7th International Conference on
Science and Development of Nanotechnology

The cytotoxicity effect of biologically synthesized bismuth oxide nanoparticles using *Vibrio* sp. *VLC* on gastric cancer cells

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Abstract

Biosynthesis of nanoparticles (NPs) is an eco-friendly, safe and cost-effective approach, specifically when bacteria are used to produce NPs. In addition, loading of NPs on minerals such as zeolite affects their diffusion in various solutions. Gastric cancer ranks as 5th for incidence and 4th for mortality among all cancer types globally. To introduce novel NPs with toxic effects on gastric cancer cells, we synthesized bismuth oxide (Bi_2O_3) NPs, alone and loaded on zeolite (Bi_2O_3-Z) using a bacterium *Vibrio* sp. *VLC*, and evaluated their cytotoxicity on MKN-45 cells. In this regard, Bi_2O_3 NPs (in two forms of heated and non-heated) and Bi_2O_3-Z nanocomposites (NCs, with 3.35 and 15.56 wt%) were synthesized, while bismuth salt and zeolite were considered as controls. For treatment of cells, 25, 50 and 100 µg/ml of all agents were freshly prepared and after 24 h, alamar Blue assay was carried out to determine cell viability. Results of this study revealed that heated and non-heated Bi_2O_3 NPs induced their cytotoxic effects in a dose-dependent manner. In addition, toxicity of Bi_2O_3-Z NCs (3.35 wt%) was more than Bi_2O_3-Z NCs (15.56 wt%), bismuth salt and zeolite. In conclusion, obtained findings indicated that toxicity of bismuth salt increased when formed as NPs and NCs. Nevertheless, more research is necessary to determine the mechanism of observed actions.

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Keywords: Bismuth oxide nanoparticles, gastric cancer, cytotoxicity, *Vibrio* sp. *VLC.*

Introduction

The production and use of materials in nanoscale, which is known as nanotechnology, has attracted a lot of attention in recent years [1]. Nanoparticles (NPs) could be synthesized by physical, chemical and mechanical processes [2]. The link between nanotechnology and biology provides a nanobiotechnology platform that involves living organisms, such as algae, cyanobacteria, actinomycetes, bacteria, viruses, yeasts, fungi, and plants, in the production of NPs [3]. Biosynthesis of NPs is an eco-friendly, safe and cost-effective approach, specifically when bacteria are used to produce NPs with biomedical applications [4]. On the other hand, loading of NPs on minerals such as zeolite, which is called nanocomposite (NC), affects diffusion of NPs in various solutions.

Cancer ranks as a leading cause of death worldwide, with increasing statistics in developing nations. Gastric cancer, ranking as 5th for incidence and 4th for mortality globally, is responsible for over one million new cases in 2020 and an estimated 769,000 deaths [5]. Since chemotherapy resistance in an important barrier to treatment of cancer, a great deal of investigation is currently focused on introducing novel NPs with anticancer effects. In this regard, the goal of present study was to synthesis bismuth oxide (Bi_2O_3) NPs, alone and loaded on zeolite (Bi_2O_3-Z NCs), by *Vibrio* sp. *VLC* and investigate their cytotoxicity effects on gastric cancer cells.

Methods

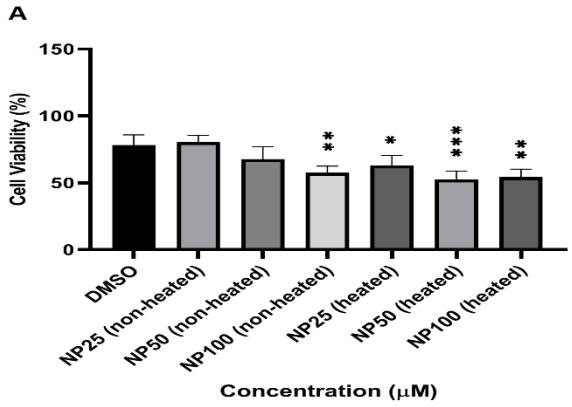
For biologically synthesis of Bi_2O_3 NPs, a bacterium (*Vibrio* sp. *VLC*) were cultured in Sea Water Complete (SWC) medium for 48 h. After centrifugation at 5000 rpm for 20 min, pellets were resuspended and incubated at 28°C for 24 h. Upon sonication of samples, they were centrifuged at 10000 rpm for 10 min, and the obtained cell lysate supernatant (CLS) was incubated with $Bi(NO)_3.5H_2O$ (10 mM) at 37 °C for 24 h. Bi_2O_3-Z NCs was prepared by combination of zeolite with $Bi(NO)_3.5H_2O$ (10 mM) for 72 h, further mixed with cell lysate supernatant. Finally, to collect Bi_2O_3 NPs and Bi_2O_3-Z NCs, centrifugation was carried out at 10000 rpm for 20 min at 4°C, followed by washing and drying at 34 °C for 24 h. Bi_2O_3 NPs and Bi_2O_3-Z NCs were characterized done by using UV-visible, FTIR, XRD, DLS, Zeta potential, TEM and FESEM analysis.

