Multimodal Imaging of Pancreatic Ductal Adenocarcinoma Using Multifunctional Nanoparticles as Contrast Agents

Ying Zhao,* Fei Ye, Torkel B. Brismar, Xuan Li, Rui He, Rainer Heuchel, Ramy El-Sayed, Neus Feliu, Wenyi Zheng, Sandra Oerther, Joydeep Dutta, Wolfgang J. Parak, Mamoun Muhammed, and Moustapha Hassan*

ABSTRACT: Late diagnosis and refractory behavior toward current treatment protocols make pancreatic ductal adenocarcinoma (PDAC) one of the most difficult cancer forms to treat. The imaging-based approach plays an important role to identify potentially curable PDAC patients in high-risk groups at the early stage. In the present study, we developed a core–shell structured gold nanorod (AuNR) as a contrast agent for multimodal imaging and investigated its application for PDAC diagnosis. The composite nanoparticles composed of a AuNR core inside a layer of mesoporous silica that was then coated with a gadolinium oxide carbonate shell (AuNR–SiO₂–Gd) are designed to be used in magnetic resonance imaging (MRI), X-ray computed tomography (CT), and photoacoustic imaging (PAI). A phantom study with the AuNR–SiO₂–Gd NPs demonstrated higher MRI contrast compared to Gadovist and higher X-ray attenuation than Visipaque. A strong, stable, and broad wavelength range signal with a peak at 800 nm was observed in PAI. The AuNR–SiO₂–Gd NPs showed significant contrast enhancement under PAI/MRI/CT in both the liver and spleen of control mice after intravenous administration. The utility in PDAC was studied in a genetically engineered mouse model carrying Kras and p53 mutations, which develops spontaneous tumors and keeps the desmoplasia and hypovascularity feature of PDAC in patients. The AuNR–SiO₂–Gd NPs were highly accumulated in the surrounding soft tissues but were sparsely distributed throughout the tumor due to dense stroma infiltration and poor tumor vascularization. Hence, a negative contrast within the tumor area in CT/PAI and a positive contrast in MRI were observed. In conclusion, AuNR–SiO₂–Gd NPs have good potential to be developed as a multimodal contrast agent for PDAC, which might improve early diagnosis and benefit the clinical outcome for PDAC patients.

KEYWORDS: multimodal, magnetic resonance imaging, computed tomography, photoacoustic imaging, pancreatic ductal adenocarcinoma

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related deaths in Europe, and it is one of the most difficult cancers to treat. The median survival time of patients diagnosed with PDAC is 4.6 months,1 and the median five-year survival rate for pancreatic cancer across Europe is 3%.2 Although several conventional and combination chemotherapy protocols exist to treat PDAC, significant side effects are observed without an increase in the patient survival rate. Currently, three major challenges hinder the treatment of PDAC. First, pancreatic cancer has an extensive tumor-stromal component that can make up more than 50% of the tumor mass, and in addition, PDAC is characterized by a disorganized vasculature, resulting in a physical barrier for drug delivery.3 Second, the majority of pancreatic cancer patients are diagnosed at an advanced stage due to a lack of biomarkers and/or vague symptoms. At last, multiple genetic alterations and dysfunctional signaling pathways in pancreatic tumor cells also lead to intrinsic drug resistance. Therefore, the development of earlier diagnostic tools in combination with new therapeutics could significantly reduce the mortality and morbidity of the disease.

Recent developments of new imaging/contrast agents together with advances in molecular imaging offer promising tools for clinical needs to address an early diagnosis and to personalize the treatment of PDAC. While the technologies used in combined modality scanners or in the fusion of imaging data collected from multiple imaging modalities have advanced, there are still limiting factors to take full advantage of the multimodal imaging techniques currently in clinical use. The main aims for the clinical use of multimodal scanners and imaging agents are to combine the advantages of different...
imaging techniques, with respect to the sensitivity of detection and image resolution. Each imaging modality has its own unique advantages and intrinsic limitations regarding sensitivity and spatial resolution; for example, magnetic resonance imaging (MRI) can provide high-resolution imaging of soft tissues; X-ray computed tomography (CT) is well suited to hard tissues, and photoacoustic imaging (PAI) has high spatial sensitivity. The recent technological revolution in molecular imaging and medical devices has allowed the commercialization of hybrid scanners combining multiple imaging modalities as well as the development of software needed to process the coregistration and postimage analysis of data obtained from multiple modalities. The integration of multimodal imaging technologies would therefore provide complementary and more complete information for subsequent decision making. To unleash the full potential of multimodal imaging technologies, one of the key prerequisites is to develop advanced and specific multimodal imaging contrast agents.

At the nanoscale, nanomaterials possess intrinsic and unique features in optical, magnetic, and other physicochemical properties. Such features enable the development of nanoparticle (NP)-based imaging agents with improved contrast enhancement, detection sensitivity, targeted biodistribution, spatial and temporal resolution, multifunctionality, and multimodal imaging capacity. Multifunctional NPs can fulfill the promise of multimodal imaging via the modification and combination of a wide variety of agents including radioisotopes and optical, CT, ultrasound (US), and magnetic contrast agents. Traditional imaging agents, such as fluorodeoxyglucose (18F-FDG), iodinated small molecules, and chelated gadolinium, are only valid for use with a single imaging modality. In addition, since such small-molecule imaging agents are rapidly distributed throughout the circulation and have short half-lives in vivo, they are not suitable for long-term follow-up.

Multifunctional NPs can be detected by multiple imaging modalities simultaneously, which improves imaging sensitivity, resolution, and diagnostic accuracy; for example, gold nanoparticles (AuNPs) are suitable for in vivo dual-modal CT/PAI. Furthermore, the optical properties and photoacoustic (PA) features of AuNPs are tunable by modification of the particle size, shape, and surface coating. Of all of the AuNP-based PA contrast agents, gold nanorods (AuNRs) have been the most prominently investigated due to their ease of synthesis and tunable aspect ratio (length/width ratio), which can lead to a shift in their absorption spectrum toward the near-infrared (NIR) region. It is well known that bioimaging in the NIR window facilitates deep tissue imaging in real time, with high sensitivity. Encapsulation of AuNRs in silica protects the nanorods from thermal deformation and thus enhances the PA signal intensity. For CT applications, AuNRs produce better CT contrast compared to iodine-based contrast agents, with a K-edge energy level of 80.7 keV. Gadolinium (Gd) is another multifunctional imaging element that can be applied to both MRI and CT. The multifunctionality of Gd originates from its powerful magnetic moment, which is due to the seven unpaired electrons in its 4f subshell and a high X-ray attenuation coefficient (lower than that of gold but higher than that of iodine) for CT imaging.

In the present investigation, we designed a trimodal contrast agent for combined MRI, CT, and PAI involving composite NPs with a hierarchical core–shell structure composed of the following: a gold nanorod core inside a shell of mesoporous silica (mSiO2) inside a gadolinium oxide carbonate shell, designated AuNR@mSiO2@Gd2O(CO3)2. AuNRs and the Gd in our composite (AuNR–SiO2–Gd) contribute to the enhancement of two imaging modalities. The contrast enhancement property of the NPs was investigated in a genetically engineered mouse (GEM) model of PDAC, which harbors tissue-specific mutations for Kras and p53 and fully represents the pathological features of PDAC patients.

