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Silica network improve the effect of fludarabine and paclitaxel on HCT8 cell line

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Abstract

This paper reports the potential of silica network to sensitize tumor cells and stimulate antitumor toxicity of fludarabine (FLU) and paclitaxel (PAC) against HCT8 cells. SiO_2, SiO_2/FLU and SiO_2/PAC nanostructured materials were characterized by X-Ray Diffraction, Scanning Electron Microscopy, InfraRed Microscopy and *in vitro* biological assays. When using SiO_2/PAC, it can be observed that the cytostatic effect of PAC is boosted only at high concentrations of this material. On the other hand, in the case of SiO_2/FLU, data showed an enhancement in the cytostatic activity of FLU by up to 25%, also when using this nanomaterial in low doses. These data represent preliminary study on the impact on silica nano-networks in targeted delivery and controlled release of antitumor drugs and they may be efficiently used for future biomedical applications in cancer therapy.

Keywords: fludarabine, paclitaxel, antitumoral, silica network, cell viability.

Introduction

Applications of nanotechnology in the treatment of cancer have attracted the attention of researchers due to the great potential of nanomaterials to be used for two or more different functions [1–10]. Multifunctional nanomaterials have been designed for various applications such as drug delivery, imaging or diagnostic sensors [11–13]. Inorganic or organic nanomaterials were involved in the development of various applications in cancer therapy [14–18]. Silica nanomaterials were successfully used in the cancer therapy due to their easy route of synthesis, easy functionalization and adjustable pore volume [19–23]. Silica networks are usually non-toxic and frequently used in biomedical applications [24, 25]. Two types of silica network were designed during recent years: core/shell nanoparticles and mesoporous nanoparticles [26, 27]. Core/shell nanoparticles are consisting of a metal oxide as a core and silica as a shell, usually prepared from tetraethyl orthosilicate or sodium metasilicate [28, 29]. Mesoporous silica nanoparticles are usually prepared by templating using various surfactants [30, 31]. In a recent study, γ-amino-butyric acid was used as templating agent for the preparation of a newly silica network able to deliver and improve the effect of therapeutic agents [32]. The dimension of particle was estimated at 10 nm from TEM analysis, while BET analysis reveal the average pores diameters of 4.6 nm, as revealed by this study [32].

Different formulations based on silica networks and antitumor agents can be found in recent literature, but no data explaining the interaction of silica networks with antitumoral agents in the light of any improvements of the antitumor effects of the active drug, was reported.

Here, we reveal the ability of silica network to deliver and improve the antitumor effect of fludarabine (FLU) and paclitaxel (PAC) against cultured HCT8 tumor cells, which may be used for future research with applications in cancer therapy.

Materials and Methods

Materials

Sodium metasilicate, sulfuric acid (ACS reagent 95–98%) and ε-amino-caproic acid were purchased from Sigma-Aldrich, and were used without any further purification.

Preparation of silica network

Silica network was prepared from sodium metasilicate by treating with 5% sulfuric acid. In a 500 mL beaker was added under vigorous stirring, 100 mL water, 20 mL $Na_2SiO_3 \times H_2O$ and 1 g of ε-amino-caproic acid. After 10 minutes of vigorous stirring, sulfuric acid solution was added drop by drop, under permanent stirring until the pH was up to 7, leading the formation of transparent hydrogel. The prepared hydrogel was filtered, repeatedly washed with deionized water, and subsequently dried at room temperature. In the next step, the dried powder was calcinated at 650^0C for 24 hours in order to remove the ε-amino-caproic acid.

Preparation of silica network/cytostatic drugs

After calcination, 80 mg of the prepared powder were homogenized with 20 mg of cytostatic drugs (Fludarabine and Paclitaxel) in chloroform until complete evaporation of the solvent. This step was repeated three times for a good distribution of cytostatic drugs into silica network.

Characterization

X-ray diffraction analysis was performed on a Shimadzu XRD 6000 diffractometer at room temperature. In all the cases, Cu Kα radiation from a Cu X-ray tube (run at 15 mA and 30 kV) was used. The samples were scanned in the Bragg angle 2θ range of 10–80^0.

