



Induction of Apoptosis and Inhibition of Invasion in Gastric Cancer Cells by Titanium Dioxide Nanoparticles

Reza Nasr¹, Hadi Hasanzadeh^{2*}, Ali Khaleghian³, Abdolvahab Moshtaghian⁴, Alireza Emadi⁴ and Shima Moshfegh⁵

¹Biotechnology Research Center, Semnan University of Medical Sciences, Semnan, Iran

²Cancer Research Center and Department of Medical Physics, Semnan University of Medical Sciences, Semnan, Iran

³Department of Biochemistry, Semnan University of Medical Sciences, Semnan, Iran

⁴Deputy of Research and Technology, Semnan University of Medical Sciences, Semnan, Iran

⁵Student Research Committee and Department of Medical Physics, Semnan University of Medical Sciences, Semnan, Iran

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ABSTRACT

Objectives: Nanoparticles induce oxidative stress in cells and damage them through the cell membrane and DNA damage, eventually resulting in cell death. This study aimed to evaluate the effect of titanium dioxide (TiO₂) nanoparticles on apoptosis induction and invasion of gastric cancer cell line, MKN-45. **Methods:** We used the MTT assay to assess proliferation of MKN-45 gastric cancer cells after exposure to different forms of TiO₂ nanoparticles including amorph, brookite, anatase, and rutile coated with polyethylene glycol (PEG) and bovine serum albumin (BSA). Ethidium bromide and acridine orange staining were used to visualize cancer cell apoptosis, and the wound healing assay technique (migration test) was used to assay cancer cell invasion. **Results:** Viability and proliferation of cancer cells in the presence of various forms of TiO₂ nanoparticles were reduced ($p \leq 0.050$). This reduction in cell proliferation and viability was directly related to concentration and duration of exposure to nanoparticles. Induction of cell death was seen in all groups ($p \leq 0.050$). Increased cell invasion was seen in PEG-amorph TiO₂ group compared to the control group. Cell invasion was decreased only in the brookite BSA group ($p \leq 0.050$). **Conclusions:** Various forms of TiO₂ nanoparticles reduced cell proliferation and induced apoptosis in cancer cells. Some forms of TiO₂ nanoparticles such as brookite BSA also inhibited cell invasion. PEG-amorph TiO₂ nanoparticles increased cell invasion. These differences seem to be due to the effects of different configurations of TiO₂ nanoparticles. TiO₂ may provide a new strategy for cancer treatment and more studies are needed.

Cancer is the second cause of death in the world,¹ and gastric cancer has a mortality of about 70% with a higher prevalence in men. In the US, the incidence of gastric cancer is 21000 cases annually, which lead to 10570 deaths.² In Iran, the percentage of gastric cancer in 2005–2006 was 15.2% and 6.8% in men and women, respectively.^{3,4} Nanomaterials are small objects with at least one dimension in the nanoscale range (1–100 nm).⁵ They can have toxic effects on cancer cells and destroy them through oxidative stress.^{6,7} This feature can be utilized to develop effective methods to destroy tumors with minimal side effects. Nowadays, using nanoparticles in the diagnosis and treatment of cancer has

been extensively studied.^{8,9} One feature of cancer cells is invasion of adjacent tissues.¹⁰ Epithelial-mesenchymal transition has a significant role in cancer cell invasion. Matrix metalloproteinases (MMPs) stimulate invasion and cancer cells can move to other tissues.¹¹ Some studies have shown that nanoparticles can inhibit the invasion of cancer cells.¹²

Titanium dioxide (TiO₂) nanoparticles have been shown to have antitumor effects, and inflammatory properties on mammalian cells through hydrogen peroxide (H₂O₂) and hydroxyl free-radical production.^{6,13–15} TiO₂ causes apoptosis in human bronchial epithelial cells through launching reactive oxygen species (ROS).^{16–18} When human epithelial

*Corresponding author: ✉Hasanzadeh.h@semums.ac.ir

cell lines were exposed to TiO₂ nanoparticles there was increased expression of Bax, cytochrome C and P53 proteins and a reduction of anti-apoptosis B-cell lymphoma (BCL2), which eventually led to apoptosis in these cells. In addition, the expression level of caspase-9 increased, but the expression of caspase-8 did not change. This suggests that TiO₂ acts through an intrinsic pathway to cause apoptosis in human epithelial cells.¹⁶

