ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

A New ability for X-ray Medical Imaging Based on Gold Nanoparticles.

Auns Q Al-Neami, Logean Q Al-Karam*, and Mohammed H Alwan.

Biomedical Engineering Department, Al-Nahrain University, Baghdad, Iraq

ABSTRACT

X-ray medical imaging is one of the most important imaging techniques because it is low-cost and reachable technique. But it has the weak ability of depict soft tissues and small details between soft tissues at the borders of interference. This limitation was overcome by using iodine-based contrast, however this X-ray contrast agent has many restrictions of utility because of its effects on the human body (side effects). One decade ago, a new variant X-ray contrast agent was found out and understudy to date. The new variance factor is the Nanomaterials and for special the gold, which overcome the restrictions because of the excellent properties of gold nano particles, where the biological apportionment of these particles is higher than iodine compounds. The interaction between bones and soft tissue is more apparent, stay longer at the targeted site which allows for a longer imaging time and all of the above factors enhance the X-ray diagnostic ability. The present study consists of three steps. The first step includes the synthesis of gold nanoparticles using a chemical method (reduction- mediated approach). The second step includes the selection of animal type, housing, preparing the tumor (Adenocarcinoma) and the tumor implantation. The third step consists of the intravenous administration of gold nanoparticles to infected mice then X-ray imaging was taken by conventional X-ray unit. The resulted X-ray images demonstrated that gold nanoparticles were attractive to move toward tumor site through the general circulation and spent more time at the tumor site (inverse the iodine contrast agent) which allows for a longer time of imaging, lower levels of toxicity and side effects. After that the X-ray images shows a lucid tumor appearance and measurable. All of above translated in X-ray diagnostic enhancement.

Keywords: Nanoparticles, X-ray medical, Gold.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

One of the best noninvasive methods of medical imaging is the X-ray imaging but it has some limitations such as its effects on the human body due to the high energy and poor ability of imaging the continuous moving parts of the body (such as lung and heart) and poor distinguish among the neighbor organs or soft tissues at the same location. To solve the last problem of imaging the organs or tissues at the same location and focus on a specific organ or soft tissue rather than the others.

A new term appears which the contrast agent is. The known X-ray contrast agent is the iodine-based X-ray contrast agent which discovered approximately before five decades. It imposes serious limitations such as the limited imaging time, obese patients need extra dose of both X-ray energy(from the X-ray unit) and X-ray contrast agent (i.e. higher side effects) to achieve satisfied X-ray image contrast level, the need for catheterization, renal diseases and toxicity. To overcome most of the mentioned restrictions, the gold nanoparticles is the new promising contrast agent[1].

In general, the nanomaterial is the material which has dimensions between one nanometer and one hundred nanometer (one nanometer= 1 x 10^{-9} meter). Nanomaterial shows different and superior features in comparison to the features of the material in micro level or above (more than one hundred nanometer) as the electrical conductivity, biodistribution inside the human body and melting point temperature and etc.[2]

Gold nanoparticles are suited to use in vivo due to its chemical properties as stability, anti-oxidation ability, inert, low toxicity and it's easy to synthesis. Also, the iodine-based contrast agents contain certain additions to increase its water solubility while the gold nanoparticles have good water solubility[3].

There are serious problems of using the iodine-based contrast agents such as poor contrast of obese patients, toxicity, arterial puncture, renal failure, short imaging time and the weak ability of molecular imaging. While the use of nanoparticles of gold as contrast agent in medical X-ray imaging is preferred because of the following reasons:

- The atomic number of the gold is higher (gold: 79, iodine: 53) which resulted in contrast level about two folds greater than iodine per unit of weight.
- The ability of absorption is higher for gold nano particles than Iodine-based contrast agent (about 5.16 squared centimeter per gram for gold nano particles and 1.94 squared centimeter per gram for Iodine-based contrast agent and 0.169 squared centimeter per gram for soft tissue and 0.186 squared centimeter per gram for bone (X-ray energy=100 Kilo Volt for all)) [4].
- At high voltage of exposure (80-120), Kv the GNP provides minimized interference between the bone (high X-ray energy absorber) and soft tissue (low X-ray energy absorber) and reduced X-ray dose to the patient.

The nanoparticles improved the diagnostics of the medical imaging techniques and allowed to investigate in higher levels of diagnosis such as the molecular imaging where each gold nanoparticle has 250 gold atoms approximately and each atom has the ability to bound the targeted tissue which resulted in X-ray image with higher accuracy while the iodine-based contrast agent reached the target tissue with insufficient concentrations.

