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RESEARCH ARTICLE

SYNTHESIS AND CHARACTERIZATION OF NICKEL MNP'S AND INVESTIGATION OF ANTICANCER EFFECTS

Hüseyin Şahin[1] and Nelisa Türkoğlu[2*]

[1]Üsküdar American Academy, Istanbul, Turkey
[2]Yıldız Technical University, Molecular Biology and Genetics Department, Istanbul, Turkey

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Corresponding author:
Dr. Safia Farooqui

ABSTRACT

This study aimed to synthesize and characterize nickel magnetic nanoparticles (MNPs), and evaluate their anticancer effects on the DLD1 colon cancer cell line and L929 mouse fibroblast cell line. The characterization of the MNPs which were synthesized by selective leaching was performed with a Zetasizer via dynamic light scattering (DLS) method, scanning electron microscopy (SEM), and atomic force microscopy (AFM). The zeta potential of Ni MNPs was determined to be -24.7(7.83) mV. The size of Ni MNPs was 764 nm with 0.486 PDI value. The cytotoxicity test was performed for L929 Fibroblast cell line and DLD1 colon cancer cell line. Cytotoxicity analyses were performed with the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The cell viability was observed to be 94.89% for L929 fibroblast cells. Then, anticancer effects on the DLD1 colon cancer cell line were evaluated. The use of Ni MNPs can be effective in reducing the effects of cancer since it decreases the cell viability overall, and it seems even more effective in the cancerous cell lines. We determined the cell viability of the DLD1 colon cancer cell line with the use of 0.001 grams of Ni MNP as 78.2%.

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INTRODUCTION

Cancer is the overall term used for the abnormal growth of cells caused by the loss of control over the cell cycle (Mader and Windelspecht, 2014). Colon cancer or colorectal cancer is a form of cancer that appears hidden in the rectal and colon regions of the large intestine in the form of a tumor more common in people above the age of 50s. Frequent symptoms include diarrhea, blood in the stool, and weight loss (Alexiusdottir, Kristin K., et al 2012). Moreover, current treatment forms are chemotherapy, radiation, and surgery (Harvard Health Publishing, 2012). The percentage of U.S. citizens who suffered colorectal cancer was seen as 68.8% in 2018 ("Centers for Disease Control and Prevention, 2019) To target colorectal cancer, magnetic nanoparticles can be functionalized. Magnetic nanoparticles (MNPs) have many applications starting from peroxidase-like activity to high-density magnetic recording (Guo et al. 2008). However, its applications on biological systems and medicinal purposes have not been explored clearly. Telomeres are located in the end parts of eukaryotic chromosomes and are made up of DNA-protein structures (Zakian, Virginia A. 2012). However, at the end of the DNA replication telomeres shorten by each cell division. Therefore, the length of telomeres is critical in determining whether the cell division is to proceed. Telomerase enzymes, though, produce sequences that are full of guanine to maintain the length of telomeres (Zvereva et al. 2008). Also, since cancerous cells have higher permeability than healthy cells (Guo et al. 2008), it is expected that cancerous cells will let more MNPs in nucleus. In this study, nickel MNPs are synthesized by the selective leaching method of Ni-Al alloy in NaOH solution (Michalcova et al. 2014). Also, the characterization of these MNPs was performed by Zetasizer (Malvern ZEN 3600 Nano ZS), atomic force microscopy (AFM) (Shimadzu SPM 9600), and scanning electron microscopy (SEM) (Zeiss, EVO® LS 10). The MNPs' average size, potential charge, and structure were observed. This study specifically concentrates on nickel MNPs' effects on biological systems and gives a comparison between the effects of nickel MNPs on the L929 fibroblast cell line and DLD1 colon cancer cell line. Since MNPs have peroxidase-like activities (Luo et al. 2019), it is expected that the MNPs should break down the telomerase enzyme, thus decrease the telomere length which in the end will decrease the mitotic division rate and decrease the cell viability of the tumor cells.

We synthesized nickel MNPs and observed the effects for various concentrations of MNPs. The effect of nickel MNPs on two cell lines or their cytotoxicity is determined by the MTT assay which involves tagging of cell lines with the MTT dye which in turn leads to the formation of formazan crystals. These crystallized molecules give rise to a unique color which by determining the absorbance of the cell lines, with an ELISA instrument, presents the cell viability since the crystallized molecules appear only in cells that have not undergone apoptosis. Although previous studies were done about Ni MNPs effects on Leukemia cells or Fe3O4 MNPs effects on Leukemia cells, there has been no study presenting the comparison between its effects on both a cancer cell line and a normal cell line which makes this study unique. Moreover, a colorectal cancer cell line has never been targeted previously in another study. In a nutshell, Ni MNPs should decrease the cell viability of cancerous colon cells due to their peroxidase-like activities, and cancerous cells should demonstrate less viability than healthy fibroblast cells.

