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Magnetic Nanoparticles of $La_{0.78}Sr_{0.22}MnO_3$, coated with SiO_2: Preparation and Cytotoxicity in Human Cell Cultures.

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Abstract

Nanomedicine is a great interesting area for use of magnetic nanoparticles. Among their main applications we can highlight, for example, its use as contrast agents for magnetic resonance imaging, or intracellular delivery, or cellular hyperthermia, which is an employed method in oncology therapy. This study aimed to determine whether the magnetic nanoparticles with $La_{0.78}Sr_{0.22}MnO_3$ (LSMO), coated with SiO_2 are cytotoxic to the cancer cell line MCF-7 (human breast adenocarcinoma). For perovskite LSMO synthesis the liquid-mix method was used and sol-gel method was used for SiO_2 coating. Cytotoxicity was determined by cell number and viability assessment as well as clonogenic assay to study chronic effect induced by nanoparticles. Concentrations of 74.88 ± 1.1 LSMO µg/ml were able to reduce 50% of cell number compared with control untreated cells and after 72 h of treatment, cells lead a slight chronic effect. We also observed morphological changes after incubation of particles.

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Keywords: perovskites, nanomedicine, breast cancer.

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1. Introduction

Nanomedicine is one of the most active fields of nanotechnology. It is can be defined as the branch of medicine that uses nanotechnology knowledge in science and medical procedures (Freitas, 2005). In particular, its combination with nanomagnetism (phenomena arising from the application of magnetic fields to magnetic particles) is of interest for this work development.

Breast cancer is one of the most common cancers and the leading cause of death from cancer among women, accounting for 23% of the total cancer cases and 14% of cancer deaths in 2008 on the world (Instituto Nacional del Cáncer, 2012). The leading cause of cancer deaths in women in Argentina, too. Epidemiology studies inform 74 cases per 100,000 women and a mortality rate of 20.1 per 100,000 women. Contrary to popular belief, 75% of women with breast cancer have no family history of the disease, and 1% of breast cancer occur in men (Instituto Nacional del Cáncer, 2012). Given the particular importance of this disease and the extensive knowledge that exists about their study tools, it is proposed as in vitro model for breast cancer cells MCF-7 (human breast adenocarcinoma).

Cell lines are a powerful tool for analyzing some aspects of cancer cell biology. The selected line, MCF-7, was developed by Soule (Soule, 1973) from a pleural effusion of human breast carcinoma and it is considered one of the quintessential breast cancer cell line (Lanari, 2003).

In the literature, no reports have been found about the study of the cytotoxicity (*in vivo* or *in vitro*) of these LSMO particles over biological systems.

The aim of this study was to evaluate the capability of $La_{0.78}Sr_{0.22}MnO_3$ (LSMO) particles to induce cytotoxic effects over human breast tumor cells and their efficacy to affect cell proliferation.

Altogether, this proposal pretends to collaborate to find better diagnostic and therapeutic approaches. By improve some characteristics as cell uptake, side effects and selectivity, the nanoparticles use could improve treatment outcome in pathologies as cancer.

2. Experimental procedure/Methodology

2.1. Perovskite Synthesis

Liquid Mix technique has been used to obtain LSMO nanoparticles; for this procedure citric acid and ethylene glycol was added to stoichiometric metal nitrates solutions under agitation and heater. After this process, the obtained resin was thermal treated to obtain the mixed oxide (Leyva, 2007).

The coating LSMO nanoparticles have been performed using the Stöber method (Leyva, 2007). This method consists in an ammonia–catalyzed hydrolysis reaction of tetraethoxysilane (TEOS), or tetramethoxysilane (TMOS), in isopropanol solution.

2.2. Cytotoxicity studies in MCF-7 cell line.

A dose-response assay was performed with LSMO nanoparticles against Michigan Cancer Foundation cell line of human mammary adenocarcinoma (MCF-7). Cells were cultured in Dubbelco´s Modified Eagle Medium (DMEM), supplemented with 10 % fetal bovine serum, 100 IU of penicillin and 100 µg/ml streptomycin. Culture conditions were 37 °C in a humidified atmosphere enriched by 5 % CO_2.

