Evaluation of the cytotoxicity of modified TiO_2 on cervical cancer HeLa cell line

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ABSTRACT

The modified TiO_2 is a substance with potential and future uses in the treatment of various cancers. The alternative photodynamic therapy with TiO_2-Au, Ti_{O2}-mod-FA, TiO_2-mod nanoparticles were studied on HeLa-CCL2 (cervical cancer) and CHO-k1 line cells. The TiO_2-mod were synthesized using a sol-gel route. The characteristic functional groups were analyzed by Scanning Electron Microscopy, Fourier Transform Infrared Spectroscopy and Energy Dispersive Spectroscopy. The structure of the each nanomaterial was studied by X-ray diffraction. The concentrations of the nanocomposite, the UV-A exposure times and UV-A presence or absence were manipulated like variables. The Cytotoxicity Detection Kit showed 99% of cytotoxicity on HeLa cells but it did not reveal cytotoxicity on CHO cells. Furthermore, compared to other traditional therapies it was only needed 40 minutes and a concentration of 200 ppm. This finding open a further possibility for the use of these nanomaterials in cancer.

Keywords: innovative, oncology, treatment

1 INTRODUCTION

The TiO_2 is a substance with a great potential and future; it is used in the treatment of various types of cancer for overlapping the limits on conventional treatments due to its possible cytotoxicity when exposed to light. TiO_2 is a metal oxide semiconductor, amphoteric and chemically stable. Heterogeneous photocatalysis is a viable option for cancer treatments, because of the ease of inducing chemical reactions with ultraviolet radiation [1].

With the extensive application of TiO_2 nanoparticles in the industry, there is a rising debate concerning the possible risk associated with exposure to TiO_2 nanoparticles[2]. The purpose of this research is to evaluate the cytotoxicity of Modified Titanium Dioxide (TiO_2-mod) on HeLa and CHO cell line.

2 MATERIALS AND METHODS

2.1 Materials

Titanium tetrabutoxide $Ti(OBu)_4$-Aldrich with 99% purity (TBT), anhidric ethylic alcohol (Mallinckrodt) and distilled deionized water were used as reagent for synthesizing nanoparticles by the sol-gel method[3].

2.2 Synthesis of modified TiO_2 nanomaterials

At room temperature and under moderate agitation, the TBT was added to the anhidric ethylic alcohol, then distilled and deionized water was added. During this process, the hydrolysis and polycondensation were presented. Further, in order to complete the condensation reaction, the gel was put in the desiccator during 72 hours. Finally, TiO_2 Anatase phase was obtained after being dried at 500 °C.

2.3 Characterization of materials

TiO_2-Au, TiO_2-mod-FA and TiO_2-mod nanoparticles were characterized by Fourier infrared spectroscopy (FTIR). The results obtained from each nanoparticle were compared by Energy Dispersive Spetroscopy (EDS) and X-ray diffraction (XRD). Finally, Scanning Electron Microscopy (SEM) was developed to observe details in structures.

3 PHOTOTHERAPY STUDY

Cells were seeded into a 24-well multiplate (5×10^4/well) for culture during one day. After washing with PBS buffer, cells were incubated with a dispersion of 100 and 200 $\mu l/ml$ of TiO_2-Au, TiO_2-mod, TiO_2-mod-FA, TiO_2-mod-FA-Au in DMEM medium for 2h . After, cells were irradiated with UV at a distance of 10 cm from the ultraviolet lamp (Blacklight blue) for 20 min or 40min. Cells were then subjected to viability assay (see Fig.1).

The cell viability was determined by Cytotoxicity Detection Kit (LDH), which membrane-damage cells results in an increase of LDH activity in the culture supernatant. This increase in the amount of enzyme activity corelates to the amount of formazan formed and in the assay is proportional to the number of lysed cells[4].

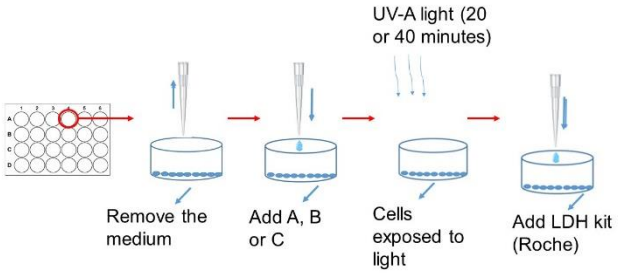


Figure 1 Phototherapy study on cancer cells

4 RESULTS AND DISCUSSION

4.1 FTIR analysis

Fig.2 shows the FTIR spectra of diferent TiO_2 . The TiO_2 spectrum (a) shows the characteristic bands of this sample between 500 and 1000 cm^{-1} reported by Wu and Cheng[5] and Tellez et al. [6]. The TiO_2 -Au spectrum (b) shows two additional bands, at 1532 cm^{-1} and at 1340 cm^{-1}, respectively, attributed to the presence of Au[7], also e) and f) show the characteristic bands of folic acid[8].

