2017 1st Pan American Conference of Nanotechnology- PanNANO-2107
Fundamentals and applications to shape the future
Guarujá, SP, Brazil, November 27-30,2017

Gold Nanoparticles synthetized with Aminolevulinic Acid for Theranostic: Photodynamic and Sonodynamic Therapies

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***Abstract*—** In this study we propose to synthesize gold nanoparticles with 5-ALA, to study cytotoxicity in cell cultures, to perform photodynamic (PDT) and sonodynamic (SDT) therapies and to compare the efficience of 5-ALA delivery. For 5-ALA:AuNPs synthesis, is used a 300 Watt Xenon lamp for photo reduction. Cytotoxicity tests were performed on 96-well plates with THP-1 human cells, which were differentiated into macrophages. For the PDT, an amber LED at 590 nm (~ 100mW) was used for 2 minutes and SDT was used with pulses of 1 MHz and 1W / cm^2, for 2 minutes. Cellular viability assays (MTS) were performed to evaluate therapies. The results have shown improved photosensitizer delivery in cells incubated with 5-ALA:AuNPs, leading to cell death and indicating potential use as a photo and sonosensitizer agent.

Keywords— gold nanoparticles, PDT, SDT, atherosclerosis.

I. INTRODUCTION

Atherosclerosis is a chronic inflammatory disease, multifactorial, slow and progressive. Some studies suggest that macrophages play an important role in the development of the disease, since they contribute to the growth of plaque, and thus its removal represents a new therapeutic strategy [1]. Photodynamic and sonodynamic therapies (PDT and SDT) are modalities of non-invasive treatments for atherosclerosis and cancer [2]. PDT involves the administration of photosensitizing molecules (PS) that bind to cancer cells and then, under irradiation with appropriate light, produce reactive oxygen species (ROS) that chemically destroy tumor cells. Sonodynamic therapy (SDT) derives from PDT and the main difference between SDT and PDT is the energy source used to activate the sensitizers [3]. However, the significant advantage of SDT over PDT is that ultrasound can penetrate deep into soft tissues [4].

Gold nanoparticles have many applications in medicine, such as photodynamic therapy and tumor screening (biomarkers) [5]. The applicability and performance of gold nanoparticles (AuNPs) depends significantly on size, shape and functionality [6]. It has recently been demonstrated in the literature that the incorporation of gold nanoparticles into the structure of aminolevulinic acid (5-ALA) improves the photodynamic properties of the therapy [7]. 5-ALA alone is already used in PDT because it is the precursor of protoporphyrin IX (PpIX), which is a fluorescent porphyrin, however, 5-ALA has difficulty in entering tissues and membranes because it is hydrophilic and low bioavailability. The delivery can be improved using nanotechnology, which allows to customize the treatments, giving dose and therapy monitored [8, 9].

In this study we propose to synthesize gold nanoparticles with 5-ALA, to study the cytotoxicity of these solutions in cell cultures, and to perform photodynamic and sonodynamic therapies to compare the effectiveness of 5-ALA release and consequently increase in singlet oxygen production.

II. MATERIAL AND METHODS

2.1 Synthesis of gold nanoparticles

The reagents used were Tetrachloroauric acid ($HAuCl_4$) and 5-Aminolevulinic acid hydrochloride ~98% were purchased from Sigma-Aldrich. For the preparation of solutions of gold nanoparticles, 15 mg de $HAuCl_4$ was mixed with 45 mg de 5-ALA and 100 mg of polyethylene glycol (PEG) in 100 mL water MilliQ. This solution was then vortexed for 5 minutes and exposed to white Xenon light 300-watt for 5 minutes. After illumination, the pH of the solution was measured and adjusted to 7.2 with addition of NaOH.

2.2 Characterization of gold nanoparticles

The characterization of the nanoparticles produced was made by UV-Vis absorption spectra measured by Shimatzu spectrophotometer, using 1-cm quartz cells.