MATERIALS AND METHODS

Synthesis of Nickel MNPs: The Nickel MNPs were synthesized with selective leaching method (Michalcova et al. 2014). 10 g of NaOH was measured with an electronic balance. Using a spatula, NaOH and 200 mL pure water were added into a beaker on a hot plate and heated up to 80 °C with a stirring rate of 900 rpm. After that, 20 g of Ni-Al (50:50 wt%) with a spatula was added to the beaker when the solution reached 80 °C and the solution was stirred for 2 hours. Then, the Ni MNPs were washed with pure water and ethanol 4 to 5 times repeatedly. Lastly, the MNPs were dried in an oven set to 50°C overnight (Michalcova et al. 2014).

Characterization of Nickel MNPs: The size and zeta potential of Ni MNPs was determined using Zetasizer (Malvern ZEN 3600 Nano ZS). Additionally, the morphological analysis of Ni MNPs was determined by using the AFM (Shimadzu SPM 9600) and SEM.

The Zeta Potential And The Particle Size: The measurements of the zeta potential and the particle size were carried out by ZetaSizer with using the technique of Dynamic Light Scattering (DLS), at 25 ° C. Nanoparticle solutions were prepared in distilled water for the measurements of the zeta potential and the size of the particles. Measurements were performed in triplicate and the average of the results was reported.AFM

SEM Analysis of Ni MNPs: The morphological characterization of Ni MNPs was evaluated by SEM (Zeiss EVO® LS 10) at an accelerating voltage of 7 kV and 20.00 KX magnification. Since the particles contain Nickel, they have conductive properties. For this reason, Au/Pd coating was not applied to the nanoparticles before the analysis.

AFM: The Atomic Force Microscope (AFM) is a kind of optical characterization technique that presents 2D and 3D images for nanoparticle morphological characterization. Nanoparticle solution dropped on agar surface and dried at room temperature. Images of Ni MNPs were scanned with 1 Hz scanning speed and by using Non-contact mode AFM (NC-AFM)(Shimadzu SPM 9600(Japan)).

In Vitro Cytotoxicity of Nickel MNPs: The in vitro cytotoxicity of Ni MNPs was determined by using the L929 fibroblast cell line.The assay was performed for different MNP concentrations (0.01g/mL, 0.005 g/mL, 0.002 g/mL, 0.001g/mL, 0.0005 g/mL). Ni MNPs concentrations were prepared by adding the required amount of NP measured with an electronic balance in a test tube and then adding the required volume of water to get each concentration.Then, dilutions were made in the DMEM medium supplemented with FBS to obtain Ni MNPs with 0.01g/mL, 0.005 g/mL, 0.002 g/mL, 0.001g/mL, 0.0005 g/mLconcentrations. 10^4 cells/well were seeded in a 96-well plate and incubated for 24 hours at 37 °C with %5 CO2. After 24h of incubation, the medium was removed and different concentrations of Ni MNP were added into the wells plate and then incubated for 24 hours at the same culture conditions. After 24h of incubation, the medium was removed and each well was treated with 100 µL methyl thiazole diphenyltetrazolium bromide (5 mg MTT/ml in medium) and incubated for a further 4h at 37°C with 5% CO2. Then, the MTT solution was replaced with 100 µL/well of DMSO to dissolve the formazan crystals. The optical density was determined at 570 nm by an ELISA reader (Thermo Scientific Multiskan Go, USA). The samples with no applied Ni MNPs were determined as the negative control group so that the cell viability percentage is determined to be 100% for the negative control group. Three samples were taken for each concentration of Ni MNPs for the L929 mouse fibroblast cell line.

The cell viability of cancer cells treated with Ni MNPs: The cell viability of the DLD1 colon cancer cell line after treatment with various concentrations of Ni MNPs was determined. The MNP concentrations were 0.01g/mL, 0.005 g/mL, 0.002 g/mL, 0.001g/mL, 0.0005 g/mL). The cell viability was determined by MTT assay. 10^4 cells/well were seeded in a 96-well plate and incubated for 24 hours at 37 °C with %5 CO_2. And the procedure mentioned above was followed. The cells with no Ni MNPs treated were the negative control. Three replicates were used for each concentration.