Intravenous administration (I.V) shows many advantages in comparison to other administration methods. As example, I.V administration expresses direct arrival to the general circulation (complete delivery with no losses in the way to the general circulation) which allow to quick response. Keep the plasma concentrations stable due to the controlled administration rate. Stopping the injection is enough to halt the unexpected side effect throughout the administration. Also the (I.V.) administration is the ideal solution for the compounds which cannot absorbed or hard to absorb by the digestive tract[5].

ISSN: 0975-8585

MATERIALS AND MATHODS

Animals and injections

The Mouse is the chosen animal for the experiment and twenty mice (gender: female) were undergone implantation of "Adenocarcinoma" subcutaneously (at the back and above the tail)[6]. After Ten days of tumor implantation, I.V. administration of gold nanoparticles were carried out. Experiment and followed protocols were supervised by the research center of cancer diseases, University of Al-Mustansaria.

Gold Nanoparticles

Size of the nanoparticles was determined by instrument produced by Brookhaven instruments Corp. and the principle depend on the Laser beam scattering and the average diameter is 49.9 nanometer. The gold concentration is 0.00346212 gm/ml and the depended ratio of dose to the weight is 0.01 ml/ gm for each mouse.

Synthesis of gold nanoparticles is according the following procedure:

Chemical Synthesis: Martin Method used to synthesize gold nanoparticles ranging from 1-10nm Protocol: Perform all steps in FUME HOOD.

1. Measure 20 ml of ddH_2O into 50 ml graduated cylinder and pour into 125mL Erlenmeyer flask.
2. Keep some extra ddH_2O on ice for later steps in a falcon tube.
3. Weigh out 1.96 mg (0.00196 g) of chloroauric acid trihydrate ($HAuCl_4$ X 3 H_2O) solid on a weigh boat.

• CAUTION: oxidizes quickly – long expose to air should be avoided.
• Plastic or wood scoopula should be used to transfer the solid
• LIGHT SENSITIVE – limit the exposure to light (i.e. short duration).
• Note: chloroauric acid is expensive - try not to be wasteful!

4. by using a micropipette, quickly add ~40 µl of the ddH_2O from the flask to the crystals of chloroauric acid trihydrate on the weigh boat. This should dissolve the solid to generate a yellow mixture on the weigh boat. Then use the micropipette to transfer the yellow liquid to a 1.5 ml microfuge tube.

• By avoiding transfer of the solid form of $HAuCl_4$, the possibility of loss of sample is lessened. Transfer as a liquid is preferable.
• Seal the tube rapidly to limit air exposure of the chemical.

5. Micropipette the $HAuCl_4$ solution directly into the flask and seal the opening with aluminum foil.
6. Add a stir bar to the flask and mix on a plate to allow the solid HAuCl4 to dissolve.

• Nanoparticle synthesis occurs at this step.
• Having the flask opening covered with aluminum foil prevents the escape of fumes from the synthesis reaction.

7. Weigh out 1.47 mg (0.00147 g) of trisodium citrate dihydrate solid.
8. Add the trisodium citrate dihydrate to the mixture in the flask with stirring.
9. On the ice, prepare 0.1 M sodium borohydride ($NaBH_4$) solution.

• Pipet 0.65 ml (650 µl) of cold ddH_2O into a 1.5mL microfuge tube kept on the ice.
• Weigh out 2.46 mg (0.00246 g) of $NaBH_4$.
• Put the measured NaBH4 into the microfuge tube containing ddH_2O and resuspend by pipetting until the solid is fully dissolved (keep on ice throughout).

10. Add 0.6 ml (600 µl) of the cold 0.1 M $NaBH_4$ solution into the mixture in the Erlenmeyer flask while stirring vigorously on the stir plate.

ISSN: 0975-8585

NOTE: the solution should turn dark red immediately - indicating particle formation.

11. The solution is then tested for presence of nanoparticles using UV spectrophotometry

• Expected absorbance peak between 510-525 nm.

12. Once nanoparticles are confirmed in solution:

• Weigh out 0.15 g of L-cysteine solid.
• Add the L-cysteine to the nanoparticle solution with stirring for 10 min.

13. Collect nanoparticles by pelleting using centrifugation at 1,000 RPM at room temperature for 50 minutes.
14. Store the solution in 4°C fridge

RADIOGRAPHS

X-ray instrument is a conventional type(country of origin: JAPAN, manufacture: Shimadzu, serial number: 3M5262F3B010) and the X-ray parameters are 40Kv and 200mAs as shown in figure (2).

