Viability assessment of human gastrointestinal cancer cells upon administration of exopolysaccharide from *luminescent Vibrio sp. VLC*

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Abstract
Colon and gastric cancers are among the top 5 most common cancers worldwide. The high mortality rate of these malignancies has made it crucial to improve clinical outcomes by exploring new chemical agents. Accordingly, the aim of present study was to evaluate toxic activity of exopolysaccharides (EPS) from *luminescent Vibrio sp. VLC* on human colon and gastric cancer cells. After biosynthesis, extraction and purification of EPS, LoVo and MKN45 cells, as well as normal fibroblasts, were treated with 25, 50, 100 and 200 µg/ml EPS and their viability was evaluated by alamarBlue assay. Results revealed that EPS from *luminescent Vibrio sp. VLC* induced its toxic effects in a time- and dose-dependent manner, as 24 h treatment of colon and gastric cancer cells reduced viability more than 48 h treatment. Furthermore, lowest viability of both cancer cell lines was detected after treatment with 200 µg/ml EPS.

Keywords: Exopolysaccharide, Cancer cells, Viability assay, *luminescent Vibrio sp. VLC.*

Introduction
Gastrointestinal malignancies account for 26% of cancer incidence and 35% of all cancer-related deaths around the world [1]. Colorectal cancer is the 3rd most common diagnosis and 2nd deadliest malignancy for both sexes combined, while gastric cancer currently ranks 4th in cancer-related mortality [2]. For both cancers, patients with advanced disease have low survival rate and palliative chemotherapy is the mainstay of treatment [3]. The high mortality rate of these malignancies has made it crucial to improve clinical outcomes by exploring new chemical agents.
Exopolysaccharides (EPS) derived from bacteria are exopolymers arranged as repeated homo- or hetero-carbohydrates as well as organic and inorganic substituents. Bacterial EPS have diverse biological effects, such as environmental protection, surface adherence, and cellular interactions [4]. Herein, toxic activity of EPS from *luminescent Vibrio sp. VLC* was evaluated on human colon and gastric cancer cells, as well as normal fibroblasts.

Experimental:

To investigate toxicity of EPS from *luminescent Vibrio sp. VLC in vitro*, at first bacterial isolates were obtained by serial dilution plating on thiosulfate-citrate-bile salts-sucrose agar and glycerol-based marine agar plates. After incubation at 28°C for 24 h, EPS-producing bacteria were screened by colony morphology. To produce EPS, *luminescent Vibrio sp. VLC* was grown in optimized mineral salts medium containing 0·2% glucose, 12·6% K_2HPO_4, 18·2% KH_2PO_4, 10% NH_4NO_3, 1% $MgSO_4 \cdot 7H_2O$, 0·6% $MnSO_4$, 1% sodium molybdate, 1% $CaCl_2 \cdot 2H_2O$, 0·06% $FeSO_4 \cdot 2H_2O$ and 1·5% of NaCl in 1000 mL distilled H_2O. To extract and purify the produced EPS, the culture was centrifuged, supernatant was precipitated by cold ethanol and solution was chilled at 4°C overnight and lyophilized.

Human colon cancer cells, LoVo cell line, were grown in Roswell Park Memorial Institute-1640 (Capricon), supplemented with 10% fetal bovine serum (FBS, Biowest). Human gastric cancer cells, MKN45 cell line, as well as human fibroblasts, were cultured in Dulbecco's modified Eagle's medium (Biowest) supplemented with 10% FBS (Biowest). All cells were incubated at 37°C and 5% CO_2 in air, and subcultured by 0.25 % trypsin-1 mM EDTA (Betacell). For treatment of cells with EPS from *luminescent Vibrio sp. VLC*, LoVo cells, MKN45 cells and fibroblasts were seeded in 96 well plates with densities of 13000, 10000 and 10000 cells/each well, respectively. Upon 24 h, cells were treated with 25, 50, 100 and 200 µg/ml EPS and incubated at 37°C for 24 and 48 h.

