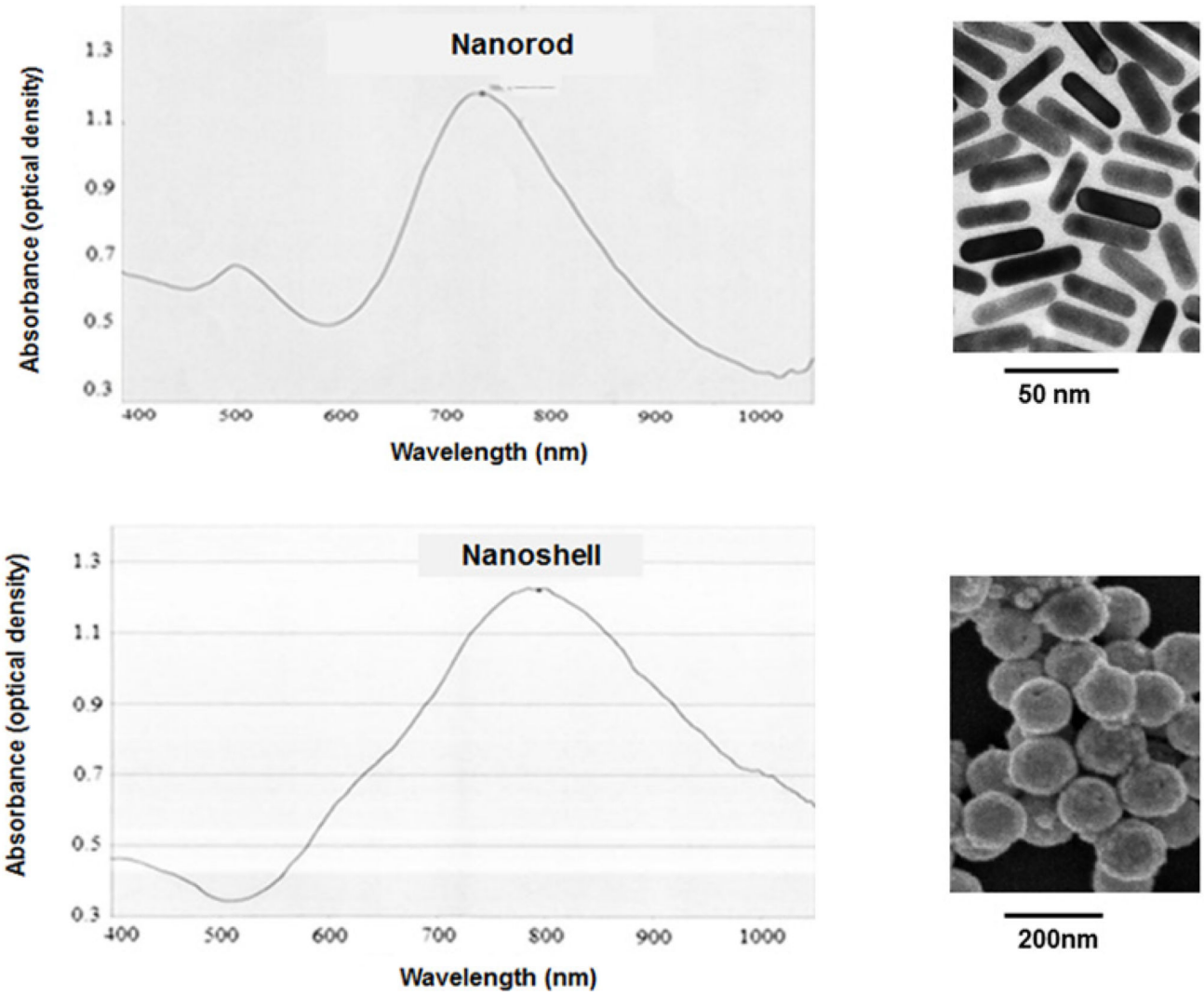

FIG. 1.

