X-ray imaging of tumor growth in live mice by detecting gold-nanoparticle-loaded cells

Chia-Chi Chien1,2, Hsiang-Hsin Chen1, Sheng-Feng Lai1, Y. Hwu1,2,3, Cyril Petibois4, C. S. Yang5, Y. Chu6 & G. Margaritondo7

1Institute of Physics, Academia Sinica, Nankang, Taipei 115, Taiwan, 2Department of Engineering and System Science, National Tsing Hua University, Hsinchu 300, Taiwan, 3Advanced Optoelectronic Technology Center, National Cheng Kung University, Tainan 701, Taiwan, 4Université de Bordeaux, CNRS UMR 5248 - CBMIA, F33405 Talence-Cedex, France, 5Center for Nanomedicine Research, National Health Research Institutes, Miaoli 350, Taiwan, 6National Synchrotron Light Source II, Brookhaven National Laboratory, Upton 11973-5000, NY, USA, 7Ecole Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland.

We show that sufficient concentrations of gold nanoparticles produced by an original synthesis method in EMT-6 and CT-26 cancer cells make it possible to detect the presence, necrosis and proliferation of such cells after inoculation in live mice. We first demonstrated that the nanoparticles do not interfere with the proliferation process. Then, we observed significant differences in the tumor evolution and the angiogenesis process after shallow and deep inoculation. A direct comparison with pathology optical images illustrates the effectiveness of this approach.

Gold nanoparticles (AuNPs) are already used as contrast agents1–5 in x-ray imaging. However, their applications to radiological cell tracing are still hindered by the limited upload in individual cells which gives insufficient image contrast. This is a serious handicap with respect to other techniques such as visible light6, X-ray7 fluorescence or Raman8,9 imaging. Such a limitation is regrettable since radiological cell tracing could otherwise provide very valuable information on physiological and pathological mechanisms. In particular, it could elucidate the mechanisms of cancer cell migration and their relations to tumor development. In this study, we present experimental evidence for the removal of the above limitation in cancer-cell-related tests in mice.

We succeeded in loading sufficient amounts of AuNPs, >50 pg/cell, in the cancer cells to effectively image them without affecting their functions and ability to proliferate. After inoculation in mice, we could detect the cells and accurately determine their positions thanks to the nanoparticle-induced contrast enhancement. This enabled us to monitor in detail the tumor growth. We could specifically analyze the relation between the primary (inoculated) cancer cells, their proliferation and the consequent tumor growth and metastasis.

The analysis was extended to the relation between cancer cell evolution and local tumor angiogenesis. For this, we imaged the tumor-related microvasculature by injecting a high-density BaSO4 nanoparticle colloid as contrast agent and following its flow in the blood system. The monitoring was also performed in three dimensions (3D) by tomographic reconstruction, with ~1 μm resolution.

Finally, particularly interesting details were revealed by ultrahigh resolution x-ray imaging using a zone plate full-field transmission hard-x-ray microscope (TXM)10,11. The instrumental resolution reached in this case ~20 nm.

Nanoparticles can find many possible applications in medicine12 as drug carriers13–16, biomarkers17–18, biosensors19 and contrast agents1–5. Surface modifications expand these applications by enabling them to targets specific sites on cell surfaces20–22, organelles, the nucleus or the extracellular matrix. Even without surface modification, AuNPs can be internalized in cells23–29.

Previous publications reported that the type of cell line and the nanoparticle size influence the amount of uptaken AuNPs per cell27. Our own research indicated that different cancer cell lines can internalize large amounts AuNPs without affecting viability30–32.

Based on these facts, we selected for the present study bare AuNPs33–35 synthesized by intense x-ray irradiation as markers for EMT-6 and CT-26 cells to be inoculated in mice. The experiments were performed for tumors in...
the thigh region, induced by shallow or deep inoculation, as well as for lung tumors induced by tail vein injection. After inoculation, the cancer cells were found not only at the injection site but also at nearby locations, revealing migration rather than just growth. Anomalous microvasculature was not confined to the injection site but primarily near the migrated cells.

We observed detectable differences in the tumor migration between shallow and deep injection of cancer cells. Specifically, there was less localized necrosis and more angiogenesis after deep injection. Finally, the tests on tail vein injection of cancer cells demonstrated the feasibility of revealing cancer development in faraway sites, specifically the lungs.