SEM analysis was performed on a FEI electron microscope, using secondary electron beams with energies of 30 keV, on samples covered with a thin silver layer.

IR mapping were recorded on a Nicolet iN10 MX FT-IR Microscope with MCT liquid nitrogen cooled detector in the measurement range 4000–700 cm^{-1}. Spectral collection was made in reflection mode at 4 cm^{-1} resolution. For each spectrum, 32 scans were co-added and converted to absorbance using OmincPicta software (Thermo Scientific, USA). Approximately 250 spectra were analyzed for each sample. One absorption peak known as being characteristic for the scanned samples was selected as spectral marker.

Biocompatibility of silica network

For cell proliferation and viability assay (CellTiter 96® Non-Radioactive Cell Proliferation Assay kit, Promega, Madison, USA), the endothelial cells (EAhy926 cell line, ATCC) were seeded in 96-well plates, at a density of 5×10^3 cells/well, in DMEM medium, supplemented with 10% fetal bovine serum. The cells were incubated in the presence of silica network for 24, 48 and 72 hours; controls were represented by endothelial cells grown in the same culture conditions, without the silica network. Cell proliferation assay was performed in triplicates, according to the manufacturer's guidelines, at different time intervals. Briefly, 15 µL of Solution I was added in each well and incubated for four hours. Furthermore, 100 µL of Solution II was added in the 96-well plate, incubated for one hour followed by spectrophotometric measurements at 570 nm using Mithras LB 940 (Berthold Technologies, Germany). The percentage of viable cells was calculated by following ratio: X=[OD treated sample/ OD untreated sample] × 100. Fluorescent microscopy was assessed using a RED CMTPX fluorophore (Life Technologies, Invitrogen, USA), a long-term living cell tracker. The RED CMTPX dye was added in the culture medium at a final concentration of 5 µM and incubated for 30 minutes to allow the dye to penetrate the cells. The cells were washed with phosphate buffered saline and visualized by fluorescence microscopy. The photo-micrographs were taken with a digital camera using the Axio-Vision 4.6 (Carl Zeiss, Jena, Germany) software.

Antitumor activity of silica network/cytostatic drugs

Quantitative determination of cytotoxicity on human HCT8 cultured tumor cells was performed using the CellTiter 96® AQueous One Solution Cell Proliferation Assay according to manufacturer's protocol. Briefly, 5×10^3 cells were seeded into each well of 96-well plates and 24 hours later binary dilutions of 1 mg/mL of hybrid materials were added. The cytotoxic effects were evaluated at 24, 48 and 72 hours. The hybrid materials were removed by gentile washing with phosphate buffered saline and 20 µL of CellTiter 96® AQueous One Solution Reagent was added into each well of the 96-well assay plate containing the samples in 100 µL of culture medium. Plates were incubated at 37^{0}C for two hours in a humidified, 5% CO_2 atmosphere. The absorbance was recorded at 490 nm using a microplate reader (HumaReader HS, Human Gesellschaft für Biochemica und Diagnostica mbH, Germany), in order to measure the amount of soluble formazan produced by cellular reduction of MTS. The percentage of viable cells was calculated by following ratio: X=[OD treated sample/OD untreated sample] × 100.

Results

Silica network was characterized by XRD, SEM, and IRM. *In vitro* biological assays were also performed. The results of the XRD analysis are presented in Figure 1. According to this figure, it can be concluded that the prepared sample has a high degree of crystalinity. The identified mineral phase was cristobalite.

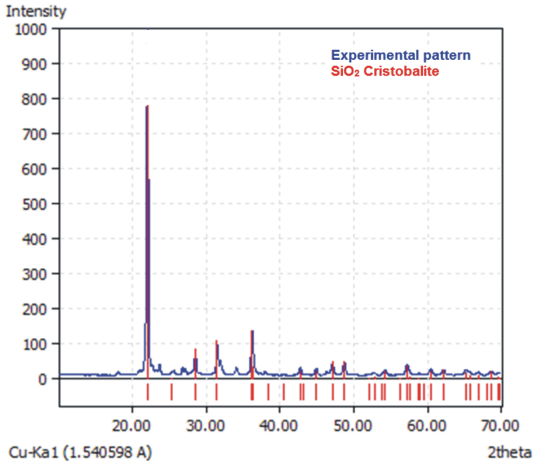

Figure 1 – ***XRD pattern of prepared silica network.***

SEM micrographs are plotted in Figure 2. It can be seen that the material is granular, with various morphology of grains and dimensions ranged between 200 and 600 nm.

