Revista Internacional de Investigación e Innovación Tecnológica
Página principal: www.riiit.com.mx

Synthesis, aminosilane-folic acid functionalization and cytotoxicity of the upconversion nanoparticle Y_2O_3:Er^{3+}, Yb^{3+} for cancer cell detection

Síntesis, funcionalización del ácido fólico-aminosilanos y citotoxicidad de la nanopartícula ascendente Y_2O_3:Er^{3+}, Yb^{3+} para la detección de células cancerígenas

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Technologic innovation: The preparation of upconversion nanoparticles to be used as biolabels to detect cancer cells.

Application: Nanoparticles able to label cancer cells.

Received: 03 June 2016.
Accepted: 10 December 2016.

Abstract

Upconversion nanoparticles (UCN) have gained interest because they can absorb near infrared radiation (NIR) and upconvert it into to higher energy light, such as the visible range, through the sequential absorption of multiple photons or energy transfer. The UCN Y_2O_3 co-doped with Yb^{3+} and Er^{3+} was synthesized by sol-gel method. These UCN find application in bioimaging, as biolabels, to diagnose and visualize cancer cells, after coating and functionalization with ligands that bind to receptors on the surface of the cell. The UCN were coated with a silica shell using Stöber method and functionalized with aminosilane (APTES/TEOS) to enable folic acid conjugation. After the folic acid conjugation, the cytotoxicity of UCN was tested on human cervix carcinoma cells (HeLa) with a colorimetric assay based on the reduction of the MTT reagent (methy-134 thiazolyltetrazolium). Different concentrations of bare and functionalized UCN between 0.001 µg/mL to 1 µg/mL were used. The MTT assays show that some concentrations of bare UCN of Y_2O_3: Er^{3+}, Yb^{3+} (1%, 1% mol) were cytotoxic for cervical

adenocarcinoma cells (HeLa); however the functionalized UCN were not cytotoxic. The bare and functionalized UCN were analyzed by transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR) and luminescence measurements. The results reveal that functionalized UNC have a particle size of 70 nm (±10 nm) with a good luminescence spectrum in comparison with the bare UCN. Finally, imaging studies were performed by epifluorescence confocal microscopy; these revealed that UCN functionalized with folic acid were internalized by HeLa cells. The fluorescence signal of folic acid-UCN was localized into the cell cytoplasm, confirming with this their efficiency to be used as biolabels.

Keywords: aminosilane-folic acid functionalization, nanoparticles, sol-gel, upconversion luminescence, biolabels.

Resumen

Las nanopartículas de conversión ascendente (UCN) han adquirido en los últimos años gran interés en la nanobiomedicina, ya que pueden tomar energía infrarroja y convertirla a un nivel de energía más alto como la luz visible, debido a una absorción secuencial de fotones o por el método de transferencia de energía. En este estudio, se sintetizaron nanopartículas de conversión ascendente (UCN) de Y_2O_3 dopado con Yb^{3+} y Er^{3+} (1%, 1% mol), por el método de sol-gel. Las aplicaciones pueden ser múltiples, una de ellas es en bioimagen, para utilizar las UCN como bioetiquetadores de células de cáncer, después de una apropiada funcionalización de las mismas, proceso en el cual se les pone un recubrimiento y ligandos compatibles con receptores de las células cancerígenas. Las UCN fueron recubiertas con una fina capa de sílica por el método de Stöber, posteriormente funcionalizadas con aminosilanos por el método APTES/TEOS. Después se realizó una conjugación de los grupos amino con el ácido fólico, para que este ligando se pudiera unir con receptores de células de cáncer de cérvix (HeLa). Se realizaron pruebas de citotoxicidad de las UCN con las células HeLa por medio del ensayo colorimétrico MTT (metil-134 thiazoliltetrazolio). Se utilizaron concentraciones de nanopartículas de 0.001 µg/mL a 1 µg/mL. Se encontró que algunas concentraciones de las UCN al desnudo fueron citotóxicas para las células, pero cuando éstas fueron funcionalizadas no hubo citotoxicidad. Las UCN al desnudo y funcionalizadas fueron caracterizadas con el microscopio de transmisión electrónica (TEM), espectroscopia infrarroja de Fourier (FTIR) y el espectrofotómetro. Las UCN tienen un tamaño promedio de 70 nm (±10 nm) con un buen espectro de luminiscencia en comparación con las nanopartículas no recubiertas. Por último, también fueron analizadas las nanopartículas internalizadas en las células HeLa con el microscopio de epifluorescencia confocal para obtener imágenes de las mismas. Se observó la presencia de las UCN dentro del citoplasma de las células, lo que confirma su uso como bioetiquetadores de células de cáncer de cérvix.