RESULTS AND DISCUSSION

Morphology, Composition, and Structure of AuNR@mSiO2@Gd2O(CO3)2 Core–Shell NPs. The morphology of the core–shell structured AuNR@mSiO2@Gd2O(CO3)2 composite NPs was examined using transmission electron microscopy (TEM). The AuNRs with an aspect ratio of approximately 4.5 (as shown in Figure 1a) were grown with a sandwiched layer of mSiO2 (denoted AuNR@mSiO2 Figure 1b) of approximately 25 nm in thickness. Figure 1c shows the morphology of the NPs after coating with the Gd2O(CO3)2 layer. High-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) was used to visualize the hierarchical structure of the composite NPs with an electron-dense AuNR core, a porous silica shell, and a layer of amorphous Gd2O(CO3)2 under low brightness (see Figure 1d). The large-area TEM image in Figure S1 demonstrates that the morphology of AuNR@mSiO2@Gd2O(CO3)2 is fairly homogeneous and of ellipsoidal shape with an average aspect ratio of ca. 1.3 (average cross section of 80 nm for the long axis and 62 nm for the short axis). Unlike a previous report in which uniform mSiO2 layers were coated on cetyltrimethylammonium bromide (CTAB)-capped gold NRs at room temperature, we found that low-temperature reactions (25 °C and 45 °C) led to interconnected core–shell particles and extra mSiO2 structures (Figure S2a,b). In contrast, a high-temperature reaction (70 °C) generated discrete, single AuNRs (Figure S2c), which might be due to the effect of the addition of ethyl acetate. Ethyl acetate can be hydrolyzed in basic conditions to gain ethanol, and the hydrolysis is accelerated at high temperatures. The small amount of ethanol
generated has the effect of slowing down the rate of TEOS hydrolysis to a silicate (with ethanol as a byproduct), by changing the equilibrium of the reaction, and therefore, mSiO₂ is formed slowly without necking. Figure S3 shows composite particles with different Gd₂O₃(CO₃)₂ shell thicknesses of ca. 2, 8, and 15 nm, formed by the addition of increasing amounts of gadolinium growth solution. The addition of a very large quantity of growth solution was found to generate additional Gd₂O₃(CO₃)₂ particles instead of increasing the coating thickness on the core particle.

The elemental composition of the composite particles was examined using energy-dispersive spectroscopy (EDS). A representative line scan (Figure 2a) shows the spectral counts corresponding to the elements Au, Si, O, and Gd, while Figure S4 indicates the overall EDS spectrum for a larger area of the composite particles. Figure 2b shows the hydrodynamic size distribution of AuNR@mSiO₂@Gd₂O₃(CO₃)₂ NPs with a mean diameter of ca. 80 nm. Optical absorbance spectra for aqueous suspensions of AuNRs, AuNR@mSiO₂, and composite particles are illustrated in Figure 2c. A clear red shift of the longitudinal surface plasmon resonance (LSPR) peaks was observed due to an increase in the local refractive index induced by the mSiO₂ and Gd₂O₃(CO₃)₂ coatings. Since the wavelength of the LSPR peaks for the composite particles is located in the NIR range (836, 846, and 853 nm for 2, 8, and 15 nm coating thicknesses, respectively), it is suitable for applications of photoacoustic imaging or photothermal therapy. The AuNR@mSiO₂ X-ray diffraction (XRD) pattern (Figure 2d) shows the crystalline peaks from gold with a broad peak centered at 22° (2θ), which corresponds to the amorphous silica shell. For AuNR@mSiO₂@Gd₂O₃(CO₃)₂ NPs, besides the peaks from the crystalline AuNRs cores, the broad peak was centered at 30° (2θ) and no further crystalline peaks were detected, indicating the amorphous nature of the Gd₂O₃(CO₃)₂ coating layers.

**Photothermal Properties.** The LSPR bands of the composite particles show a red shift for increasing shell thicknesses due to the increased local refractive index. Since the LSPR peak of the composite particles is located at approximately 850 nm and has a stronger absorbance than the transverse ones (Figure 2c), a tunable laser with the wavelength adjusted to 850 nm was used to irradiate the composite particles suspended in Dulbecco’s modified Eagle’s medium (DMEM) to allow for the best photothermal conversion efficiency (Figure S5a). Upon photothermal heating of the different NP samples, the suspension of AuNRs reached the highest temperature elevation of approx. 47 °C. The temperature of the suspension of composite particles increased to a maximum of approximately 43 °C. This is ascribed to the scattering of light by the coating shells, which restricts the photothermal conversion. The global (i.e., bulk) temperature increase of the composite particle suspensions barely fulfills the requirement for hyperthermia treatment (under 42 °C), considering the low thermal conductivities of silica and water. However, the local temperature close to the surface of a single AuNP in liquid under laser irradiation can be very high (a few hundred degrees), and therefore, in principle, the composite NPs could still be used for photothermal therapy. Upon laser excitation by both 850 and 532 nm lasers, the temperature increase reached a plateau after 10 min irradiation for all samples. Figure S5b shows the morphology of the composite particles after laser irradiation. Most of the particles have an empty core, and small AuNPs generate outside the composite particles. This could be attributed to the local melting of AuNRs caused by laser irradiation, which could have increased the temperature of gold to that approaching its melting point. After the molten gold has diffused out through the porous coating layer, it may form small-sized spherical particles once cooled. Such good photothermal properties and the strong optical absorption coefficient of AuNR@mSiO₂@Gd₂O₃(CO₃)₂ NPs in the NIR make the multifunctional NPs suitable as PA contrast agents for deep tissue imaging.

**Determination of Free Gd³⁺ Ions and Cytotoxicity Evaluation.** Owing to the toxic properties of Gd³⁺ ions, we measured the concentrations of free Gd³⁺ ions leached from the composite particles in aqueous suspension using a colorimetric method. A complexometric indicator dye, xylene orange, was employed as it reacts only with free Gd³⁺ ions but not with chelated ions due to the low thermodynamic stability of Gd–xylene orange. By comparing the absorbance results with a standard curve (Figure S6), we calculated the concentrations of free Gd³⁺ ions in the suspension of the composite NP samples with variable Gd₂O₃(CO₃)₂ shell thicknesses. The total concentration of elemental Gd in the composite samples was examined by inductively coupled
plasma atomic emission spectroscopy (ICP-AES) and was found to take up 20.0, 36.0, and 48.0% of the total weight for composite particles with 2, 8, and 15 nm Gd$_2$O(CO$_3$)$_2$ shell thicknesses, respectively. The leached free Gd$^{3+}$ ions contributed to 2.08, 0.47, and 0.17% of the total Gd concentrations for these three samples (see Table S1). Even though the composite particles with thin coating layers had a higher percentage of released Gd$^{3+}$ ions from the composite particles with a 2 nm thick shell might be due to a higher proportion of surface atoms than in the other samples with thicker shells.

