Gold nanoparticles produced by laser ablation in liquids for diagnostic imaging improvements

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Abstract

Pulsed Nd:YAG laser at 10^{10} W/cm^2 intensity was employed to induce gold ablation in liquids. Gold nanoparticles are generated with 10-100 nm size and different solution concentrations. Nanoparticles were characterized using different physical analyses (optical spectroscopy, SEM, TEM, XRF,…). The solution was monitored through optical transmission spectroscopy in the visible wavelength range at concentrations from 1 mg/ml to 100 mg/ml. Surface plasmon absorption resonance was investigated. The biocompatible solution was injected by Biologists in living mice in order to control the organs uptake and decay by using 45 keV X-rays imaging.
The increment of the contrast imaging was investigated and measurements and theoretical aspects are present and discussed.

Introduction

The Surface Plasmon Resonance (SPR) is one of the main optical properties of metal nanoparticles. It consists of a collective oscillation of the conduction electrons excited by the incident electromagnetic field of the light. A metallic nanoparticle can be described as a pattern of nuclei with electrons of quasi-free conduction of moving in its surface. When a particle is illuminated, the light of the electromagnetic field exerts a force on moving conduction electrons toward the surface, thus creating a dipole[1]. This dipole in turn generates an electric field opposite to that of the light, which will force the electrons to return to their equilibrium position. After removing the external electric field the electrons will oscillate with a characteristic frequency called plasmon frequency. The surface plasmons are affected by various factors, first of all the size and shape of the nanoparticle, then the surrounding medium and finally the influence of the electric field of the other near nanoparticles. Recently, Au nanoparticles (NPs) are finding many potential applications in different scientific fields, from Physics to Chemistry and from Material sciences to Bio-Medicine, particularly thanks to the variety of their peculiar properties.
Particular interest is devoted to Au NP due to:

- Their high atomic number and atomic weight;
- It is easy to synthesize stable AuNPs;
- Exhibition of excellent biocompatibility and chemical passivation;
- High electronic density;
- High mass absorption coefficient.

Au significantly changes the absorption of X-rays during radiography, therefore, if suitably introduced into liquids and biological tissues, it may produce high-contrast imaging of

biological systems enhancing the organ image having the higher uptake of the element.
This improvement given by the high mass absorption coefficient of the Au NPs enhances also the absorbed dose of the organs containing the gold solution and find high interest for applications in radiotherapy expositions. Au nanoparticles increases the dose released by ionizing radiation during traditional radiotherapy using X-rays or electrons or innovative protontherapy [2]. Moreover the SPR effect can be employed also to localize heating during photothermal therapy induced by lamps or laser light.
Au nanoparticles are also widely investigated as particles conveyed by special functionalized molecules or drugs to be transported in special organs to be treated. Au NPs form various structures, including nanorods, nanoclusters, nanoshells, nanocages, nanocubes, nanoprisms and nanospheres, which can be well conveyed and spread in the different organs, liquids, tissues and living cells.

Materials and methods

The synthesis of the nanoparticles is one of the top-down methods using laser ablation in liquids. It is the process of removing material from the surface of a solid sample (in our case gold) by means of erosion and vaporization due to the energy released by a repetitive high energy laser pulse. The experimental set-up consists of a high intensity pulsed laser, a set of optics that focus the laser beam on the Au-target surface immersed in a liquid (in our case 2.5 ml of distilled water), as reported in Figure 1.
The procedure was performed at the Laboratory of plasmas laser of Messina University.
A Nd:YAG laser operating at a fundamental frequency of 1064 nm with a pulse energy of 150 mJ, 3 ns pulse duration and with a pulse repetition of 10 Hz was employed. The laser beam is deflected by a prism and focused on the gold sample, placed in a polyethylene holder, through a lens placed at 50 cm distance, under 10 mm water. The laser-generated plasma, containing gold ions, expands in the liquid and finally condenses to form spherical nanoparticles[3]. Au NPs are produced subsequent to laser irradiations from 10 to 30 minutes, in order to obtain different solution concentrations, from 1 mg/ml to 10 mg/ml of gold nanoparticles in distilled water.

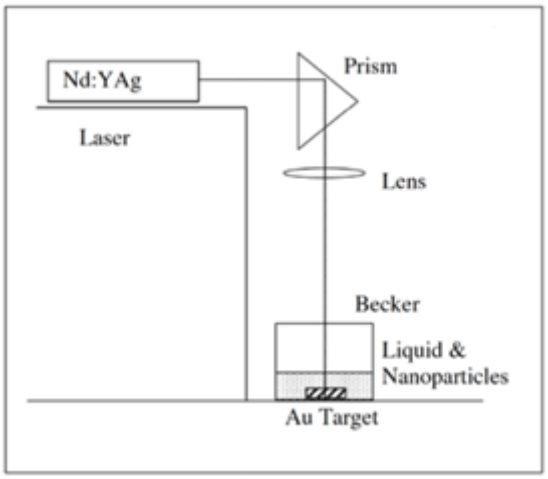

Figure 1: Experimental set up for laser ablation in liquids.