Palabras claves: funcionalización con ácido fólico-aminosilanos, nanopartículas, síntesis sol-gel, luminiscencia de conversión ascendente, bioetiquetadores.

1. Introduction

Upconversion luminescence can be applied to a wide range of biomedical applications such as biolabels for cancer detection, cancer therapy, fluorescence imaging, magnetic resonance and drug delivery [1-2]. Luminescence upconversion nanoparticles (UCN) were synthesized by combining two rare earth ions in a proper host (usually an oxide); the first ion (sensitizer) absorbs near infrared (NIR) energy and transfers the energy to the second ion; then the nanoparticle emits the excess of energy as photons in the visible range. This phenomenum is called energy transfer upconversion (ETU) [3-4]. Several authors have prepared different UCN with the host Y_2O_3, Chen [5] used the host Y_2O_3 codoped with Er^{3+}/Yb^{3+} (1%, 10% mol) and Kong [6] compared the hosts Y_2O_3, La_2O_3 and Gd_2O_3 codoped with several concentrations of Er^{3+}/ Yb^{3+}. In this work, the host Y_2O_3 was codoped with Yb^{3+} (1% mol), as sensitizer, and Er^{3+} (1 % mol) by sol-gel method [7]. The Yb^{3+} absorbs NIR radiation (980 nm) and transfers a first photon to Er^{3+}, and then the electron in the $^4I_{15/2}$ level is promoted to $^4I_{11/2}$ level. The Yb^{3+} transfers a second photon to Er^{3+}; the same electron is raised to a higher level $^4S_{3/2}$. From here it rapidly decays to its base state, emitting green light [4]. The advantage of using NIR radiation is that the phototoxicity is reduced compared with UV light, and can have a major penetration into biological tissues [8]. Our objective was to create an efficient UCN biolabels for cancer cell detection with good luminescence spectra and biological penetration into cancer cells.

In order to have efficient biolabels in cancer therapy and detection, the delivery of intracellular agents is an important issue. On this context, several authors worked with the functionalization of nanoparticles with specific biological ligands such as folic acid (FA) and antibodies [9-10]. The FA can have and interaction with a folic acid receptor (FR) located on the surface of the cancer cell [10]. The binding of FA-FR activates the internalization of the functionalized UCN-FA via endocytosis [11]. The FR is overexpressed on a variety of human cancer cells such as ovary, kidney, lung, mammary gland, brain and endometrium. This is an advantage because the UCN-FA can easily target the specific cancer cells [10-11].

The functionalization of the UCN began with the coating of a thin silica layer by Stöber method [12], then functionalization with amine group (APTES/TEOS), known as silanization process, to enable folic acid conjugation [13]. The silanization process was done by several authors to target specific ligands overexpressed in cancer cells. The targeting of cervix carcinoma (HeLa) cells was done before via FR using UCNs functionalized with the ligand 6-aminohexamonic acid [14]. Also the coating of the UCN using a thin layer of polymer, instead of silica, called PEI (polyethyleneimine) was done. This polymer has amino groups for further functionalization [15].

Cytotoxicity of UCN bare and functionalized was tested on human cervix carcinoma (HeLa) cell line, by a cell viability assay based on the reduction of the MTT reagent (methy-134 thiazolyltetrazolium) [16]. The functionalized UCN obtained had strong luminescence emission and adequate size also they were non-cytotoxic. Also, imaging studies obtained reveal that the UCN were uptaken by HeLa cells, confirming by confocal microscopy, thus, the UCN-FA synthetized in this work can be further used as biolabels.