The cytotoxicity of AuNR@mSiO$_2$@Gd$_2$O(CO$_3$)$_2$ composite particles was evaluated in AS49 cells and mouse pancreatic stellate cells (representing normal cells) using cell viability assays (MTT and WST-1) that measure the metabolic activity of mitochondria. As shown in Figures S7 and S8, upon exposure to various concentrations of composite particles ranging from 1 to 200 μg/mL for 24 and 48 h respectively, cell viability decreased, in both cell lines, in a dose-dependent manner; however, the decrement was not significant. At the highest concentration tested, 200 μg/mL, cell viability was only modestly impaired compared to that of the control. Moreover, cell viability was similar after 24 and 48 h after exposure to the composite particles. The contrast enhancement properties of the composite particles in MRI and CT were quantified using agarose gel phantoms (Figure 3). The composite particles with different shell thicknesses were
shown to generate MR contrast on both $T_1$- and $T_2$-weighted images. The MR contrast was evaluated by estimating the relaxivity of the particles, which is defined as the gradient of the linear plot of the relaxation rates ($1/T_1$ or $1/T_2$) versus Gd concentrations (Figure 3a–d). We found that the $T_1$ and $T_2$ relaxation times were not affected by the thickness of the Gd$_2$O(CO$_3$)$_2$ shell. The obtained magnetic relaxivities, $r_1$ and $r_2$, were estimated to be 46.40 and 63.37 s$^{-1}$ mM$^{-1}$ for NPs with 2 nm Gd$_2$O(CO$_3$)$_2$ shell thickness. The obtained magnetic relaxivities are more than seven fold higher compared to those observed using the clinical contrast agent (Gadovist, Table 1). For CT imaging, both AuNRs and gadolinium in the coating layers contributed to the CT contrast (Figure 3e). The X-ray attenuation of the composite particles containing 2 mM Au and 42 mM Gd was 342 Hounsfield units (HUs). This is higher than that of the commercially available CT contrast agent, Visipaque, at the same concentration of iodine (188 HUs), suggesting that the composite particles are more efficient at attenuating X-ray signals.

### In Vitro and In Vivo PA Imaging.

The PA spectrum of AuNR@SiO$_2$-Gd NPs in the range 680–970 nm was examined in a PA phantom to test the contrast enhancement features for PAI and to identify the spectral features compared to endogenous PA contrast in vivo. AuNR-SiO$_2$–Gd NPs showed strong PA contrast enhancement within the range 680–970 nm with a peak absorbance at around 800 nm (Figure 4a). Quantification of the PA signal of AuNR-SiO$_2$–Gd NPs (Au 4.5 mg/mL) and a phosphate-buffered saline (PBS) control resulted in a signal-to-noise ratio (SNR) at 800 nm of 23.11 (Figure 4b). There was an obvious PA spectral pattern difference between exogenous contrast, AuNR-SiO$_2$–Gd NPs, and endogenous PA contrast, oxygenated hemoglobin and deoxygenated hemoglobin (Figure 4a). Oxygenated hemoglobin has a peak absorbance at around 850 nm and deoxygenated hemoglobin has a peak absorbance at around 750 nm. This PA spectral pattern makes AuNR–SiO$_2$–Gd NPs especially suitable for in vivo PA applications since the contrast of NPs can be more efficiently separated from the endogenous PA background by multiple-wavelength PAI and spectral unmixing.

After intravenous injection of AuNR–SiO$_2$–Gd NPs at a dose of 22.50 µg/g for Au and 168.50 µg/g for Gd in normal albino C57BL/6 mice, single-wavelength (800 nm) PAI (Figure 4c) and multiple-wavelength (680, 804, 850, 900, 924, and 954 nm) PAI (Figure 4d) were acquired over the regions of the spleen, liver, and kidneys, separately. The wavelength of 800 nm was chosen for single-wavelength PAI as both forms of hemoglobin have a low PA signal, while AuNR–SiO$_2$–Gd NPs have the maximum PA signal. Before NP administration, only hemoglobin in the blood gave a PA background signal for the spleen, liver, and kidneys. Regions of interest (ROIs) were defined using B-mode images as anatomy references. A B-mode image is a two-dimensional diagnostic image representing the ultrasound echoes, which therefore provides anatomic information about various organs. Single-wavelength PAI at 800 nm quantified the total PA signal of AuNR–SiO$_2$–Gd NPs, oxygenated hemoglobin, and deoxygenated hemoglobin. Multiplexed PAI at multiple wavelengths quantified the PA signal from AuNR–SiO$_2$–Gd NPs, oxygenated hemoglobin, and deoxygenated hemoglobin separately after the process of spectral unmixing, resulting in a significant decrease of the PA background signal in the AuNR–SiO$_2$–Gd NP channel. The PA background signal (PA before IV) in multiplexed PAI of the liver, spleen, and kidney regions is only around 10% of that for 800 nm PAI. Figure 4c,d shows that right after systemic administration (10 min post IV), PA contrast enhancement caused by AuNR–SiO$_2$–Gd NPs could be easily detected by both 800 nm PAI and multiplexed PAI in the liver, spleen, and kidneys. Benefiting from such contrast enhancement induced by AuNR–SiO$_2$–Gd NPs, the anatomic structures of the spleen and liver were well defined and easily recognized in both 800 nm PAI and multiplexed PAI. The PA signal enhancement (PA after IV/PA before IV) as acquired by 800 nm PAI is 5.28, 1.03, and 1.93, respectively, in the spleen, liver, and kidneys. In multiplexed PAI, the PA signal enhancement is 12.97, 3.66, and 3.44 respectively. Therefore, the magnitude of PA contrast enhancement due to AuNR–SiO$_2$–Gd NPs is significantly improved by spectral unmixing. At 60 min postinjection, there was a decrease in the PA signal in the spleen and kidneys and an increase in the PA signal in the liver, indicating that NPs had accumulated in the liver (Figure 4e).

### In Vivo MR Imaging.

For MR applications, AuNR–SiO$_2$–Gd NPs can be applied as a contrast agent for both $T_1$-weighted MRI and $T_2$-weighted MRI.

Before and after IV injection of AuNR–SiO$_2$–Gd NPs at a lower dose (0.23 µg/g Au and 1.69 µg/g Gd) in normal albino C57BL/6 mice, $T_1$-weighted MRI was acquired using a GRE sequence over the whole body of each mouse. An overall increase in the MR signal (brighter region) due to a $T_1$ shortening effect was observed over the whole body at 30 min, 60 min, and 24 h (Figure 5a). The $T_2$ contrast enhancement was detectable at 30 min postinjection and was maintained at 24 h. No obvious change in the $T_1$ contrast was observed from $T_1$-GRE images acquired at different time points up to, and including, 24 h. The SNRs for the liver, spleen, kidney-cortex, kidney-medulla, left ventricle wall, and bladder were measured at different time points postinjection (Figure 5b). At 30 min, there was a general increase in SNR in all of the ROIs. At 60 min, the SNR was unchanged in all of the organs, while at 24 h postinjection, a further $T_1$ contrast enhancement was detected in the liver and left ventricle walls. Therefore, longitudinal magnetic relaxation ($T_1$ shortening effect) is dominant and the mild transverse magnetic relaxation ($T_2$ shortening effect) is overwhelmed at a lower dose of
Figure 4. In vitro and in vivo PA imaging of AuNR@mSiO2@Gd2O3(CO3)2 NPs. (a) PA spectra of the phantoms containing composite NPs (Au 4.5 mg/mL), together with PA spectra of endogenous oxygenated hemoglobin and deoxygenated hemoglobin. (b) PA imaging of phantoms containing composite NPs (Au 4.5 mg/mL) at the single wavelength, 800 nm. (c) Representative in vivo single-wavelength (800 nm) PA images of mice before and after IV injection of composite NPs at a dose of 22.50 μg/g Au and 168.50 μg/g Gd. Images were taken preinjection and at 10 min and 60 min postinjection. (d) Representative in vivo multiwavelength PA images of mice before and after IV injection of composite NPs. Images were processed by spectral unmixing. Green, AuNR@mSiO2@Gd2O3(CO3)2 NPs; blue, deoxygenated hemoglobin; red, oxygenated hemoglobin. (e) Quantification of PA contrast enhancement in the spleen, liver, and kidneys in the AuNR@mSiO2@Gd2O3(CO3)2 NP channel (mean ± SD, n = 3).
AuNR−SiO2−Gd NPs, which results in an MR signal increase in \( T_1 \)-weighted MRI.