Theoretical studies have evaluated the absorption of 45 keV X-rays in different biological materials (water, soft tissue and cortical bone) without and with different concentrations of Au NPs. By using the NIST database [4] that provides the mass-absorption coefficients of the X-rays for the various materials and elements, it was possible to compare the absorption value in pure materials and the material with the addition of Au nanoparticles. The calculations were carried out starting from the formula of Lambert-Beer (eq. (1)) [5]. The Bragg rule (eq. (2)) has been employed to evaluate the absorption in the compounds (water, soft tissue and bone) with and without Au NP as a function of the percentage of the Au content. Calculations were performed using three cases with the addition of a solution containing 1 mg/ml, 10 mg/ml and 100 mg/ml of Au nanoparticles.

$$I_{trasm} = I_0 \cdot e^{-\frac{\mu_{en}}{\rho}\rho \cdot \Delta x} \qquad (1)$$

$$\varepsilon_{A+B} = \frac{A}{A+B}\varepsilon_A + \frac{B}{A+B}\varepsilon_B \qquad (2)$$

Where in eq. (1) I_{trasm} is the intensity of the transmitted beam, $-\frac{\mu_{en}}{\rho}$ is the mass absorption coefficient as a function of energy, ρ is the density and Δx is the thickness. In eq. (2) ε_{A+B} is the mass absorption coefficient of the compound A+B, ε_A is the mass absorption coefficient of element A, ε_B is the mass absorption coefficient of element B.

The prepared solution was submitted to optical spectroscopy to evaluate the absorbance as a function of the wavelength. To this was used a mercury-argon lamp showing peaks of light in the 200-1000 nm wavelength range, a system of optical fibers and a portable spectrophotometer (Horiba Joben-Yvon, VS70). In addition, the solution was subjected to TEM and SEM microscopy. Finally the solution was injected intravenously into living systems (mice) to assess its possible use as a contrast medium[6,7]. The X-ray imaging diagnostics uses a desktop imaging system at the University of Messina, so called "In Vivo MS-FX PRO" by Bruker[8].

It was possible to perform the X-rays images of the healthy mice by verifying the effectiveness of the gold nanoparticles as a contrast medium. The photo of the X-ray instrumentation is reported in the Fig. 2. The *in-vivo* MS-FX Pro is the first multispectral imaging system with a true microfocus X-ray head, it delivers high resolution X-ray images for co-registration taking 1.2 s for single image.

It is characterized by unmatched imaging versatility with rapid multimodal acquisitions, rapid imaging of fluorescent and radioisotopic labeled molecules in combination with luminescent and X-ray imaging, automatic co-registration of optical images with high resolution X-ray images, and four imaging modalities. Co-register signals from any imaging modality 360° imaging with multimodal animal rotation system (MARS). To maximize the sensitivity the animal position can be moved to adjust the angle position. Very high spatial resolution and superior image quality can be obtained. Exclusive wide angle emission filters enhance the detection sensitivity and image quality. The instrument uses an imaging geometry to improve the image clarity by providing a flat focal plane, an advanced 4.2 megapixel camera, a cooled CCD technology and a powerful 400 W Xenon illumination source. The voltage range is 20-45 kVp, the maximum current 500 µA, the spot Size < 60 µm and tungsten as anode material.

Figure 2: In VIVO MS-FX Pro

Results

Immediately after the laser preparation of the solution containing the Au MPs with a know concentration, directly in a glass test tube, the solution was subjected to absorbance spectroscopic analysis as a function of the wavelength. To this it was employed the mercury-argon lamp, the optical fibers and the spectrophotometer. Figure 3 shows the absorption curve of our solution that has a concentration 1 mg / ml of Au nanoparticles in distilled water. The solution absorbance has a peak due to the surface plasmon resonance centered at 510 nm in the visible region. Subsequently, the solution is subjected to SEM and TEM microscopy.

