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SYNTHESIS OF BISMUTH NANOPARTICLES FOR BIOMEDICAL APPLICATIONS

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ABSTRACT. Bismuth is a biocompatible material that can be employed in the form of nanoparticles to be used as a possible alternative contrast medium for diagnostics. In particular, it has been prepared as spherical nanoparticles in liquid solution by using the technique of the pulsed laser ablation in water. The high atomic number Bi nanoparticles in the solution show surface plasmon resonance effects, high mass absorption coefficient for soft X-ray interaction and high electron and nuclear stopping powers for electrons and ion beams. This biocompatible solution can be injected into living systems, such as mice, in order to study the up-take effect in different organs with a high contrast spatial localization of the X-ray images in the tissues in which the nanoparticles are confined. The high contrast medium for high-resolution imaging in biological systems can be also used as targeting for successive exposure to ionizing radiation during radiotherapy or hyperthermia of diseased cells. The obtained results demonstrate high contrast images of Bi nanoparticles in biological environment by using the X-ray fluorescence in the electron microscopy and the X-ray absorption in mice.

1. Introduction

Recently, nanoparticles are used for many applications in different scientific fields, such as physics, chemistry, materials science and bio-medicine. Nanotechnology applied to life sciences has led to a better understanding of the cellular and molecular processes and of factors that cause a disease thus by developing more and more the nanomedicine. Advances in this field increase the accuracy and specificity of medical diagnosis. Unfortunately, for most chronic degenerative diseases the most effective treatments are available only in the early stages of the disease. So the early detection is the key to improving patients' survival rates. Among all the diagnostic imaging modes, X-ray imaging techniques are the most used for their simplicity, high spatial resolution and low cost. The improvement of these techniques strongly depends on the development of new X-ray contrast agents. Up to now, the clinically used contrast media consist of small iodine molecules in liquid solutions that allow highlighting the functionality of particular organs. However, the iodine medium can cause serious side effects due to excessive repeated doses necessary to achieve good image contrast. Recently, metallic nanoparticles (NPs), such as Ag, Au and Bi, have been used as alternative contrast agents for radiological imaging (Torrisi *et al.* 2017) owing to their high atomic number and high X-ray absorption. The interest for gold and bismuth nanoparticle solutions is not only for the contrast medium use but also for the radiotherapy efficiency enhancement, in order to maximize in this case the delivered radiation dose to tumours and at the same time minimize the level of damage to surrounding healthy tissues. A complementary approach to potentially achieve this goal involves the localization of high atomic number (Z) elements and compounds in the target tumour tissue before irradiation. The presence of high Z atoms in the target leads to an increase in the cross-section of the photoelectric effect and to the generation of low-energy free radicals by photoelectrons and Auger electrons. These low-energy free radicals have high rates of linear energy transfer (LET) and, consequently, can cause the DNA damage through the generation of reactive oxygen species. Bi NPs have several advantages over iodine because they have a higher Z-number and therefore a higher probability of photoelectric interactions, which depends roughly on Z (Rabin *et al.* 2006; Zhang *et al.* 2014). Bismuth is a heavy metal; it has an unusually low toxicity and high biocompatibility; it has an atomic number of 83; it is a brittle metal with white colour when freshly produced, but its colour turns pink because of the surface oxidation. Bismuth, as a dense element of high atomic weight, is used in bismuth-impregnated latex shields to protect from X-ray in medical examinations, such as computed tomography (TC), mostly as it is considered non-toxic. The Bi mass absorption coefficients of X-ray and the stopping power for electrons and ions are high demonstrating the best behaviour for its use as contrast medium and as targeting element in biological cancer tissues for appropriate radiotherapy expositions (Torrisi 2015). The CT contrasts based on nanoparticles bearing bismuth, in particular bismuth sulphide nanoparticles, have received special attention due to a combination of low price, low toxicity and high X-ray attenuation coefficient (Cormode *et al.* 2010; Veintemillas-Verdaguer *et al.* 2015). The present paper aim is to demonstrate the usefulness of Bi nanoparticles as contrast medium for microscopy images in biological environment studying the uptake and decay in different organs of mice in which they are injected, and as target of tumour tissues in which the radiotherapy or the hyperthermia can be more effectively applied.

