

Photo-activated N-doped/TiO₂ nanoparticles with anticancer properties

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Abstract

The aim of this study was the synthesis of titanium dioxide (TiO₂) nanoparticles with photo-induced anticancer properties, under visible light irradiation. It is well established that when pure TiO₂ nanoparticles are excited by ultraviolet light, the formed reactive oxygen species (ROS) can significantly damage cancer cells by inducing apoptosis and, thus, promoting TiO₂ as a promising photosensitizer against cancer. Surface modification by TiO₂ doping with nitrogen (N-doped) is proved to improve TiO₂ photocatalytic activity, according to recent studies. This doping process is associated with the reduction of electron-hole recombination, resulting in efficient separation and stronger photocatalytic reactions, upon irradiation with visible light. Thus, synthesis of N-doped TiO₂ was conducted, followed by surface and morphological characterization realized by micro-Raman spectroscopy, SEM (Scanning Electron Microscopy) microscopy and DLS (Dynamic Light Scattering) techniques. Additionally, cultured MCF-7 (non-metastatic) and MDA-MB-231 (highly malignant) breast cancer epithelial cells were irradiated, using visible light, in the presence of N-doped TiO₂ aqueous dispersions. Cell proliferation was estimated, and growth rates were prepared in order to investigate the biological effect of N-doped TiO₂. In conclusion, experimental

findings indicate that there is an important percentage of cancer cell population which partly loses their functionality, with a significant effect on cell proliferation.

Keywords: N-doped TiO₂, photocatalysis, visible light, anticancer

1. Introduction

Proposing alternative cancer treatments, focusing on minimizing the side effects of the conventional choices, like radiotherapy, chemotherapy would be a very promising scientific challenge (Tran et al., 2017). Nanomaterials have been proven to be a promising candidate for filling this scientific gap, as many studies indicate various types of them, to be used in drug delivery systems (Patra et al., 2018). The use of photo-activated titanium dioxide TiO₂ nanoparticles has highlighted the importance of photocatalytic process in anticancer applications (Behnam et al., 2018, Lagopati et al, 2010). It is supported that when pure TiO₂ nanoparticles are excited by ultraviolet light, the photon energy generates pairs of electrons and holes which can react with water and oxygen, forming reactive oxygen species (ROS). The produced ROS can significantly damage cancer cells by inducing apoptosis (Chen et al., 2014, Wang et al., 2007, Kang et al., 2019, Magalhães et al., 2017).

The energy gap of TiO₂, which is approximately 3-3.2 eV, can allow its photoactivation by ultraviolet (UV) radiation. Although UV is widely used, there are reported harmful effects, related to DNA damage (Suh et al., 2009). Thus, when it is possible, it is important to avoid UV [12]. For this reason, doping of TiO₂ with non-metal or metal elements is usually applied, in order to enhance its photocatalytic properties under visible light irradiation. In this study, nitrogen was selected, as the dopant of TiO₂ (Hashimoto et al., 2005) and the synthesis followed by surface and morphological characterization, realized by micro-Raman spectroscopy, SEM (Scanning Electron Microscopy) microscopy and DLS (Dynamic Light Scattering) techniques

Many studies support that TiO₂ could be used against melanoma cancer cells and others have shown that TiO₂ particles can affect leukemia tumors (Huang et al., 2012). In this study, breast cancer epithelial cells have been chosen in order to investigate the biological effect of doped TiO₂ particles on cell proliferation, since this is a continuation of our previous studies

on breast cancer cells (Lagopati et al., 2010, Lagopati et al., 2014) allowing a direct comparison between pure and doped TiO₂. The experimental findings indicate that there is an important percentage of cancer cell population which partly loses their functionality, with a significant effect on cell proliferation.

2. Materials and Methods

2.1 Preparation of N-doped/TiO₂ Nanoparticles

In order to synthesize N-doped/TiO₂ nanoparticles, 100 mL of deionized water was acidified by adding an amount of nitric acid (HNO₃), in order to adjust the pH ensuring the evolution of hydrolysis. Afterwards, Titanium Butoxide (Titanium (IV) butoxide, C₁₆H₃₆O₄Ti) alkoxide was added under vigorous stirring (Galata et al., 2019). After 5 h, the addition of 30 mL 2-propanol (C₃H₈O) followed and the initially whitish solution, became transparent. In the formed titania sol-gel, 30 g of urea (CH₄N₂O) were added. The heating of this sol-gel allowed the complete evaporation of the solvent. The resulting gel was calcinated at 450 °C for 4 h and the produced powder was triturated and purified (Moustakas et al., Kontos et al., 2008).