Vis-NIR absorption spectra and electron micrographs of pegylated AuNR (top) and pegylated AuNS (bottom)

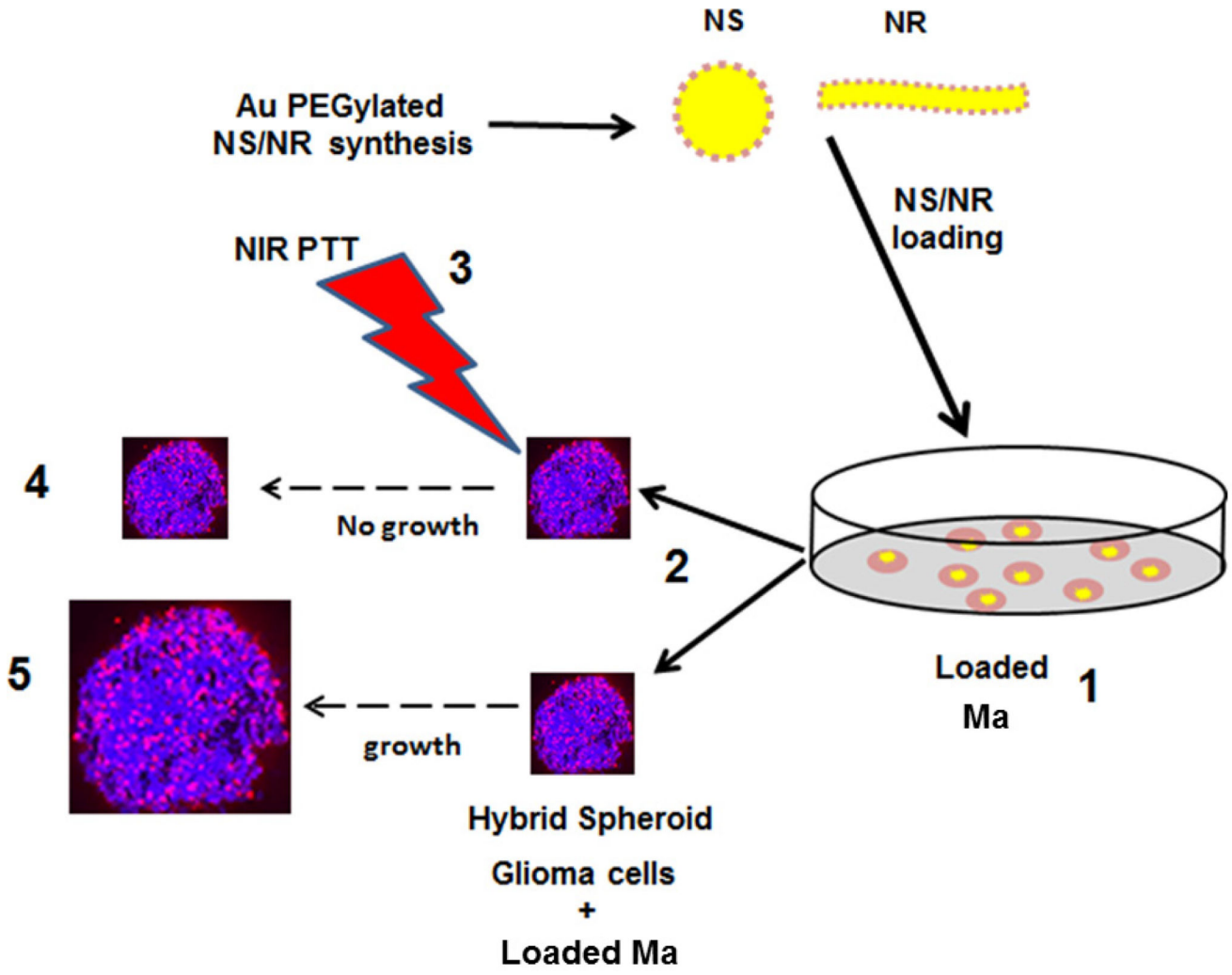

FIG. 2.

Basic experimental setup, (1) Ma loaded with AuNS or AuNR by 24 h co-incubation; (2) Hybrid spheroid consisting of ACBT glioma cells and a variable number of Ma^{NS} or Ma^{NR}; (3) One group of hybrid spheroids irradiated with variable levels of NIR laser light; Control groups received no irradiation; (4) Growth of experimental group followed for 14 days; (5) Growth of control group followed for 14 days.