Before and after intravenous injection of AuNR−SiO2−Gd NPs at higher doses in normal albino C57BL/6 mice, both \( T_1 \)-weighted and \( T_2 \)-weighted MRI were performed over the whole mouse. \( T_1 \)-weighted MRI was acquired using a \( T_1 \)-GRE sequence, and \( T_2 \)-weighted MRI was acquired using a \( T_2 \)-FSE sequence. Figure 5c shows that when AuNR−SiO2−Gd NPs were administrated systemically at higher doses, (1) 2.25 \( \mu g/g \) Au and 16.90 \( \mu g/g \) Gd; (2) 4.50 \( \mu g/g \) Au and 33.70 \( \mu g/g \) Gd, there was a significant decrease in the MR signal (darker region) in the liver and spleen in both \( T_1 \)-GRE and \( T_2 \)-FSE images. This MR signal decrease is due to the \( T_2 \) shortening effect caused by Gd at higher concentrations, which leads to an MR signal decrease in both \( T_1 \)-weighted and \( T_2 \)-weighted MRI.

AuNR−SiO2−Gd NPs accumulated mainly in the liver and spleen, resulting in an observably lower MR signal as compared to the surrounding organs in the \( T_1 \)-GRE and \( T_2 \)-FSE images (Figure 5d). The MR signal decreased further at 24 h compared to the signal at 1 h. A higher dose of Gd gave a greater decrease in the MR signal in the liver and spleen. Thus, the transverse magnetic relaxation (\( T_2 \) shortening effect) is dominant, and the longitudinal magnetic relaxation (\( T_1 \) shortening effect) is overwhelmed in all organs at a higher dose of AuNR−SiO2−Gd NPs.

**In Vivo CT imaging.** The contrast enhancement properties of AuNR−SiO2−Gd NPs for CT imaging were investigated by intravenous injection of NPs at a dose of 11.25 \( \mu g/g \) Au and 84.25 \( \mu g/g \) Gd in normal albino C57BL/6 mice. The CT attenuation level was measured before and after contrast...
administration by respiration-gated CT scans (Figure 6a). Before contrast administration, CT imaging was unable to differentiate soft tissues within the abdominal area since different organs had similar CT attenuation. After systemic administration, NPs mainly accumulated in the liver and spleen, which correlates to the previous results from MRI and PAI, resulting in higher attenuation and a brighter CT contrast in these organs (Figure 6b). In contrast to conventional iodinated contrast media, no obvious accumulation in the kidneys was observed after 30 and 60 min.

To explore the possibility of simultaneous multimodal CT/MR/PA imaging, we performed MRI and PAI on the same animals at 1 and 24 h after contrast administration. Results from MRI and PAI were consistent with CT imaging (Figure 6c,d). However, at the dose of 84.25 μg/g Gd, there was a decrease in the MR signal (darker region) in the liver and spleen on the T₁-GRE images due to the T₂ shortening effect as discussed above. In PAI, PA signals from AuNR–SiO₂–Gd NPs, oxygenated hemoglobin, and deoxygenated hemoglobin were well separated after spectral unmixing and there was strong PA contrast enhancement in the liver and spleen due to NP accumulation. All three modalities showed a change in contrast medium distribution over time, with the highest effect observed in the liver and spleen 24 h postinjection.

The kinetics and biodistribution of AuNR–SiO₂–Gd NPs, expressed as the concentrations of Au and Gd, were further evaluated in animals on 1, 24, 48 h, and 7 days postinjection of the contrast agent using inductively coupled plasma atomic emission spectroscopy (ICP-AES). ICP measurements of Au and Gd were consistent with the results obtained from MRI, PAI, and CT imaging (Figure S9a). AuNR–SiO₂–Gd NPs were mainly accumulated in the lungs, liver, and spleen after...
systemic administration. Rapid distribution of the particles in the lungs at 1 h was observed, which was then cleared until day 7. The concentration of Au and Gd in the spleen was the highest on day 2, significantly decreased on day 7. The amounts of Au and Gd in the liver were relatively stable over the examination period, showing a slow clearance from day 1 to day 7.

In Vivo CT/MR/PA Imaging on a PDAC GEM Mouse Model. Following the confirmation and characterization of AuNR–SiO$_2$–Gd NPs in normal albino C57BL/6 mice with all of the three imaging modalities, we investigated the contrast enhancement, depth of penetration, and imaging capacity of AuNR–SiO$_2$–Gd NPs in a GEM model of PDAC.

Of the available Kras mutant PDAC mouse models, the KPF model phenocopies human PDAC in terms of preneoplastic changes, malignant progression, tumor metastases, and chemoresistance. In KPF mice, the histological, molecular, genetic, and clinical hallmarks of PDAC patients are recapitulated through the tissue-specific expression of oncogenic Kras and inactivation of the tumor suppressor p53 in the pancreas. The Flp-FRT recombinase system was utilized to generate pancreas-specific Kras and p53 mutations. The mechanism underlying the Flp-FRT system involves flippase (Flp), a yeast-derived recombinase, which recognizes a pair of Flp recombinase target (FRT) sequences in the genome that flank the region of interest. The Pdx1-Flp transgenic mouse lineage was generated to express Flp recombinase under the

Figure 7. In vivo CT/MR/PA imaging of AuNR@mSiO$_2$@Gd$_2$O(CO$_3$)$_2$ NPs in KPF mice. KPF mice received IV injection of composite NPs at a dose of 11.25 μg/g Au and 84.25 μg/g Gd. (a) Representative CT images. Images were taken preinjection and at 24 h and 48 h postinjection. Dorsal views of the whole body are shown. (b) CT attenuation measurements over the tumor, liver, and spleen in CT images (mean ± SD, n = 3). (c) Ratio of CT attenuation over the tumor and liver (mean ± SD, n = 3). (d) Representative T$_2$-FSE MR images. Images were taken pre-injection, and at 24 h and 48 h postinjection. Dorsal views of the whole body are shown. (e) SNR in T$_2$-FSE, over the tumor, liver, spleen, kidney-cortex, and kidney-medulla (mean ± SD, n = 3). (f) Ratio of SNR over the tumor and liver (mean ± SD, n = 3). (g) Representative T$_1$-GRE MR images. Images were taken preinjection and at 24 h and 48 h postinjection. Dorsal views of the whole body are shown. (h) SNR in T$_1$-GRE, over the tumor, liver, spleen, kidney-cortex, and kidney-medulla (mean ± SD, n = 3). (i) Ratio of SNR over the tumor and liver acquired by T$_1$-GRE (mean ± SD, n = 3). (j) Representative in vivo multwavelength PA images of KPF mice before and after intravenous injection of composite NPs. Images were processed by spectral unmixing. Green, AuNR@mSiO$_2$@Gd$_2$O(CO$_3$)$_2$ NPs; blue, deoxygenated hemoglobin; red, oxygenated hemoglobin.
control of the mouse Pdx1 (pancreatic and duodenal homeobox gene-1) promoter, which is a pancreatic tissue-specific promoter, active during embryonic development.28 The FSE-KrasG^{G12D/4} transgenic mouse lineage was generated by the knock-in of an FRT-stop-FRT (FSF) cassette, silencing a Kras allele containing the oncogenic codon 12 mutation (Kras^{G12D}). KPF mice were generated by two-step cross breeding. To achieve the conditional activation of oncogenic Kras in the pancreas, KF (Pdx1-Flp; FSE-Kras^{G12D/4}) mice were generated by cross breeding Pdx1-Flp lineage mice with FSE-Kras^{G12D/4} lineage mice. The KF mouse model can already recapitulate human PDAC disease progression, from well differentiated to undifferentiated tumors.26 To accelerate PDAC development, KPF mice were generated by cross breeding KF mice with a mouse lineage carrying an FRT-flanked Trp53 allele (Trp53ff), which leads to the tissue-specific inactivation of the p53 tumor suppressor in the pancreas in addition to oncogenic Kras^{G12D} expression. Starting at two months after birth, KPF mice were examined regularly by palpation and tumor formation was confirmed by high-frequency ultrasound B-mode scanning. KPF mice with tumor sizes above 5 mm were enrolled in imaging experiments.