To evaluate the size and shape of the nanoparticles obtained, transmission electron microscopy (TEM) images obtained by Joel (Zeiss, Germany) with images captured by MegaView III camera and processed by the software ITEM (Universal TEM Imaging Plataform – Olympus Soft Imaging Solutions GmbH, Germany).

The Fourier transform infrared spectroscopy (FTIR) were obtained on a spectrometer Shimadzu Prestige-21 (Shimadzu Corp., Kyoto, JP) with a resolution of 2 cm^{-1} in the range of 4000 cm^{-1} a 400 cm^{-1}. The nanoparticles were pressed into the hydraulic press for formation of a KBr pellet.

2.3 Cell culture

Human monocytic leukemia THP-1 cells were cultured in RPMI-1640 medium. Cells were seeded at 5000 cells per well in 96-well plates and incubated at 37°C in a humidified atmosphere of 5% CO_2 in 95% air. THP-1 cells were treated with 75 ng/mL of Phorbol-12 myristate-13- acetate (PMA, Sigma-Aldrich Co., St, Louis, MO, USA) for 48 hours to induce differentiation of the cells into macrophages. After differentiation, non-attached cells were removed by aspiration and the adherent macrophages were washed with RPMI-1640 medium 3 times and then incubated in cell culture medium at 37°C.

2.4 Study of cytotoxicity

Cytotoxicity studies were made for 5-ALA and 5-ALA:AuNPsNPs. The THP-1 cells were incubated with 5-ALA and 5-ALA:AuNPs which were diluted. Dilutions of the test compound were made using RPMI culture medium, and the wells were filled with solutions. Test compounds were suitably diluted on average, not exceeding 5% of the total well volume (100 µL). Final dilutions contained 40, 30, 20 or 10 µL / mL nanoparticle solution supplemented with RPMI for a final volume of 500 µL.

Cell viability was assessed by MTS (CellTiter 96® AQueous MTS Reagent) reduction into formazan. The quantity of formazan product as measured by the amount of 490 nm absorbance, and is directly proportional to the number of living cells in culture. Results were statistically compared (ANOVA and Bonferroni post-test) to negative (control cells, NaCl 0,9%) or positive (latex powder suspension, 0,5 g/L).

2.5 PPIX extraction of cells

Around 50.000 THP-1 cells and 75 ng/mL of PMA were added in each well of a 96 wells plate. After 48 hours the macrophages, the solution of 5-ALA:AuNPsNPs (10 and 20 µL) and the control group with cells.

The contents of each well were collected and added to tubes containing 3 volumes of acetone. These solutions were then centrifuged for 15 minutes at 4000 rpm, and the supernatants were analyzed in 3 Fluorolog of

Fluorimeter Jobin Yvon. The samples were excited at 400 nm, and emission spectra were measured between 415 and 785 nm.

2.6 Therapies

The therapies were performed on differentiated cells in macrophages. After differentiation, macrophages were divided into groups presented in Table I:

TABLE I: Groups, incubation times and solution concentrations of 5-ALA and 5-ALA: Au.

Groups	***Incubation Time (hours)***	***Concentration***	***Therapies***
5-ALA	24	40 µL in 60 de RPMI	PDT / SDT
5-ALA:AuNPsNPs	24	10 µL in 90 µL de RPMI	PDT / SDT
NaCl	24	25 µl in 75 µL de RPMI	
Cells - CC	24	100 µL de RPMI	PDT / SDT
Cells - CC	24	100 µL de RPMI	

The solutions were incubated for 24 hours, then the plate was washed with PBS and the therapies were done. For the PDT it was used amber LED at 590 ± 10 nm (Laser VENUS OMEGA-MM OPTICS), P ~ 100 mW and exposure time of 2 minutes, and for the SDT, the transductor of ultrasonic generator, Sonic Compact from HTM Brazil - 1 W/cm^2 and 1 MHz. The cells groups were irradiated separately during 2 minutes.