RESULTS AND DISCUSSION

Characterization of Ni MNPs: It was observed that the synthesized Ni MNPs demonstrated a polydisperse structure and a negative zeta potential.

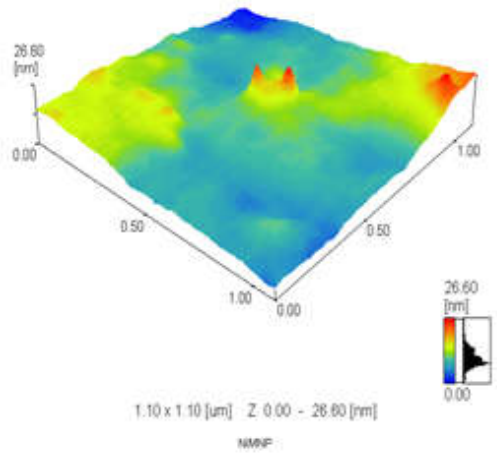

Figure 1. 3-D profile analysis of Ni MNPs reported by the atomic force microscopy. Red peaks indicate greater sizes as blue ones indicate the lowest size

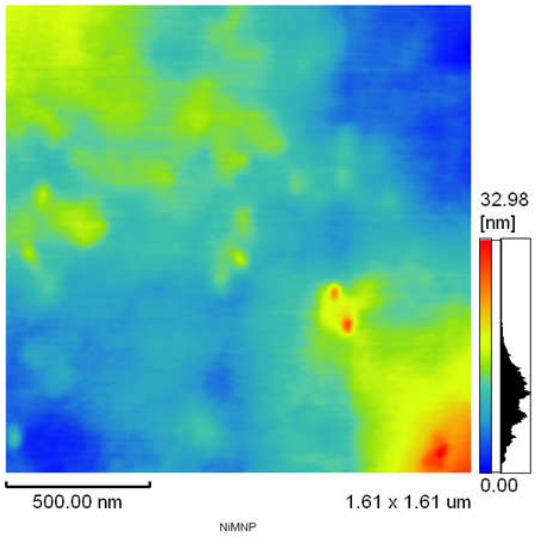

Figure 2. The 2-D profile analysis of Ni MNPs' size distribution is given

The zeta potential of Ni MNPs was determined to be -24.77.83 mV and three peaks were observed in the size of the Ni MNP with first peak 732.9273.8(83.8% intensity), second peak 5184484.1(10% intensity), third peak 103.925.8(6.2% intensity) and an average of 764 nm with a PDI of 0.486. In a study by Akhtar et al. the characterization of MNPs in that study was performed through SEM, TEM and X-ray diffraction (Akhtar et al. 2016) whilst in this study AFM, SEM and Zetasizer are utilized in order to characterize the MNPs.

The average size of MNPs in that study was determined to 30 nm which differs from the average size of MNPs in this study which is 764 nm. The predicted limitation of using MNPs of smaller size is that cells that aren't tumorous would also face apoptosis in higher percentages. The zeta potential of Ni MNPs was determined to be -24.77.83 mV (Figure 4) by the measurements done with the Zetasizer via dynamic light scattering (DLS) method. There were mainly 3 different sizes among the Ni MNPs. However, the average was determined to be 764 nm (Figure 2). Also, the profile analysis of MNPs done with the atomic force microscopy (Figure 1) has demonstrated a polydisperse structure (where larger MNPs were shown by red color and smaller ones with blue) explaining why the Zetasizer gave rise to 3 peaks. Furthermore, the SEM image of the MNPs gave a clear image of the sample (Figure 7).

In Vitro Cytotoxicity of Nickel MNPs: The in vitro cytotoxicity of Ni MNPs was determined by using the L929 fibroblast cell line. The assay was performed for different MNP concentrations (0.01g/mL, 0.005 g/mL, 0.002 g/mL, 0.001g/mL, 0.0005 g/mL). As for the cytotoxicity test, it is apparent that for the Fibroblast cell line as the MNP amount increases, the toxicity also increases. However, since it doesn't harm the cells dramatically, it is possible to use Ni MNPs in cancer-related research without much concern about its side effects on normal cells (Figure 5).

The cell viability of cancer cells treated with Ni MNPs: The cell viability of the DLD1 colon cancer cell line after treatment with various concentrations of Ni MNPs was determined.