To evaluate the feasibility of AuNR−SiO2−Gd NPs as a multimodal contrast agent for PDAC, triple modality imaging was performed on tumor-bearing KPF mice. After intravenous injection at a dose of 11.25 μg/g Au and 84.25 μg/g Gd, the time-dependent biodistribution of AuNR−SiO2−Gd NPs was tracked by MRI, CT, and PAI.

As shown in Figure 7a, without contrast administration, CT imaging is unable to differentiate a pancreatic tumor from the surrounding soft tissue within the abdominal area. This is because both tumor and nontumor tissues have similar levels of X-ray attenuation in CT scans. After systemic administration of AuNR−SiO2−Gd NPs, accumulation was mainly observed in the liver and spleen. PDAC tumors in KPF mice are highly fibrotic and stroma-enriched. Nests of tumor cells are surrounded by dense collagen deposits from numerous myofibroblasts with poor vascularization. This results in a very limited uptake of NPs in the pancreatic tumor. The CT attenuation of the tumor was thereby lower than that of the surrounding tissues, providing a negative contrast. Before the intravenous injection of NPs, the liver, tumor, and spleen had similar levels of attenuation; 24 h postinjection, there was a significant increase in CT attenuation in the liver and spleen, while no obvious change was observed in the tumor (Figure 7b). At 48 h postinjection, there was further distribution and accumulation of NPs in the liver and spleen as indicated by an increase in CT attenuation, while CT attenuation in the tumor stayed at the same level. The negative CT contrast in the PDAC tumor was calculated as the ratio of CT attenuation between the tumor and the liver (Figure 7c). The CT contrast for tumor-to-liver decreased from 0.7 before IV to 0.29 at 24 h and to 0.27 at 48 h.

A similar biodistribution pattern of AuNR−SiO2−Gd NPs in KPF mice was observed by both T_1-weighted GRE MRI and T_2-weighted FSE MRI (Figure 7d-g). As described previously, AuNR−SiO2−Gd NPs at a Gd dose of 84.25 μg/g gave a T_2 shortening effect, dominating the MR signal in normal mice. In KPF mice, we observed that the liver and spleen became darker in both T_1-GRE and T_2-FSE images at 24 and 48 h postinjection due to NP accumulation in these two organs. At the same time, PDAC tumors became relatively brighter in T_1-GRE and T_2-FSE images because of the poor distribution of NPs and the low concentration of Gd within the tumor mass. This finding is consistent with our previous in vitro data. In addition, the necrotic regions of the tumor gave a brighter contrast in T_2-FSE due to the low concentration of Gd. SNRs over the ROI in the tumor, liver, and spleen were measured at different time points postinjection (Figure 7e-h). At 24 h and 48 h postinjection, liver and spleen showed a significant SNR decrease for both T_1-GRE and T_2-FSE, while tumors showed an SNR increase at T_2-FSE but not at T_1-GRE. The positive MR contrast in PDAC tumors at T_2-weighted MRI, calculated as the ratio of SNRs between the tumor and liver, increased from 1.75 before IV to 5.43 at 24 h and to 6.10 at 48 h (Figure 7f).

In PAI, at 60 min postinjection, a higher level of NP distribution in the area surrounding the tumor mass was illustrated by a stronger PA signal outside the tumor area (Figure 7j). The separation of PA signals between AuNR−SiO2−Gd NPs and the endogenous PA background in KPF C57BL/6 mice was not as successful as that observed in albino C57BL/6 mice. This is probably due to PA signal interference between AuNR−SiO2−Gd NPs and the melanin present in black-skinned C57BL/6 mice. Melanin has a broad PA absorbance spectrum in the wavelength range 680–970 nm,29 which overlaps the PA absorbance spectrum of AuNR−SiO2−Gd NPs. After hair removal, the melanin in the skin of C57BL/6 mice probably reduced laser penetration, resulting in a high PA background.

Organs and tumors were collected from KPF mice at 1 and 48 h postadministration. Quantification of Au and Gd was performed using ICP-AES. Only low concentration of Au and Gd were detected in the tumors ([Au] 0.10 μg/g; [Gd] 1.43 μg/g) at 1 h, ([Au] 0.15 μg/g; [Gd] 2.7 μg/g) at 48 h, respectively). The organ/tumor ratio of both Gd and Au was calculated for the surrounding organs, i.e., liver, spleen, and kidneys. As can be observed in Figure S9b, significantly higher ratios (fold) of Au and Gd concentration were found in the spleen, liver, and kidneys, which is consistent with the results obtained from MRI, PAI, and CT imaging.

To our knowledge, this is the first time a triple modality imaging contrast agent was developed and evaluated in the Kras/P53 PDAC transgenic mouse model. The preparation and application of core−shell structured gold nanorods have been described.30,31 However, our in vivo imaging data suggested that AuNR−SiO2−Gd NPs as a multimodal imaging agent has great potential to be applied in the PDAC diagnostic imaging. The clinical imaging-based approach to detect primary PDAC tumors in the pancreas includes ultrasound endoscopy, CT, and MRI.32 Due to the difficulty in distinguishing PDAC from the surrounding pancreatic parenchyma and other soft tissues, an intravenously administrated contrast agent is required to reach satisfactory performance, for example, US with microbubbles, CT with iodine contrast, and MRI with gadolinium contrast. It is worth noting that the distribution pattern of the imaging contrast in PDAC is different from the other types of cancers that are better vascularized. The contrast-highlighted anatomical difference between PDAC and surrounding soft tissues (pancreatic parenchyma, liver, and spleen) is based on a lower contrast agent uptake by PDAC and a higher contrast agent uptake by the surrounding soft tissues. Such differences in the distribution pattern are most probably due to the extremely low perfusion into the tumor mass, contributed by the extensive tumor-stromal/extracellular matrix component and
low vascularity. Therefore, most of the described enhanced permeability and retention (EPR) effect and higher accumulation of NPs in tumor tissue compared to normal tissues cannot be applied to PDAC tumors. Unfortunately, most of the previous investigations have reported NP-based imaging agents for PDAC based on orthotropic pancreatic cancer models. The orthotropic models are well established; however, they do not represent the disease features in PDAC patients. The contrast enhancement property of our AuNR−SiO₂−Gd NPs in KPF mice is consistent with that of the microbubble, iodine contrast, and gadolinium contrast in PDAC patients. Compared to transplantation pancreatic cancer mouse models, KPF mice fully represents the genetic, molecular, andpreneoplastic/malignant progression characteristics of human PDAC. Therefore, this study provides direct evidence to translate the application of AuNR−SiO₂−Gd NPs into clinically used CT, MRI, and photoacoustic endoscopy, which is under development for clinical usage. CT scan as first-line PDAC diagnostic imaging allows rapid acquisition with a high spatial and temporal resolution. Compared to that of CT, MRI has a lower resolution, while it provides good soft tissue contrast. Ultrasound/photoacoustic endoscopy is highly expertise-dependent and provides good resolution for small lesions and additional molecular information such as oxygen saturation/hypoxia. The comprehensive imaging characterization of PDAC by multimodal imaging in combination with a blood-based biomarker, genetic information, a history of pancreatitis/pancreatic cyst/diabetes will allow us to identify potentially curable PDAC patients in high-risk groups at the early stage. Furthermore, multimodal imaging of PDAC using AuNR−SiO₂−Gd NPs as a contrast agent will provide not only anatomical information such as tumor size but also the degree of vascularity as a readout to be associated with the differentiation and grading of the tumors. In addition, the accumulation of AuNR−SiO₂−Gd NPs in the liver may also provide the imaging possibility to identify PDAC patients with hepatic metastasis, which is one of the major causes of mortality in PDAC.