We also implemented this imaging strategy taking advantage of a new type of AuNP which emits photoluminescent red light when their core size is reduced to ~1 nm and capped by MUA (11-mercaptoundecanoid acid).

The first part of the study concerned the uptake of sufficient quantities of AuNPs by the cells, and specifically their biocompatibility and their effects on cell proliferation. We used two types of AuNPs: bare AuNPs and MUA-AuNPs; both types we extensively tested.

The optical image of Fig. 1c shows cells in the mitotic phase, indicating that AuNPs pass from the first cell generation to the next generations. The partition of the nanoparticles between daughter cells explains the decrease in the AuNP number per cell. We did not detect significant excretion of AuNPs during the cell culture period of 48 hours; however, we cannot rule out their possible excretion from cells via the lymphatic system or by other metabolic mechanisms after inoculation. The excretion would affect our quantitative assessments but not change the qualitative description of cell proliferation.

Results

The first part of the study concerned the uptake of sufficient quantities of AuNPs by the cells, and specifically their biocompatibility and their effects on cell proliferation. We used two types of AuNPs: bare AuNPs and MUA-AuNPs; both types we extensively tested. The one-pot, reducing agent free synthesis produced clean and monodisperse AuNPs with high colloidal stability. Optical microscopy and TEM tests on cell specimens in vitro confirmed that the AuNPs were internalized by endocytosis and did not penetrate into the cell nuclei. The nanoparticles were biocompatible with the cell lines we tested up to a concentration of 2.0 mM in the culture media. We also confirmed that 24 hours after exposure to the bare-AuNP colloid, a large number of AuNPs accumulated in the cytoplasm (Fig. 1a). After 7 days and as many as six cell cycles, the AuNPs were still present in >20% of the cells although the number per cell decreased (Fig. 1b).

The optical image of Fig. 1c shows cells in the mitotic phase, indicating that AuNPs pass from the first cell generation to the next generations. The partition of the nanoparticles between daughter cells explains the decrease in the AuNP number per cell. We did not detect significant excretion of AuNPs during the cell culture period of 48 hours; however, we cannot rule out their possible excretion from cells via the lymphatic system or by other metabolic mechanisms after inoculation. The excretion would affect our quantitative assessments but not change the qualitative description of cell proliferation.

Figure 1 | (a) Optical images show 500 µM AuNPs internalized in all primary EMT-6 cells. (b) and (c): optical images at day 7 of culture of daughter EMT-6 cells produced by proliferation; note that the AuNP number is reduced with respect to Fig. 1a; specifically, (c) shows EMT-6 cells, after 5 or 6 proliferation generations, still carried AuNPs in the mitotic phase. The arrows in Fig. 1b mark AuNP containing daughter cells. (d) Cell proliferation curve, i.e., the ratio between the cell numbers of AuNP-containing cells and control cells; this curve indicates that the AuNPs do not influence the proliferation process. (e) Tumor size for shallow (black and red) and deep (green and blue) inoculation, for AuNP and control cells; this curve indicates that the AuNPs do not influence the proliferation process. Note the similarity of the results for AuNP-loaded cells and control cells. The ANOVA statistical analysis was performed by GraphPad Prism 4 software.
The experiments revealed interesting differences in the tumor growth between deep and shallow inoculation in the thighs. Figure 4 shows results for deep inoculation sites. Overall, there is more proliferation than for shallow inoculation, resulting in less contrast in the tumor core, and more angiogenesis; these are indications of a more aggressive tumor development.

We still see in Fig. 4a regions with dead cells in the core (left arrow), but their density is much less than for shallow inoculation. On the contrary, a larger number of cells proliferated (right arrow). The proliferation decreased the AuNP content per cell because of the partitioning between daughter cells. This resulted in weaker image contrast.

We see in Fig. 4b and 4d that the proliferating cells are in deep locations around the inoculation site (but not confined to it as dead cells). However, Figs 4d and 4e show that proliferating cells are also present in shallow locations. In summary, these findings indicate, for deep inoculation, less localized necrosis and more proliferation than for shallow inoculation.

