Radiotherapy of the Excretable Radioactive Gold Nanocomposite with Intratumoral Injection

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***Abstract*—we synthesized excretable radioactive gold nanoparticles at gum arabic (^{198}AuNPs@GA) for radiotherapy. The nanocomposite of AuNPs@GA was first synthesized by synchrotron X-ray. After neutron activation, the nanocomposite of AuNPs@GA formed radioactive ^{198}AuNPs@GA in which the radioisotope of ^{198}Au can generate β particles (β_{max} = 960 keV) and gamma ray (E_{gamma} = 412 keV) to kill cancer cells and suppress tumor growth. The efficacy of radiotherapy was evaluated with H460 tumor model by intratumoral injection. At day 7 after intratumoral administration of ^{198}AuNPs@GA, the tumor was significantly suppressed over 90% ($P < 0.01$), compared to the controls. Intratumoral injection with ^{198}AuNPs@GA did not cause serious weight loss of mice. After 2-weeks observation period, ^{198}AuNPs@GA was still mainly accumulated in the tumor. Interestingly, excretion of ^{198}AuNPs@GA in feces and urine was observed from first day to the endpoint of experiment. In this study, the radioactive ^{198}AuNPs@GA nanocomposite not only successfully suppressed tumor growth but also could be excreted through urine and feces, eliminating possible toxic concerns of nanomaterial accumulation in vivo.**

***Index Terms*—Gold nanoparticles, gum arabic, radiotherapy, synchrotron X-rays irradiation.**

I. Introduction

Radiotherapy has been applied as a part of treatment and prevents tumor recurrence before and after a surgery for a primary malignant tumor, respectively [1], [2]. Various radiation sources from machines and radionuclides are practically used in clinical treatments, which can be further classified into external beams and radioactive seed implants for radiotherapy. For deep tumors, the external radiation has to go through normal tissues that may cause damages and health hazards, and therefore radioactive implants are developed to treat the tumors in limited spatial localization [3]. Since the nanotechnology was studied for decades, the nanomaterials have been designed to target a tumor by the unique physical-chemical properties [4]. By combination of nanotechnology and radiotherapy, various radioisotopes, such as ^{64}Cu, ^{90}Y, ^{111}In and ^{166}Ho, can be fabricated to form nanoparticles or incorporated with nanoparticles as nanocomposites for radiotherapy [5], [6].

Gold is a well-studied element that can be synthesized to form nanomaterials from subnanometer clusters to more than 100 nm nanoparticles [7]. After neutron activation, gold-198 (^{198}Au) is also one of the promising radionuclides for radiotherapy [8]-[10]. The radioisotope of ^{198}Au can generate β particles (β_{max} = 960 keV) to kill cancer cells and gamma ray (E_{gamma} = 412 keV) for gamma imaging or single photon emission computed tomography (SPECT). However, when the gold nanoparticles (AuNPs) are used in biological environment, surface modifications are necessary for stabilizing the nanoparticles and preserving their properties. After the in vivo use of nanomaterials, long-term accumulation of the nanomaterials may increase conceivable hazards of the living body [11], [12]. In order to minimize the toxicity concern, the quantum dot with size smaller than 5.5 nm has been reported that can be rapidly and efficiently excreted in urine [13]. Therefore, the design of nanocomposites with the organic template for stabilizing excretable nanomaterials is expected to maintain functions and to increase the potential uses as nanomedicines [14]-[16].

In this study, we first synthesized a nanocomposite of gum arabic stabilized AuNPs (AuNPs@GA) by exposing the mixture of $HAuCl_4$ and GA solution under synchrotron X-ray. The resulting AuNPs@GA was further neutron-activated to generate radioactive ^{198}AuNPs@GA which was evaluated with radiotherapy and toxicity by intratumoral injection. The endpoint biodistribution and excretion of ^{198}AuNPs@GA were measured to assess the problem of nanomaterial accumulation.

II. Experimental Section

A. Preparation of ^{198}AuNPs@GA

Briefly, AuNPs@GA was first synthesized by irradiating the mixture that contained 1 mL of 0.2% $HAuCl_4$ solution and 1 mL of 4 % (w/w) aqueous GA in 5 mL deionized water. The reaction was accomplished in 5 minutes by irradiated with synchrotron X-rays (beam-line BL01A of the National Synchrotron Radiation Research Center, Hsinchu, Taiwan) at room temperature. No chemical reducing agents or catalysts were used for the reaction.