Figure 8. H&E staining and Sirius Red staining on tumors collected from KPF mice. Panels (a−f) correspond (have the same field of view (FOV)) to panels (g−l). Slides (a−f) were stained using H&E, while slides (g−l) were stained using Sirius Red and Fast Green counterstaining to recognize the fibrotic tissues. Pancreatic morphological features can be observed by H&E staining at 40× (a−c) and 100× (d−f). The extent of fibrosis within the tumor mass is shown by Sirius Red staining at 40× magnification (g−i) and 100× magnification (j−l). (d) PanIN 1 lesions (black arrow) and PanIN 2 lesions (white arrow) were illustrated. (e) PanIN 3 lesions (black arrow) and carcinoma cells (white arrow) were illustrated. Dashed zones in 40× images are shown at higher magnification (100×) below. 40× images, scale bar 200 μM; 100× images, scale bar 100 μM.
Histological Analysis and Blood Analysis. H&E staining was carried out to assess tissue morphology and histopathological lesions. Collagen detection was carried out using Sirius Red and Fast Green counterstaining. Sirius Red stains all types of collagen proteins, whereas Fast Green stains all noncollagenous proteins.\(^\text{33}\) Histological analysis showed that the PDAC tumors developed in KPF mice were highly heterogeneous, covering the full range of lesions as observed in patients, i.e., from the pancreatic intraepithelial neoplasia (PanIN) precursor lesion to progressed PDAC (Figure 8). The tumors had ductal morphology, abundant stroma with collagen deposition, and a necrotic core at the center. The outer region of the tumor sections showed PanIN lesions at various stages surrounded by abundant fibrosis (Figure 8a,g). PanIN 1 lesions (black arrow) and PanIN 2 lesions (white arrow) were observed at the tumor edge together with a few normal acini and significant fibrosis (Figure 8d,j). The middle regions of the sections were comprised of carcinoma and abundant fibrosis (Figure 8b,h). PanIN 3 lesions (black arrow) and carcinoma cells (white arrow) were surrounded by extensive fibrosis (Figure 8e,k). The inner region exhibited vast areas of necrosis and fibrosis (Figure 8c,i). The majority of the central zone was occupied by necrotic and fibrotic areas (Figure 8f,l). There was an increase in the level of collagen deposition and stromal composition from the tumor edge to the tumor center. Such histological data are consistent with the previously described data from in vivo imaging in the present investigation. The poor distribution of NPs in PDAC was most probably due to the high fibrotic content and poor vascularity of the tumor. The heterogeneity of the lesions within the tumor mass and the central necrotic core led to heterogeneous patterns of contrast enhancement in multimodal imaging.

To evaluate the toxicity of NPs with respect to the various organs, a histopathological examination was performed on the heart, lungs, liver, spleen, pancreas, and kidneys at 48 h postinjection (Figure S10). Heart sections showed normal architecture of cardiac myocytes with centrally placed nuclei. No obvious evidence of a toxic effect on either the renal tubules or glomeruli was observed in the kidneys. The alveolar areas of the lungs retained their normal structure, with no sign of inflammation or of increased cellularity in the pulmonary septa. The white pulp and the hematopoietic red pulp of the spleen showed normal structure and organization. Non-tumorous pancreatic tissue demonstrated a normal islet morphology surrounded by an exocrine portion of the pancreatic tissue without obvious signs of inflammation. Finally, the liver retained a normal hepatic architecture with homogenously sized nuclei.

To investigate the hepato- and hematological toxicities of NPs, hematological analyses and liver enzymes including alanine transaminase (ALT) and aspartate transaminase (AST) assays were performed on day 1, day 2, and day 7 postadministration of the contrast agent. The white blood cell (WBC) count, red blood cell (RBC) count, and ALT and AST enzyme activity are shown in Figure 9. A decrease in WBC counts on day 1 and day 2 postinjection can be observed, which was normalized on day 7. RBC and other hematological parameters (Figure S11), such as hemoglobin (HGB), hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), and red cell distribution width.
Scheme 1. Schematic Illustration of the Procedure for the Synthesis of AuNR@mSiO2@Gd2O(CO3)2 Composite NPs as the Contrast Agent for Multimodal Imaging

**CONCLUSIONS**

In summary, we have developed a multimodal imaging agent, which can provide enhanced MRI, CT, and PAI contrast properties. Our in vitro and in vivo data demonstrated that AuNR@mSiO2@Gd2O(CO3)2 NPs are highly effective as a multimodal (MRI/CT/PAI) contrast agent for PDAC and might facilitate an early diagnosis of PDAC patients, which certainly would benefit the clinical outcome.