2. Materials and methods

2.1 Sol-gel synthesis

The precursors for this method were: $Y(NO_3)_3$ (Alfa Aesar 99.9965%), $Yb(NO_3)_3$ (Alfa Aesar 99.9%), $Er(NO_3)_3$ (Alfa Aesar 99.9%). Reagents were diluted with HNO_3 (14% mol), also tartaric acid ($C_4H_6O_6$ Aldrich, USA) was dissolved in de-ionized water. Each individual solution was mixed under constant stirring. The molar concentration of tartaric acid was 1:2 for the metal ions present in the Y_2O_3. Both solutions were mixed and stirred for 24 h at room temperature. Thereafter the pH of 5.0 was verified and the mixture was heated under constant stirring at 80°C for 2 h. This yielded a denser sol. Subsequently, the sol was heated at 120°C until a gel was produced and dried to form the xerogel [7]. The xerogel was collected and annealed at 1200◦C for 2 hours.

2.2 Ultrasonication

The UCN used were ultra-sonicated with high intensity ultrasonic processor (Sonics & Materials, Inc.) for about 30 min with 20 ml of isopropanol/ethanol, before the analysis for TEM or functionalization to avoid agglomeration of the nanoparticles.

2.3 Silica and Aminosilane functionalization

In order to conjugate the UCN with biological molecules, the UCN need a proper functionalization with aminopropyltrimethoxysilane (APTES), with these process, the amino groups formed on the surface of the UCN are able to bind with folic acid. The folic acid recognizes cell surface receptors (folic acid receptors), the ligands of the UCN need to have high affinity and specificity, so it can bind to the cell and internalize into the cell to identify it. Silica coating of the nanoparticles was done by Stöber synthesis [9]. Distilled water (100 ml) was mixed with 10 mmol concentration of the UCN under constant magnetic stirring for 20 min. Separately, 30 ml of ethanol were mixed and agitated for 20 min with 0.6 ml of TEOS (Tetraethyl orthosilicate, Sigma Aldrich 98%). Another solution containing 1 mol ammonium hydroxide (NH_4OH, Sigma Aldrich), 0.2 ml of surfactant IGEPAL (Sigma Aldrich) and distilled water was stirred; this surfactant was needed in order to minimize the UCN agglomeration during the coating process. The solutions were mixed together and agitated for 24 h. The silica coated UCN were precipitated with acetone and centrifuged three times. Finally the UCN were filtered and then annealed at 900°C for 2 h. They were ultrasonicated again for 15 min. The aminosilane functionalization process was done by mixing the silica-coated UCN with ethanol, 0.02 ml of 3-Aminopropyl- trimethoxysilane (APTES, 98% Sigma Aldrich), 0.14 ml of TEOS and 0.2 ml of ammonium hydroxide, for 4 hours approximately at 24°C [13]. The functionalized UCN-NH_2 were dried and collected. FTIR analysis was performed to confirm the amine-folic acid functionalization and TEM analysis were done to verify the core-shell on the UCN.

2.4 Folic acid conjugation

For biological applications, UCN solubility in aqueous environments can be very important; the silica shells provide chemical durability to the UCN and protection for cytotoxicity to the cells. The surface can be tailored with more complex functional groups to modulate the UCN intermolecular interactions. These functional groups can range from simple ligands to biologically active components, including peptides, proteins, and DNA [16]. The amino groups added with the APTES/TEOS functionalization, enable further folic acid (FA) conjugation. Some cancer cells, such as cervical adenocarcinoma (HeLa) over-express FA on the surface of the cell