By use of dimethylsulfoxide (DMSO) as solvent, a solution of LSMO was added to the culture medium at different concentrations (from 37.5 up to 300 µg/ml, particles weigh/culture media volume). Maximal concentration used of DMSO was assayed and did not determine cytotoxicity by itself. After 24 h of cellular plate, working solutions were dissolved directly in culture medium and the final concentration of nanoparticles was calculated to add 100 µl/dish of the solution treatment, without remove the culture medium.

After 72 h of treatment, without any change of medium, all cells were collected, including supernatants and those adhering to the bottom. Then, cells were centrifuged, washed and suspended in a solution of phosphate buffered saline (PBS). After trypan blue staining, cell number was quantified using a Neubauer chamber. Assays were performed in triplicate. Obtained values were analyzed using Prism 5.0 software to calculate IC_{50}.

Morphological changes induced by LSMO treatment were observed by optical microscopy. In accordance to this aim, cells were grown adhering to a sterile cover slip, placed into six wells plate. At 72 h (end of experiment), the slide was removed by forceps from the well bottom. Subsequently, cells were rinsed with sterile PBS, fixed with methanol and GIEMSA stained. To microscopic analysis and photographic register, samples were mounted.

Finally, to evaluate the chronic effect induced by perovskite treatment, a clonogenic assay was performed. In accordance to the current practice, 500 viable cells obtained after treatments, were plated in a fresh media without nanoparticles. Cells capability to attach and proliferate was registered 10 days later and colonies containing ≥ 30 cells were considered positive (cells with non-chronic damage). At the end of the assay, cells were rinsed with PBS, fixed with methanol and stained with crystal violet; percentage number of treated cells was compared with control cells (treated only with solvent), in which clonogenic capability was considered as 100 %.

2.3. Statistical analysis

Two-Way ANOVA test followed by Dunnet´s post-test were performed to determine the differences between control and experimental groups. Statistical analyses were performed using the Prism computer program (Graph Pad Software, San Diego, CA) and a $p < 0.05$ was considered statistically significant.

3. 3. Results and discussions

3.1. Perovskite´s characterization

The X Ray Difraction (XRD) patterns of the powder samples for x = 0.22 composition at several temperatures are shown in the Figure 1. Samples were found to be a single phase without any trace of impurities and this pattern was consistent with the perovskite crystal structure. The Scherrer formula was applied to determine the particle size using the medium high width peaks (MHW). The average particle size for T= 700 ºC was around 22 nm, this temperature was used for subsequent treatments.

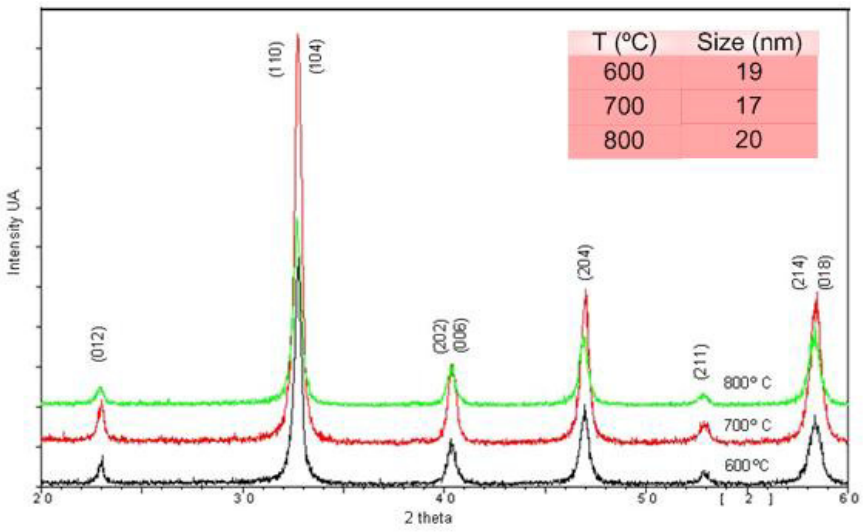

Fig. 1: Average crystallite size of perovskite is 22 +/-1 nm by DRX assay

Transmision electron microscopy (TEM) showed that particles were forming agglomerates as is shown in Figure 2. LSMO dispersed particles in isopropanol were coated using the previously described method.