X-ray film printer characteristics (country of origin USA, manufacture: KODAK, type: CR MAX, serial number: 59580328) and the computed radiography is illustrated in the figure (3).

The X-ray image shows excellent details after a long time (10 min.) and all of mice stayed alive after the gold nano particles administration and housed for seven days then they returned to the research center of the cancer diseases.

RESULTS

Gold nanoparticles of 49.9 nanometer were administrated via tail vein of mouse bearing "Adenocarcinoma" which is special type of tumor invade the mammary glands. X-ray imaging carried out 10 minutes after I.V administration. More distinguished details of the tumor were obtained as shown in the Figure (4) b. Gold nano particles contrast agent enables measurable detection of tumor.

The X-ray images were carried out at different timings after intravenous to proof the fact of that the small (GNP) don't concentrate in spleen and liver and toxicity is an important issue for study in the Medical X-ray imaging.

The mice intravenously administrated with gold nano particles 2.7 micro gram of gold to gram of mouse weight were stayed alive for seven days without any Syndrome.

3.2 milligram of gold to gram of mouse weight represent The LD50. The ratio of gold nano particles absorption to tumor against muscle about 3.4 after 10 minutes of administration then improve to 9.6 after twenty four hours which enable a clear plan of tumor.

As well as detection of tumor, the ability of the tumor to absorb the X-ray energy will increase due to the existing of gold nano particles which resulted in enhancement of radiotherapy abilities[7].

The gold nano particles solution have viscosity similar to water viscosity (even concentrated solution) while the high level of contrast needs high viscosity of iodine based X-ray contrast agent. Also dried gold nano particles can be resuspend in water or any aqueous buffer later easily, gold nano particles are stable and its features not affected due to storage at suitable temperature.

DISCUSSION

- This study proofed the capability of gold nano particles to use in X-ray medical imaging as a contrast agent as shown in Figure (4).

ISSN: 0975-8585

- The Fig. (4) (a) shows an infected mouse (bearing tumor) without gold nanoparticles administration and the Fig.(4) (b) shows an infected mouse after 10 minutes post intravenous injection of spherical gold nanoparticles and the Fig.(4) (c) shows an infected mouse after 10 min after Intravenous injection of Rod Gold Nanoparticles. Rod gold nanoparticles show enhancement in the contrast level as shown in figure (4) (c) but it is lower than the enhancement of spherical gold nanoparticles as shown in Fig. (4) (b) where the gold nanoparticles attracted to bind with specific proteins, which found at the surface of the tumor and the area of contact between the rod gold nanoparticles and the surface of the tumor is lesser than the contact area of the spherical gold nanoparticles.
- The size of spherical gold nanoparticles effects the enhancement where the size of the spherical gold nanoparticles is proportional inversely with the enhancement the X-ray images.
- The volume of the injection is 0.2 ml for I.V administration while the I.P administration needs a bigger volume. So, according to the high cost of the gold and the X-ray images diagnostic enhancement. The I.V administration is better than the I.P administration.
- The X-ray imaging was carried out after 2, 10 and 60 minutes after the administration and after comparison of the X-ray images for the same animal show the best contrast level after ten minutes of administration.