To determine whether EPS affects viability of cells, alamarBlue assay was used. This method is based on the reduction of oxidized blue resazurin to a pink fluorescent resorufin. In summery, 20 µl alamarBlue (0.1 mg/ml, Sigma) was added, and after 2 h of incubation at 37°C, absorbance of each well was measured at 600 nm in microplatereader (Epoch). At the end of the procedure, cell viability (%) was calculated as 100-((AT-AU)/(AB-AU)×100), in which AT and AU were absorbance of treated and untreated cells, respectively, and AB was absorbance of blank control.

Results and discussion

As presented in Figure 1, viability of LoVo cells after 24 h treatment with 25, 50, 100 and 200 µg/ml EPS from *luminescent Vibrio sp. VLC* was as 80%, 71%, 74% and 63%, respectively. However, assessment of cell viability revealed that 88%, 85%, 85% and 86% of cells were alive after 48 h treatment with 25, 50, 100 and 200 µg/ml EPS, respectively. Accordingly, EPS from *luminescent Vibrio sp. VLC* showed a time-depend toxicity on LoVo cells, as longer treatment reduced its effects.

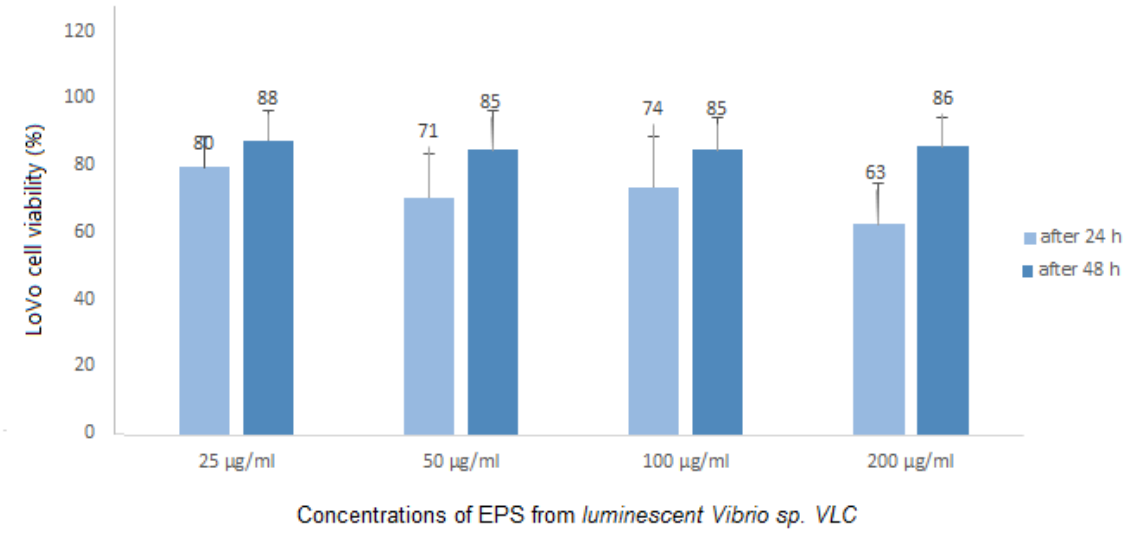

Figure 1. Viability of LoVo cells after 24 and 48 h treatment with EPS from *luminescent Vibrio sp. VLC*.

After treatment of MKN45 cells with 25, 50, 100 and 200 µg/ml EPS from *luminescent Vibrio sp. VLC*, viability assay indicated 88%, 81%, 81% and 80% viability after 24 h, respectively. In addition, 94%, 96%, 88% and 96% of cells were alive after 48 h treatment with 25, 50, 100 and 200 µg/ml EPS, respectively (Figure 2). Similar to obtained results on colon cancer cells, longer treatment period did not increase toxicity of EPS from *luminescent Vibrio sp. VLC* on gastric cancer cells.