The X-ray produced solution of AuNPs@GA was ultrafiltered with a Vivaspin 500 centrifugal concentrator (molecular weight cutoff 100 kDa) and washed three times with deionized water to remove residual gold ions. Neutron activation of AuNPs@GA was carried out in an Open-Pool

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Reactor (National Tsing Hua University, Hsinchu, Taiwan) with thermal neutrons (7.42×10^{12} n cm^{-2} s^{-1}) and fast neutrons (1.02×10^{13} n cm^{-2} s^{-1}). The AuNPs@GA after neutron activation formed ^{198}AuNPs@GA in which the radioactive ^{198}Au can generate β particles (β_{max} = 960 keV) for radiotherapy.

B. Animal Experiments of Radiotherapy

Male NU/NU mice of 7 weeks old weighing 25–30 g were used for the studies. Tumor-bearing mice were performed by subcutaneous injection with 2×10^6 cells of H460 which were suspended in RPMI medium contained 10% FBS and kept the tumor growing to around 200 mm^3 for radiotherapy of ^{198}AuNPs@GA. The injections were well tolerated and no adverse effects were observed during the 24 h observation period. The ^{198}AuNPs@GA suspension was injected intratumorally to nude mice (N = 4) at a dose of 103.00 ±1.31 μCi/mouse for tumor suppression. The volume of injection was adjusted to 100 μL per mouse. The nude mice were euthanatized until two weeks. Blood was collected through retro orbital plexus region in the heparinized glass tube. Further, the mice were sacrificed by 100% CO_2 and tissues including heart, liver, lung, spleen, kidney, stomach, pancreas, brain, intestine and carcass were collected. Urine and feces were collected at each time point. Organs, urine and feces were collected in a bottle and analyzed by γ counter (2480 WIZARD^2, PerkinElmer). All the irradiation data were deduced from 2.7 days of ^{198}Au half-life to obtain the accurate amount.

III. Results and Discussion

The synthesis of AuNPs@GA is a one-step reaction by exposing $HAuCl_4$ and GA solution under synchrotron X-ray. Briefly, gold ions can be efficiently reduced by the hydrogen radicals and solvated electrons that are generated from photolysis of water under X-ray irradiation [17]. During the reduction, GA serves as a template to confines the growth of AuNPs [18]. This synthesized nanocoposite of AuNPs@GA comprises 2 nm AuNPs in GA and the hydrodynamic size is about 60 nm. Fig. 1 shows the transmission electron microscopy (TEM) images of AuNPs@GA observed by negative stain with uranyl acetate. The size of AuNPs@GA observed in TEM is about 50 nm that is slightly smaller than hydrodynamic size in dynamic light scattering (DLS) because of the dehydration of the nanocomposite in TEM observation.

The solution of AuNPs@GA synthesized by X-ray was ultrafiltered to remove the trace gold ions that could be possibly interfered with the data assessments. The AuNPs@GA was subsequently activated by hot neutrons to form radioactive ^{198}AuNPs@GA for radiotherapy. The ^{198}Au is dominantly following β decay to produce β particles for tumor treatment and has been approved by FDA. The efficient radius of therapy is 1-10 mm in tissue [10]. Tumor suppression was evaluated by intratumoral injection of ^{198}AuNPs@GA (103.00 ± 1.31 μCi/mouse) (Fig. 2a). The radioactive ^{198}AuNPs@GA can significantly suppress the growth of tumor more than 90% (P <0.01) at day 7 after injection (Day 17), compared to the control and the non-radioactive AuNPs@GA treatment. Additionally, toxicity was evaluated by estimation of body weight loss (Fig. 2b). After injection of ^{198}AuNPs@GA, the mice showed a slight decrease (< 20%) during 4 days. At day 7 after injection (Day 17), the body weight was recovered and showed continuous increases in the following days, indicating that ^{198}AuNPs@GA is safe for treatment.