Fig (1) samples of Gold Nanoparticles

Fig (2) Conventional X-ray unit

ISSN: 0975-8585

Fig. (3) Computed radiography

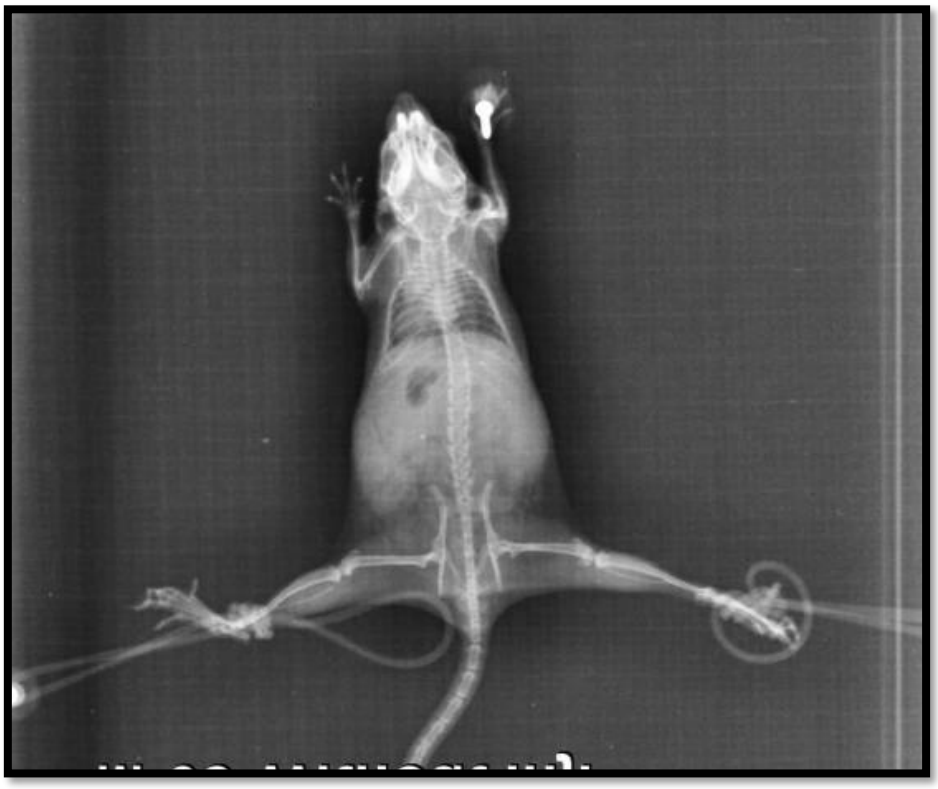


Fig (a)

ISSN: 0975-8585

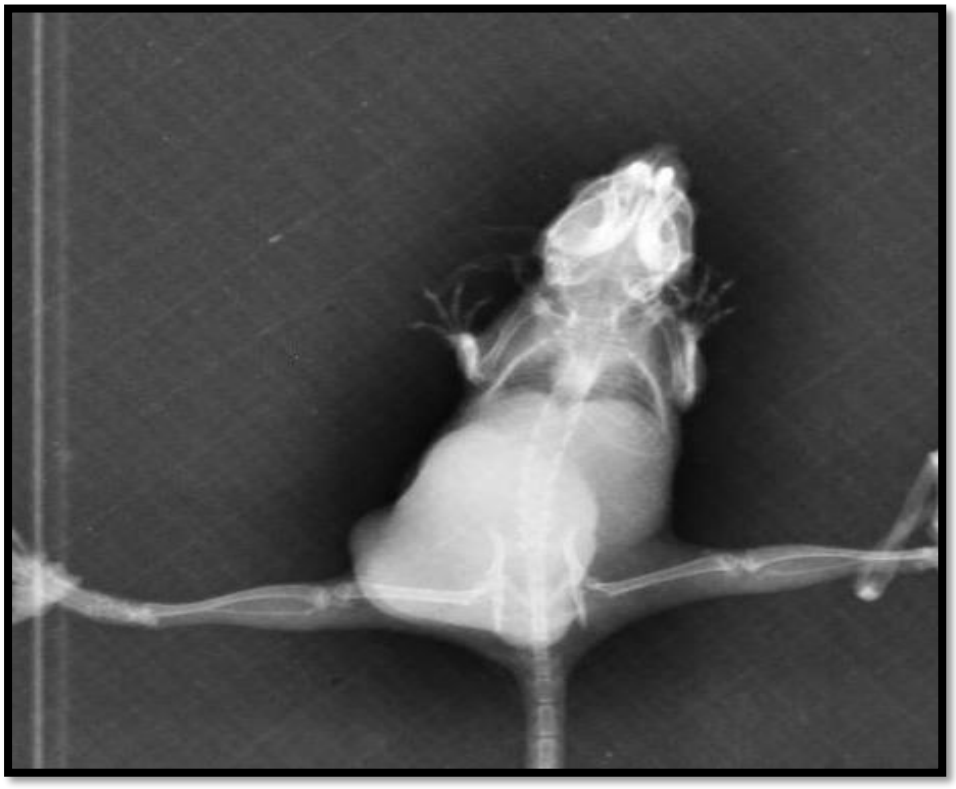
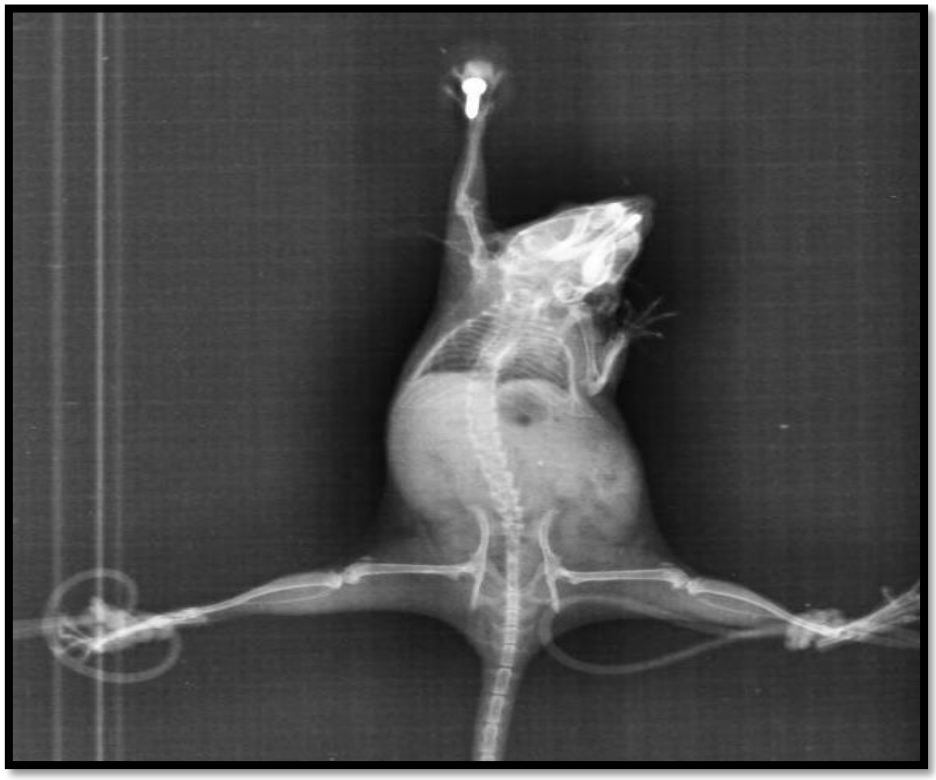