The smaller number of AuNPs per cell makes the proliferating cells not as easy to observe as dead cells. However, we could track the tumor development by imaging the microvasculature by microangiography. Significantly more microvasculature could be observed in the tumor core for deep inoculation (Figs. 4f–h) than for shallow inoculation (Figs. 3d–h): this could be linked to the faster tumor growth.

In addition, tomographically reconstructed images (Figs 4f–h) of the leaky areas (blood pools) also revealed a lymphatic microvasculature network. This might be an interesting point since the possible involvement of the lymphatic vasculature in the tumor development is still not an entirely clarified issue.

The above results were cross-checked with pathologic images (Figs. 5a–5e) showing that the AuNP distribution in the tumor induced by shallow inoculation is consistent with them. High-resolution TXM images (Figs. 5f–5n) further confirmed the distribution of AuNPs: the highest concentration of AuNPs was found at the inoculation site (Figs. 5m and 5n) and decreased at faraway locations (Figs. 5k and 5l). Once again, we saw that parts of the cells were affected by apoptosis at the inoculation site -- see the pathology images of Fig. 5c and 5d and the TXM image of 5m and 5n exhibited a characteristic morphology with shrunken cytoplasm. No angiogenesis microvessels were found at the inoculation site as it is clear in the 3D reconstructed images of Figs. 2g and 2h.

The fast concentration decrease of AuNPs away from the core cannot be explained only by cell migration, whereas it can be justified by proliferation. This is quite evident from TXM images that directly reveal AuNP aggregates; in particular, almost no aggregates are observed away from the core (Fig. 5).

In addition to tumor development near the inoculation site, we also traced AuNP labeled tumor cells in areas very far from the inoculation site. Specifically, we studied in the metastasis mouse model\cite{27,28} the lungs after tail vein injection of CT-26 colon cancer cells. The cancer cells were passively transported to the lungs and then developed lung tumors mimicking metastasis. In our tests, we found that initially the cells were attached to vessel walls, and then
started to proliferate. We did not detect any AuNP-loaded cells in other organs, thus confirming that this image strategy can detect such cells far from the inoculation site.

The x-ray microradiography images of Fig. 6 a and b show the related distribution of AuNP aggregates (>3 μm) in the lung tissue. The TXM micrographs of Figs. 6 c, d and f were able to detect <60 nm nanoparticles in tumor cells and reveal cancer cells near the lung capillary vessels. Such small nanoparticle aggregates are not visible in the pathology optical images (Fig. 6e) whose detection is limited to aggregates larger than 200 nm. Figures 6f and 6g show tomography results: 6f is a projection image part of a tomography set and 6g a reconstructed image, showing cells with AuNPs together with erythrocytes.

We also exploited a new, recently developed type of AuNP36 to extend this tracing approach to visible light. Using again fast x-ray synthesis but with MUA as capping agent, the nanoparticles were found to become photoluminescent, emitting light at ~602 nm under 290 nm UV irradiation, when the Au core size was smaller than 1.2 nm. The MUA-AuNPs are highly biocompatible up to a concentration of 1 mM and can produce strong red fluorescence when a sufficient quantity is internalized into cells. We specifically measured by ICP-MS (inductively coupled plasma mass spectrometry), that an average of 57 pg, equivalent to $2.1 \times 10^{10}$ of AuNPs was loaded to each cell during 48 hr of culture with 1 mM of MUA-AuNP. The strong fluorescence is visible in subcutaneous inoculation cases even after 30 days. Supplementary data Fig. S7 shows preliminary results of this approach. Right after the inoculation, Figs. S7 a and S7 b the red fluorescence is very bright and remain visible 7 days after the inoculation (Figs. S7 c and S7 d). At the same time, the x-ray microscopy images of Fig. S7 e and S7 f indicate that the amount of MUA-AuNP loaded into EMT-6 cells is sufficient to affect the transmitted x-ray intensity so that we can locate the cell in the mouse thigh after inoculation as well as with bare AuNPs.

Pathology examination of tissue removed from the tumor shows that the photoluminescence is still present long after the inoculation and therefore allows the identification at the cellular level of the presences of MUA-AuNPs. Those cells loaded with fluorescent MUA-AuNPs can still similarly strong red fluorescence more than 30 days after inoculation to mouse tissues (supplementary data Fig. S7 g) which facilitate a convenient confirmation of the pathological examination as well as visual observation.

