Preparation and optimization of aminolevulinic acid with gold nanoparticles for photothermal and photodynamic therapies applications

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ABSTRACT

The use of gold nanoparticles (AuNps) as the vehicle for 5-Aminolevulinic acid (ALA) delivery for photodynamic and photothermic plasmonic therapies is a promising approach, especially with the recent demonstration that this photosensitizer immobilization on the particle surface improves reactive oxygen species (ROS) formation, increasing its cytotoxicity. Gold nanorods (AuNRs) present an absorption spectrum shifted to 700 nm, within the tissue transparency window, which allows excitation of the nanoparticles situated deeper in the tissues. Here, we describe a new synthesis method that was applied to control the shape of the gold nanoparticles during its synthesis. To obtain ALA:AuNRs, precursor ALA:AuNps were irradiated by ultrashort laser pulses. The variation of the laser parameters such as pulse energy and duration and irradiation time was assessed. The relevant mechanisms are discussed.

Keywords: Gold nanoparticles, ultrafast phenomena, photodynamic therapy, photothermic therapy, aminolevulinic acid

1. INTRODUCTION

Aminolevulinic acid (ALA) is a precursor of tetrapyrroles in the biosynthesis of chlorophyll and heme [1]. The conversion of ALA to protoporphyrins (PPIX) within tissues induces a photosensitive target that produces reactive oxygen species upon exposure to light [2]. For this reason, it is used in photodynamic therapy (PDT) for diseases as cancer and atherosclerosis [3], the leading causes of death in the world. The oral administration of ALA leads to the preferential accumulation of the fluorescent molecule PPIX within certain types of tissues with inflammatory reaction, cell proliferation and vascular remodeling. This allows fluorescence-based identification of tissue borders during the surgical procedure [4].

The association of ALA with gold nanoparticles (AuNPs), increase the applications possibilities of this drug [3, 5, 6]. The unique surface plasmon resonance features of AuNPs make them candidate materials for photothermal plasmonic therapy (PPTT) [7]. Unlike photodynamic therapy, PPTT does not require oxygen to interact with the target cells or tissues. The photothermal therapy is able to use longer wavelengths, which are less energetic, and therefore less harmful to other cells and tissues [8-10]. The absorbed light is converted into heat to form a hot metallic lattice by two processes: electron-electron relaxation occurring on the femtosecond time scale, and electron-phonon relaxation occurring on picoseconds. The nanoparticles dissipate the generated heat into the surrounding environment, and when they are attached to cancer cells, this heat can change the function of the cells and even destroy them depending on the amount of heat generated [11].

Spherical gold nanoparticles have absorption bands limited to the 520 to 580 nm range for sizes ranging from 10 to 100 nm [12]. These wavelengths are not effective in the therapeutic window from 650 to 1350 nm, where light has its maximum depth of penetration in tissue. On the other hand, when in the nanorods shape, the peak absorption gold nanoparticles can be tuned from 550 nm up to 1 μm by changing their aspect ratio [13].

Biophotonics South America, edited by Cristina Kurachi, Katarina Svanberg, Bruce J. Tromberg, Vanderlie Salvador Bagnato, Proc. of SPIE Vol. 9531, 95314C · © 2015 SPIE
CCC code: 1605-7422/15/$18 · doi: 10.1117/12.2180017

In general, gold nanorods can be synthesized in either rigid templates or in the presence of surfactants. In the former case, the metal ions are reduced inside cylindrical pores of oxide or polymeric membranes [14]. In this case, neutral or charged surfactants are used for growth of the nanoparticles. In aqueous media, hexadecyltrimethylammonium bromide (CTAB) has been one of the most popular molecules in the synthesis gold silver nanorods (NRs) [15].

Femtosecond laser-based irradiation is of particular interest for nanoparticle synthesis, and provides controllable size characteristics [12]. The high intensities originated by femtosecond laser pulses produces nonlinear effects causing ionization of the surface, charge repulsion and transformation of nanorods into the most thermodynamically stable form, which is spherical [16]. The addition of polyethylene glycol (PEG) is found to significantly accelerate the laser-induced reduction processes, as well as control the AuNPs diameter and size distribution in strong laser fields [16].

The reshaping of spherical gold nanoparticles from spheres into rods by femtosecond pulses has not been demonstrated in the literature, to our knowledge. In this work we demonstrate that changing the irradiation parameters, nanorods can be produced from colloidal solutions prepared with Aminolevulinic acid and Polyethylene glycol water medium. The dependence of the nanorods shape on the laser parameters such as irradiation time, pulse energy and pulse duration was assessed. The relevant mechanisms are discussed.