2.2 Characterization Techniques

The structural properties of N-doped/TiO₂ were studied by various techniques, such as micro-Raman spectroscopy, SEM (Scanning Electron Microscopy) microscopy and DLS (Dynamic Light Scattering) techniques. For the Raman spectroscopy (inVia, Renishaw) two excitation sources were used, a solid-state laser ($\lambda = 532$ nm) and a high power near infrared (NIR), diode laser ($\lambda = 785$ nm). The measurements were undergone at room temperature (RT) and the laser was focused by means of an $\times 50$ short distance magnification lens. Three spots were measured for each sample with a 10-s exposure time and 2–10 accumulations. Additionally, the morphological characteristics of N-doped TiO₂ were analyzed by scanning electron microscopy. The size and the zeta potential were estimated by Dynamic light scattering (Malvern, UK).

2.3 Biological Effect

2.3.1 Cell cultures

The biological effect of N-doped/TiO₂ particles was examined on two different cancer cell lines: MDA-MB-231 (human breast adenocarcinoma, highly malignant, ATCC - LGC Standards GmbH, Germany) and Michigan Cancer Foundation MCF-7 (low metastatic cells, ATCC - LGC Standards GmbH, Germany), which are both derived from breast epithelium. The cells were cultured in 75 cm² flasks, in the appropriate medium (Dulbecco's modified Eagle's medium (DMEM)), supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine, 1% sodium pyruvate, and antibiotics and incubated at 37 °C in a 5% CO₂ incubator (Lagopati et al., 2010, Lagopati et al., 2014).

2.3.2 Effect on Cell Proliferation

For the estimation of the effect of N-doped/TiO₂ nanoparticles on the cell proliferation, the cancer breast cells (~100,000 cells/well) were cultured in 6-well plates. Twenty-four hours after plating, increasing concentrations of dispersions of the N-doped/TiO₂ nanoparticles were added to the appropriate plates, and the samples were irradiated with visible light for 2 h. In the following three days, the cells were stained with Trypan Blue and counted using a hemocytometer (Neubauer, Corning, Amsterdam, The Netherlands) and an Optical Microscope (Olympus Deutschland GmbH, Germany) (Piccinini et al., 2017). The experiment was repeated at least five times in triplicate. In all instances, similar results were obtained. Values of the cell number are presented as means ± standard deviation. Statistically significant differences between values were evaluated by one-way analysis of variance and the nonparametric Kruskal–Wallis method in the SPSS program. $p < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1 Characterization of N-doped/TiO₂ nanoparticles

Evaluating the results, obtained from Raman spectroscopy, it seems that all the peaks observed correspond to the Raman fundamental modes of pure anatase crystal phase, located

at 143 (Eg(1)), 197 (Eg(2)), 395 (B1g(1)), 514 (A1g), and 640 (Eg(3)) cm^{-1} (Figure 1). No other crystalline form of TiO_2 was detected. These findings are in agreement with the relevant literature for Raman analysis (Li et al. 2011).

The morphology of the produced N-doped/ TiO_2 was examined by SEM. The images are shown in Figure 2.

The hydrodynamic diameter (Dh) of N-doped/ TiO_2 was determined via DLS. All measurements were conducted by using a red laser line operating at 633 nm. The DLS data reveals that the hydrodynamic diameter is ~ 129.2 nm with a coefficient of variation CV=0.7%, meaning that the sample is quite homogenous. The zeta potential was measured as -20mV which means that the sample is steady.

Figure 1: Raman spectrum of N-doped/ TiO_2 nanoparticles.