Fig (b)



Fig(c)

Figure (4). X-ray images of living mice hind legs. (a) without administration (b) 10 minutes after I.V. administration of spherical gold nano particles; (c) 10 minutes after I.V. administration of Rod Gold nano particles

ISSN: 0975-8585

CONCLUSION

This study demonstrate four important facts:

First fact: The high contrast and extended imaging time which resulted by the utility of gold nano particles after I.V. administration might open the gate of investigation in minute applications as X-ray medical imaging of minute blood vessels, renal angiography, delineation of stroke, aneurysms, blood vessels malformations, virtual colonoscopy and enhancement the diagnostic ability of mammography[1].

The Second fact is the new X-ray contrast agent offer pharmacokinetic advantages and significant physical advantages in comparison to iodine based contrast agents hence its low toxicity, longer imaging times and higher contrast level than iodine based contrast agents.

The Third fact is the I.V. administration is more control than other administration routes and resulted in X-ray images with more details because of direct path (via general circulation) and volume of injection is lesser than volume of administration routes.

The Fourth fact is the utility of gold nano particles (rod aspect) is less accurate than utility of gold nano particles (spherical).

REFRANCES

[1] J. F. Hainfeld, D. N. Slatkin, T. M. Focella, and H. M. Smilowitz, "Gold nanoparticles: A new X-ray contrast agent," *Br. J. Radiol.*, vol. 79, no. 939, 2006,pp. 248–253.

[2] A. Nouailhat, " *An introduction to nanoscience and nanotechnology*. " 2010.

[3] N. Li *et al.*, "Gold nanoparticles for biology and medicine," *Small*, vol. 1, no. 1, 2010, pp. 3280–3294.

[4] J. H. Hubbell and S. M. Seltzer, "Tables of X-Ray mass attenuation coefficients and mass energy-absorption coefficients (version 1.4)," p. , 2004.

[5] K. Nebendahl, "Routes of Administration," *The Laboratory Rat*. 2000, pp. 463–483.

[6] L. J. T. Young, D. Medina, K. B. DeOme, and C. W. Daniel, "The influence of host and tissue age on lifespan and growth rate of serially transplanted mouse mammary gland," *Exp. Gerontol.*, vol. 6, no. 1, 1971,pp. 49–56.

[7] J. F. Hainfeld, D. N. Slatkin, and H. M. Smilowitz, "The use of gold nanoparticles to enhance radiotherapy in mice," *Phys. Med. Biol.*, vol. 49, no. 18, 2004, pp. N309–N315.