**EXPERIMENTAL SECTION**

_Synthesis of AuNR@mSiO2@Gd2O(CO3)2 Core−Shell NPs and PEGylation._ All chemicals were purchased from Sigma-Aldrich Chemie GmbH (Munich, Germany) and used as received without further purification, except when mentioned specifically. AuNRs were prepared using a nonseeded method. Typically, 1.2 L of an aqueous solution of cetyltrimethylammonium bromide (CTAB; 0.1 M) was mixed with HAuCl4 (1 mM) and stirred until the solution was homogeneous. To this mixture, 1.2 mL of AgNO3 (0.2 M) was added, followed by the sequential addition of ascorbic acid (1 M, 2.4 mL) and NaBH4 (0.01 M, 1.2 mL) as a reducing agent. After 1 h, the reaction vessel was moved to a water bath (25 °C) and was gently shaken overnight. To coat individual AuNRs with a uniform layer of mSiO2, using CTAB, 10 mL of as-synthesized AuNR suspension were diluted with 10 mL of deionized DI water and the pH was adjusted to ca. 12 using NaOH. Finally, tetraethyl orthosilicate (TEOS; 0.1 M) was slowly introduced when the temperature of the solution reached 70 °C. The AuNR@mSiO2 NPs obtained were redispersed in 20 mL of DI water. Various amounts (0.5, 2.0, or 5 mL) of growth solution containing 38 mM Gd(NO3)3·6H2O and 2 M urea were added to achieve different coating thicknesses of gadolinium oxide. The reaction was conducted at 80 °C for 2.5 h. Afterward, the composite NPs were washed twice with DI water with centrifugation and were then dispersed in ethanolic NH4NO3 (20 mg/mL) solution at 60 °C for 1 h to extract CTAB from the mesopores. The AuNR@mSiO2@Gd2O(CO3)2 composite NPs obtained were then modified with the linker 3-(triethoxysilyl)-propylsuccinic anhydride (TESPS, 94%, abcr GmbH, Karlsruhe, Germany) to allow grafting with a copolymer of poly(ethylene glycol) (PEG), which provides antibiofouling features. The PEG copolymer was synthesized separately through the reaction between poly(lactic-co-glycolic) acid (PLGA; PURASORB PDLG 5002A, 15 kDa, Purac Biomaterials, Gorinchem, Netherlands) and bis(3-aminopropyl)-terminated PEG via carbodiimide conjugation using 1-ethyl-3-(3-dimethylaminopropyl) (EDC) carbodiimide) and N-hydroxysuccinimide (NHS) at room temperature. The PLGA-PEG copolymer obtained was precipitated, washed, and freeze-dried. AuNR@mSiO2@Gd2O(CO3)2 composite NPs modified with TESPS were redispersed in dimethyl sulfoxide (DMSO) and reacted with the PLGA-PEG copolymer via carbodiimide conjugation (Scheme 1). To differentiate between the contribution from gold and that from gadolinium on CT attenuation, mSiO2@Gd2O(CO3)2 NPs were also prepared and tested as a reference. Mesoporous silica particles were first synthesized by adding TEOS to a mixture of H2O, CTAB, and NaOH (with a molar ratio of 2000 H2O/0.125 CTAB/0.3 NaOH/1 TEOS) at 80 °C with stirring for 2 h. The Gd2O(CO3)3 layer was then coated onto the surface of the mSiO2 NPs using the same method mentioned above.

_Characterization of Nanoparticles._ The morphologies of the NPs were examined by high-resolution transmission electron microscopy (HRTEM) using a JEM-2100F electron microscope (JEOL Nordic AB, Sweden) operating at an accelerating voltage of 200 kV. Dark-field imaging was performed using high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM), and elemental analysis was carried out by energy-dispersive X-ray (EDX) spectroscopy in a JEM-2100F HRTEM. Optical absorbance spectra of particle suspensions were recorded using a PerkinElmer Lambda 750 UV/vis/NIR spectrometer. X-ray diffraction (XRD) patterns were obtained by a PANalytical X’Pert Pro powder diffractometer with Cu Kα radiation (45 kV, 35 mA). The hydrodynamic diameters of the particles in suspension were measured with a DeltaNano Zetasizer (Beckman Coulter, Inc.). Elemental concentrations were measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES, ICAP 650, Thermo Scientific Inc.). Laser irradiation experiments were conducted using a tunable, pulsed Q-switched Nd:YAG laser (repetition rate of 40 Hz and pulse duration of 2.2 ns) and a 532 nm pulsed Nd:YAG laser (repetition rate of 20 Hz and pulse duration of 3 ns) with a spot area of ca. 0.25 cm² and a power density of 0.8 W/cm². To quantify Au and Gd in organs, organs were weighed and digested in 5 mL of (67%) HNO3 for 24 h at room temperature. This liquid solution was then further digested by mixing 0.1 mL of sample with 0.3 mL of aqua regia and finally diluted until 10 mL (e.g., 100 times dilution) for ICP-AES measurements.

_Spectrophotometric Determination of the Concentration of Free Gd³⁺ Ions._ The concentrations of free Gd³⁺ ions leached from AuNR@mSiO2@Gd2O(CO3)2 composite NPs in a aqueous suspension were determined based on the differences in optical absorbance between free and complexed xylene orange dye in the visible spectrum. To prepare a calibration curve, 1 mL of xylene orange (50 mM) in acetic buffer solution (pH ca. 5.80) was added to 0.1 mL of gadolinium standard solution at different Gd³⁺
concentrations (0/10/20/30/40/50 μM, respectively), and the absorbance spectra of these solutions were collected in the range 350–800 nm. The ratios between the two absorbance maxima at 573 and 433 nm (A573/nm/A433/nm) were plotted against the gadolinium concentration, leading to a linear relationship, which was used as a calibration curve. Before taking the measurements, AuNRs@SiO2@Gd2O3(OCO2)3 composite NPs with different thicknesses of the Gd2O3(OCO2)3 layer were incubated with xylene orange in acetate buffer for 4 days. Then, the optical absorbance at 573 and 433 nm was measured for all samples at least three times. The calculated absorbance ratios were used to estimate the concentration of free Gd3+ ions released from the composite NPs by correlation with the calibration curve.

**Cytotoxicity Evaluation by the MTT Assay.** Cytotoxicity evaluation was performed using the A549 human lung adenocarcinoma epithelial cell line (obtained from the American Type Culture Collection) and the immortalized mouse pancreatic stellate cell line (gift from Dr. Raul Urrutia and Dr. Angela Mathison, Mayo Clinic College of Medicine, Rochester, Minn.). Cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 1 mM sodium pyruvate, penicillin–streptomycin (100 U/mL penicillin, 100 μg/mL streptomycin), and 10% fetal bovine serum and were grown under 5% CO2 and 95% relative humidity at 37 °C. Short-term cell viability was determined by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay, which reflects the mitochondrial function of cells. Briefly, cells were seeded at a density of 1 × 104 cells/well in 96-well plates. After 24 h culture, the medium was changed to fresh DMEM without phenol red and serum. Three replicates were used for each sample, and three independent experiments were performed. After exposure to the NPs for 24/48 h, the supernatant was removed and cells were washed once with phosphate-buffered saline (PBS) (pH 7.4). One hundred microliters (100 μL) of the MTT solution (0.5 mg/mL) was added and incubated for 3 h at 37 °C. Finally, 50 μL of DMSO was added to dissolve the formazan crystals. MTT conversion was quantified by measuring the absorbance at 570 nm using a spectrophotometer (SpectraMax 250, Molecular Devices). Cell viability was determined by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay, which reflects the mitochondrial function of cells. Briefly, cells were seeded at a density of 1 × 104 cells/well in 96-well plates. After 24 h culture, the medium was changed to fresh DMEM without phenol red and serum. Three replicates were used for each sample, and three independent experiments were performed. After exposure to the NPs for 24/48 h, the supernatant was removed and cells were washed once with phosphate-buffered saline (PBS) (pH 7.4). One hundred microliters (100 μL) of the MTT solution (0.5 mg/mL) was added and incubated for 3 h at 37 °C. Finally, 50 μL of DMSO was added to dissolve the formazan crystals. MTT conversion was quantified by measuring the absorbance at 570 nm using a spectrophotometer (SpectraMax 250, Molecular Devices). Cell viability was quantified in terms of absorbance at 570 nm and was normalized to the absorbance recorded from cells that had not been exposed to NPs.38 The WST-1 assay was performed according to the kit description as previously described.39

**In Vivo Phantom MR Imaging and CT Phantom.** Phantom samples were prepared by fixing a suspension of AuNRs@SiO2@Gd2O3(OCO2)3 composite NPs in 3% agarose gel matrix loaded into polypropylene tubes. Phantom samples for MRI contained composite NPs with a series of fixed concentrations of 0.005, 0.01, 0.05, 0.1, 1, and 2 mM. Contrast agents used in clinical practice, including contrast-enhanced MRI, were coregistered with gray-scale B-mode imaging. Within an ROI, quantification of the PA average from AuNRs@Gd2O3(OCO2)3 and oxygen saturation were performed using the Spectro unmixing tool (VisualSonics, Inc.) and the OxzyZed tool (VisualSonics, Inc.), respectively.