III. RESULTS AND DISCUSSION

After the synthesis of the nanoparticles, the absorption spectrum of the obtained solution was measured, and the absorbance spectrum is shown in Fig. 1. An absorption peak at ~ 522 nm appears due to the SPR (surface plasmon resonance) effect, indicating the formation of gold nanoparticles.

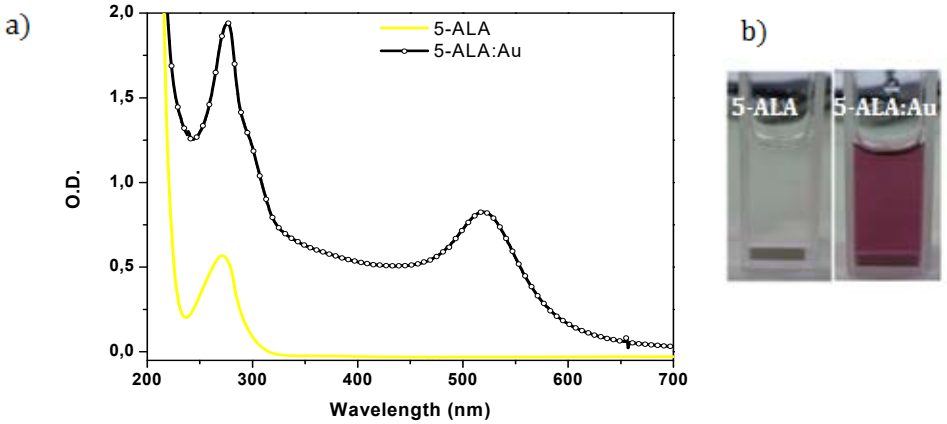

Fig. 1. a) UV/Vis spectra of 5-ALA and 5-ALA:AuNPs. b) Color of 5-ALA and 5-ALA:AuNPs solutions.

The morphology of the sample was observed through Transmission Electron Microscopy. Fig. 2 shows the images of sample 5-ALA:AuNPs.

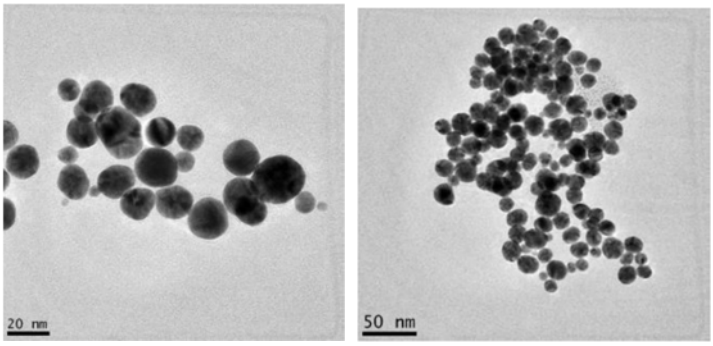

Fig. 2. TEM images of synthesized 5-ALA:AuNPs.

The presence of functional groups in the nanoparticle solutions was determined by FTIR. Fig. 3 shows the spectrum of the 5-ALA:AuNPs sample. The band at ~ 1650 cm^{-1} confirms the presence of bonds of carbonylic compounds (C = O), in ~ 1222 cm^{-1} amine group (C–N) which refer to 5-ALA. In 3314 cm^{-1} a strong and wide band referring to water (O-H).