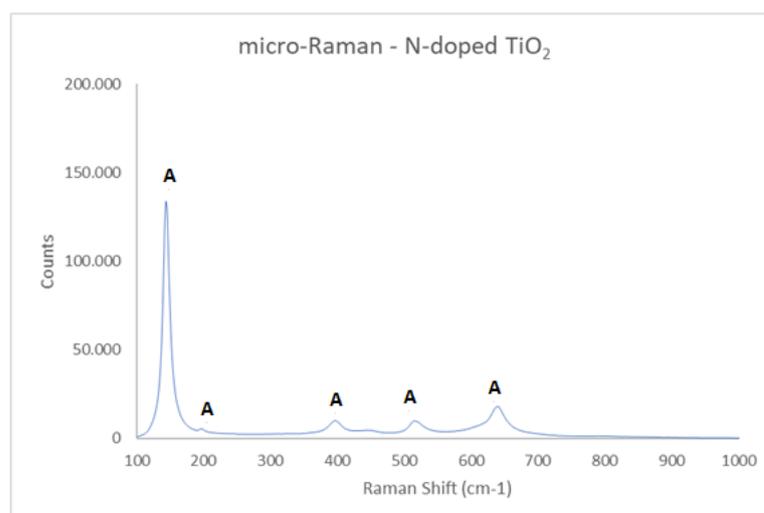
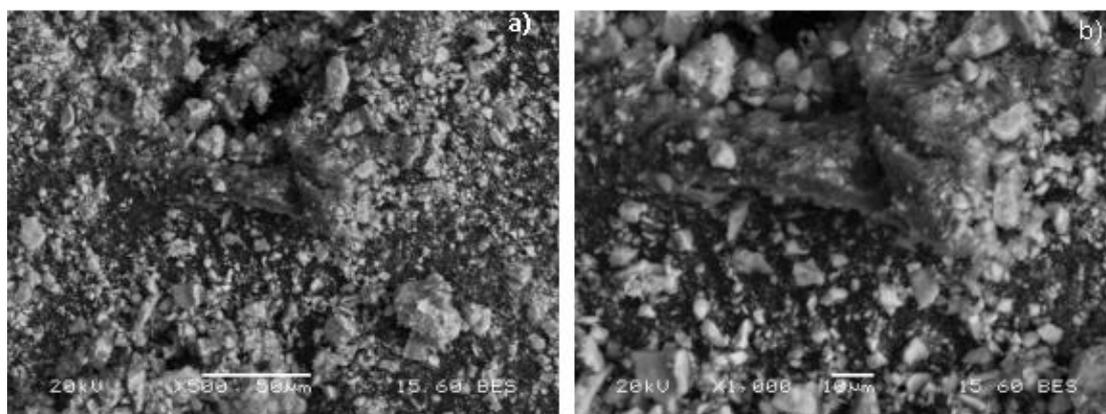


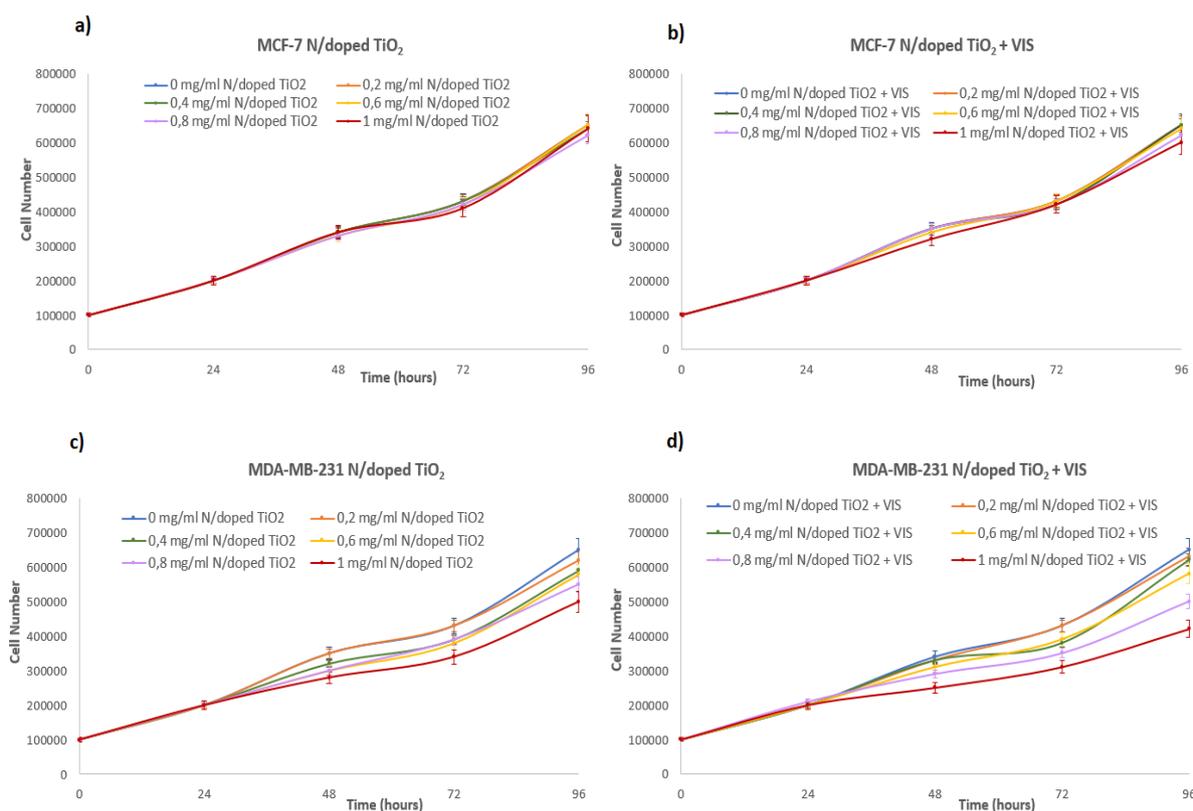
Figure 2: SEM images of N-doped/ TiO_2 , a) at $\times 500$ magnification, b) at $\times 1500$ magnification.