We sought to determine the effect of TiO₂ nanoparticles on apoptosis and invasion of gastric cancer cell line, MKN-45. We used the MTT assay and a wound healing assay to assess invasion and acridine orange and ethidium bromide (AO/EB) staining to test cell death. We used seven different TiO₂ structures: Polyethylene glycol (PEG)-amorph TiO₂, amorph TiO₂, PEG-brookite, rutile, anatase, amorph bovine serum albumin (BSA), and brookite BSA. Anatase and rutile are two important TiO₂ structures. Rutile is more stable and bigger than anatase.¹⁹ All nanoparticles were used at different concentrations (0, 10, 20, 30, 40, and 50 µg/mL), and the cells were exposed to the nanoparticles for 24, 48, and 72 hours).

METHODS

Human gastric cancer cell line MKN-45 (NCBI No.C615) was purchased from Iran Pasteur Institute. The cells were cultured in RPMI 1640 (Gibco, Invitrogen GmbH, Darmstadt, Germany) included 10% fetal bovine serum (Gibco, Invitrogen GmbH, Darmstadt, Germany), 100 unit/mL penicillin and 100 µg/mL streptomycin at 37 °C in a humidified incubator with 5% CO₂. Amorph, brookite, anatase, and rutile forms of TiO₂ nanoparticles (Grafen Chemical Industries, Ankara, Turkey) were coated with PEG and BSA.

We used the MTT assay to examine cell survival. MKN-45 cells were seeded in a flask; when the concentration reached to 60%, cells were trypsinized and moved to a 96 well plate. Each well contained 5000 cells in 200 µL RPMI 1640 medium. Cells were cultured for 24 hours after which they were exposed to the nanoparticles. Different concentrations of nanoparticles (0, 10, 20, 30, 40, and 50 µg/mL) were used for three different times (24, 48, and 72 hours). On the day of testing, 20 µL of MTT reagent was added to cells and the contents of each well were replaced with 100 µL dimethyl sulfoxide. Absorbance

in each well at 570 nm was recorded using the Elisa reader system (Rayto software). Each experiment was triplicated. The results of the MTT assay were used to calculate the 50% inhibitory concentration (IC₅₀).

MKN-45 cells were seeded in six-well plates and incubated with nanoparticles at the IC₅₀ value for 48 hours. AO/EB reagent at 100 µg/mL was prepared, and cells were trypsinized and stained with 100 µL of AO/EB solution for 15 minutes and washed twice with phosphate buffered saline (PBS) solution and assessed by fluorescence microscopy. Green color indicated live cells, red represented apoptotic cell, and orange indicated necrotic cells.

A wound healing assay to examine the migration of MKN-45 cells was done. MKN-45 cells were seeded in six-well plates. After 24 hours, the center of well was scraped with a sterile micropipette tip to make a straight scratch with the same width. Each well was washed with PBS solution, and MKN-45 cells were exposed to TiO₂ nanoparticles (at the IC₅₀ value) and moved to an incubator for 48 hours. Wound closure was recorded with an inverted microscope.

Each assay was triplicated, and data expressed as the mean. Statistical analyses were performed using SPSS Statistics (SPSS Inc. Released 2008. SPSS Statistics for Windows, Version 17.0. Chicago: SPSS Inc.). Graphs were prepared using Microsoft Excel 2007. After normality and homogeneity evaluation of variables, analysis of variance (ANOVA) was done with a 95% confidence interval ($p \leq 0.050$); Tukey's test was used for multiple comparisons.

RESULTS

MKN-45 cells were exposed to various forms of TiO₂, and cell viability at 24, 48, and 72 hours with different concentrations of nanoparticles was evaluated using MTT [Figure 1]. In the PEG-amorph TiO₂ nanoparticles group, increasing concentration and exposure time led to an increase in cell toxicity ($p \leq 0.050$). For PEG-amorph TiO₂, the most toxic effect of nanoparticles was at concentrations of 50 µg/mL at 24, 48 and 72 hours. In the amorph TiO₂ group, nanoparticle toxicity was the highest at a concentration of 30 µg/mL for 72 hours. In the PEG-brookite group, the highest toxicity levels on cancer cells were observed at 40 µg/mL for 72 hours. In rutile, anatase, amorph BSA and brookite BSA groups, the highest toxicity to cancer cells was