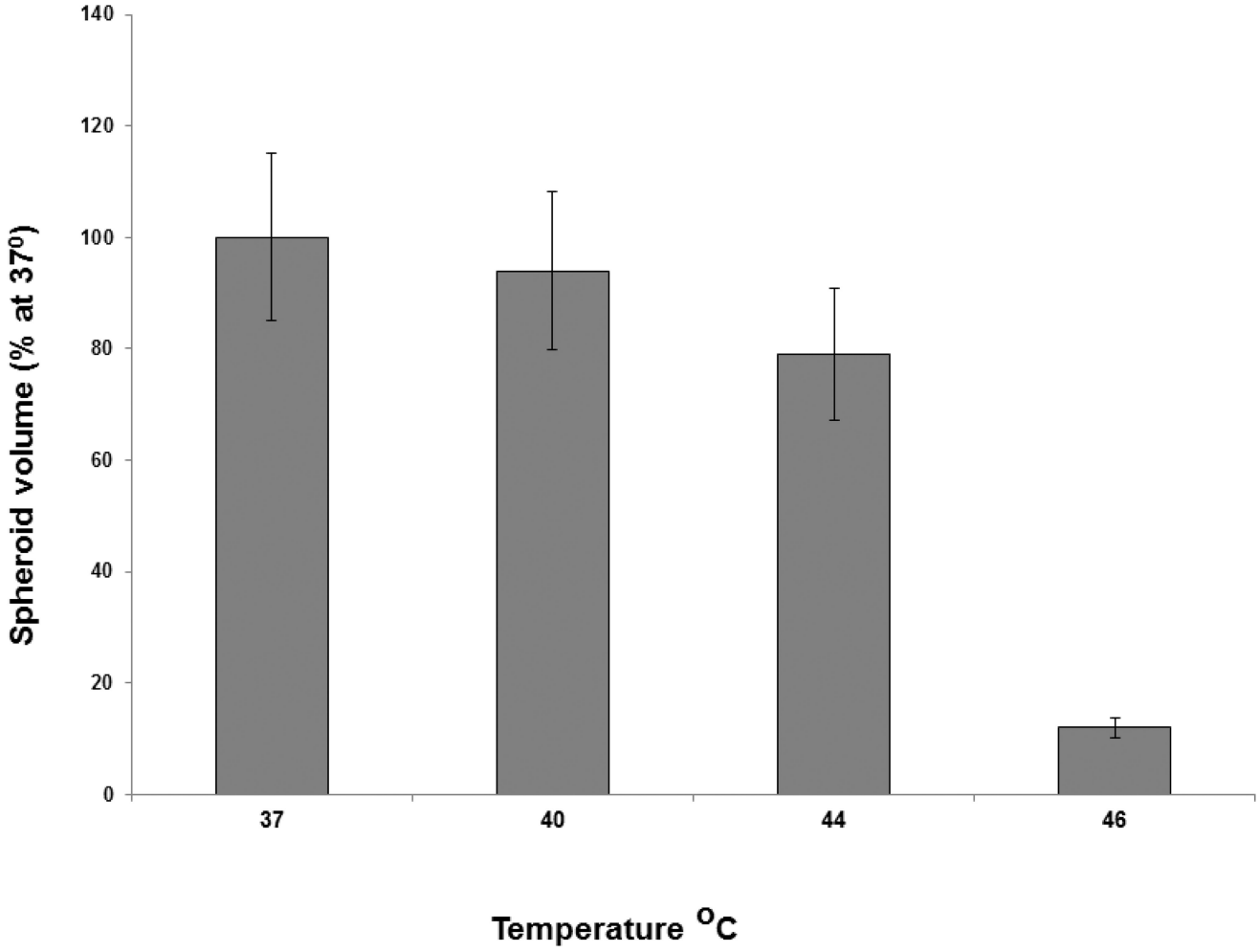

FIG. 3.

Effects of temperature on ACBT spheroid growth. Spheroids 48 h after initiation were incubated at temperatures ranging from 37 to 46°C, for 45 minutes at each temperature. Each data point represents spheroid volume after two weeks in culture as a % of 37°C controls and is the mean of three experiments. Error bars denote standard errors.