membrane [2]. In this context, the UCN were functionalized with folic acid ligands to bind to the surface receptors of the HeLa cells. The process was done by mixing N-hydroxisuccinimide (NHS, 99% PS) ($C_4H_5NO_3$) in the presence of N,N' Dicyclohexylcarbodiimide (DCC) ($C_{13}H_{22}N_2$). The reaction was done on a Schlenk system with 0.500 g of FA y 500 µL de distilled Triethylamine (TEA) in 10 mL of dry Dimethyl sulfoxide (DMSO) into a N_2 atmosphere. The mixed was agitated by 2 h at 37°C, and then 0.260 g of NHS and 0.470 g of DCC were added. They were agitated all night at 37°C into the darkness, and then filtered. A sample with an intense brown color was obtained. The UCN-NH_2 were dispersed in 25 ml of a carbonate / bicarbonate buffer (0.01M pH = 9.0). They were ultrasonicated for 5 min and then a 3 mL of a FA-NHS solution were added. They were agitated 2 h into the darkness. The resulted UCN-NH_2-FA were centrifuged (6000 rpm for 15 min), washed with DMSO (3 x 45 ml) and ethanol (5 x 45ml). Finally they were vacuum dried overnight at 30°C. The material turned into a yellow color.

2.5 Characterization

The crystallinity of the UCN were analyzed by XRD with the Phillips X'Pert-MPD, equipped with Cu Kα radiation (λ= 0.15406 nm). Measurements scanned over 2θ range of 10–80° were taken with a step size of 0.1° and a 1 s dwell per point. The results obtained were compared with the database PCPDFWIN [17]. Transmission electron microscopy (TEM) used was JEOL JEM-2100-F to study the morphology, the nanoparticle size and the presence of the silica-coating. The photoluminescence of the UCNP was analyzed with a fluorescence spectrometer (PL, Hitachi® FL-4500) with 980 nm for excitation. FTIR analysis was done to confirm the folic acid functionalization (Thermonicolet 1700).

2.6 Cell culture

Human cervix carcinoma HeLa cells (ATCC CCL-2) were obtained from the American Type Culture Collection (ATCC). Cells were cultivated in RPMI-1640 media supplemented with 10% Fetal Bovine Serum (FBS, BenchMark, Gemini Bio Products), 1% Penicillin streptomycin (Sigma-Aldrich), 1% L-glutamine and 1.5 g/l sodium bicarbonate. Cells were propagated in growth medium and maintained at 37°C and 5% CO_2.

2.7 Cytotoxicity assay

Cytotoxicity assay was used to test the viability of a cell with the bare and functionalized UCN. Viability of HeLa cells was analyzed by a colorimetric assay based on the reduction of the MTT reagent (methy-134 thiazolyltetrazolium) by using the TOX1 in vitro toxicology assay kit (Sigma-Aldrich). Cytotoxicity test was performed in a 96-well plate containing 10,000 cells per well. UCN were ultrasonicated and diluted at different concentrations ranging from 0.001 µg/mL to 1 µg/mL in RPMI-1640 media. Cells were incubated with different amounts of UCN for 24 h at 37°C and 5% CO_2. Incubation of cells in complete RPMI-1640 media without UCN were taken as a positive control, simulating cell behavior under ideal conditions. DMSO was used to induce cell death. After incubation time, cells were washed with phosphate buffer solution pH 7.4 (PBS 1x) and MTT reagent was added to the plate following the instructions of the manufacturer. Cytotoxicity was assessed by absorbance measurements with an ELISA plate reader (Thermo Scientific, USA) at 570 and 690 nm. All data obtained from incubated UCNP were normalized to data from three positive control wells with no Y_2O_3:Er^{3+}/Yb^{3+} in each triplicate from three independent experiments [18].