2. MATERIALS AND METHODS

All the reagents used had analytical grade. Chloroauric acid ($HAuCl_4$) and 5-Aminolevulinic acid hydrochloride ~98% (A3785) were purchased from Sigma-Aldrich. To prepare ALA Gold Nanoparticles (ALA:AuNPs) solutions, $HAuCl_4$ were mixed with ALA and Polyethylene glycol (PEG) in Mili-Q water. The process was accompanied by vigorous stirring for 5 minutes. This solution was exposed to a 150-watt xenon lamp during 5 min. Details of the prepared solutions are shown in table 1.

Table 1. Concentrations of the reagents, light exposure time for the prepared solutions.

Solution	Reagents	Ligth Exposure Time
ALA:AuNPs	~15.0 mg of $HAuCl_4$ ~45.0 mg of ALA ALA in 100 mL	5 min
ALA:PEGAuNPs and pH control	~15.0 mg of $HAuCl_4$ ~45.0 mg of ALA ALA in 100 mL PEG (10 mg/ml)	5 min

The ALA:AuNPs and ALA:PEGAuNPs solutions were irradiated with ultrashort pulses from an amplified Ti:Sapphire laser system (Quantronix Odin seeded by a Coherent Mira-Seed-R). The pulses were centered at 800 nm, with duration from 80 to 150 femtoseconds (FWHM), and 1 kHz repetition rate. The laser beam was focused by a 50-mm converging lens inside a 1-cm acrylic cuvette containing the solution. Many irradiations with different parameters were done: to determine the influence of the irradiation timethe pulse energy was kept constant at 450 μJ and the repetition rate at 1 kHz, while the irradiation times were fixed at 2, 4, 16 and 32 minutes. To verify the impact of the pulses energy solutions were irradiated for 16 minutes at 1 kHz, and the pulse energies of 110, 210, 306 and 350 μJ were used. To study the influence of the pulses duration the solutions were irradiated for 16 minutes and 300 μJ, with pulse temporal width of 80, 100 and 150 fs.

The solutions absorption spectra were measured with a UV-Vis Spectrophotometer Shimadzu Multispec-1501, using 1-cm quartz cells. A LEO 906E (Zeiss, Germany) transmission electron microscope (TEM), with images captured by a MegaView III camera and processed by the software ITEM – Universal TEM Imaging Platform (Olympus Soft Imaging Solutions GmbH, Germany) was used for obtaining micrographies. A drop of gold nanoparticles, dispersed in distilled water, was placed onto a carbon-coated copper grid. The excess liquid was removed using a paper wick and the deposit was dried in air prior to imaging.

3. RESULTS AND DISCUSSION

ALA:AuNPs

The UV-vis absorption spectrum of the initial ALA solution presents an absorption band around 270 nm, as shown in the figure 1. In the presence of $HAuCl_4$ and PEG, new bands are observed at 223 nm and 310 nm, corresponding to the Chloroauric complexes in water. Setting the solution pH to 7.2 and illuminating it with the Xe lamp results in nanoparticles with SPR absorption bands around 540 nm. The production of gold nanoparticles after illumination was verified in the TEM micrograph shown in figure 2, which shows nearly spherical particles with sizes ranging from 5 to 40 nm.

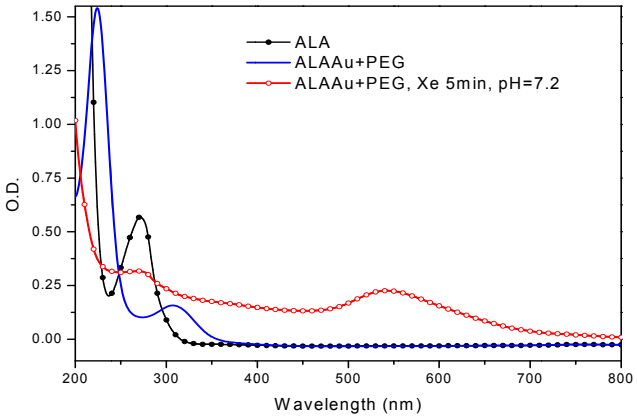

Figure 1: Abosrption spectra of ALA, ALA:AuNPs (Xe for 5 min), and ALA:PEGAuNPs with pH 7.2.

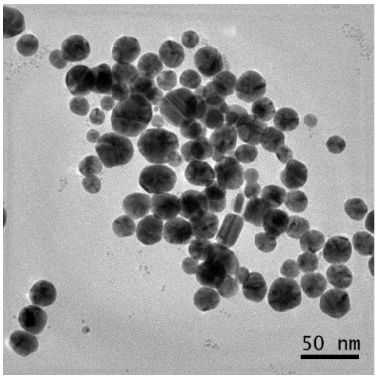

Figure 2. TEM of the gold nanoparticles solutions illuminated for 5 minutes with light xenon (pH ~7.2): ALA:PEGAuNPs.