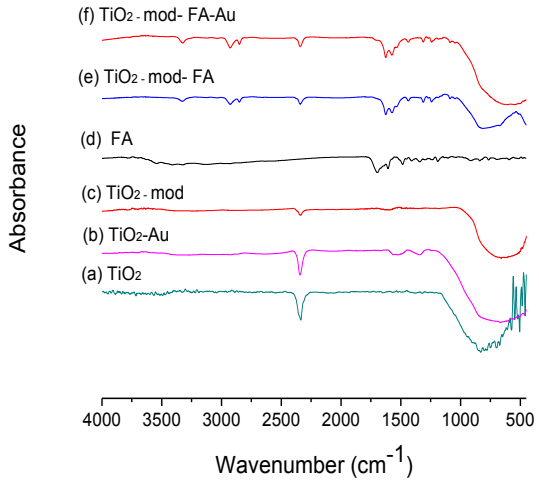


Figure 2 Fourier infrared spectroscopy a) TiO_2 b) TiO_2 -Au, c) modified TiO_2 , d) Folic acid (FA), e) TiO_2 - mod- FA f) TiO_2 - mod- FA-Au

4.2 XRD analysis

Fig 3. shows the XRD pattern of the synthesized pure Anatase TiO_2 nanomaterial[9]. Using the Rietveld method with the GSAS refinement program[10], it was determined that the anatase TiO_2 nanomaterial correspond to the reported Crystallographic Information File with reference code 01-071-1166 [11],with single tetragonal structure and space group I41/amd. The main peaks are at 2θ = 25.25°, 37.73°, 48.02°, 55.01°, 75.05°, and 62.67°

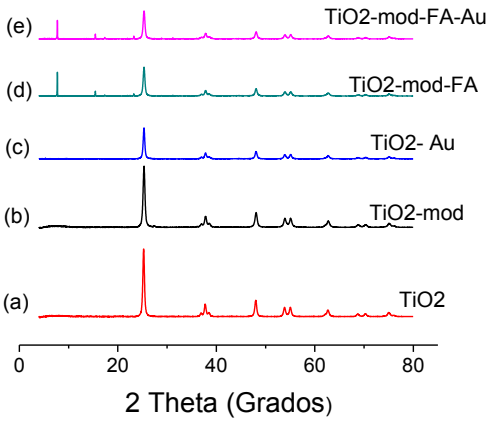
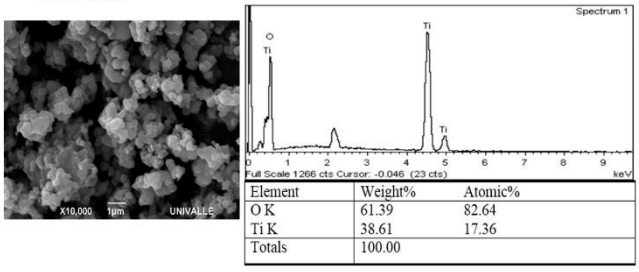


Figure 3 X ray difraction a) TiO_2 b) modified TiO_2 c) TiO_2-Au d) mod - TiO_2 - FA, e) mod -TiO_2 -FA- Au

4.3 SEM and EDS analysis

a)

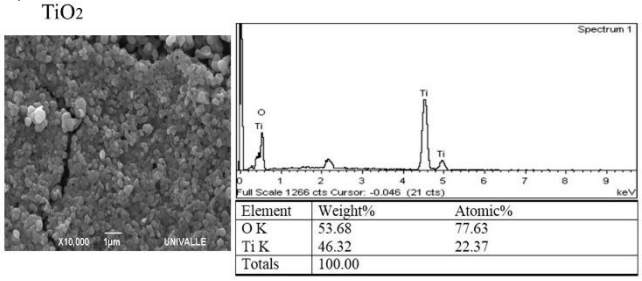
TiO_2-mod



Element	Weight%	Atomic%
O K	61.39	82.64
Ti K	38.61	17.36
Totals	100.00	

b)

TiO_2

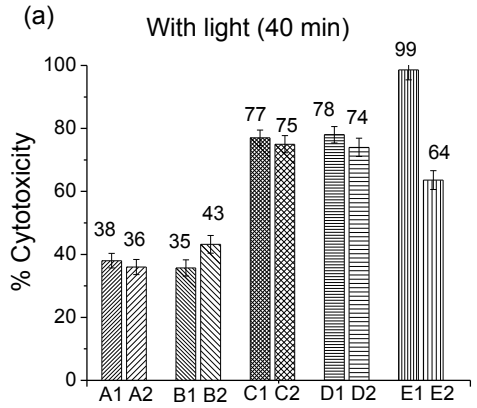


Element	Weight%	Atomic%
O K	53.68	77.63
Ti K	46.32	22.37
Totals	100.00	

Figure 4 SEM images and EDS of TiO_2 and TiO_2-mod

Fig.4 shows the SEM images of TiO_2 and modified TiO_2 nanoparticles, respectively. It can be noted that they are forming clusters among them. The average size of these clusters are 370 ± 100 nm. The clusters size (z-average) were calculated using the ImageJ software (free license).