2.8 Confocal microscopy cell imaging

DAPI (4', 6-diamidino-2-phenylindole) is a fluorescent stain that binds strongly to A-T (adenine- thymine) rich regions in DNA. It is used extensively in fluorescence microscopy. As DAPI can pass through an intact cell membrane, it can be used to stain both live and fixed cells. In this context, DAPI was used to dye the nucleus of the cells. Cell culture Petri dishes coated with Poly-d-lysine (Mat-Tek P35GC1.5-10C) were used to seed 300,000 HeLa cells in RPMI-1640 media and incubated overnight at 37 °C and 5 % CO_2. HeLa cells were incubated with 1 µg/mL of UCN for 24h at 37 °C and 5 % CO_2. Afterwards, cells rinsed with PBS 1x and then were fixed with 4 % formaldehyde-PBS solution at 4 °C for 15 min. After fixation, cells were permeabilized with 0.5 % Triton X/PBS 1x for 15 min at 4 °C. Nuclear staining was achieved by incubated the cells with DAPI at 0.5 ng/µL in darkness for 10 min at RT, followed by five washes with PBS. Nuclear staining with DAPI was also visualized with an inverted laser-scanning microscope Olympus FluoView FV1000 (Japan) using an argon ion laser for excitation at 405 nm wavelength and filters for emission of DAPI. UCN fluorescent was detected using the NIR laser (980 nm) and EGFP filter channel (excitation at 488 nm and emission at 515–530 nm). Cells were visualized with a 63 × (DIC), 1.4 N.A. planapochromatic oil immersion objectives. The imaging parameters used produced no detectable background signal from any source other than from UCN and DAPI. Confocal images were captured using MetaMorph software for Olympus [19].

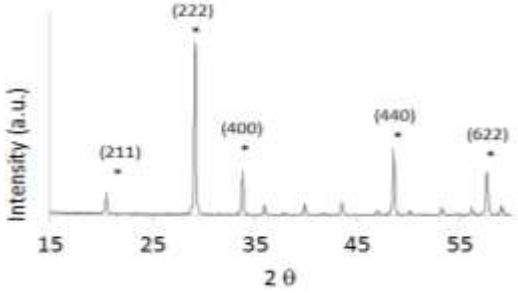
3. Results

This section shows crystallinity of the UCNP by XRD and the morphology analysis performed by TEM, the photoluminescence of all UCN synthesized in this work and the FTIR analysis. The cytotoxicity effect of the UCN was also analyzed in human cervix carcinoma (HeLa) with a colorimetric assay based on the reduction of the MTT reagent (methy-134-thiazolyltetrazolium). Also the images of the UCN internalization into the cell, confirm their use of biolabels.

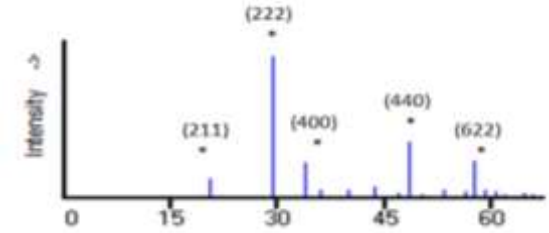
3.1 XRD and morphology of $Y_2O_3:Er^{3+}/Yb^{3+}$

The composition obtained for the UCN synthesized by and sol-gel method was and $(Y_{0.98}Yb_{0.1}Er_{0.1})_2O_3$. The XRD patterns of the UCN are in Figure 1. All the diffraction peaks are consistent with the database JCPDS No. 89-5592 (Y_2O_3) and demonstrate that the samples have a cubic structure (figure 1-a). The peaks for the planes (211) were in 2θ=20.514°, for (222) in 29.171°, for (400) in 33.809°, for (440) in 48.564° and for (622) in 57.659°[17].

Figure 2 shows the TEM images of bare (figure 2-a) and functionalized (figure 2-b) $Y_2O_3:Er^{3+}/Yb^{3+}$ (1%, 1% mol) UCN prepared by sol-gel. UCN obtained were mostly of spheroidal shape. The average size of UCN $Y_2O_3:Er^{3+}/Yb^{3+}$ were 70 nm (+/- 10 nm). Agglomerates were observed mostly on the all the UCN. In figure 2-b the amorphous silica shell (black arrow) is shown in contrast with the crystal lattice of the UCN.

