Synthesis of Hybrid AuFe Nanoparticles by Photoreduction and Methyl Aminoluvinate

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***Abstract*— The nanohybrid gold/iron (AuFe) system is particularly interesting for biomedical applications such as targeted drug delivery, hyperthermia and photodynamic therapy (PDT) mainly due to its magnetic combined to the plasmonic properties. In this study, we aimed to synthesize nanohybrids to be used as a vehicle to deliver Methyl Aminoluvinate (MAL) for selective and efficient PDT. MAL is a prodrug that is metabolized to protoporphyrin IX (PpIX), photosensitizer in PDT. The nanohybrids were synthesized by photoreduction of solutions containing iron powder, polyethylene glycol (PEG), chloroauric acid ($HAuCl_4$) and MAL as a reducing agent in water solution. The products were characterized by UV-Vis, zeta potential and transmission electron microscopy. The synthesized nanoparticles had the optical properties of metallic nanoparticles and the magnetic properties of iron nanoparticles. Their characteristic absorption bands, in the visible light range, became become broader and red - shifted in the presence of iron nanoparticles. Cell viability of cancer cell lines in the presence of nanohybrids studies were performed.**

***Keywords*— Gold nanoparticles, Iron nanoparticles, Photodynamic Therapy,* B16-F10.**

I. INTRODUCTION

Selective destruction of tumor cells with minimal damage to surrounding healthy cells is the goal in photodynamic therapy (PDT) [2]. In this therapy, three components are combined to induce tumor destruction: a photosensitizer, light, and oxygen[3]. This therapy is based on the accumulation of a porphyrin sensitizer in tumor cells or pathogens and its subsequent destruction when exposed to radiation. Singlet oxygen species are produced, which damage membranes and organelles, causing cell death[4].

Methyl Aminolevulinate (MAL) is a produg that is metabolized to protoporphyrin IX (PpIX)[1]. PpIX is used as photosensitizer for PDT.

The focus of this paper was to obtain strategies for the fabrication of multifunctional nanostructures, combining either the magnetic or the plasmonic properties to improve the effectiveness of the methyl aminolevulinic acid - mediated PDT[5]. Indeed, these nanohybrids can generate heat under an external magnetic field, making them useful for magnetic hyperthermia therapy [6]. Besides, they can be guided and immobilized close to a specific biological target for magnetically assisted drug delivery. The nanohybrids were synthesized by photoreduction method and MAL, as reducing agent. Toxicity of synthesized nanohybrids were evaluated.

II. MATHERIALS AND METHODS

A. Synthesis of MAL:AuFeNps

To synthesize MAL:AuFe nanoparticles, 15 mg of $HAuCl_4$, 100 mg of polyethylene glycol (PEG) and 45 mg of Methyl Aminolevulinate (MAL), obtained from Sigma Aldrich, and 45 mg of iron powder from Cidepe - Centro Industrial de Ensino e Pesquisa Ltda, were added to 100 mL of distilled water and agitated in the vortex by few seconds . The pH was adjusted to 12 in solutions containing iron powder. Following, 10 mL of the resulting solutions were exposed to a 300 Watt Cermax xenon lamp by duration times from 1 to 10 min. The Xe lamp was positioned 10 cm away from the recipient containing the solution, and the illuminated region covered exactly the recipient diameter, with a 3.6 W/cm^2 estimated intensity. The pH was adjusted to ~7.0 before illumination.

B. Nanoparticles Characterization

The nanohybrids UV-Vis absorption spectra were measured by the UV-2600 spectrophotometer, (Shimatzu Japan) using 1-cm quartz cells.

Transmission Electron microscopy was obtained with a Jeol (Zeiss, Germany) transmission electron microscope (TEM), with images captured by a Gatan camera and processed by the software Digital Micrograph.

The hydrodynamic size and zeta potential distribution of the synthesized nanoparticles were determined using Nano-ZS90 (Malvern) instrument (5 mW HeNe laser at 632 nm).