Figure 4 shows the microscope TEM image

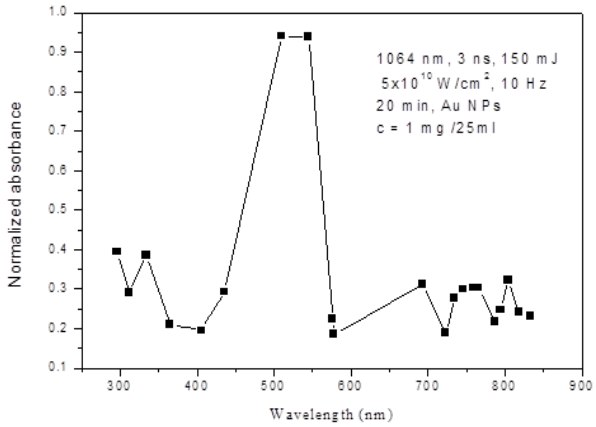

Figure 3: Typical absorbance curve as a function of the wavelength of the solution of gold nanoparticles in water

that indicates that there are two types of nanospheres having mainly 5-7 nm and 20 nm diameter. The TEM images have been performed 48 h after the preparation of the solution at the Charles University in Prague (Czech Republic).

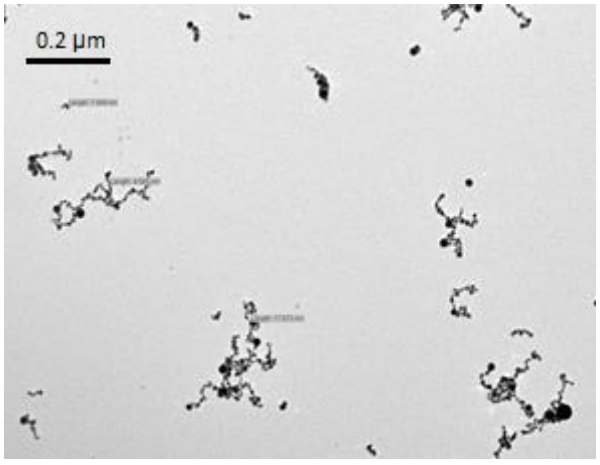

Figure 4: TEM image of the Au NPs

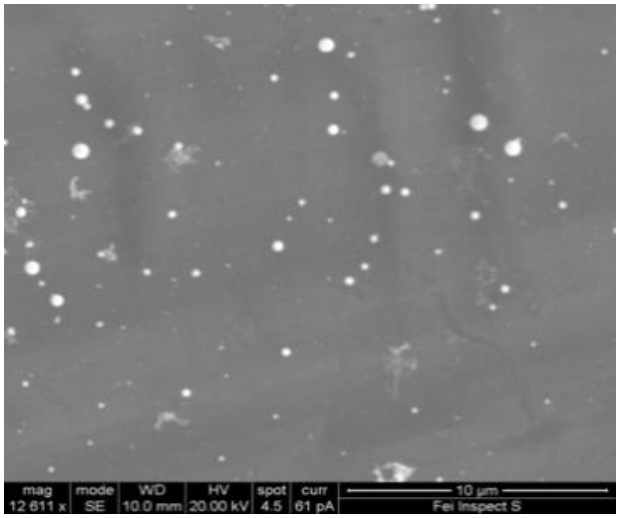

Figure 5: SEM image of Au-NP

Figure 5 shows the microscope SEM image which identifies the phase of the individual nanoparticle spheres. Furthermore, the SEM electron microprobe was used to distinguish the nanoparticles using the characteristic X-ray fluorescence of the Au nanoparticles. Three plots represent the theoretical evaluation of the absorption coefficients in the water, soft tissue and cortical bone.

Each graph shows four curves, the first is relative to the pure material, the second to the material with the addition of Au nanoparticles in a concentration of 1 mg/ml, the third to the material with the addition of Au nanoparticles in a concentration of 10 mg/ml, and finally the fourth to the material with the addition of Au nanoparticles in a concentration of 100 mg/ml (Figure 6).

One hour after the laser preparation of the solution containing Au nanoparticles, the latter is injected via the tail vein in healthy mice.