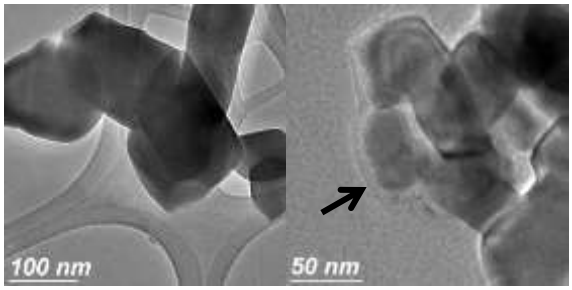


b)



a)

Figure 1. XRD patterns of a) JCPDS No. 89-5592 (Y_2O_3) database and b) Y_2O_3: Er^{3+}/Yb^{3+} (1%, 1% mol).



a) b)

Figure 2. TEM images of UCN Y_2O_3: Er^{3+}/Yb^{3+} a) bare and b) functionalized UCN-NH_2-FA. The black arrow indicates the silica-coating.

3.2 Upconversion luminescence

Upconversion luminescence spectra of bare and UCN-NH_2-FA, under 980 nm laser excitation, are shown in figure 3. For Y_2O_3:Er^{3+}, Yb^{3+} (1%, 1% mol) transitions $^2H_{11/2} \rightarrow {}^4I_{15/2}$ (550 nm), $^4S_{3/2} \rightarrow {}^4I_{15/2}$ (564 nm) and $^4F_{9/2} \rightarrow {}^4I_{15/2}$ (660 nm) are present. The emission of the UCN is green (564 nm).

3.3 FTIR analysis

The Fourier transform infrared spectroscopy analysis (FTIR) was used to verify the functionalization APTES-TEOS-FA of the UCN, to confirm the presence of the amine groups and folic acid on the surface of the nanoparticle. Figure 4-b shows the FTIR spectra and also for comparison purposes, the FTIR analysis for bare UCN was done as depicted in figure 4-a.

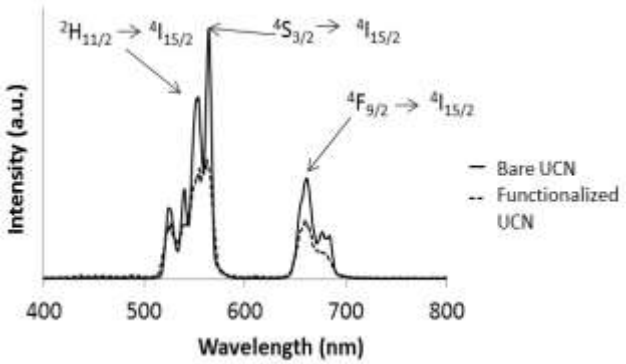
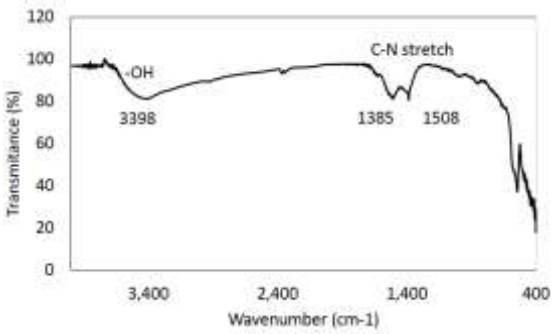
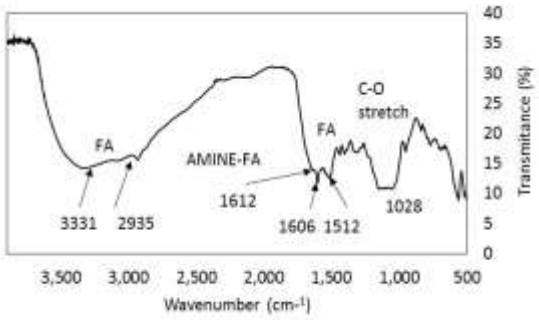


Figure 3. The upconversion emission spectra, under 980 nm excitation of bare and functionalized UCN Y_2O_3:Er^{3+}/Yb^{3+} (1%, 1% mol) green emission (564 nm) in $^4S_{3/2} \rightarrow {}^4I_{15/2}$ transition is present.



a)



b)

Figure 4. FTIR spectra of a) bare UCN Y_2O_3:Er^{3+}/Yb^{3+} (1%,1% mol) and b) functionalized UCN-NH_2-FA.