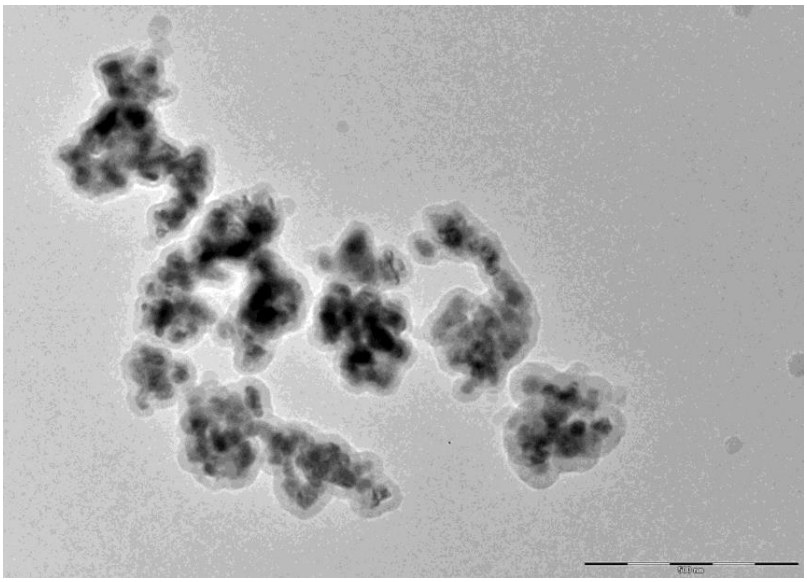

Fig. 2: LSMO Nanoparticles TEM Micrography, with X= 0.22 (Bar: 500nm)

In order to analyse the coating layer; Fourier Transformed Infrared Spectroscopy (FTIR) was used to identify the existence of SiO_2 layer. In the FTIR spectrum (see Figure 3) of the core shell NP.

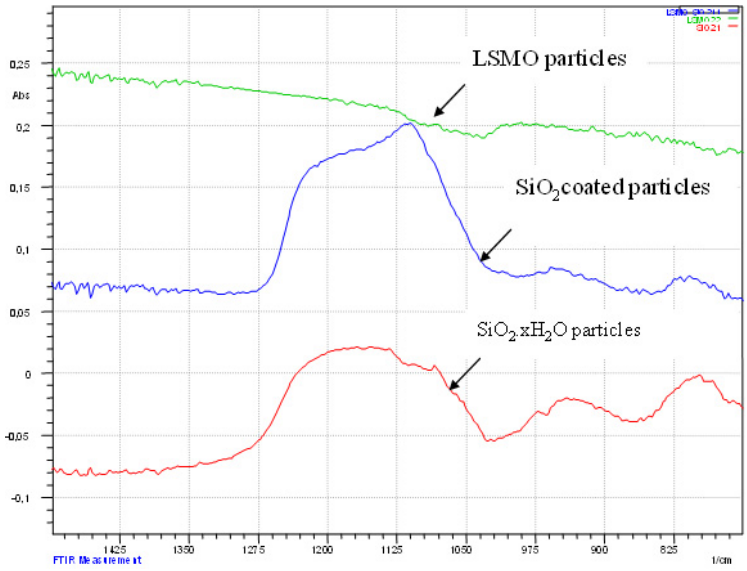

Fig. 3: FTIR spectrum of the core shell NP the 1100 cm- 1 band corresponds to the O-Si-O stretching vibrations and the 800 cm^{-1} band corresponds to Si-OH, according to the signals of pure SiO2.XH2O nanoparticles.