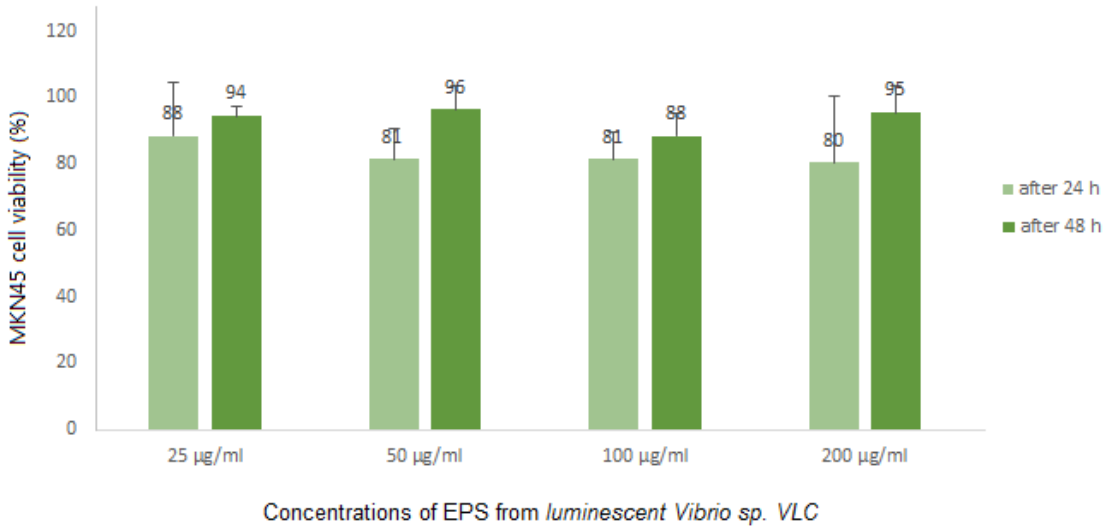

Figure 2. Viability of MKN45 after 24 and 48 h treatment with EPS from luminescent Vibrio sp. VLC.

Since EPS from *luminescent Vibrio sp. VLC* induced more toxic effects on cancer cells after 24 h rather than 48 h treatment, we investigated viability of human normal fibroblasts after 24 h as well. As shown in Figure 3, 96%, 70%, 83% and 70% of cells were alive upon treatment with 25, 50, 100 and 200 µg/ml EPS, respectively.

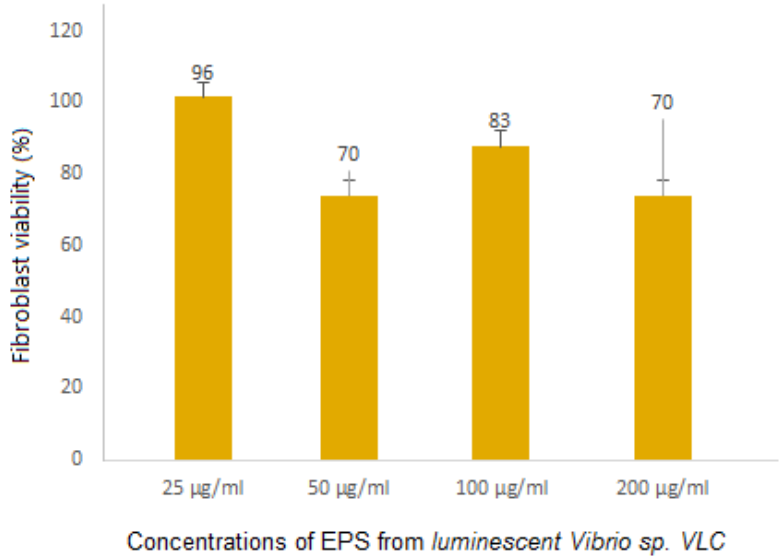

Figure 3. Viability of fibroblasts after 24 h treatment with EPS from *luminescent Vibrio sp. VLC*.

Conclusion

Obtained findings revealed that EPS from *luminescent Vibrio sp. VLC* induced its toxic effects in a time- and dose-dependent manner, as 24 h treatment of colon and gastric cancer cells reduced viability more than 48 h treatment. Furthermore, lowest viability of both cancer cell lines was detected after treatment with 200 µg/ml EPS.

Acknowledgements

This study was financially supported by a grant from Ferdowsi University of Mashhad (No. 54060).

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