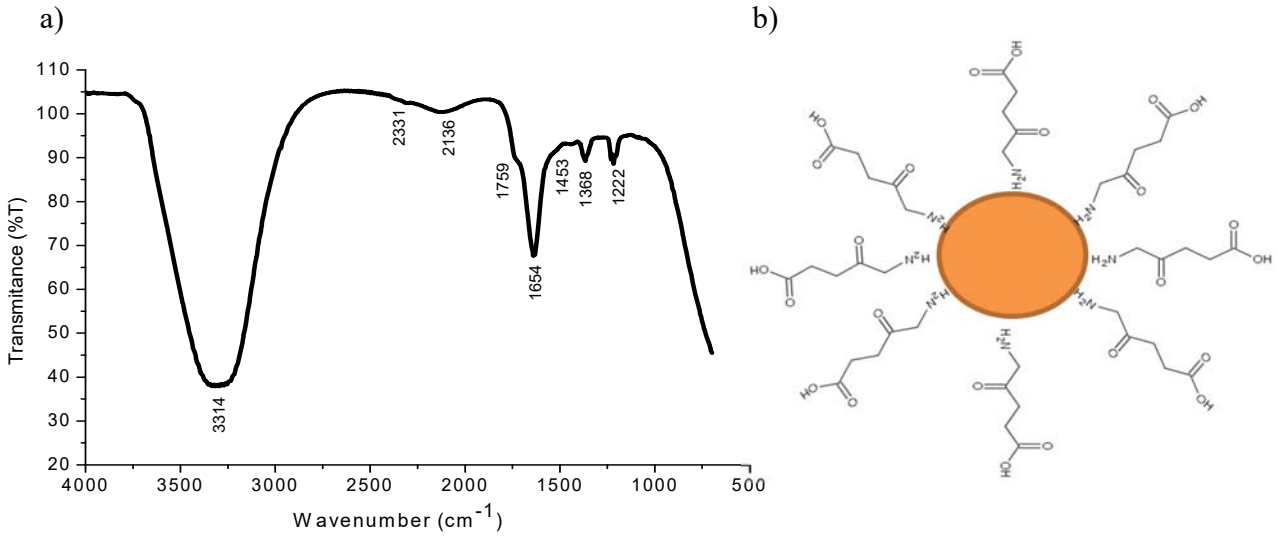

Fig. 3. The FTRI of nanoparticles. a) Spectrum with functional groups. b) Possible binding of 5-ALA with Au via amino group.

To evaluate cell toxicity of 5-ALA:AuNPs the MTS test is done. Fig. 4 shows the result, that viability analysis indicated values of ~20 % after 24 h exposure of 5-ALA:AuNPs for concentrations > 10 µL. For concentration of 10 µL, the viability becomes ~76%, showing that there is no high degree of toxicity.

Therefore, it can be concluded that the 5-ALA:AuNPs nanoparticles exhibits good biocompatibility which make them suitable for biomedical applications. Gold is inert, do not exhibit high cytotoxicity.The 5-ALA solution showed no toxicity, even though it was used in the concentration of 40 µL.

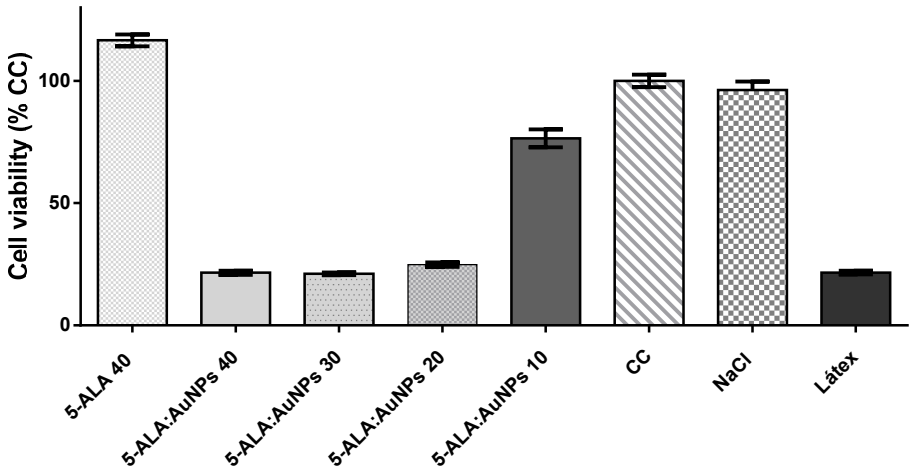

Fig. 4. Cell viability results for 5-ALA and 5-ALA:AuNPs.