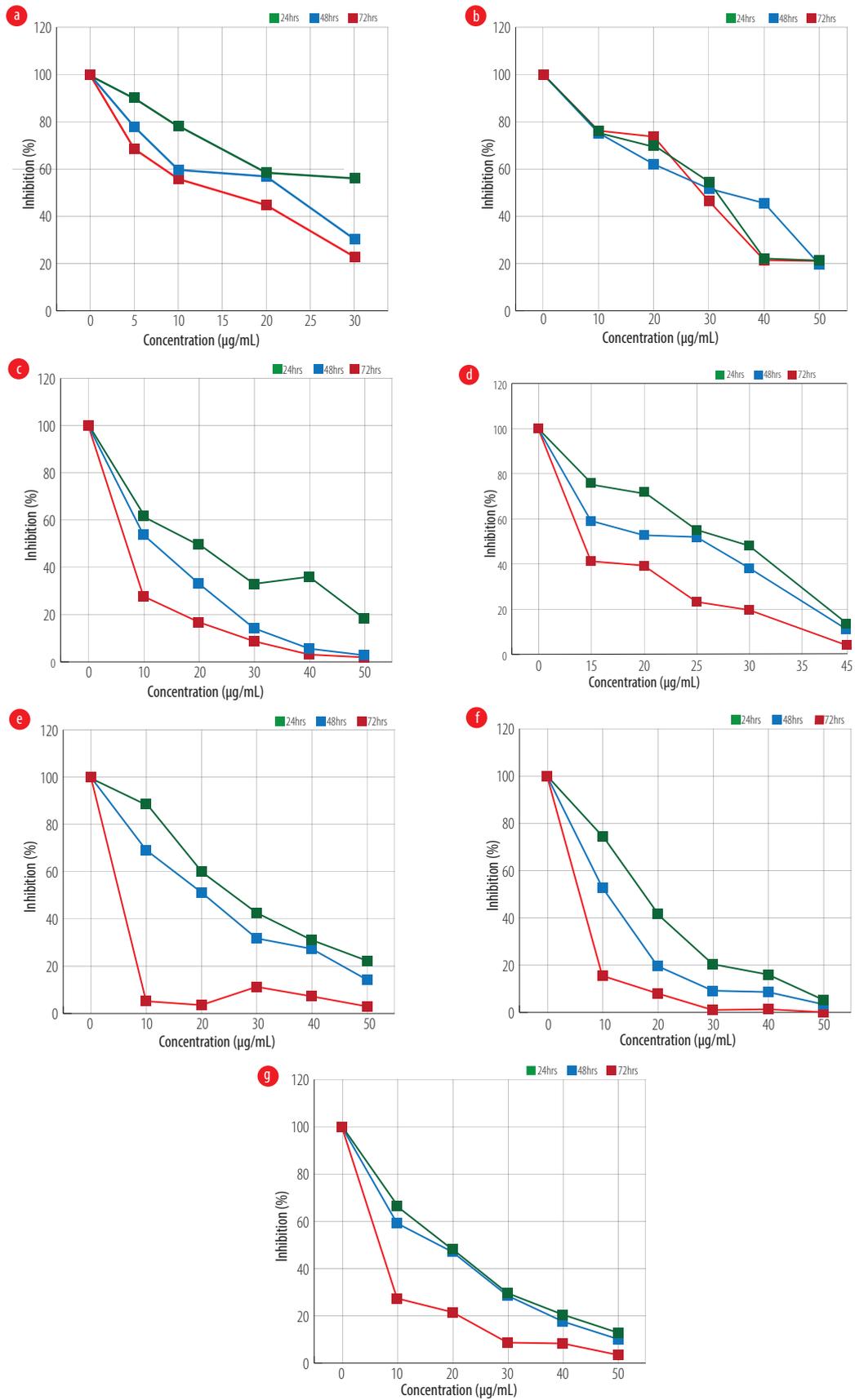


Figure 1: Effect of different configurations and coating of nanoparticles on the proliferation of gastric cancer line, MKN-45 (a) amorph titanium dioxide (TiO₂), (b) polyethylene glycol (PEG)-amorph TiO₂, (c) anatase, (d) PEG-brookite, (e) rutile, (f) brookite bovine serum albumin (BSA), and (g) amorph BSA.