Note that the fluorescence in Fig. S7 d is much weaker than in Fig. S7 b since the originally inoculated cells are buried under the thick tumor tissue (as seen in the x-ray image of Figs. S7 e and S7 f) as the tumor grows. Correlated the observed fluorescence in Fig. S7 d and x-ray contrast in Fig. S7 e, we can conclude that those fluorescent red light remaining in the Fig. S7 d are not likely those from the originally inoculated cells buried >1 mm under the skin tissue. The fluorescent light still visible in Fig. S7 d, therefore, must be due to daughter cells carrying decreasing amounts of MUA-AuNPs, which is no longer detectible by X-rays, after a few generations of cell divisions, but located at a much shallower position beneath the skin.

**Discussion**

The successful development of AuNP based contrast agent for X-ray imaging was augmented by a novel approach at cell level imaging. We first confirm that the AuNPs are highly stable and biocompatible by demonstrating that the proliferation rate of the tumor cells loaded with a high concentration of AuNPs did not differ from the value with no nanoparticles. These loaded tumor cells therefore maintain their ability to develop tumor after inoculation into animals. The partition of AuNPs with respect to cell proliferation provides means to estimate the generation in the proliferation from their x-ray contrast. Combined with the development of X-ray based microangiography imaging techniques, whose ability to perform complete profiling of microvasculature of tumor angiogenesis was recently...
trations did not affect the cell capability to proliferate; (3) for shallow inoculation we imaged cancer cells and followed their behavior; (2) such concentrations in the tumor microangiogenesis. Specifically, the main findings are: (1) the AuNP concentrations obtained with our method enabled very small AuNPs and the possibility for additional surface conjugation with the sub-cellular level. The discovery of the strong photoluminescent in tissues confirmed the role of AuNPs as contrast and tracing agents at the nanoresolution projection TXM images with two different magnification levels of lung cancer tissues with AuNPs (some marked by arrows) of CT-26 cells. The optical pathologic image in (e) does not show the AuNPs. (f) and (g): tomography results; specifically, (f) is a projection image from a tomography set and (g) the corresponding tomography reconstructed image of the rectangular area of (e) (supplementary data S6 g), revealing <60 nm nanoparticles in tumor cells, seen as black dots in (f) and red dots in (g). The scale bars in (a) and (b) are 50 and 25 μm, in (c)–(d) 24 and 12 μm, and in (f) 1.7 μm.

Figure 6 (a) and (b): projection x-ray images at two magnification levels of unstained lung tissues with AuNPs in formalin solution. The black dots reveal AuNPs in CT-26 cells after injection in the tail. (c) and (d): nanoresolution projection TXM images with two different magnification levels of lung cancer tissues with AuNPs (some marked by arrows) of CT-26 cells. The optical pathologic image in (e) does not show the AuNPs. (f) and (g): tomography results; specifically, (f) is a projection image from a tomography set and (g) the corresponding tomography reconstructed image of the rectangular area of (e) (supplementary data S6 g), revealing <60 nm nanoparticles in tumor cells, seen as black dots in (f) and red dots in (g). The scale bars in (a) and (b) are 50 and 25 μm, in (c)–(d) 24 and 12 μm, and in (f) 1.7 μm. This demonstrated imaging technology offers a reliable imaging method to trace tumor cells, their proliferation and development and, most interestingly, their relation with respect to the tumor angiogenesis in animal models.

Although there were no morphological changes in the animals during in vivo imaging, the current radiation level is quite high and radiation effects on the tumors and their growth cannot be ruled out without more comprehensive tests.

The nanotomography analysis of the locations of the AuNPs in tissues confirmed the role of AuNPs as contrast and tracing agents at sub-cellular level. The discovery of the strong photoluminescent in very small AuNPs and the possibility for additional surface conjugation could promote multimodality imaging with x-ray and visible light microscopy.

With these technical developments, we were able target key questions in the tumor microangiogenesis. Specifically, the main findings are: (1) the AuNP concentrations obtained with our method enabled us to image cancer cells and follow their behavior; (2) such concentrations did not affect the cell capability to proliferate; (3) for shallow inoculation, there was substantial necrosis at the inoculation site, accompanied by proliferation away from it; (4) for deep inoculation, proliferation increased and local necrosis was less relevant; (5) finally, for deep inoculation we also observed migration to large distances from the inoculation site.