3.2 Cell Proliferation

The effect of N-doped/TiO₂ nanoparticles on the cell proliferation of both MCF-7 and MDA-MB-231 cells is depicted in the growth rates of the figure 3. Cells were incubated in the presence of increasing concentrations of N-doped/TiO₂ dispersions and the cell number is shown as a function of time, upon irradiation with visible light. The range of concentration of dispersions which was selected was 0–1 mg/mL. As a positive control of the experiments, cells treated for 24 h with cisplatin (1 mg/mL) were used. One more internal negative control was used, namely the cells treated with visible light without TiO₂, in order to ensure that the effect is not relevant to the irradiation itself. Thus, the phenomenon of hyperthermia as a possible mechanism, which results in the inhibition of cell proliferation, can be excluded. As shown in Figure 3a and 3b, in the case of MCF-7 cells, there is no significant effect on cell proliferation, even after the photo-activation with visible light. However, in the figure 3d, it is obvious that the cell proliferation of the highly malignant MDA-MB-231 is gradually decreased in the presence of photo-activated N-doped/TiO₂, since there is no remarkable effect in the absence of irradiation (Figure 3c) . It seems that the concentration of 0.8 mg/mL allows the detection of a significant difference on the biological effect among the two different cell lines.

Figure 3: Growth Rates of: a) MCF-7 cells in the presence of N-doped/TiO₂, b) MCF-7 in the presence of photo-activated N-doped/TiO₂ with visible light, c) MDA-MB-231 cells in the presence of N-doped/TiO₂, d) MDA-MB-231 cells in the presence of photo-activated N-doped/TiO₂



These results indicate that the highly malignant MDA-MB-231 cancer cells are more susceptible to the inhibition of cell proliferation when exposed to photo-activated N-doped/TiO₂, compared to non-metastatic MCF-7 cells. This different behavior could be explained by considering the differences between these cell lines and possibly the different membrane receptors, which could interact with the TiO₂ nanoparticles in a different way. It is well known that MDA-MB-231 cells demonstrate stem cell characteristics, such as high expression of cancer stem cells (SCCs) markers CD44/CD133 and high activity of aldehyde dehydrogenase (ALDH), compared to MCF-7 (Crocker et al., 2009). The low toxicity which is observed on MCF-7 cells could be attributed to the resistance of these cells, due to their xenobiotic transporter (BCRP), which plays a crucial role in the multi-drug resistance.

4. Conclusion

N-doped/TiO₂ was synthesized by the sol-gel method in order to enhance the photocatalytic activation of the produced TiO₂, broadening under the visible light spectrum due to the doping with nitrogen. Characterization of the produced material verified the physical-chemical properties. The study of the effect of N-doped/TiO₂ on cell proliferation, using visible light irradiation has shown a decrease in cell proliferation on MDA-MB-231 cells. In the case of MCF-7 cancer cells, no significant effect on cell proliferation was observed, highlighting the selectivity of the synthesized nanoparticles toward specific cancer cells. Further investigation of the mechanism provoking this selectivity is in our perspectives. These preliminary results are very promising for the development of an alternative photodynamic cancer therapy upon irradiation with visible light.

Acknowledgment (TNR 12pt. bold)

This research is implemented through IKY scholarships programme and co-financed by the European Union (European Social Fund - ESF) and Greek national funds through the action entitled "Reinforcement of Postdoctoral Researchers", in the framework of the Operational Programme "Human Resources Development Program, Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) 2014 – 2020 (MIS-5001552).

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