3.2. Evaluation of the DMSO´s effect

To treat attached cell lines, a convenient solvent to prepare perovskites solution was investigated. DMSO was able to resuspend the solid LSMO particles and allow the proper solution preparation. However, DMSO was reported as a toxic by itself to cultured cells. Consequently, a study of specific compound induced toxicity was designed at the maximal concentration presents in the assays, and cellular response was quantified. Figure 4 shows

the control growth rate of MCF-7 cell line compared with the cells cultured in presence of 10 µl/ml DMSO. By statistical analysis with Student's t test there was not significant ($p < 0.05$) difference in growth when DMSO reached a maximal doses used in perovskites biological studies.

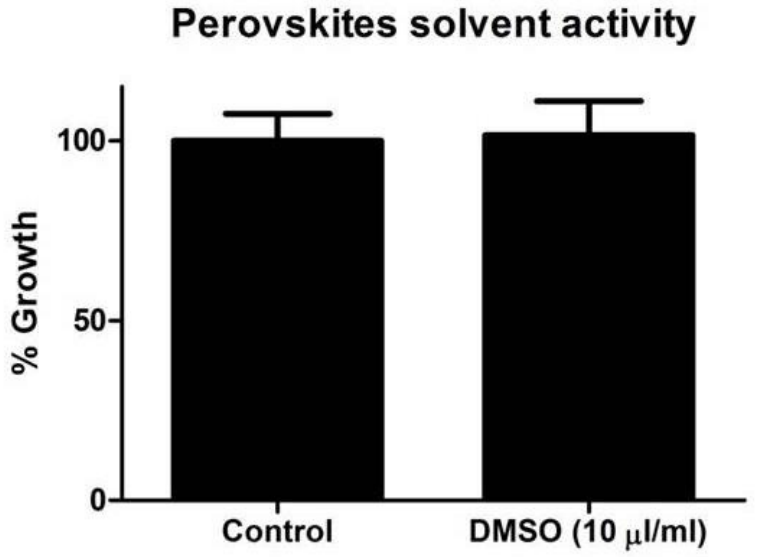

Fig. 4. Growth interference induced by DMSO at maximal concentration

3.3. Efect of perovskites on proliferation of MCF-7

To evaluate changes in MCF-7 cell line proliferation induced by LSMO particles presence, a dose-response experimental design was performed. In accordance to cell line duplication time (data not shown) treatment time was fixed at 72 h. Briefly, 5 x 10^5 cells were plated in six well plates and, 24 h later (to avoid the cells attach), treatment at the convenient doses was applied. Three days later, total cells present in wells were collected, trypsinized and quantified. To evaluate treatment capability to interfere with normal cells proliferation, IC_{50} (50% of growth inhibition) was calculated (see Figure 5) with Prism 5.0 software.

Analysis demonstrates a dose-dependent growth inhibition and 74.88 ± 1.1 µg/ml (mean ± SEM) was the IC_{50} estimated dose. Decreases values found in cell number can be attributable to cell arrest and/or cell death. Trypan blue exclusion dye was used to evaluate cell viability but LSMO particles presence did not allow a precise determination.

3.4. Clonogenicity assay

Clonogenic survival assays or clonogenicity is an experimental approach to evaluate cell effects not related to experimental treatment presence. Frequently, this design is interpreted as compound capability to induce chronic effects. In short, cells were treated during 72 h and then, a small number of viable cells were re-plated and cultured for 10 days. Cells capability to attach, proliferate and reach ≥ 30 cells colonies are considered as positive colony and its percentage expression compared with control is utilized to evaluate chronic effect of compounds. Specifically to LSMO particles, chronic damage appears as dose-effect dependant (see Figure 6), but one way ANOVA and Dunnet´s post-test analysis were not able to demonstrate statistical difference (significance $p < 0.05$).