We use EDS to determine the elements present in the samples and their concentrations, as it is illustrated in Fig.4 shows the percentages of elements present in the samples.



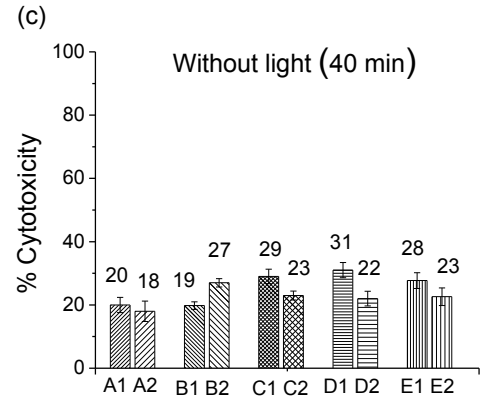
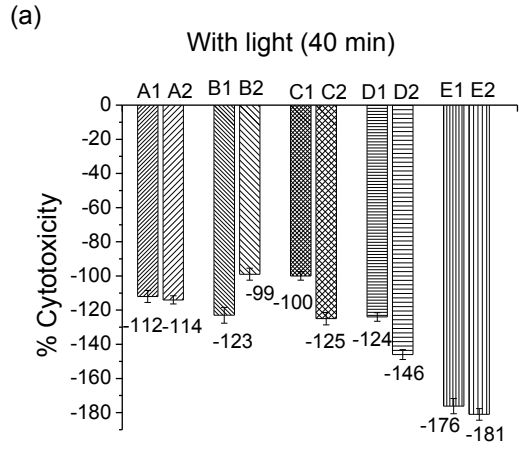


Figure 5 Graph of cytotoxicity assay on HeLa-CCL2 line cells (ATCC). Assay was carried out (a) with and (b) without irradiation of UV light in 40 minutes, with TiO_2 in two concentration A1 = 200 µg/ml and A2= 100 µg/ml, TiO_2-Au (B1, B2), TiO_2 -mod-FA (C1, C2), TiO_2-mod-FA-Au (D1, D2) and TiO_2-mod (E1, E2).



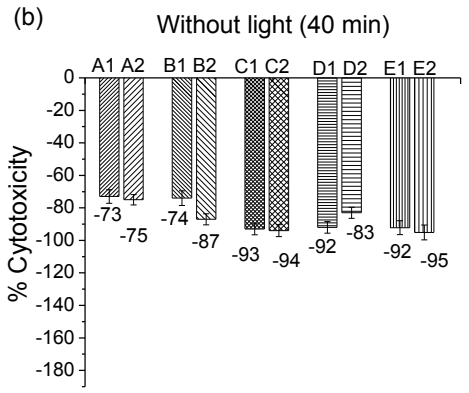


Figure 6 Graph of cytotoxicity assay on CHO-k1 line cells (ATCC). Assay was carried out (a) with and (b) without irradiation of UV light 40 minutes, with TiO_2 in two concentration A1 = 200 µg/ml and A2= 100 µg/ml, TiO_2-Au (B1, B2), TiO_2-mod-FA (C1, C2), TiO_2-mod-FA-Au (D1, D2) and TiO_2-mod (E1, E2).

Fig.5 and Fig.6 show the results of cytotoxicity assay on Hela and CHO cell line respectively. The concentrations of the nanocomposite, the UV-A exposure times and UV-A presence or absence were manipulated like variables. The Cytotoxicity Detection Kit (LDH) showed the maximum percentage was 99% of cytotoxicity on HeLa cells, when we used a concentration of 200 µg/ml of TiO_2-mod nanoparticles and the cells were exposed to UV light to 40 minutes; showing that this alternative therapy is much more effective[12]. In contrast, the nanoparticles did not reveal cytotoxicity in CHO cells. This finding open a further possibility for the use of these nanomaterials in cancer.

CONCLUSIONS

The selectivity for cancer cells lines was the principal finding, this finding open a further possibility for the use of these nanomaterials in cancer.

ACKNOWLEDGEMENTS

The authors would like to thank the financial support given to this work by COLCIENCIAS, Colombian agency, and to Universidad del Valle.

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