InfraRed Microscopy is a powerful technique that allows evaluating the presence of functional groups on the scanned surface. Prepared samples were scanned and the IR maps were realized by monitoring the absorption band at 2930 cm^{-1} and 2943 cm^{-1}, characteristic to cytostatic drugs. By comparing the Figures 3 and 4, it can be seen that characteristic bands of FLU can be identified easily in the IR spectra of SiO_2/FLU. In Figures 5 and 6 are plotted the IR maps and spectra of PAC and SiO_2/PAC. In this case, absorption bands at 1793 cm^{-1} (characteristic to C=O) and 2943 cm^{-1} (characteristic to C–H) can be observed in the IR spectra of PAC and SiO_2/PAC, too. Also, in the case of SiO_2/PAC and SiO_2/FLU, absorption bands characteristic to silica network can be seen. Regarding IR maps, cytostatic drugs are distributed homogenous into the entire silica network (Figures 4 and 6).

The cytotoxicity test based on the reduction of the

MTT tetrazolium salt revealed that the silica network has a good biocompatibility in contact with cultured endothelial cells, and no cytotoxic effects were observed. The silica network does not significantly influence the growth and normal metabolism rate of these cells for up to 72 hours incubation (Figure 7).

Figure 8 represent the results obtained after five days of treatment of endothelial cells with tested silica network. Through the comparative analysis of the cells grown on the silica network and cells grown in standard conditions it we observed that there are no significant differences among samples, as revealed by the microscopy images. These results demonstrate that the tested nanostructurated silica network has a good biocompatibility *in vitro*. The microscopy evaluation was performed after RED CMTPX cell staining (Figure 8). Due to its ability to penetrate within viable, metabolically active cells and because of the long-term persistence of this vital dye, it allows highlighting morphological changes and growth of the analyzed cells for several generations.