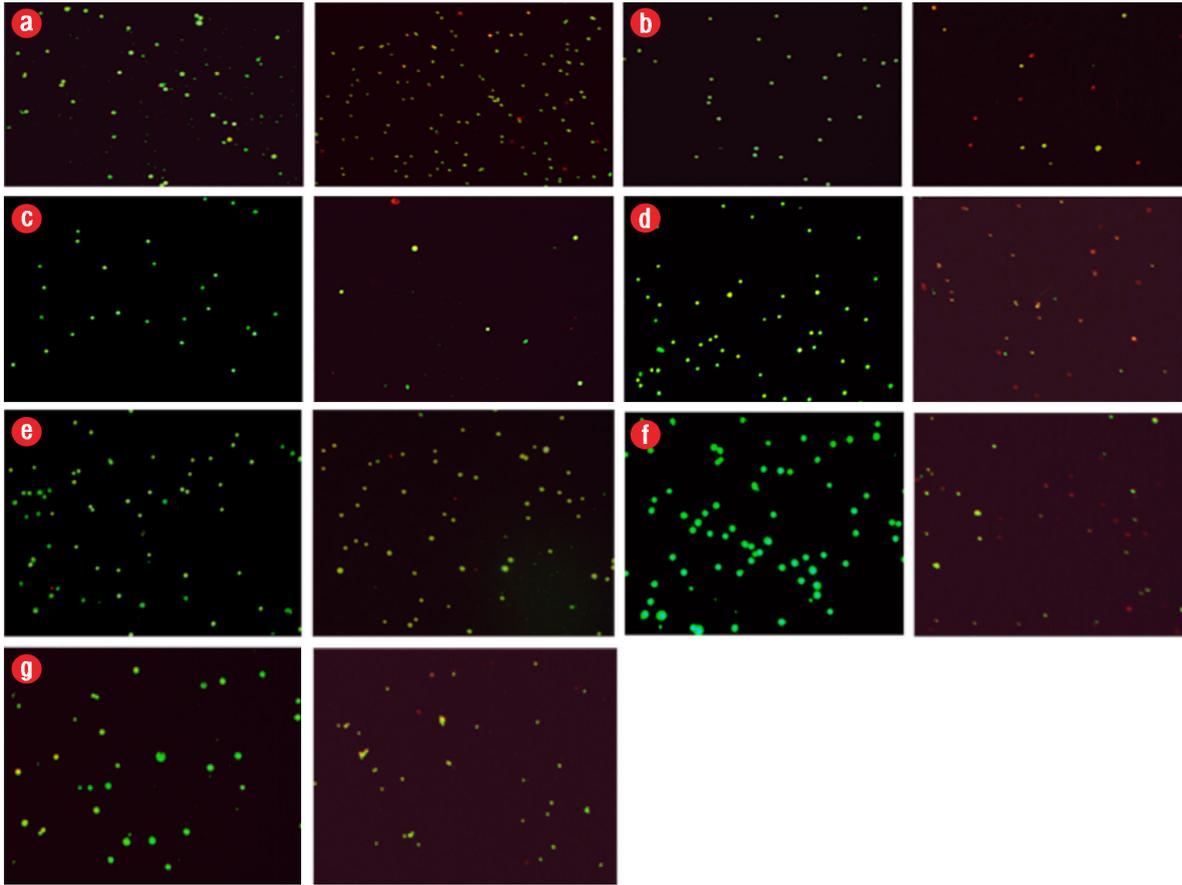


Figure 2: Induction of apoptosis in cancer cells after exposure to different configurations and coatings of nanoparticles for 48 hours. In all groups, the concentration used was the 50% inhibitory concentration (IC₅₀) value. Apoptosis, left (control), and right (IC₅₀ dose at 48 hours). Green color indicated live cells, red apoptotic cells and orange necrotic cells. (a) polyethylene glycol (PEG)-brookite, (b) amorph titanium oxide (TiO₂), (c) PEG-amorph TiO₂, (d) rutile, (e) anatase, (f) brookite bovine serum albumin (BSA), and (g) amorph BSA.

at a concentration of 50 µg/mL nanoparticles for 72 hours.

Nanoparticles caused cell death in the MKN-45 cancer cells. In previous studies, nanoparticles caused cell death by activating the intrinsic apoptotic pathway.¹⁶ Apoptosis in MKN-45 cells was tested using AO/EB staining [Figure 2]. After exposure to TiO₂ nanoparticles, we saw cell death ($p \leq 0.050$). In all cases, the nanoparticles concentration was 50 µg/mL and MKN-45 cells were exposed to nanoparticles for 48 hours. The percentages of apoptotic and live cells in the control group were 10.0% and 90.0%, respectively, and the percentages of apoptotic and live cells following exposure to TiO₂ nanoparticles are given in Table 1.