3.4 Cytotoxicity assay and cellular imaging.

Figure 5 shows the cytotoxicity results for bare and functionalized UCN-NH_2-FA incubated on HeLa cells. The bare Y_2O_3:Er^{3+}/Yb^{3+} (1%,1% mol) tend to agglomerate and precipitate after the

addition to the well of the 96-well plate, even after been ultrasonicated. The mechanical treatment of the cells and the agglomeration may contribute to damage the cells. Lower concentrations of bare UCN (0.001 µg/mL) showed less cell viability than higher concentrations (0.1 µg/mL); this is explained by agglomeration of nanoparticles in the bottom of the plate that may interfere with the absorbance reading of the plate. But the functionalized UCN have no cytotoxic effects on HeLa cells.

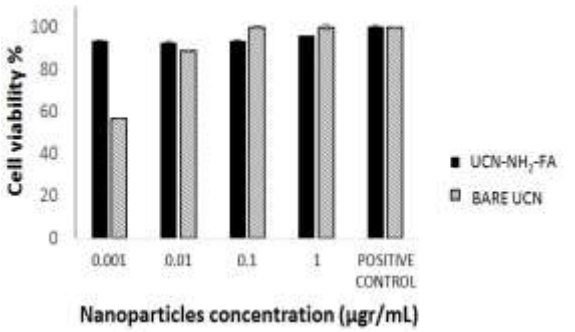


Figure 5. Cell viability assay of bare UCN $Y_2O_3:Er^{3+}/Yb^{3+}$ (1%,1%) (gray bars) and functionalized UCN-NH_2-FA (black bars) with HeLa cells.

A numerous studies have reported the application of UCN for *in-vitro* cellular and tissue imaging. In vitro cellular imaging involves targeting of UCN to some subcellular components [19-20]. In this work, the UCN were functionalized with folic acid ligands that bind to the receptors overexpressed on the surface of the HeLa cells. The excitation of the modified UCN in cell culture medium with 980 nm light results in the characteristic green upconversion emissions. A concentration of 1 µg/mL were incubated 24 hours into the cells, they were properly fixed and prepared in order to obtain imaging with the confocal microscope. The nucleus (N) stained with DAPI, was excited with 405 nm to obtain 461 nm of emission of the DAPI dye as shown on figure 6-a. Figure 6-b shows the localization of functionalized UCN only excited with NIR 980 nm + EGFP (green fluorescent protein) and figure 6-c shows the merge of the images. The UCN were clearly localized in the cytoplasmic region as expected, since folic acid ligands were used for UCN internalization, confirming their applications as biolabels.

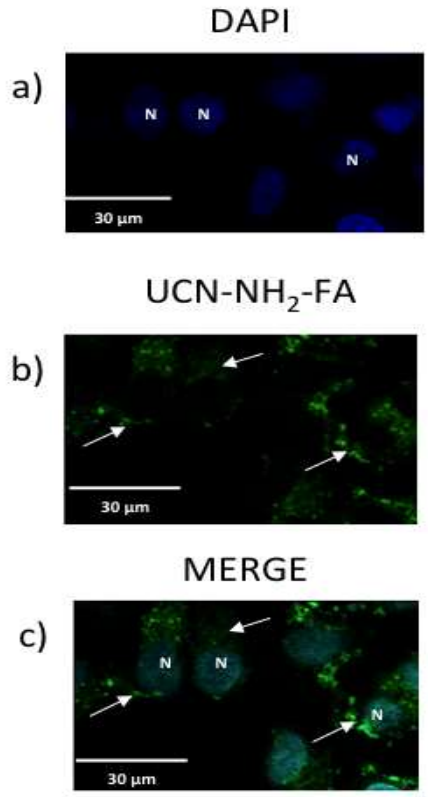


Figure 6. Fluorescence emission by $Y_2O_3:Er^{3+}/Yb^{3+}$ (1%,1% mol) incubated with HeLa cells (concentration 1 µg/mL), incubation time 24 h. (a) Staining with DAPI show nuclei of HeLa cells labeled as "N" (b) Laser NIR 980 nm + EGFP shows the functionalized UCN into the cell. (c) Overlay DAPI, Laser 980 nm +EGFP localize the UCN activity in the cytoplasm of the HeLa cells (white arrows).