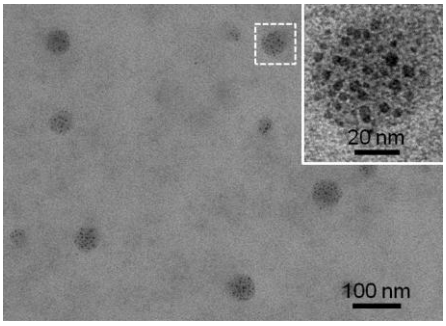

Fig. 1. TEM images of the AuNPs@GA nanocomposite observed by negative staining with uranyl acetate (inset, magnified image).

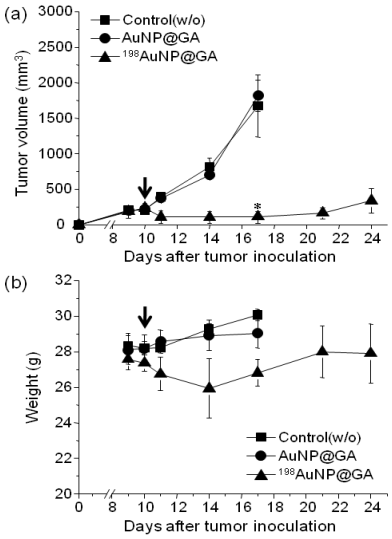

Fig. 2. Intratumoral injection of ^{198}AuNP@GA was evaluated with radiotherapy and toxicity. Tumor suppression curve (a) and body weight loss of mice (b) were shown for therapeutic efficacy and health of mice, respectively. The symbols of square, circle, and triangle stand for the control and the treatment of non-radioactive AuNP@GA and ^{198}AuNP@GA, respectively. The arrow indicates the injection of ^{198}AuNP@GA. (* P < 0.01)

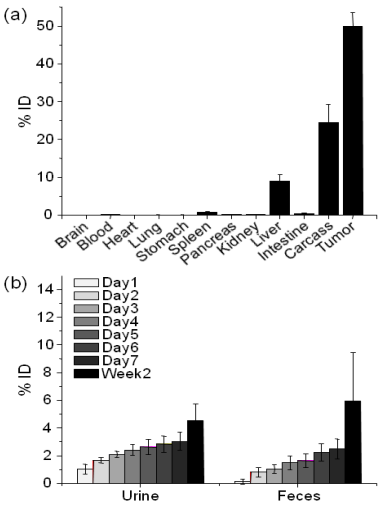

Fig. 3. (a) Biodistribution of ^{198}AuNPs@GA was analyzed after 2-weeks observation period. (b) Accumulated excretion of ^{198}AuNPs@GA in urine and feces were measured at each time point during the treatment.

Fig. 3a shows the endpoint biodistribution of ^{198}AuNPs@GA which was monitored after intratumoral injection up to two weeks. The major accumulation was 50.0% in the tumor, following by 8.9% in the liver. Meanwhile, the clearance increase of ^{198}AuNPs@GA was observed in urine and feces. Fig. 3b shows accumulated amount of excretion in urine and feces at each time point. The ^{198}AuNPs@GA with a large hydrodynamic size can efficiently accumulate in the tumor by intratumoral injection [15]. We proposed that the fine-sized AuNPs (< 2 nm) were released into circulation system following the excretion pathway after GA degradation. The results demonstrated that the ^{198}AuNPs@GA not only had excellent efficiency to suppress tumor growth but also could be excreted possibly by the renal and hepatobiliary systems.

IV. Conclusions

In this study, we synthesized the radioactive ^{198}AuNPs@GA via intratumoral injection for tumor suppression and excretion of the fine-sized AuNPs were observed after the treatment. Intrarumoral injection of ^{198}AuNPs@GA was capable to keep the materials in the tumor site, and then the radioactive ^{198}AuNPs could generate β particles to kill tumor cells and suppress the tumor growth. After the treatment, the fine-sized AuNPs were observed in urine and feces, which were possibly excreted by renal and hepatobiliary systems. The nanocomposite of ^{198}AuNPs@GA can take advantages in therapeutic level for cancer treatment, and their body-excretion can be expected to minimize toxicity concerns from long-term accumulation in vivo.

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