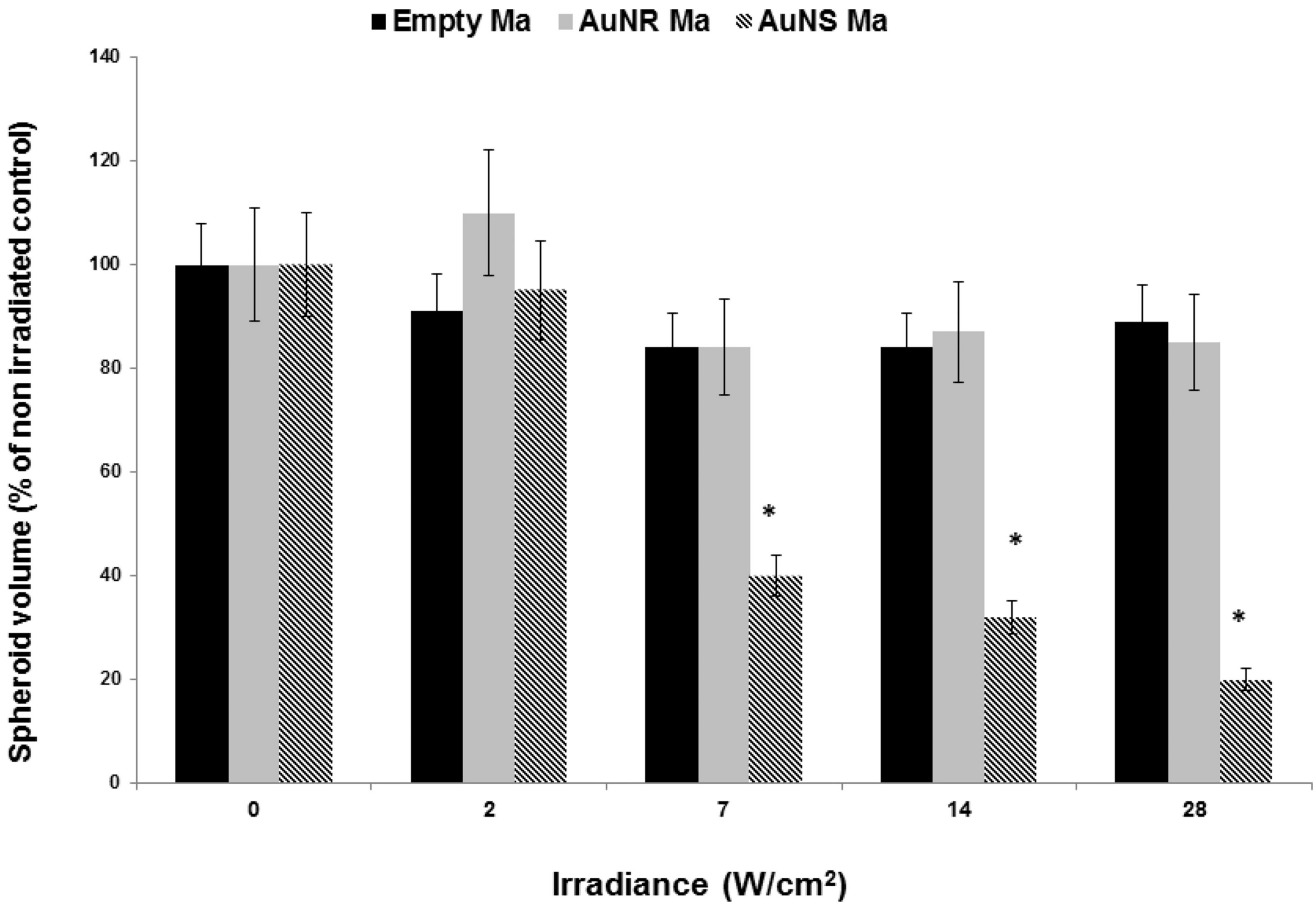

FIG. 4.

Growth of hybrid spheroids as a function of 808-nm laser irradiance. Ma^{E} denotes hybrid control spheroids consisting of empty macrophages and Tc denotes tumor cells. The ratio of Tc: Ma^{NS} or Tc: Ma^{NR} was 5:1. Each data point corresponds to the mean of three trials as a % of controls on day 14. Error bars denote standard deviations. * denotes significant difference from nonirradiated controls ($P < 0.05$).