Methods

AuNP synthesis and characterisation. Two types of AuNP were used in this study: bare and MUA-coated. The synthesis and characterization procedures were reported previously31–32. In short, these AuNPs are synthesized by a one-pot, reducing-agent-free method taking advantage of the extremely intense x-rays from synchrotron sources. Compared to previous studies exploring AuNPs as contrast agents, our synthesis method offers reducing agent-free surfaces and excellent size control (because of the fast reaction). These factors positively affect the colloidal stability and biocompatibility and allow high-level loading of AuNPs as required for this imaging strategy. The specific photoluminescent properties and the cytotoxicity of MUA-AuNPs were characterized previously33–35. The intense red (602 nm) fluorescent emission with UV irradiation is a result of the high quantum efficiency (>5%) and increased the effectiveness of our imaging strategy, allowing tests with UV confocal microscopy.

Cell culture. EMT-6 cells and CT-26 cells were obtained from American Type Culture Collection (ATCC) and cultured at 37°C in humid air with 5% CO2. EMT-6 cells were incubated with Dulbecco’s Modified Eagle’s Medium: Nutrient Mixture F-12 (DMEM/F12)/10% fetal calf serum (FCS). CT-26 cells were incubated with RPMI-1640/10% FCS. All media were supplied by Gibco.

Proliferation tests. EMT-6 cells were co-cultured with 500 μM AuNPs36–38 for 24 hr, after 1x trypan blue test. EMT-6 cells with and without AuNPs were separately co-cultured with Dulbecco’s Modified Eagle’s Medium: Nutrient Mixture F-12 (DMEM/F12)/10% fetal calf serum (FCS). CT-26 cells were incubated with RPMI-1640/10% FCS. All media were supplied by Gibco.

Tumor development. To load the AuNPs, the EMT-6 cells and CT-26 cells were cultured with 500 μM AuNPs or 500 μM MUA-AuNPs for 24 hr, after 1x trypan blue test. The amount of Au loaded per cell was measured by ICP-MS, obtaining 20 pg for bare AuNP and 28 pg for MUA-AuNP. Then, harvested cells were added to PBS. 50 μl of 1×10⁶ cells/ml EMT-6 cell solution were incubated in the subcutaneous tissue of the left leg region using a 29 gauge needle and an injection rate of 10 μl/sec. To investigate the effect of the inoculation site to tumor growth, two different sites were tested. For shallow inoculation, the needle was inserted into the subcutaneous tissue nearly parallel to the skin. Insertion at an angle ~15 degree with respect to the skin surface resulted in an inoculation point ~1 mm below the skin surface, with the needle tip entering the thigh muscle region. For the model, 100 μl of 1×10⁶ cells/ml of the CT-26 cell solution was introduced by tail vein injection at an injection rate of 10 μl/s with a 29 gauge needle.

All procedures involving animals were approved by the Academia Sinica Institutional Animal Care and Utilization Committee (AS IACUC). BALB/cByJNarl mice were provided by National Laboratory Animal Center, Taiwan. All mice were housed in individual ventilated cages (five per cage) with wood chip bedding and kept at 24 ± 2°C with a humidity of 40–70% and a 12-hour light/dark cycle. The subcutaneous tumor volume was estimated with the formula

\[ v = 0.5 \times a \times b^2 \]

where \( a \) and \( b \) are the smallest and the largest diameters. Tumor imaging started after ~7 days, when they reached a size of 100 to 120 mm³. Overall, 18 mice were used in this experiment: 7 were shallow-inoculated with AuNP loaded cells and 3 were deep-inoculated. Control experiment in which the injected cells were without AuNP concerned 10 mice, 6 for shallow and 4 for deep inoculation. The results were statistically analyzed with the ANOVA by GraphPad Prism 4 software.