C. Citotoxicity assay

For the cytotoxicity analysis, the cell treatments and preparation parameters were based on the MTS assay. The B16-F10 murine metastatic melanoma cells were grown in

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density of 5 x10^4 cel/ml in culture medium RPMI 1640 containing 10% FBS, 1% of penicillin/streptomycin and cultured in an incubator (37 °C , 5% CO_2). The cells were routinely subcultured every 3 days with 70-80% confluency and harvested using 0.25% trypsin. Cells were trypsinized and cells suspensions were seeded on 96 well plates (5000 cells/well). B16-F10 cells were incubated with MAL:Au and MAL:AuFe (5, 10, 20, 30, and 40 mL diluted in RPMI medium to a final volume of 500 µL). The plates were then incubated for 24 hours at 37 ° C and 5% CO_2 atmosphere. Subsequently, the supernatant was removed, and the cells were washed with PBS. Cell viability was evaluated by MTS (CellTiter 96® AQueous MTS Reagent) in formazan. The amount of product is absorbable at 490 nm and is directly proportional to the number of live cells in culture. The results were statistically compared (ANOVA and Dunnett test) or negative (control cells, NaCl 0.9%) or positive (suspension of latex powder, 0.5 g / L).

III. Results

The Figure 1 shows the results of the UV-Vis spectra measurements obtained for MAL+PEG+$HAuCl_4$ (Fig.1a) and MAL+PEG+$HAuCl_4$+iron powder (Fig.1b) illuminated with Xe light until times of 10 min. In this figure it is possible to observe the surface plasmon resonance peak around 536 nm for MAL:Au in solutions from illumination by 6 min, characteristic of the spherical gold nanoparticles. The presence of iron in plasmon coupling of gold results in an enlargement of the extinction peak.

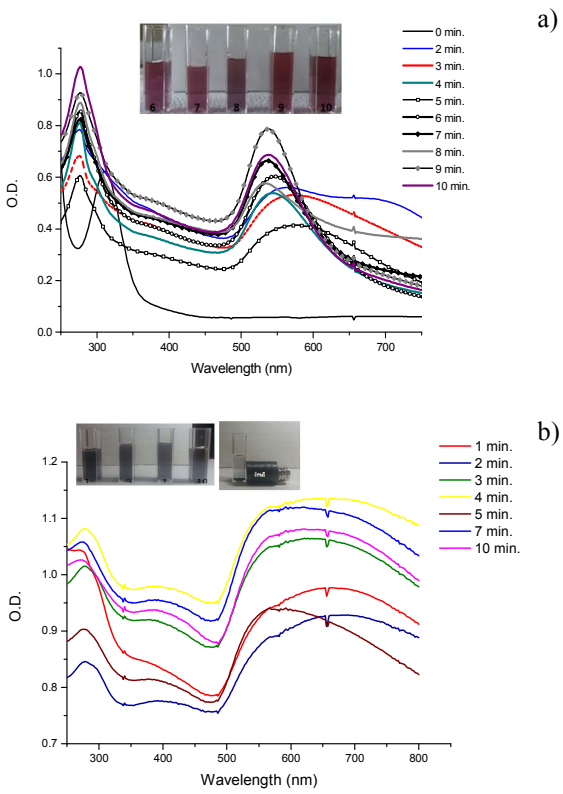

Fig. 1: Uv-Vis spectra of solutions with illumination times from 0 to 10 min: a) MAL:AuNPs and b) MAL:AuFeNPs. In the inset figures it is possible to observe the color aspect of solutions and effect of a magnet placed next to the cuvette containing MAL:AuFe solution for 10 minutes, evidencing the magnetization of nanoparticles.

The morphology of the NPs was observed through the images of transmission electron microscopy. The MAL:AuNps showed in Figure 2a, present spherical shape and size ranging from 10 to 30 nm. For the solution of MAL:AuFeNPs, Fig. 2b, it is observed larger spheres (probably AuNPs) surrounded by smaller spheres of around 5 nm (probably FeNps).

The stability of MAL:Au and MAL:AuFe was estimated by Zeta Potential. MAL:AuFe NPs have potential zeta of ~ -25 mV, and PDI ~0.577 confirming good stability of nanoparticles.

TABLE I. Size and Zeta potential values.