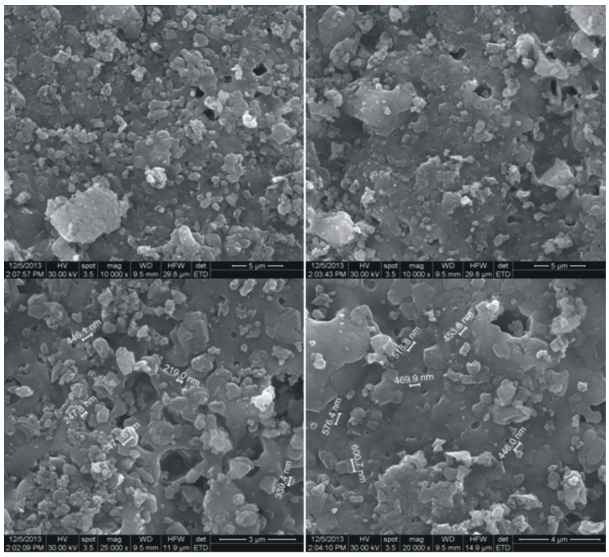

Figure 2 – *SEM micrographs of prepared silica network.*

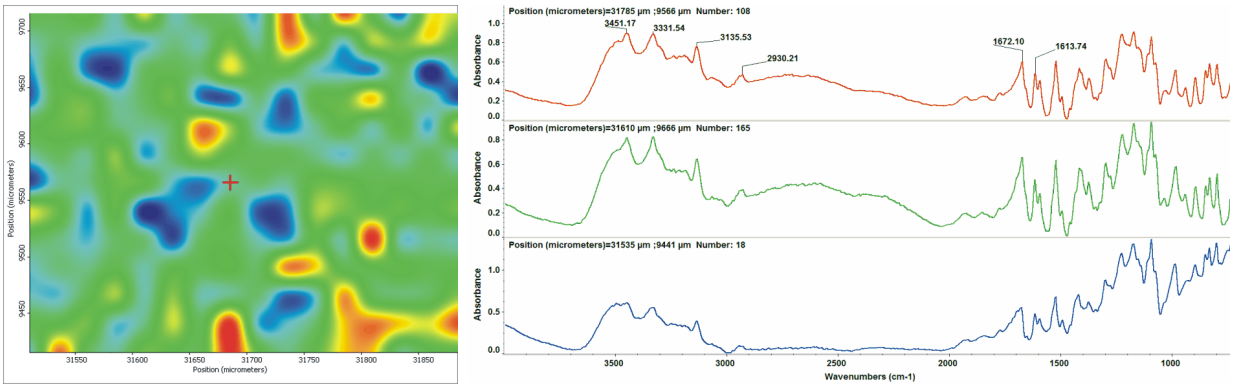

Figure 3 – *IR map (2930 cm^{-1}) and IR spectra of FLU.*

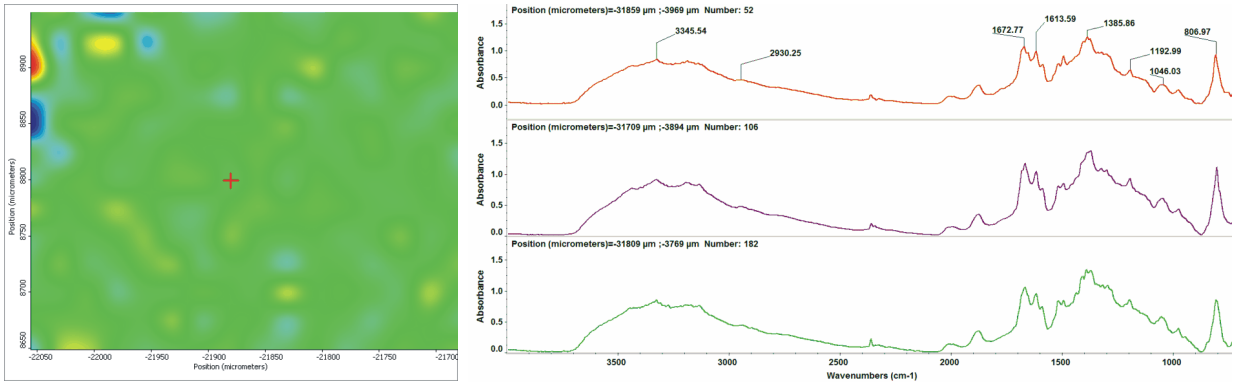

Figure 4 – *IR map (2930 cm^{-1}) and IR spectra of SiO$_2$/FLU.*

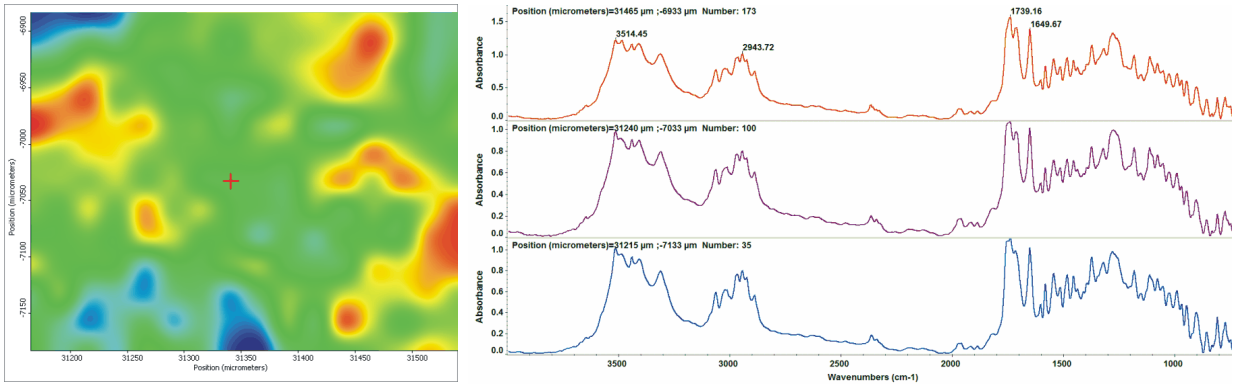

Figure 5 – *IR map (2943 cm^{-1}) and IR spectra of PAC.*

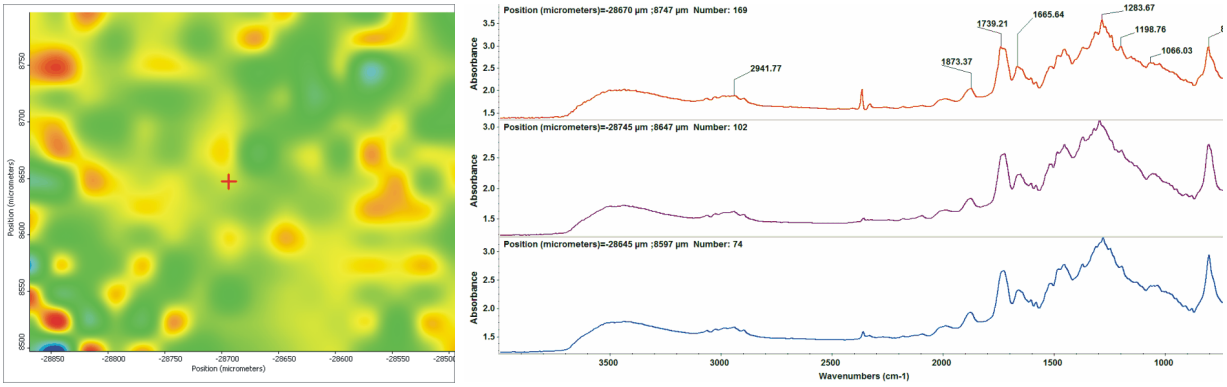

Figure 6 – ***IR map (2943 cm^{-1}) and IR spectra of SiO_2/PAC.***

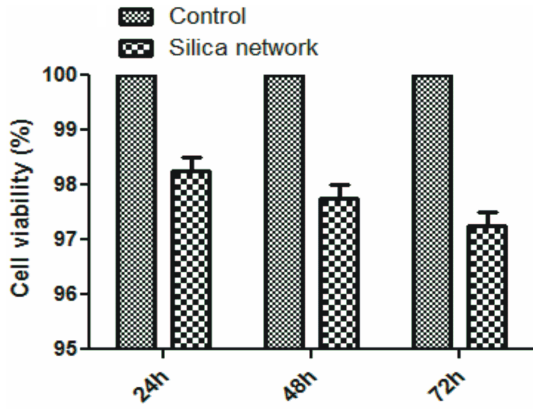

Figure 7 – ***Biocompatibility of the silica network on human endothelial cells after 24, 48 and 72 hours incubation.***

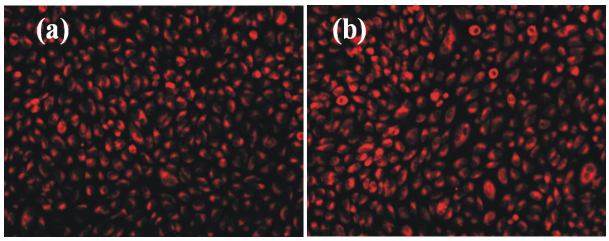

Figure 8 – ***Micrographs of endothelial cells after five days of incubation in control conditions (a) and in the presence of the silica network (b).***

The antitumor potential of the fabricated hybrid nanomaterials has been tested for a period of up to 72 hours. The results obtained after 24 hours of treatment showed that none SiO_2/FLU or SiO_2/PAC showed significantly higher antitumor effect as compared with PAC and FLU cytostatics used individually at the same concentrations.

However, after 72 hours of treatment, SiO_2/FLU showed a significantly higher antitumor effect at concentrations between 15.63 and 0.96 µg/mL as compared with the same concentrations of the plain drug. At these concentrations, SiO_2/FLU nanosystems enhanced the cytostatic activity of FLU by 15–25% (Figure 9).