2. Materials and Methods

Metallic bismuth nanoparticles (Bi-NPs) were prepared using the technique of the pulsed laser ablation in liquids. A Nd:YAG laser was employed at the 1064 nm wavelength, $3ns$ pulse duration, $100mJ$ maximum pulse energy, 1 mm^2 focused spot and 10 Hz repetition rate. The metal, in form of sheet of about $3cm^2$ surface and 1 mm thickness, was placed on the bottom of a glass beaker, covered by $3-4mm$ distilled water. Generally, a volume of 5 ml of distilled water was employed. Using a 70 mJ pulse energy and an irradiation time of 10 min at a 10 Hz repetition rate and covering the Bi sheet surface with $4mm$ liquid, the ablated mass was of 4 mg; thus in these conditions a solution concentration of $4mg/5ml$, that is $0.8mg/ml$, was obtained. The ablation yield, in terms of removed mass per laser shot, was $0.67\mu g$/pulse and it can be increased rising the laser pulse energy. Longer irradiation times than 20 min were not used because they produce laser absorption in the same solution and consequent decrement of the ablation yield. A scheme of the used set-up is reported in Figure1 a, a photo in Figure1 b and the colour of the solution at the $C = 0.8mg/ml$ concentration in the inset (Figure1 c). The removed atoms in the laser-generated plasma and in the water environment condense in a nanometric particulate. The nanoparticles tend to aggregate forming micrometric particles but the presence of a surfactant liquid (sodium citrate), added to the solution at the minimum concentration of 1 μg/ml, stops the aggregation phase. The produced solution of Bi-NPs in water changes colour during the laser ablation from white transparent to white opaque, to brown and to dark brown colour at high concentration. The Bi-NPs were characterized at the MIFT and CHIBIOFARAM Departments laboratories of Messina University and at the Messina IPCF-CNR laboratories by using FESEM microscopy, X-rays and UV-Vis absorption. In particular the bismuth nanoparticle solutions were diluted with deionised water before taking the UV-Vis absorption spectra. Deionised water was used as reference.

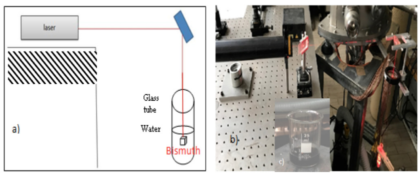

FIGURE 1. Laser Ablation in liquid set up scheme (a), photo of set up in our laboratory (b), and the Bi-NPs solution after the laser ablation (c).

To observe the Bi-NPs morphology and composition a drop of the resulting solution was dried onto a silicon or glass substrates and a field emission scanning electron microscope (FESEM), operating at $5-30kV$, was used. The prepared Bi-NPs were injected in specific organs of living systems (mice) and their spatial location and contrast were controlled using the fluorescence induced by a $(20 \div 45)kV$ X-ray tube of the Bruker-in vivo MS-FX PRO system.

3. Results

The presence of the metallic nanoparticles in the solution induces surface plasmon resonance (SPR) absorption effects showing high absorption at specific wavelength bands, depending strongly on the particle shape, dimensions, concentration and nature of liquid medium.

The resonant absorption is due to the light induction of dipole and electron oscillations on the surface of the metallic nanoparticles, according to the literature (Garcia 2011). Figure 2 reports the UV-Vis absorption spectra vs. wavelength of the concentrated and diluted aqueous solution of Bi-NPs containing the surfactant stabilizer. The absorbance spectra shows a poor resolved peak at about $269nm$; this feature can be assigned to the SPR absorption peak of the Bi NPs that gradually broadens as the size of Bi NPs decreases. Since the Bi clusters with diameter below $10nm$ do not show any SPR absorption band, while the larger nanoparticles ($> 100nm$) show a strong absorption peak at about 280 nm (Wang *et al.* 2005), our spectrum seems to indicate that the Bi-NPs shape is a sphere, with a diameter greater than $10nm$ but smaller than $100nm$, in agreement with the SEM

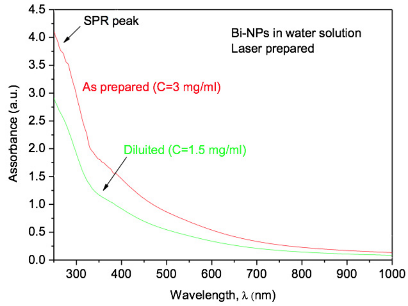

FIGURE 2. Absorbance vs. wavelength of the Bi-NPs at two different concentrations in water.

investigations. Figure 3 (a, b) display typical SEM images of the Bi-NPs solution deposited onto a Si substrate after prolonged drying (6 h). The nanoparticles have spherical shape with a diameter distribution going from about 10*nm* to 100*nm* and an average value of about 25*nm*.