4. Discussion and conclusions

The UCN with lanthanide ions have an upconversion process that absorb NIR light and emit in the visible spectra, also the nanoparticles have a long luminescence life if the source of energy is maintained, in this

case an infrared laser with 980 nm of wavelength [20-21].

They were prepared by the sol-gel method with molar concentrations of Er^{3+} and Yb^{3+} ions of 1% into the host lattice of Y_2O_3, which resulted to show the characteristic green emission [17]. Hemmer [18] used the same molar concentrations with the host Gd_2O_3, but with different functionalization for UCN as biolabels.

As seen on the TEM images, the shape of the nanoparticles is mostly spheroidal with an average diameter of 70 nm (±10 nm) for Y_2O_3. As verified by a detailed XRD analysis the structure of the UCN is cubic. Surface modification by silica-coating was approximately 5 nm thick on all the UCN. The nanoparticles tend to agglomerate after and during coating, therefore a surfactant was added to have a homogeneous coating on all UCN.

The functionalized UCN-NH_2-FA showed good emission spectra in comparison with bare nanoparticles, they yielded high intensity green luminescence at 564 nm. The peaks can be attributed to the transitions $^2H_{11/2} \rightarrow {}^4I_{15/2}$ (525-550 nm), $^4S_{3/2} \rightarrow {}^4I_{15/2}$ (564 nm) and $^4F_{9/2} \rightarrow {}^4I_{15/2}$ (660-670 nm). The FTIR spectra showed the prescence of the NH_2-FA groups on the surface of the UCN (1612 to 1512 cm^{-1}), the FA ligands (3331 cm^{-1}) and the wagging $–NH_2$ (669-793 cm^{-1}) [17]. The advantage of these UCNs is that the NIR light is safer to the body and can penetrate several inches of tissue [22].

On the cytotoxicity assay, MTT reagent is taken up by cells and reduced to its insoluble form formazan, showing a violet color and can be measurement by spectroscopy. The amount of MTT reduced by the living cells is quantified by a colorimetric method, thus ability of cells to reduce MTT is an indicator of mitochondrial integrity and its functional activity is interpreted as measure cell viability. Determining the ability of cells to reduce MTT to formazan after exposure to a compound provides information about the toxicity of the UCN evaluated. Cytotoxicity assays based on MTT, revealed that HeLa cells incubation with different bare Y_2O_3:Er^{3+}/Yb^{3+} (1%,1%), at 0.001 µg/mL concentration, caused a 40% of cell death. However, after functionalized of nanoparticles with APTES/TEOS/FA, all UCN had more than 80% of cell viability on HeLa cells, thus the cell death and the cytotoxic effect was reduced for all of the tested concentrations.

The UCNs Y_2O_3; Er^{3+}, Yb^{3+} (1%, 1% mol) functionalized with folic acid have the capability to be taken up by the cells and they are clearly localized into the cell cytoplasm with no cytotoxicity effects to other possible cells. With these results we confirm that functionalized UCN-NH_2-FA not only show a good emission spectra but also are able to be visualized in the intracellular part of cervix adenocarcinoma cells HeLa. These functionalized UCN may represent a good technology to be used as biolabels in cancer diagnosis.

5. Acknowledgements

The authors wish to acknowledge financial support from DGAPA-UNAM (Grant No. 109913). Technical support provided by E. Aparicio, F. Ruiz. M. Ponce, Dr. F. Castillón, Leobardo Pérez and Dr. Cristian Campos (Facultad de Ciencias Químicas, Universidad de Concepción, Chile) are gratefully acknowledged. Karla Juarez-Moreno is a member of the International Network of Bionanotechnology with impact in Biomedicine, Food and Biosafety (CONACYT, Mexico).

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