On the other hand, in the case of SiO_2/PAC it was observed that the effect of PAC is boosted only at high concentrations of the nanomaterial (above 125 µg/mL), demonstrating that this controlled release system is less effective for delivering lower concentrations PAC (Figure 10).

MTT results were also confirmed by optical microscopy analysis. These results show that the use of high concentrations of chemotherapy drugs, used both as plain solutions and cytostatics embedded in the fabricated SiO_2 matrix, causes a small percentage of viable cells, which have an altered morphology. When using SiO_2/FLU, higher cytostatic effects can be seen at almost all tested concentrations (Figure 11), whereas if SiO_2/PAC is used, more pronounced cytotoxic effects can be observed only when using higher concentrations of the bioactive material (Figure 12).

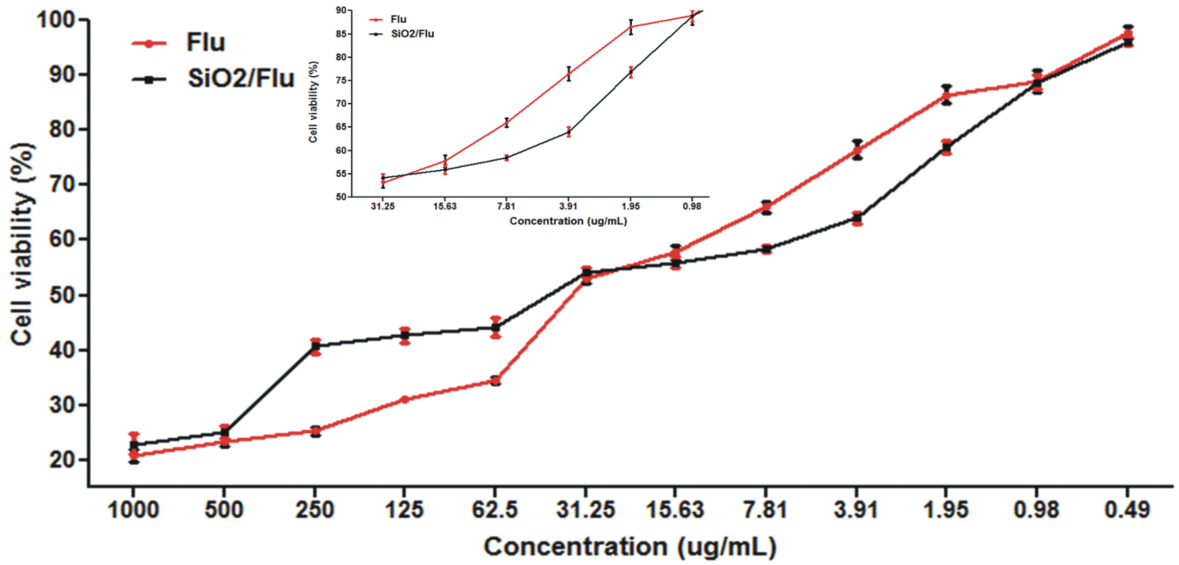

Figure 9 – ***Graphical representation of the MTT results after 72 hours of incubation of human tumor cells with FLU and SiO_2/FLU.***