**In Vivo MR Imaging.** MR images were acquired by a Vevo PHANTOM imaging chamber (VisualSonics, Inc.). NP samples were loaded into PE-20 medical polyethylene tubing using a syringe with a 27 gauge needle. PA spectra were analyzed under Spectro Mode scan from 680 to 970 nm with a step size of 5 nm. During PAI, a tunable laser (680–970 nm) was used.25 Pulse-to-pulse energy fluctuation was continuously monitored, and variations exceeding 25% resulted in the recalibration of the system before further imaging. Multimodal imaging object was acquired at a frequency of 21 MHz (MX250, VisualSonics, Inc.) combined with a high-efficiency Vevo Optical Fiber (medium). Multispectral and volumetric photoacoustic images were exported for offline analysis using Vevo LAB. In the multimodal PA mode, PAIs at 680, 804, 850, 900, 924, and 954 nm created images of oxygenated hemoglobin, deoxygenated hemoglobin, and AuNRs@SiO2@Gd2O3(OCO2)3, which were coregistered with gray-scale B-mode imaging. Within an ROI, quantifications of the PA average from AuNRs@SiO2@Gd2O3(OCO2)3 and oxygen saturation were performed using the Spectro unmixing tool (VisualSonics, Inc.) and the OxzyZed tool (VisualSonics, Inc.), respectively. **In Vivo CT Imaging.** CT imaging was performed using a high-speed μCT scanner (Quantum FX, PerkinElmer, Waltham, MA). CT images were acquired at an X-ray source current of 200 mA, a voltage of 90 kV, a field of view (FOV) of 73 × 73 mm2, a scan time of 4.5 min with respiration gating. Acquired CT images were visualized and analyzed via a 3D Viewer using existing software within the Quantum FX system. During scanning, mice were anesthetized using 2.5–3% isoflurane to minimize motion artifacts from respiration. Following the scanning process, mice were revived under a heating lamp and returned to their home cages.

**US and PA Imaging.** All US and PA scans were performed on a VisualSonics Vevo LAZER Imaging System (VisualSonics, Inc., Toronto, Canada) as previously described.25

**In vitro phantom PAI was performed using a Vevo PHANTOM imaging chamber (VisualSonics, Inc.). NP samples were loaded into PE-20 medical polyethylene tubing using a syringe with a 27 gauge needle. PA spectra were analyzed under Spectro Mode scan from 680 to 970 nm with a step size of 5 nm. During PAI, a tunable laser (680–970 nm) was used.25 Pulse-to-pulse energy fluctuation was continuously monitored, and variations exceeding 25% resulted in the recalibration of the system before further imaging. Multimodal imaging object was acquired at a frequency of 21 MHz (MX250, VisualSonics, Inc.) combined with a high-efficiency Vevo Optical Fiber (medium). Multispectral and volumetric photoacoustic images were exported for offline analysis using Vevo LAB. In the multimodal PA mode, PAIs at 680, 804, 850, 900, 924, and 954 nm created images of oxygenated hemoglobin, deoxygenated hemoglobin, and AuNRs@SiO2@Gd2O3(OCO2)3, which were coregistered with gray-scale B-mode imaging. Within an ROI, quantifications of the PA average from AuNRs@SiO2@Gd2O3(OCO2)3 and oxygen saturation were performed using the Spectro unmixing tool (VisualSonics, Inc.) and the OxzyZed tool (VisualSonics, Inc.), respectively.
with a horizontal bore magnet, a solenoid coil (diameter of 35 mm), and a 450 mT/m gradient. Mice were anesthetized using 1.5% isoflurane with medical air at a flow rate of 2 L/min and placed on the MRI scanner bed. T₂-weighted MR scans were performed using a fast spin echo (FSE) sequence with the following parameters: TR/TE = 10960/55.6 ms, flip angle 90°, slice thickness 1 mm, FOV 100 × 35 mm², 300 × 90 matrix. T₂-weighted MR scans were performed using a gradient echo (GRE) sequence with the following parameters: TR/TE = 702/6.4 ms, flip angle 90°, slice thickness 1 mm, FOV 100 × 35 mm², 300 × 100 matrix. MRI images were visualized and analyzed via InterView Fusion software (Mediso, Budapest, Hungary). In vivo signal-to-noise ratios (SNRs) were calculated for different ROIs. SNRs were calculated using the equation SNR = 0.655 × S/σ, where S denotes the average signal of the ROI and σ is the noise defined as the standard deviation of the signal in an ROI placed in background air (free of ghosting artifacts).

**Necropsy and Histology.** The mice were sacrificed at 48 h after the NP injection. The histological analysis of the tumor, heart, lungs, liver, spleen, pancreas, and kidneys was performed using light microscopy. The histological analysis of the tumor, heart, lungs, liver, spleen, pancreas, and kidneys was performed using a light microscope after hematoxylin and eosin (H&E) staining. Collagen detection in PDAC tumors was performed using Picosirius Red (Histolab, cat. no. H-2175.0500)/Fast Green counterstaining (CertiStain, Merck, cat. no. 1.04022).

**Blood Analysis.** The hematologic analysis was performed using VETSCAN HMS (ABAXIS) according to the manual instruction. Alanine transaminase (ALT) and aspartate transaminase (AST) activity in serum were measured using the ALT and AST assay kit (Certistain, Merck, cat. no. 1.04022). The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsami.0c15430.

**ASSOCIATED CONTENT**

1. Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsami.0c15430.

TEM images, EDS spectra, temperature evolution curves, absorbance spectra, cell viability assays, Au and Gd concentrations by ICP-AES, H&E staining, and concentrations of leached free Gd³⁺ (PDF)

**AUTHOR INFORMATION**

**Corresponding Authors**

Ying Zhao — Division of Experimental Cancer Medicine, Department of Laboratory Medicine (LABMED), Karolinska Institutet, SE-141 86 Stockholm, Sweden; Clinical Research Center, and Center for Allogeneic Stem Cell Transplantation (CAST), Karolinska University Hospital, Huddinge, SE-141 86 Stockholm, Sweden; Email: ying.zhao.1@ki.se

Moustapha Hassan — Division of Experimental Cancer Medicine, Department of Laboratory Medicine (LABMED), Karolinska Institutet, SE-141 86 Stockholm, Sweden; Clinical Research Center, and Center for Allogeneic Stem Cell Transplantation (CAST), Karolinska University Hospital, Huddinge, SE-141 86 Stockholm, Sweden; Email: moustapha.hassan@ki.se

**Author Contributions**

Y.Z. and F.Y. contributed equally to this work. Y.Z., F.Y., and M.H. conceived the study. Y.Z. and F.Y. performed experiments and analyzed data. T.B.B. contributed to the acquisition and interpretation of MRI/CT phantom data. X.L. contributed to histological analysis. R.He, W.Z., R. Heuchel, and S.O. contributed to KPF line breeding. R.E.-S., N.F., W.J.P., and J.D. contributed to histological analysis. R.He, W.Z., R. Heuchel, and S.O. contributed to NP characterization. M.M. supervised the study. M.H. wrote the manuscript with input from all authors.

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Notes
The authors declare no competing financial interest. Animal studies were approved by the Stockholm Southern Ethical Committee and performed in accordance with the Swedish Animal Welfare law.

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