Invasion of cancer cells after exposure to nanoparticles was evaluated using wound healing assay. We saw an increase in cell invasion in the PEG-amorph TiO₂ group compared to the control

Table 1: The percentage of apoptotic and live cells following exposure to titanium oxide (TiO₂) nanoparticles.

Nanoparticle type	Apoptotic cells, %	Live cells, %
Control	10.0	90.0
PEG-amorph TiO ₂	40.0	60.0
Amorph TiO ₂	75.0	25.0
PEG-brookite	72.0	28.0
Rutile	75.0	25.0
Anatase	40.0	60.0
Amorph BSA	80.0	20.0
Brookite BSA	85.0	15.0

PEG: polyethylene glycol; BSA: bovine serum albumin.

group, and a decrease in cell invasion in the brookite BSA group ($p \leq 0.050$). Our results suggest that different configurations of nanoparticles have

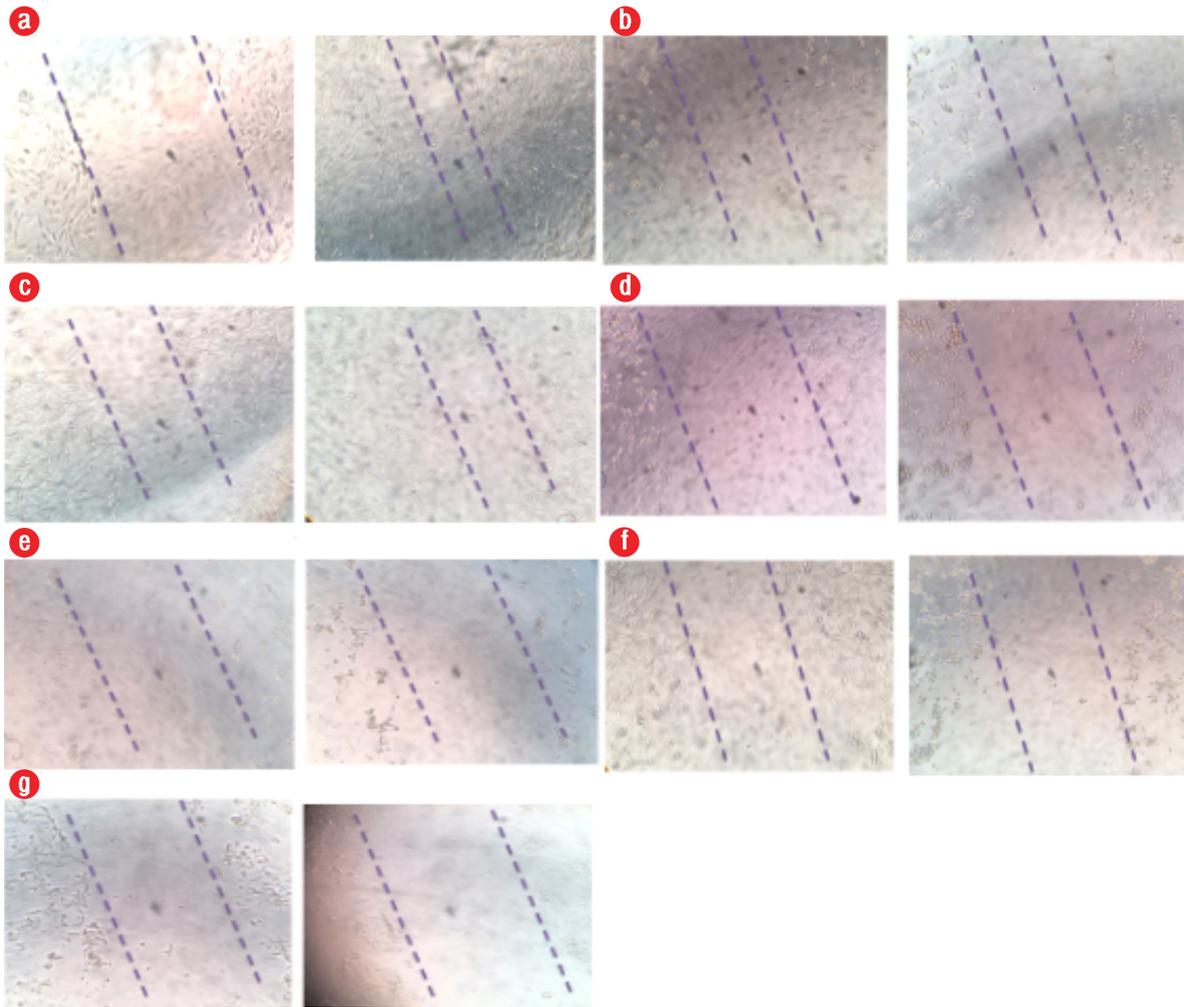


Figure 3: Effects of different configurations and coatings of nanoparticles on cell invasion. In all groups, concentration was 50% inhibitory concentration (IC₅₀) and cells were exposed to nanoparticles for 48 hours. Migration test (qualitative test) left (control) and right (IC₅₀ dose at 48 hours). (a) polyethylene glycol (PEG)-amorph titanium oxide (TiO₂), (b) rutile, (c) amorph TiO₂, (d) anatase, (e) PEG-brookite, (f) amorph (bovine serum albumin) BSA, and (g) brookite BSA.

different effects on cancer cell invasion. We saw no significant changes when using amorph TiO₂, PEG-brookite, rutile, anatase, and amorph BSA nanoparticles [Figure 3].

DISCUSSION

We studied the effect of TiO₂ nanoparticles on gastric cancer cell line MKN-45. Different configurations and different coatings of nanoparticles were used. Cell proliferation was significantly decreased in all nanoparticle groups. The reduced proliferation and increased concentration and nanoparticle exposure time have a direct relationship.

In the rutile and brookite BSA groups, the cell death rate was higher ($p \leq 0.050$), which shows the greater impact on the induction of apoptosis in

these configurations. Cell invasion declined only in the brookite BSA group. Some nanomaterials can prevent metastasis and invasion of cancer cells through inhibition of matrix metalloproteinase; these nanoparticles such as gadolinium metallofullerenol prevent MMP enzymes production.²⁰

Nanotechnology has been introduced as a new tool for the treatment of diseases like cancer.²¹ Nanomaterials can be used for cancer detection and imaging.²² Nanomaterials are used as vector for drug delivery for cancer treatment.²³ For example, gold nanoparticles can be implemented as drug carriers in cancer treatment.²⁴ Nanomaterials are also used in gene therapy as a carrier for DNA and RNA.²⁵