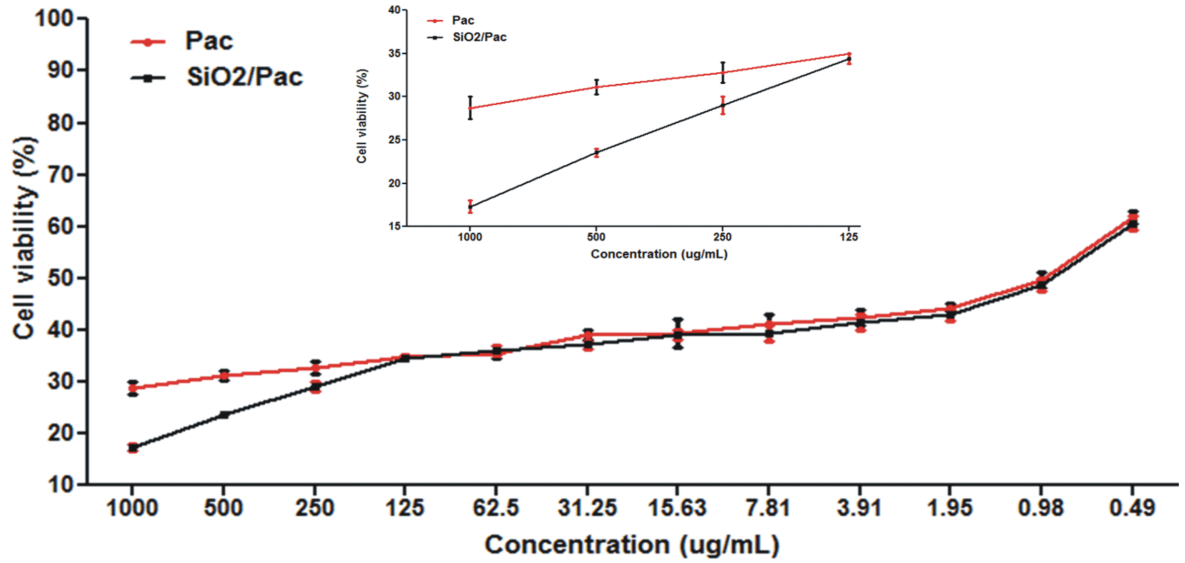

Figure 10 – ***Graphical representation of the MTT results after 72 hours of incubation of human tumor cells with PAC and SiO_2/PAC.***

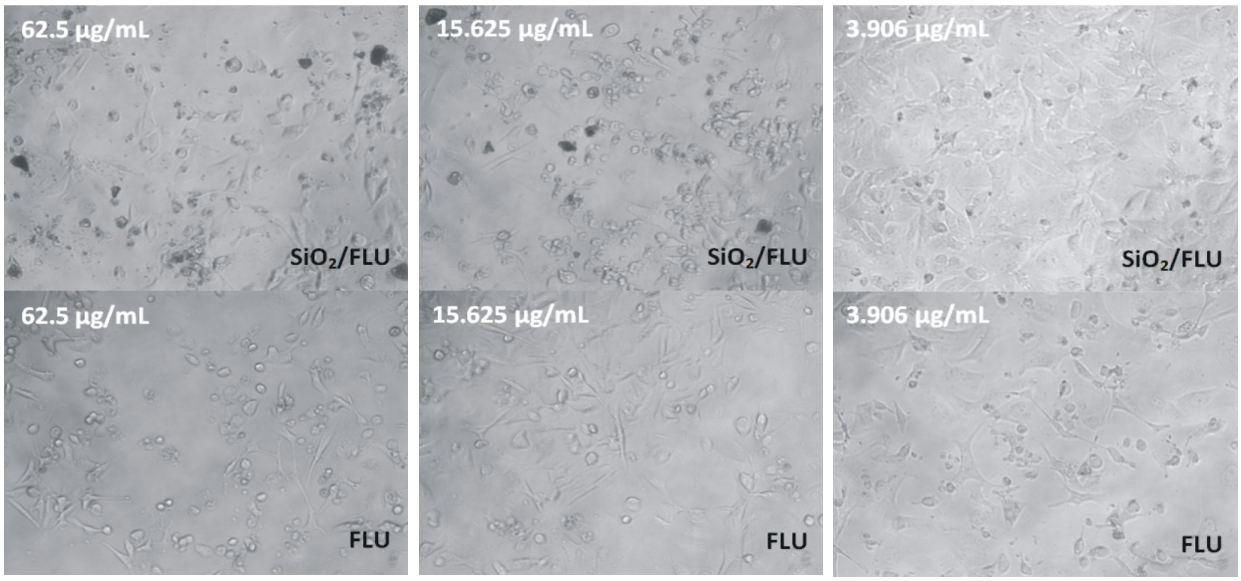

Figure 11 – ***Microscopy images highlighting the morphology of tumor cells treated with various concentrations of FLU and SiO_2/FLU.***