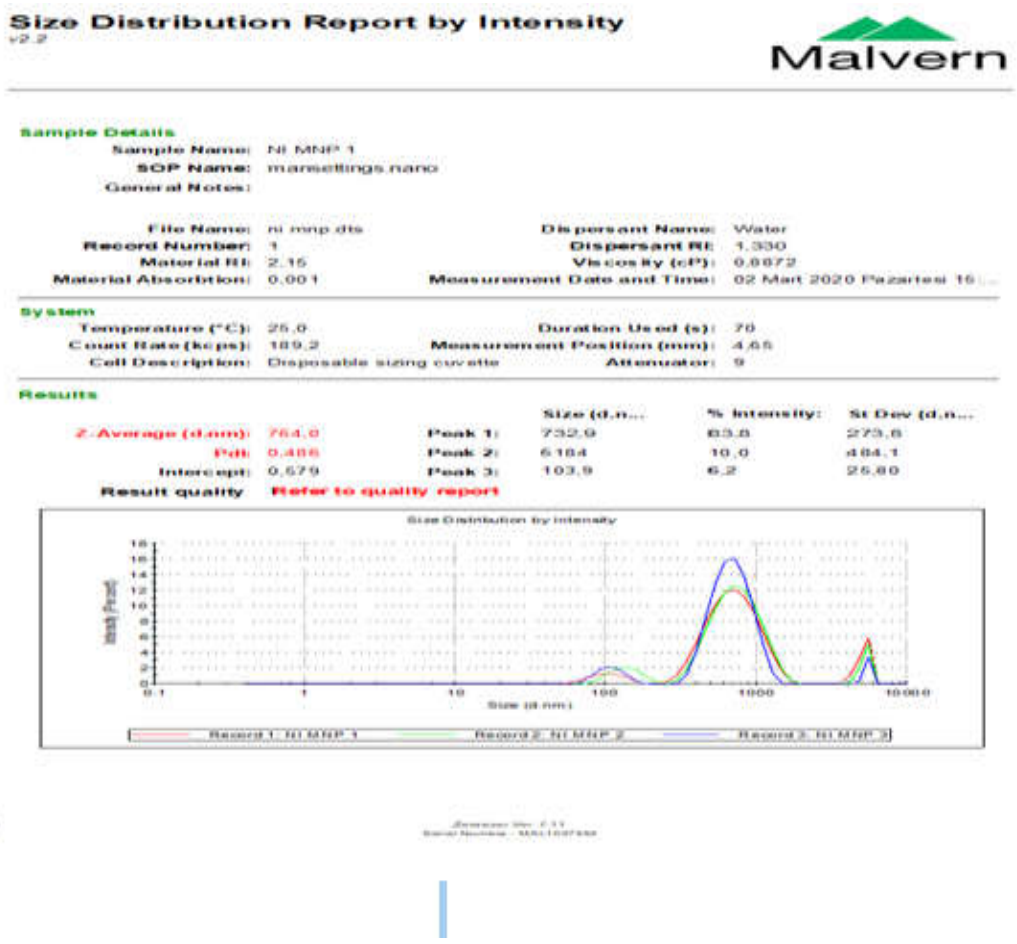

Figure 3. The size distribution graph of Ni MNPs

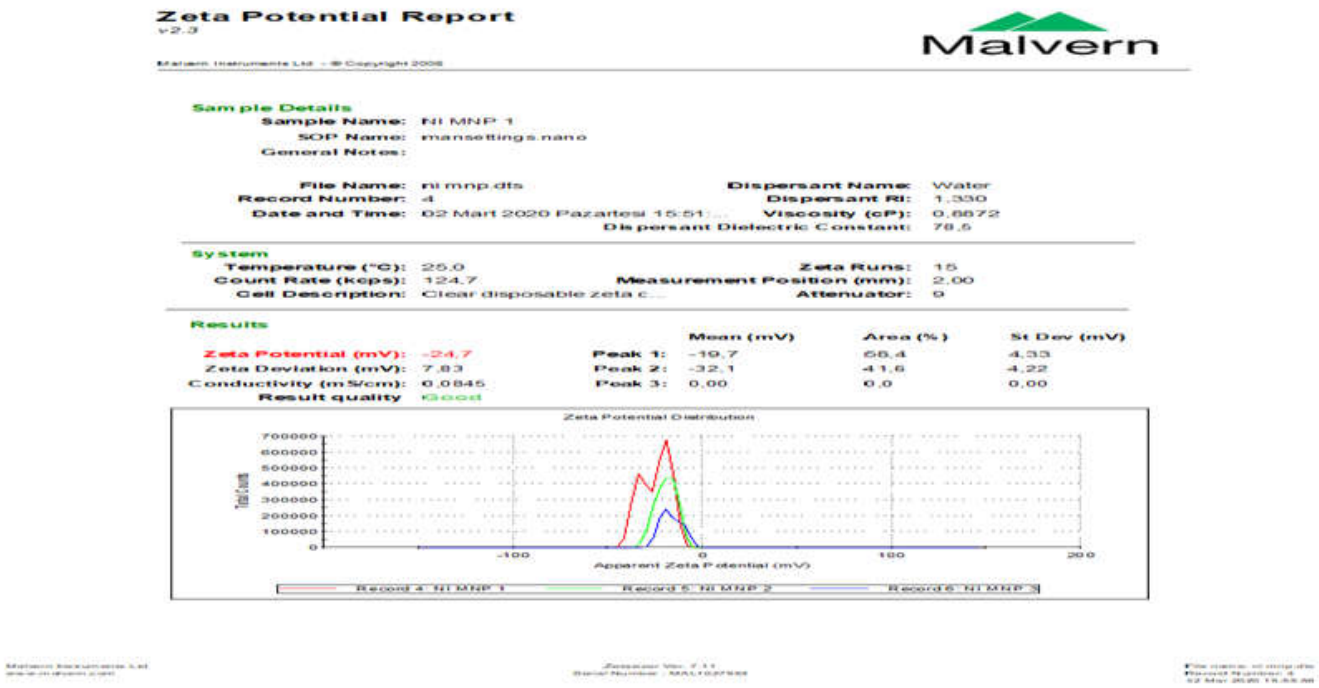

Figure 4. The surface charge potential report of the Zetasizer

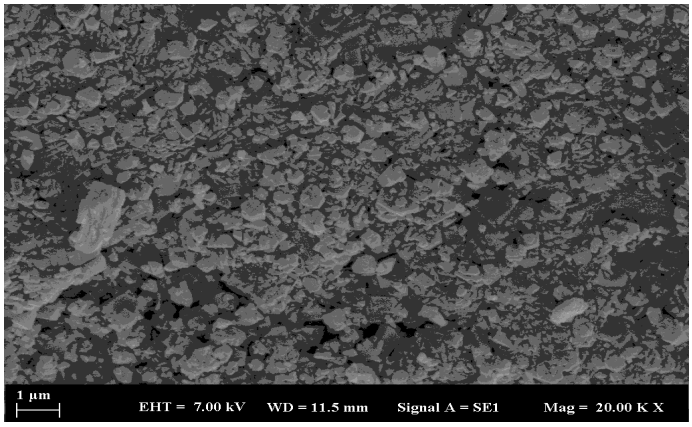

Figure 7. The SEM image of the MNPs

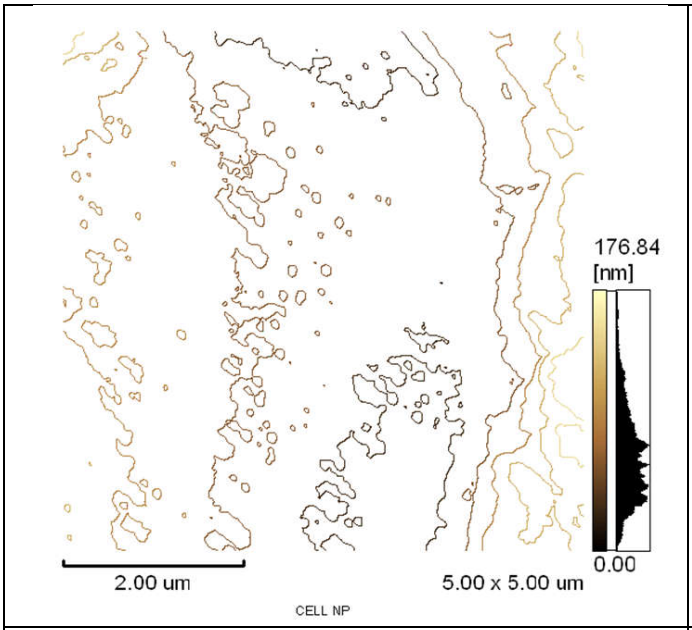

Figure 8. AFM analysis of cell with nanoparticles applied

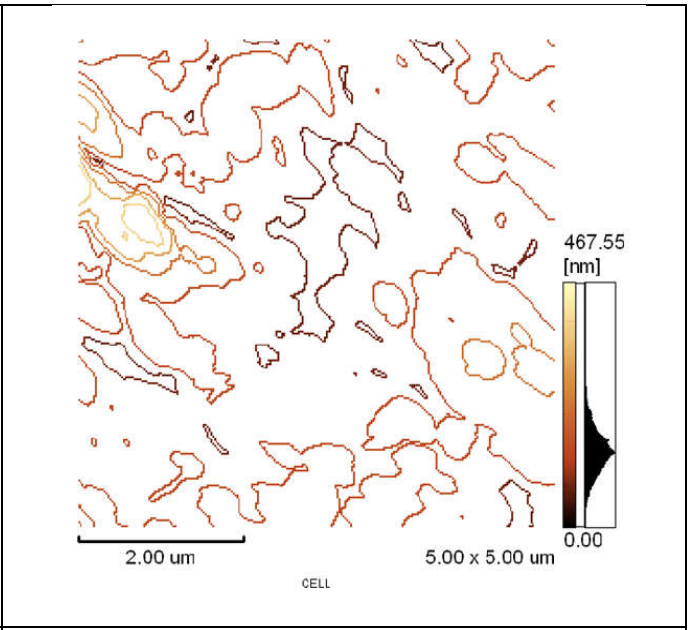

Figure 9. AFM analysis of cell without MNP applied

The MNP concentrations were 0.01g/mL, 0.005 g/mL, 0.002 g/mL, 0.001g/mL, 0.0005 g/mL). The cell viability was determined by MTT assay. A significant trend of a decrease in cell viability with the decrease in concentration is not observable and is rather stable. The cell viability was given for DLD1 colon cancer cell line in Figure 6. Still, though, it is observable that the cell viability of the DLD1 colon cancer cell line decreased with respect to the cell viability of the normal cell line. For instance, whilst the cell viability for fibroblast cells was 96.4%+-0.7%, the cell viability for DLD1 colon cancer is 82.6+-0.3% for the same concentration of of Ni MNPs. Basically, the most interesting result was that even in very low concentrations, although Fibroblast cell line's cell viability was very high (94.8+-0.4% for 0.001g of Ni MNPs the cell viability of the cancerous cell line stably demonstrated a low value such as 78.2+-0.3% for 0.001g of Ni MNPs.