ALA:AuNRs

ALA:AuNPs with and without PEG solutions were irradiated by 450 μJ, 100 fs ultrashort laser pulses, at 1 kHz repetition rate with different irradiation times of 2, 4, 16 and 32 min. The results are shown in Figure 3. The non-irradiated ALA:AuNP solutions without PEG (figure 3a) present SPR bands centered at 555 nm. After irradiation for 2 min, the SPR band shows a blue shift to 547 nm, and after 4 min the absorption peak displaces to 537 nm, indicating the particles size reduction with rising irradiation time. The increase in the irradiation time to 16 or 32 min, results in the observation of the suppression of the absorption band. For ALA:AuNP solutions with PEG (Figure 3b) without irradiation, the SPR band is centered at 541 nm, and after irradiation during 4 min a blue shift to 531 nm is observed. After irradiation during 16 and 32 min an evidence of two bands around 530 and 625 nm is seen, indicating that the particles are no longer spherical and have two dominant sizes [17]. It is known that the spectrum of gold nanospheres smaller than 20 nm in diameter shows a characteristic strong absorption band at ~530 nm that is assigned to the dipole resonance of the nanospheres [12]. On the other hand, the spectrum of gold nanorods is characterized by two bands: the shorter wavelength band located at around 530 nm is attributed to the transverse surface plasmon resonance, while the longer wavelength band (~698 nm) is attributed to the longitudinal SPR [18].

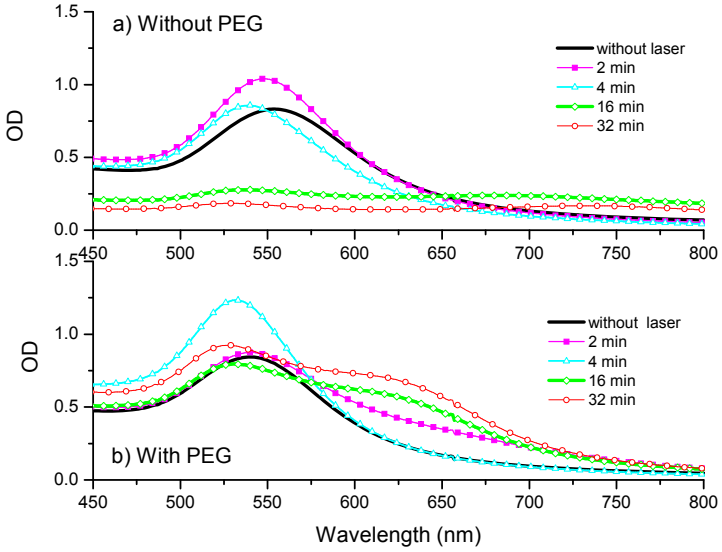

Figure 3. Influence of irradiation time. Absorption spectra of ALA:AuNP without PEG (a) and with PEG (b) irradiated with femtosecond laser pulses 1 kHz, 100 fs, 450 μJ, by 2, 4, 16 and 32 min.

To investigate the possibility of obtaining a decrease of the spherical gold nanoparticles number and an increase of nanorods, we fixed the irradiation time at 16 min and changed the laser energy and the pulse duration. The results are shown in Figures 4 and 5. The increase in the energy produces changes in the nanosphere/nanorod formation rate, and Figure 4 shows the displacement of the SPR bands to the infrared for 375 μJ pulses. This displacement is observed also in the irradiations with 80 fs, according to the figure 5, indicating that shorter pulses increase the probability of obtaining nanorods.

TEM micrographs are shown in figure 6. In Figure 6a the presence of spherical nanoparticles (~20 nm) in the solutions without PEG irradiated for 16 min can be observed. Figure 6b shows that, under the same irradiation condition, solution with PEG give rise to nanorods.

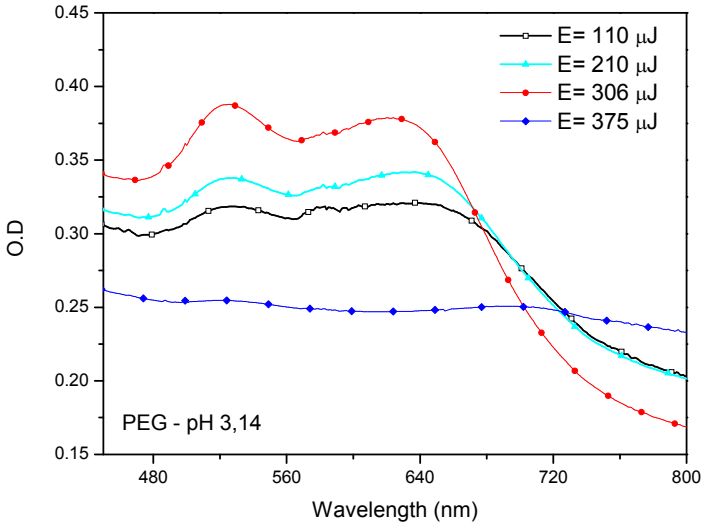

Figure 4: Influence of laser energy. Absorption spectra of the ALA:PEGAuNPs solutions after laser irradiation for 16 min and 1kHz with 110, 210, 306, and 375 μJ energies,100 fs pulses.