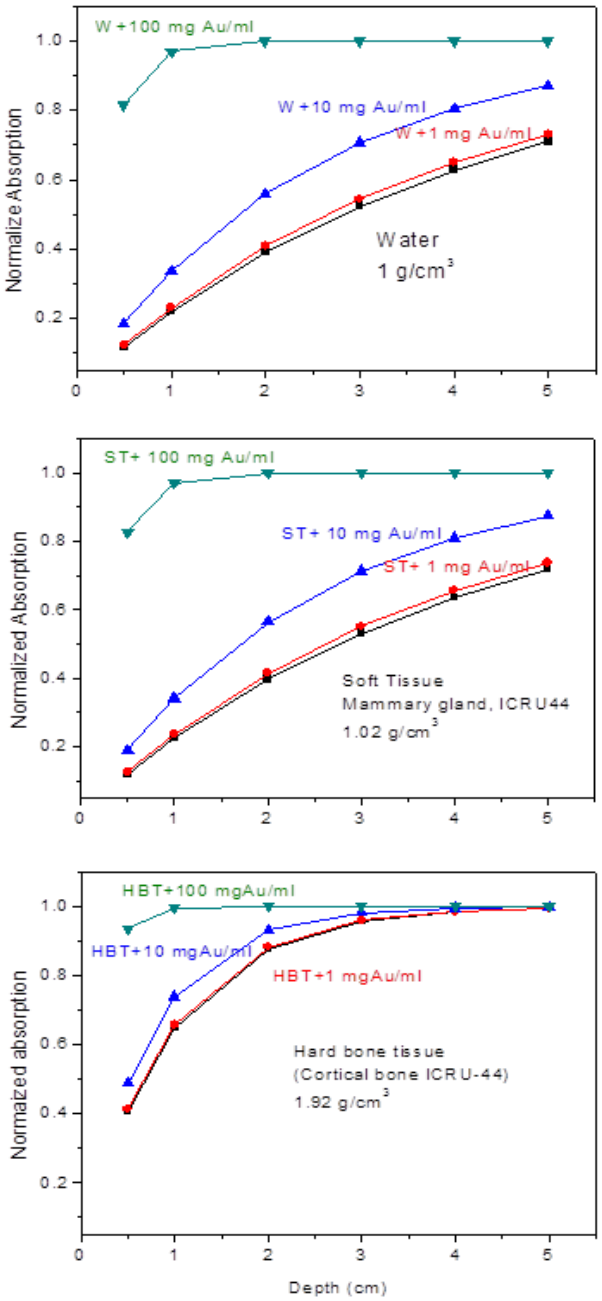

Figure 6: Absorption of 45 keV X-rays as a function of the depth in water (a), soft tissue (b) and cortical bone (c) for different Au NPs concentrations, from 0 up to 100 mg Au/ml.

We have injected 0.01 mg per gram weight of the mouse. The X-ray image measurements are made on a control test subject without admission of Au NPs and mice with the injection of Au nanoparticle solution, in order to have a useful comparison.

The X-ray images were acquired at different times (Figure 7); those reported here are the first after a few minutes, the second after approximately 45-60 minutes and the third after 24 hours.

The image acquisition was continued up to three days after the injection, in order to verify the non-toxicity of the solution and its total expulsion from the organism. The total images have demonstrated that after 45-60 minutes the colon of the mouse appears clearly thwarted thanks to the presence of Au nanoparticles. After 24 hours there is the decay of the solution and the disappearance of the image contrast of the colon.

The mouse lives and the solution seem to be biocompatible.

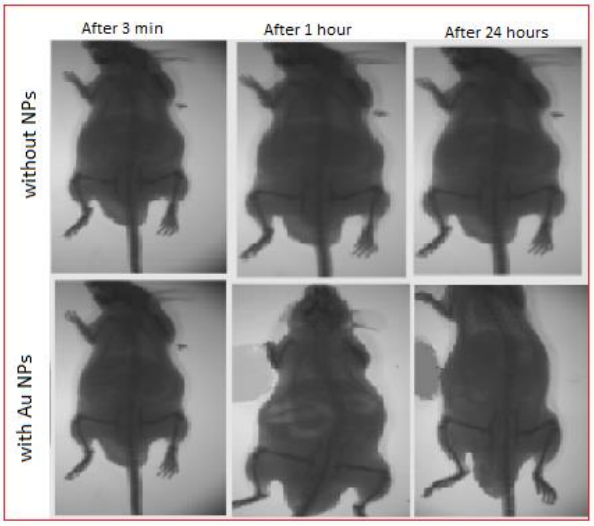

Figure 7: X-ray images of a rat at 3 minute (a), at 45 min (b) and at 48 hours (c) from the injection time in the tail vein

Discussion and Conclusions

The reason why gold has collected so much attention to the development of new contrast media in biological tissues and organs is due to its peculiar properties (biocompatibility, chemical inertia, high atomic mass and absorption coefficient for X-rays, …) that make it suitable for medical imaging applications. In addition it has a massive absorption coefficient greater than the traditional iodine contrast element. In fact, gold has a dynamic contrast of image 2.7 higher than iodine. Furthermore, the Au nanoparticles exhibit good biocompatibility and marginal levels of toxicity. Thanks to their small size can easily travel through the capillaries of the circulatory system, and though the cell membrane. This means that

when the tissues contains Au NPs the absorbed dose of ionizing radiations is lower with respect to that need to a pure tissue, without NPs, in order to give the same level of image contrast.

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