In vivo x-ray imaging. Microradiography was implemented with unmonochromated (white) synchrotron x-rays emitted by the 0.1 A beamline wavelength shifter of the National Synchrotron Radiation Research Center (Taiwan)39–40. The photon energy ranged from 4 keV to 30 keV with a peak intensity at energy ~12 keV and the beam current was kept constant at 30e6 C with the top-up operation mode. To obtain 4.5×3.4 mm images, the x-rays were first converted to visible light by a CdWO₄ single crystal scintillator and then captured by an optical microscope with a CCD camera (model 211, Diagnostic instruments, 1600×1200 pixel). The radiation dose was reduced by attenuating the emitted x-ray beam with two pieces of 550 μm single crystalline silicon wafers placed before the animal. During x-ray imaging, the mice were kept under anesthesia using 1% isoflurane in oxygen. Tissues and organs were removed from sacrificed animal right after microradiology tests for tomography and additional TXM imaging.

The exposure time was ~100 ms with a dose of ~34 Gy and the distance between the sample and the scintillator was ~5 cm; a 2x lens in the optical microscope was used to obtain the desired field of view. The size of each pixel in the final image taken with the 2x lens was ~2.8×2.8 μm². To follow the circulation dynamics of the contrast agents, 5–10 images were recorded with further attenuation of the x-rays to keep the accumulated dosage below 100 Gy.

Tissue sample preparation. After developing subcutaneous or lung tumors, the mice were sacrificed by an overdose of Zoletil 50 (50 mg/kg; Virbac Laboratories, Carros, France) administered by intramuscular injection (weight ~20–25 g); then, subcutaneous tissues and lungs were removed. Tissue specimens were immersed in the 3.7% paraformaldehyde for 24 hr. After fixation, the tissues were washed by PBS (pH 7.4) and then dehydrated with the same procedure to remove the remaining wax. Then, the specimens were dehydrated with an acetone series and embedded in the resin or paraffin. The specimens for nano resolution were sliced to 3 μm thick and immersed in Xylene for three times 5 minutes to remove the remaining wax. Then, the specimens were dehydrated with the same procedure.
described above and immersed in distilled water. The specimens were then processed with heavy metal staining (osmium), washed with distilled water 3 times for 5 minutes, dehydrated as above and embedded in Embed-812 Resin (EMS, Hatfield, PA). The same procedure as for x-ray nanoimaging was followed to prepare specimens for optical microscopy except that the heavy metal staining was replaced by H&E staining.

**Tomography.** Thick samples in resin were used to take sets of 1000 images at equal angular distance within 180 degrees; tomographic reconstruction was then performed with the IDL software. All reconstructed images were processed with the Amira 5.2 software to obtain three dimensional pictures.

**High resolution x-ray imaging.** These tests were performed on the 32-ID microscopy beamline of the Advanced Photon Source (APS) at the Argonne National Laboratory. Our full-field x-ray transmission microscope (TXM) uses a set of capillary condensers to precisely illuminate the object by a numerical aperture matched to the Fresnel zone plate (FZP) objectives. The condensers are elliptically shaped glass capillaries. The inner diameter of 0.9 mm was chosen to maximize the vertical acceptance of the APS undulator beam at 65 m from the source.

The estimated monochromatic x-ray flux through a Si (111) double crystal monochromator focused by the condenser was 2 x 10^17 photons/s at 8 keV. The high brightness of the APS and the optimized condensers design yielded an excellent imaging rate of 50 ms/frame with ~1 x 10^11 CCD (charge coupled device) counts per pixel. We used for all images phase contrast with a Au Zernike phase ring placed at the back focal plane of the FZP objective.