		Size (nm)	Zeta Potential (mV)
MAL:Au	pH 7.3	16	-23,4 ± 0,98
MAL:AuFe	pH 7.2	5-22	- 24,6 ± 0,4

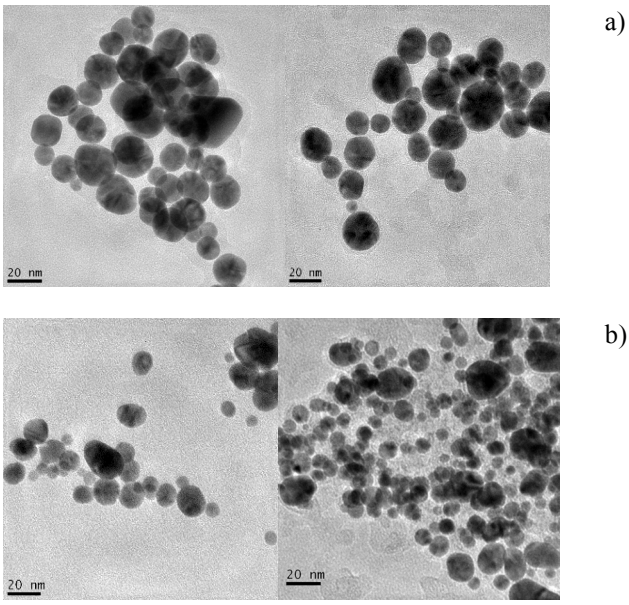

Fig. 2: Transmission electron microscopy of solutions: a) MAL:Au and b) MAL:AuFe.

The toxicity of MAL:Au and MAL:AuFe nanoparticles to B16-F10 skin melanoma cells was therefore examined as shown in Figure 3. MTS assay showed that the MAL:AuNps does not have significant cell toxicity up to 30 µM. MAL:AuFeNPs present no toxic effects (dark toxicity) on the cells between 10 and 40 µM (Figure 5b).

a)

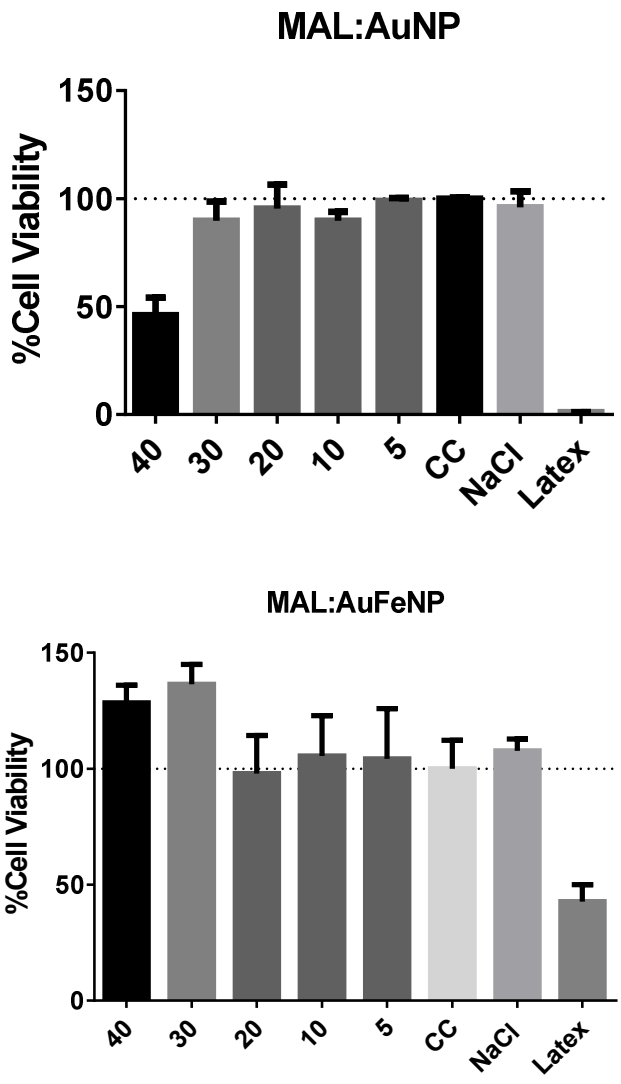

Fig. 3: B10-F16 viability test for a) MAL:AuNPs and b) MALA:AuFeNPs. The volume of nanoparticles in each well containing cells varied from 5 to 40 µL. The final volume in each well was kept constant adding serum free RPMI-1640.

b)

IV. Conclusions

We have demonstrated a photoreduction method to synthesize AuFe nanoparticles in aqueous medium using Methyl Aminolevulinate as photoreducing agent, capping and effective stabilizing agent. The stable synthesized nanohybrids have iron particles surrounded by gold nanoparticles. MTS assay showed that nanohybrids present similar toxicity compared to MAL:Au nanoparticles. Nanohybrids offer a new modality for selective and efficient destruction of tumor cells to PDT, with minimal damage.

Acknowledgment

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