The cytotoxicity test demonstrates that for small concentrations (≤ 10 µL for 5-ALA:AuNPs) there was no significant cell death, indicating that this concentration can be used for tests with photodynamic and sonodynamic therapies.

To evaluate the amount of PpIX produced by cells with and without the addition of nanoparticles, THP-1 cells were cultured in a 96-well plate and after differentiation into macrophages, 10 and 20 µL of 5-ALA:AuNPs were added. Also the control group was done, with only cells. After 24 hours of incubation with the test solutions, the PpIX was extracted with acetone and the fluorescence intensities were measured by exciting the samples at 400 nm. The results are shown in Fig. 5. There is an increase in the emission signal around 630 and 700 nm, characteristic of PpIX, with incubation of cells with 5-ALA:AuNPs for a period of 24 h. The results suggest that 5-ALA:AuNPs was incorporated by the gold nanoparticles, which in turn entered the cells, where conversion of 5-ALA to endogenous porphyrins occurred, which led to the accumulation of PpIX.

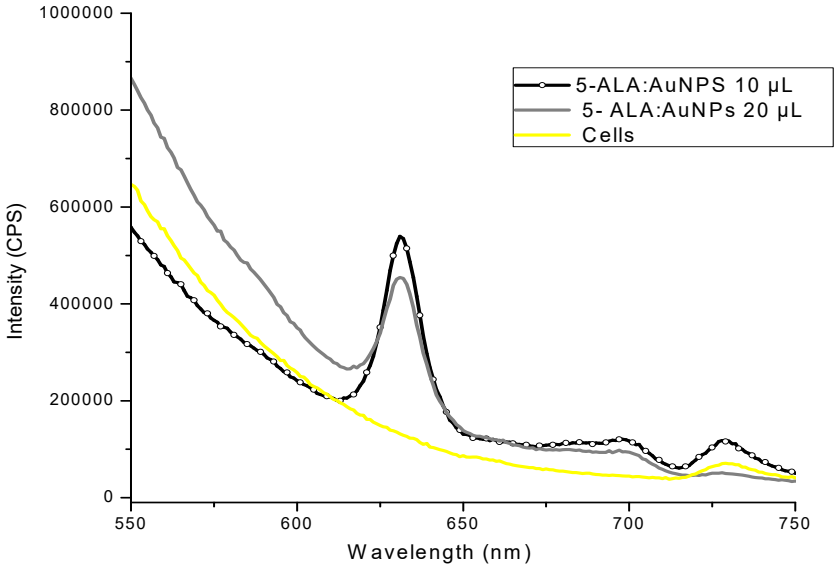

Fig. 5: Fluorescence of PpIX extracted samples of groups of cells and 5-ALA:AuNPs (10 and 20 µL), after incubation for 24 hours.

PDT and SDT therapies were done with groups shown in Table 1. Cell viability after Amber LED therapy is shown in Fig. 6. The therapy with the Amber LED alone does not lead to cell death, since the cells of the CC + LED group present viability of 93.6%. The 5-ALA: AuNPs proved to be effective because without the therapy, cell viability was ~ 76% and with therapy it dropped to ~ 58% viable cells.