1. Chien, C. C. et al. Synchrotron microangiography studies of angiogenesis in mice with microemulsions and gold nanoparticles. *Anal Bioanal Chem* **397**, 2109–2116 (2010).
2. Cai, Q. Y. et al. Colloidal gold nanoparticles as a blood-pool contrast agent for X-ray computed tomography in mice. *Invest Radiol* **42**, 797–806 (2007).
3. Hainfeld, J. Gold nanoparticles: a new X-ray contrast agent. *The British Journal of Radiology* **79**, 248–253 (2006).
4. Menk, R. H. et al. Gold nanoparticle labeling of cells is a sensitive method to investigate cell distribution and migration in animal models of human disease. *Nanomedicine: Nanotechnology, Biology and Medicine* **7**, 647–654 (2011).
5. Reuveni, T. et al. Targeted gold nanoparticles enable molecular CT imaging of cancer: an in vivo study. *International Journal of Nanomedicine* **6**, 2859–2864 (2011).
6. Chen, H. et al. Optical imaging in tissue with X-ray excited luminescent sensors. *Analyst* **133**, 5045–5049 (2011).
7. Lewen, D. V. et al. Intravital synchrotron nanoimaging and DNA damage/genotoxicity screening of novel lanthanide-coated nanovectors. *Nanomedicine* **5**, 1547–1557 (2010).
8. Jiang, W., Papa, E., Fischer, H., Mardyani, S. & Chan, W. C. Semiconductor nanofabrication achievement. *ChemMedChem* **8**, 4593–4596 (2008).
9. Taylor, U. et al. Nonendosomial cellular uptake of ligand-free, positively charged gold nanoparticles. *Cytometry A* **77**, 439–446 (2010).
10. Sadauskas, E. K. et al. Kupffer cells are central in the removal of nanoparticles from the organism. *Part Fibre Toxicol* **4**, 10 (2007).
11. Bohlool, L. et al. Significant effect of size on the in vivo biodistribution of gold composite nanodevices in mouse tumor models. *Nanomedicine* **3**, 281–296 (2007).
12. Chen, H. H. et al. Quantitative analysis of nanoparticle internalization in mammalian cells by high resolution X-ray microscopy. *J Nanobiotechnology* **9**, 14 (2011).
13. Lai, S. F. et al. Size control of gold nanoparticles by intense X-ray irradiation: the relevant parameters and imaging applications. *KSC Adv.* DOI:10.1039/C2RA20260C.
14. Lai, S. F. et al. Very Small Photoluminescent Gold Nanoparticles for Multimodality Biomedical Imaging. *J. Biotech. Adv.* 10.1016/j.jbiotechadv.2012.05.005.
15. Wang, C. H. et al. Aqueous gold nanostols stabilized by electrostatic protection generated by X-ray irradiation assisted radical reduction. *Materials Chemistry and Physics* **106**, 323–329 (2007).
16. Yang, Y. C., Wang, C. H., Hwu, Y. K. & Je, J. H. Synchrotron X-ray synthesis of colloidal gold nanoparticles for drug delivery. *Mater Chem Phys* **100**, 72–76 (2006).
17. Zhang, Q., Hitchins, V. M., Schrand, A. M., Hussain, S. M. & Goering, P. L. Uptake of gold nanoparticles in murine macrophage cells without cytotoxicity or production of pro-inflammatory cytokines. *Part Fibre Toxicol* **5**, 284–295 (2011).
18. Taylor, U. et al. Nonendosomial cellular uptake of ligand-free, positively charged gold nanoparticles. *Cytometry A* **77**, 439–446 (2010).
19. Zeelenberg, I. S., Ruuls–Van Stalle, L. & Roos, E. The Chemokine Receptor CXCR4 Is Required for Outgrowth of Colon Carcinoma Micrometastases. *Cancer Research* **63**, 3833–3839 (2003).
20. Chia-Chi, Chien et al. Complete microscale profiling of tumor microangiogenesis: A microradiological methodology reveals fundamental aspects of tumor angiogenesis and yields an array of quantitative parameters for its characterization. *Biotechnology Advances*. doi:10.1016/j.biotechadv.2011.12.001 (2012).
21. Hwu, Y. et al. Synchrotron microangiography in live mice by detecting gold nanoparticle-loaded cells. *Sci. Rep.* **2**, 610; DOI:10.1038/srep00610 (2012).

**Acknowledgements**

Work supported by the ANR NSIC French-Taiwan bilateral program n° ANR-09-BLAN-0385, the National Science and Technology Program for Nanoscience and Nanotechnology, the Thematic Research Project of Academia Sinica, the biomedical Nano-Imaging Core Facility at National Synchrotron Radiation Research Center (Taiwan), the Fonds National Suisse pour la Recherche Scientifique and the CIBMS. Imaging work performed at Advanced Photon Source is supported by the U. S. Department of Energy, Office of Basic Energy Sciences, Office of Science, Office of Basic Energy Sciences, under Contract No. DE-AC02-06CH11357.

**Author contributions**

CCG designed and performed experiments, analysed data and wrote the paper; HHC, YC and SFL performed experiments; YC, CP, CSY developed analytical tools and analysed data; YH and GM designed experiments, developed analytical tools, analysed data and wrote the paper.

**Additional information**

Supplementary information accompanies this paper at http://www.nature.com/scientificreports