Some nanomaterials selectively target cancer cells. For example, zinc oxide nanoparticles (ZnO) have toxic effects on human myeloblastic leukemia

cells (HL60) more than normal peripheral blood mononuclear cells. This is a therapeutic benefit for using nanomaterials in cancer treatment since common chemotherapy drugs cannot distinguish cancerous and healthy cells and causes side effects. ZnO nanoparticles induce apoptosis in cancer cells by creating free oxygen radicals.²⁶

TiO₂ is a kind of metallic nanomaterials and has special chemical characteristics. TiO₂ has several forms with different chemical and physical properties. TiO₂ interacts with water molecules inside cells and creates free radicals, especially ROS.²² Free radicals damage DNA and cell membrane leading to cell death.⁶

It has shown that TiO₂ nanoparticles can enhance the sensitivity of tumor cells to radiation therapy through ROS production *in vivo*¹⁹ and they have the potential to be implemented in the near future in clinical trials.

TiO₂ nanoparticles have antitumor properties.²¹ Research shows that TiO₂ nanoparticles are environmentally friendly and have low toxicity *in vivo* and *in vitro* as they have several industrial applications.²⁷ In addition to the low toxicity, nanoparticles can be controlled in term of size and charge. Also, nanoparticles have low off-target toxicity, and therefore, are useful for cancer therapy.²⁸ Nanoparticles can act as a carrier in tumor therapy. Nanoparticles protect drugs from degradation and prolong drug circulation time in body.²⁹

CONCLUSION

Different forms of TiO₂ nanoparticles reduce cell proliferation and viability in gastric cancer cells. The inhibitory effect was enhanced by increasing the nanoparticle concentration and exposure time. As nanoparticles induced cell death in cancer cells and decreased cell invasion in the brookite BSA group. Brookite BSA has the most inhibitory effect on cancer cells, and we suggest that this group be investigated further in *in vivo* models. According to these results, TiO₂ nanoparticles have the potential to be used as a new therapeutic agent for cancer treatment in the future.

Disclosure

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