Even though statistics did not support colonies reduction, treatments of 150-300 µg/ml were related to increased incidence of abortive and smaller colonies.

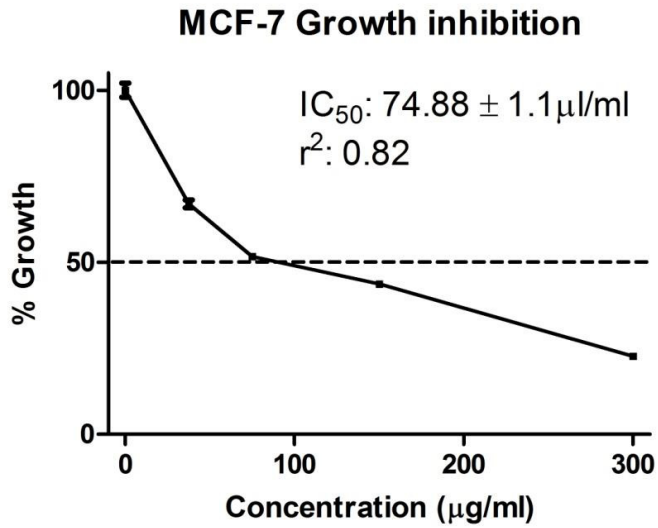

Fig. 5. LSMO dose response against MCF-7.

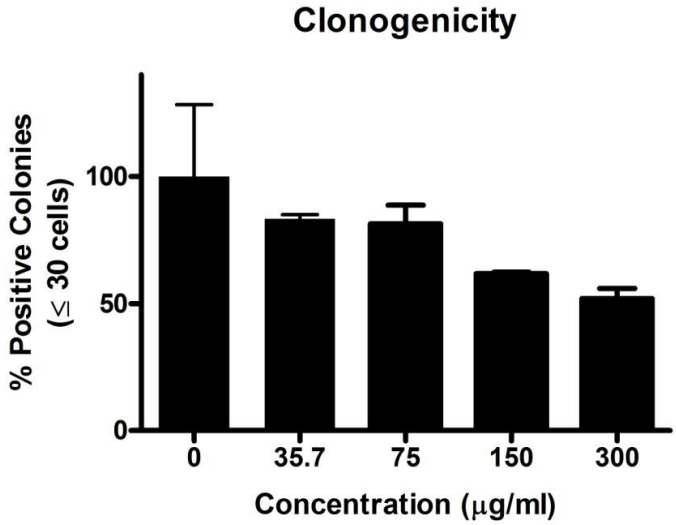

Fig. 6. Clonogenic survival assay after 72 h of LSMO treatment.

3.5. Changes in morphology induced by treatments with LSMO

Afterward proliferation interference was established, cell content of LMSO particles needs to be demonstrated. Morphological changes induced by perovskites treatment were observed and optical microscopy photographs are shown in Figure 7. Panels A and B show a general appearance of perovskites treatment against MCF-7 cell line, and panels C to E show intracellular location of particles. Treatment-induced cell damage is characterized by cells with large and flat cytoplasm containing multiple vacuoles with nanoparticles content. Vacuoles characterization present a general pattern which is multiple vacuoles, located close to nucleous (perinuclear).