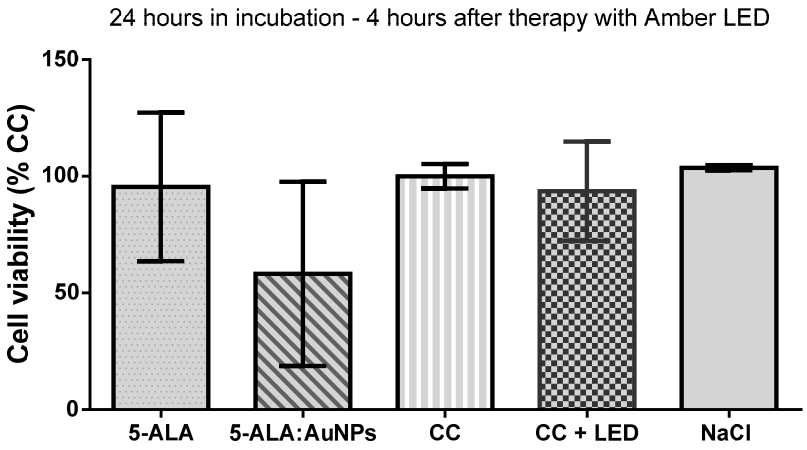

Fig. 5: Cells viability results for 5-ALA, 5-ALA:AuNPs and control groups (CC, CC + LED and NaCl), after therapy with amber LED.

For SDT, a high rate of cellular death occurs for 5-ALA (20.47%) and 5-ALA: AuNPs (12.96%), which demonstrates the efficacy of the therapy (Fig.6).

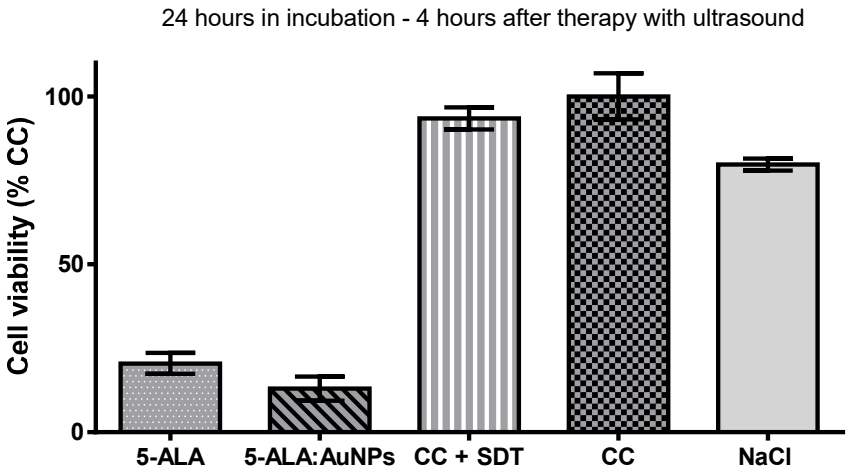

Fig. 6: Cells viability results after ultrasound.

IV. Conclusions

The synthesis method of nanoparticles with 5-ALA and gold is quite reproducible, simple and compatible for therapeutic applications. The characterization of the solution showed that it possesses good stability and

narrow size distribution. The synthesis is considered to be green, since it does not use toxic reducing agents, since xenon light heats up and supplies photons to the solution, functioning as a metal reduction catalyst (photoinduced oxidation / reduction), PEG acts as a stabilizing agent and biocompatible nanoparticles. 5-ALA is important in the stabilization and reduction of metals because it has carboxyl (-COOH) and amino (-NH2) groups in its structure. At the same time, not all 5-ALA participate in the reaction, and thus a portion of these reactants are loaded by the nanoparticles, and then are converted into the cells in PpIX, which is a photosensitizer.

Cytotoxicity assays have shown that at low concentrations the nanoparticles are non-toxic, but when combined with therapies they can cause a high rate of cell death.

The use of the therapies potentiated the action of the 5-ALA:AuNPs solution in the cells, which may be related to an increase in the delivery of 5-ALA to the macrophage cells. SDT provides an advantage over PDT, where used light less penetrating, because the SDT can penetrate in deeper tissues, generating ROS, that leads to the cellular death.

The 5-ALA:AuNPs combined with PDT or SDT induced macrophage apoptosis, indicating a useful and promising photosensitizer / sonosensitizer for atherosclerosis.

V. Acknowledgment

The Biotechnology Center, Energy and Nuclear Research Institute, the Multiuser Experimental Center of Federal University of ABC and Capes.

VI. References

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