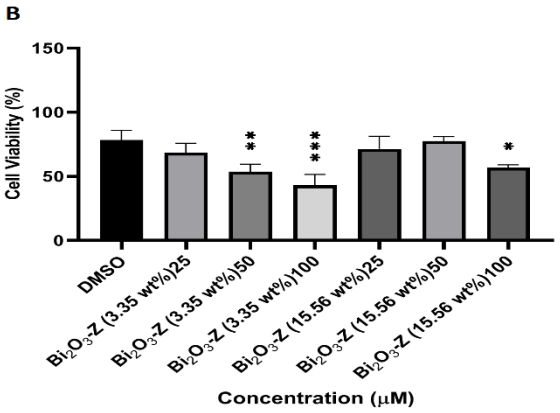
To prepare different the various concentrations (25, 50 and 100 µg/ml) of heated and non-heated Bi_2O_3 NPs, Bi_2O_3-Z NCs (with 3.35 wt% and 15.56 wt%), bismuth salt and zeolite were prepared in fresh medium using 8 mg/ml stock solution (dissolved in DMSO and stored at 25°C).

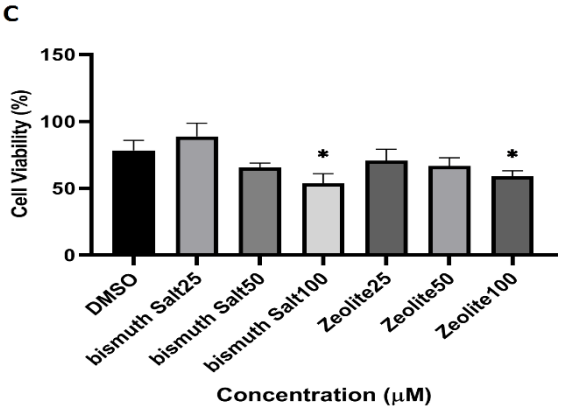
MKN-45 cells, a human gastric cancer cell line, were cultured in DMEM culture medium containing 10% fetal bovine serum incubated at 37 °C and 5% CO_2 in air. The grown cells were seeded with a density of 6000/well in 96-well plates. After 24 h, cells were treated with freshly prepared agents (mentioned above) and incubated at 37 °C for 24 h. Then, viability was evaluated by alamar Blue assay. In which 18 µl alamarBlue (0.1 mg/ml) was added to each well and further incubated at 37°C for 3 h in the dark. Finally, optical density (OD) of each well was measured at 600 nm and cell viability (%) was calculated by the following formula: $100-((OD_T-OD_U)/(OD_B-OD_U)\times100)$, in which T, U and B stand for treated cells, untreated cells and blank control, respectively.

Results and Discussion

Results of viability assay indicated that non-heated Bi_2O_3 NPs induced its cytotoxic effects in a dose-dependent manner, as only 100 µg/ml Bi_2O_3 NPs significantly (p <0.01) reduced cell viability. In addition, heated Bi_2O_3 NPs







induced more toxic effects on MKN-45 cells, as viability was significantly (p <0.01 and p <0.001) decreased down to 62.9%, 52.5% and 54.2%, after treatment with 25, 50 and 100 µg/ml heated Bi_2O_3 NPs, respectively (Figure 1-A). Also, Bi_2O_3-Z NCs significantly induced cytotoxicity (p <0.01 and p <0.001) (Figure 1-B). The viability of cells was reduced down to 68.4%, 53.7% and 43% upon administration of 25, 50 and 100 µg/ml Bi_2O_3-Z NCs (3.35 wt%), respectively. However, effects of 25 and 50 µg/ml Bi_2O_3-Z NCs (15.56 wt%) was not considerable, and only upon treatment with 100 µg/ml Bi_2O_3-Z NCs (15.56 wt%) cell viability was significantly (p <0.05) decreased. Worth to note, 25 and 50 µg/ml bismuth salt and zeolite did not induce significant toxicity, and viability of MKN-45 cells upon treatment with 100 µg/ml of these agents were 53.7% and 59%, respectively (Figure 1-C).

Previous studies have also reported the cytotoxic effects of Bi_2O_3 NPs on other cell lines. For instance, Abudayyak et al. treated hepatocellular, renal, colon and lung carcinoma cells with Bi_2O_3 NPs and reported their cytotoxic and genotoxic effects; the highest and lowest toxicity was on renal and hepatocellular carcinoma cell, respectively [6].

Figure 1. Viability of MKN-45 cells after treatment with different concentrations of Bi_2O_3NPs (in two forms of heated and non-heated), Salt, Zeolite, Bi_2O_3-Z NCs (with 3.35 wt% and 15.56 wt%) during 24 h. Viability assessment was carried out for at least 3 times and results are presented as mean ± SD.

Conclusion

Obtained findings indicated that toxicity of bismuth salt on gastric cancer cells increased in the forms of NPs and NCs. Nevertheless, more research is necessary to determine the mechanism of observed actions.

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