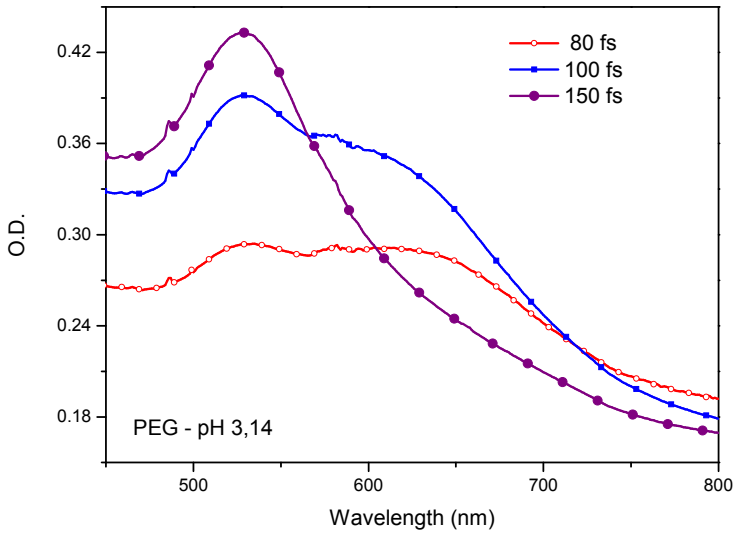

Figure 5. Influence of laser time duration. Absorption spectra of the ALA:PEGAuNPs solutions measured after 5 min laser irradiation with 300 μJ pulses pulse durations of 80, 100 and 150 fs.

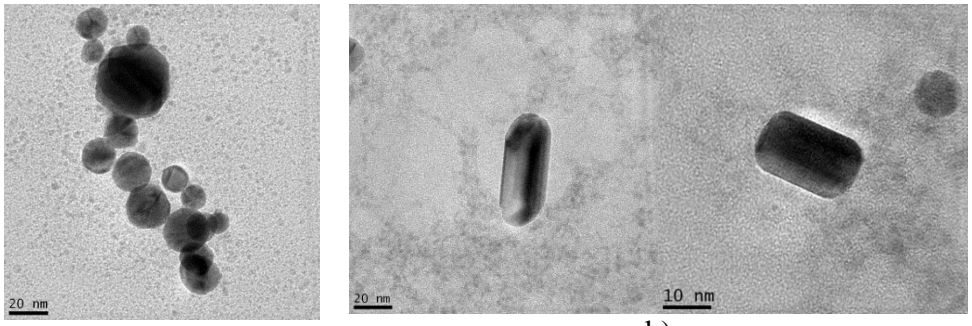

Figure 6. TEM microscopies of ALA:AuNPs without PEG (a) and with PEG (b) irradiated for 16 min, 450 μJ and 100 fs.

Based on the presented results, we propose that the production of metallic nanoparticles in the mixture of $HAuCl_4$ and Aminolevulinic acid water solution occurs according to the following steps: the of Xenon light absorption promotes the reduction of gold ions (Au^{3+}) into metallic gold (Au^0) (in ALA/ Chloroauric acid solution). The PEG presence suppresses the agglomeration of gold particles stabilizing the colloidal suspension. Simultaneous to this reduction, the solution heating via the absorption of the IR spectrum of the lamp enhances the growth process, leading to the production of nanoparticles with sizes between 5 and 40 nm. In the subsequent laser irradiation, the ultrashort pulses induce fusion and fragmentation processes [12], and the materials ejected from the particles are composed of ions, atoms, and nanoparticles of different sizes, depending on the laser pulse characteristics (energy, duration). At the longer irradiation times (32 min), thermal electron diffusion produces stronger interactions, particles agglomeration and crystal growing. Actually, the higher number of incident photons is expected to generate smaller species, thus creating a greater number of gold seeds that facilitate the ripening of particles, increasing the particle size. Reducing time duration and consequently increasing in the power energy appears to be important in the rod synthesis.

4. CONCLUSIONS

The possibility of obtaining of gold nanorods with ALA:PEG:AuNps changing ultrashort laser irradiation parameters as irradiation time, energy and pulse duration was studied. The best parameters obtained were irradiation for 32 min, with 80fs, 375 μJ pulses. With femtosecond laser irradiation, SPR band are shifted to wavelengths shorter than 650 nm, allowing deeper penetration of light in tissues, improving PDT efficiency. Simultaneously, the ALA:PEG:AuNRs excitation around 650 nm can act in photothermal plasmonic therapy.

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