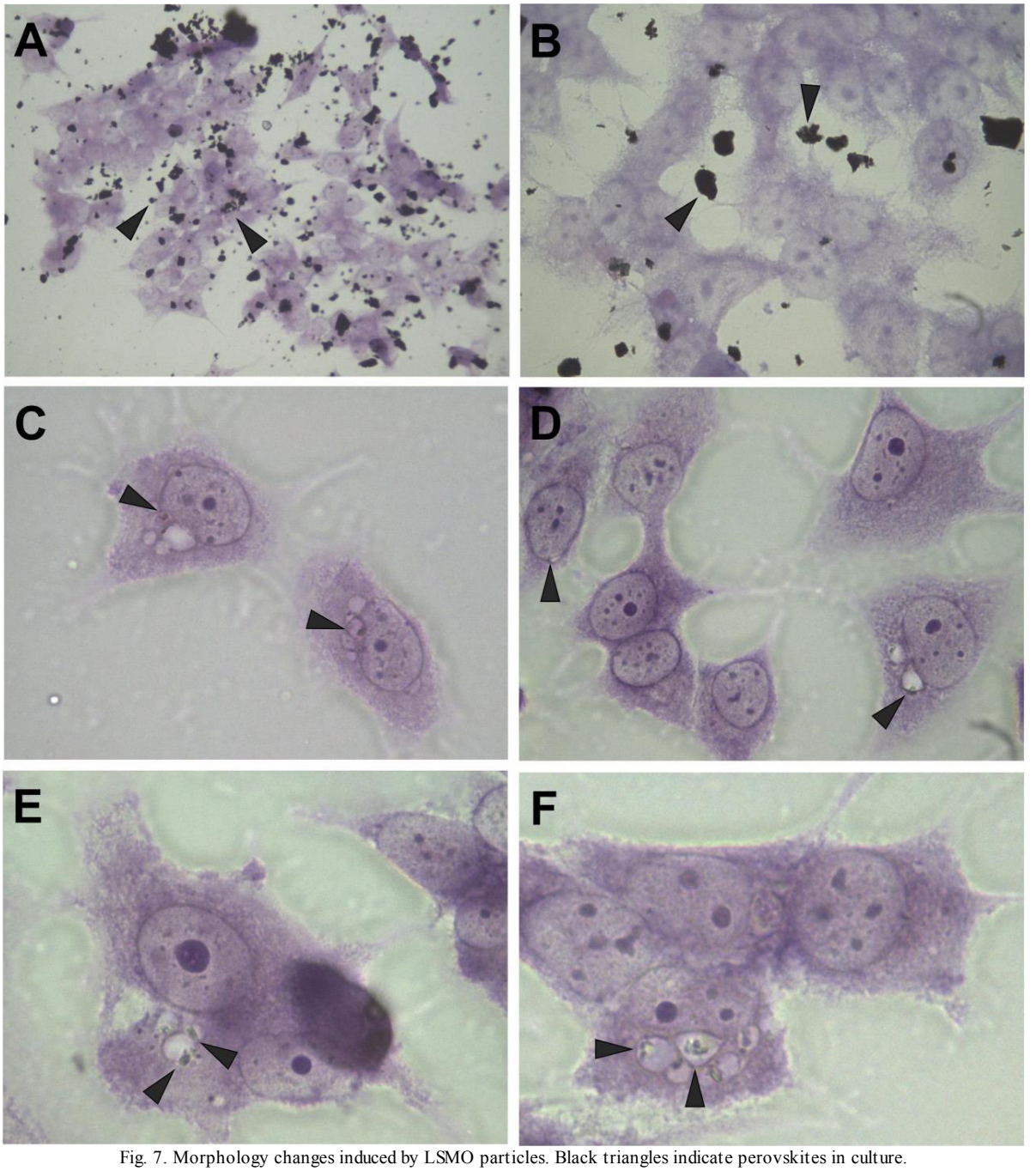

Fig. 7. Morphology changes induced by LSMO particles. Black triangles indicate perovskites in culture.

4. Conclusions

DMSO is an adequate solvent with allow LSMO dissolution and cell culture treatment. LSMO particles induce changes in cell proliferation evaluated by cell number reduction after treatment. Concentrations of 74.88 ± 1.1 LSMO μg/ml are able to reduce 50% of cell number compared with control untreated cells. After 72 h of treatment,

cells lead a slight chronic effect as the clonogenic assay results evident. The LSMO particles treatment induces morphological changes which can be observed by optical microscopy. According to the evidence provided by optical microscopy, the biological effects observed may be attributed to the intracellular localization of LSMO into cytoplasmic vacuoles.

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