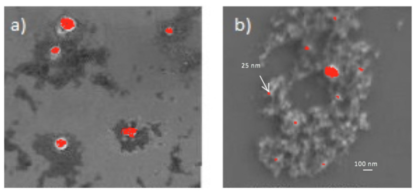

FIGURE 3. SEM photos of the dried Bi-NPs solution deposited on a silicon surface (a, b).

Further physical investigations on the prepared Bi-NPs have indicated that they are crystalline (from the XRD analysis), have metallic characteristics (from the XPS analysis) and contains a small amount of oxygen, mainly due to substrate's contamination (from the EDX analysis). Figure 4 illustrates a typical EDX spectrum of the Bi-NPs observed using a $5keV$ electron beam of the SEM microscope. The qualitative analysis shows the $K\alpha$ lines of C and O elements and the $M\alpha$ line of Bi. The $SiK\alpha$ line comes from the substrate and can be subtracted. Thus, the quantitative analysis indicates Bi, O and C peaks corresponding to the composition of 85%, 10 % and 5%, respectively. The use of Bi-NPs as a contrast medium in living tissues has been tested in mice, injecting them the prepared biocompatible solution in some organs or in the blood flux through their tail vein.

The Bi X-ray fluorescence image was investigated by the Bruker X-ray "In Vivo" acquisition system. Figure 5a shows the image when the solution was injected in the heart. The image shows very well the organ (observed in dark gray scale such as X-ray transmission) and the nearest blood irrigated tissues are very bright due to the X-ray induced fluorescence. Figure 5b reports the image when the solution was directly injected in the Sx kidney. The image illustrates very well the organ (observed in gray scale such as X-ray transmission)

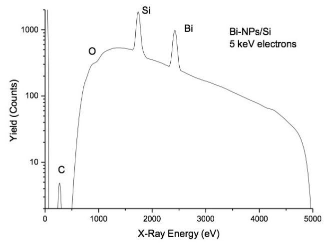

FIGURE 4. Typical EDX spectrum of Bi-NPs induced by 5 keV electrons.

and the nearest blood irrigated tissues are very bright due to the X-ray induced fluorescence. The obtained bio-images are well contrasted and the interested organ shows high spatial resolution of the zones where the Bi-NPs are localized. The images permit to evaluate the up-take and decay of the different organs in healthy and in diseased organisms. The aim of the study was to distinguish the interested organ in order to be observed in detail before that a successive radiotherapy procedure can be applied on the diseased tissues.

FIGURE 5. X-ray fluorescence images showing the Bi-NPs whose solution was injected in heart (a) and Sx kidney (b).

4. Conclusions

The obtained results have showed that Bi-NPs have good biocompatibility and low toxicity and do not affect the normal functioning of the organs. Mice are alive after injection of the solution into the blood through the tail vein. Measurements are being performed on the up-take and the decay of Bi-NPs. These nanoparticles can be used with success as contrast medium for bio-images of diseased and healthy organs and can be employed to targeting tumour tissues to be submitted to radiotherapy with high efficiency. However, to this aim, the nanoparticles must be injected only in the tumour tissues and not in the adjacent healthy ones. In the future we plan to test the laser-synthesized Bi-NPs using the M13 phage as a scaffold. The p8 protein of the phage is functionalized with thiol groups of different lengths and these thiolate regions act as nucleation centres for the Bi^{3+} ions. The size, shape and resistance to the oxidation of Bi-NPs depend on the length of the used thiol group. Thanks to this type of approach it will be possible to direct the Bi nanoparticles in the affected organs, to obtain their bio-image using X-ray fluorescence or absorption, and to proceed to irradiation for the development of radiotherapy. On this subject our group of research has published different scientific articles (Restuccia and Torrisi 2018; Torrisi *et al.* 2018)

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