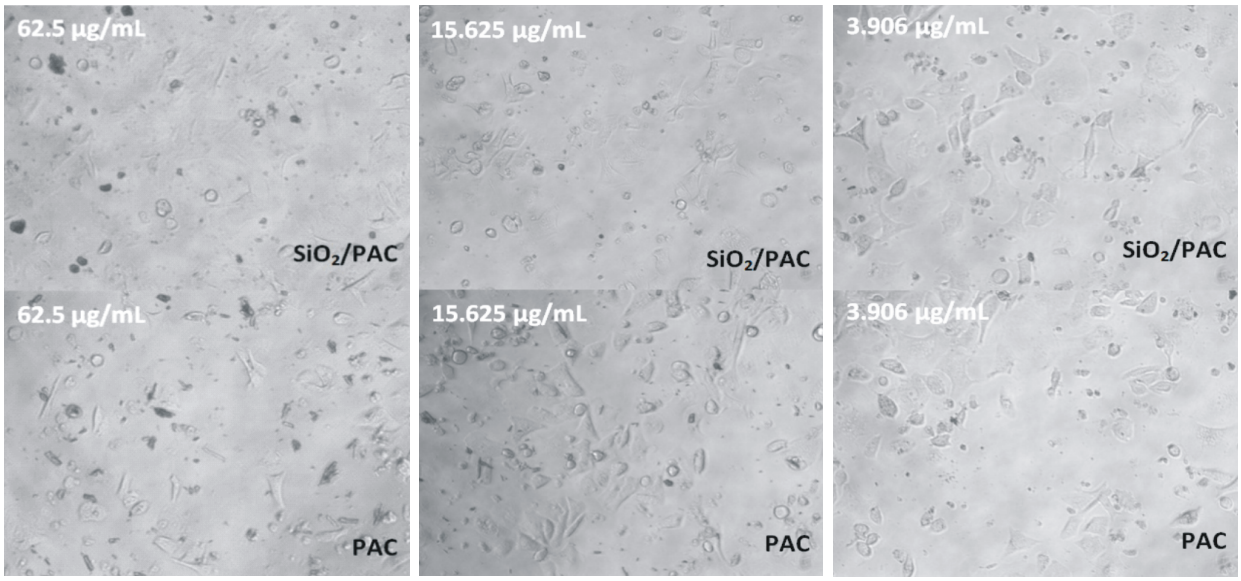

Figure 12 – ***Microscopy images highlighting the morphology of tumor cells treated with various concentrations of PAC and SiO_2/PAC.***

Discussion

Fludarabine is an anti-metabolite frequently used in chemotherapy for the treatment of chronic lymphocytic leukemia (CLL), including CLL that has not responded to or reoccurred after standard therapy, but also for salvage therapy for non-Hodgkin's lymphoma and acute leukemias [33]. Paclitaxel is a mitotic inhibitor used in cancer chemotherapy, being approved for treating ovarian, breast and lung, bladder, prostate, melanoma, esophageal, and other types of solid tumor cancers as well as Kaposi's sarcoma [34]. Since both drugs represent first-line and second-line treatment of many cancers, their use in the medical field is increased, despite the severe side effects caused in most patients [35]. In this context, research aiming in improving the efficacy of these drugs, but in the same time keeping the necessary therapeutic concentrations low, are of a great interest. Our study represents the first research on this field and demonstrates that nanostructurated silica networks may be efficiently used as efficient carriers and efficient release systems for fludarabine and paclitaxel drugs. The results demonstrated that the fabricated silica network improve the antitumor activity of these cytostatic drugs when used at certain concentrations. These data represent preliminary study on the impact on silica nano-networks in targeted delivery and controlled release of antitumor drugs and they may be efficiently used for future biomedical applications in cancer therapy. Further optimizations may be necessary in order to obtain the best formulation for a specific type of cancer cell, depending on the required concentration and the particularities of the cell line.

Conclusions

The obtained results highlights the remarkable potential of silica networks, in addition to those described in the literature, to sensitize tumor cells and stimulate antitumor toxicity of therapeutic agents against HCT8 cells.

Acknowledgments

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