Comparisons of cells with MNP treated and non-treated can be seen in fig. 8 and 9 as the AFM images are presented. It can be concluded, the use of Ni MNPs can be invaluable in reducing the effects of cancer since it decreases the cell viability overall, and is even more effective in the cancerous cell lines. Also, it was determined that increasing amounts of Ni MNPs caused a decrease in the cell viability. In explanation, it can be suggested the cancerous cell's nuclear membrane must be more permeable than normal cells' nuclear membrane so that the MNPs entered the nuclear membrane of the cancerous cell's nuclear membrane more easily and interacted with the DNA and the telomerase enzyme so that the cancerous cell's led to apoptosis. The cell damage can be caused by two possible reasons. The first reason can be the destruction of the telomerase enzyme due to MNPs' catalytic activities leads to a decrease in the telomere length and thus lead to lesser mitotic index and cell viability. On the other hand, it is also possible that the interaction of Ni MNPs with the DNA caused the cell to undergo apoptosis due to the damage of ROS caused by NPS (Guo 2008). However, the first theory is more likely since the DNA damage might not affect the cancerous cells (Alhmoud, Jehad F., et al. 2020) very much since they already underwent mutations considering the DNA. Therefore, it is probable that the telomere length is decreased due to the Ni MNPs which were then useful in terms of reducing the cell viability. Additionally, no study has targeted colorectal cancer with MNPs. Moreover, Guo's explanation on this matter states that the reason for leukemia cells' apoptosis is triggered by DNA damage (Guo 2008). However, being damaged already, it would not be very effective for cancer cells' DNA to be further damaged to undergo apoptosis. Therefore, this study offers a different explanation which is the telomere shortening. The MNPs which entered the nuclear membrane since it is more permeable in cancerous cells interacted with the telomerase enzyme, and thus bring about telomere shortening which resulted in a decrease in cell viability. This study's findings show that Ni MNPs can be functionalized and synthesized with another agent to target colorectal cancer cells.

CONCLUSION

In this study, the anticancer effects of Ni MNPs which were synthesized by the selective leaching method were observed along with the demonstration of cancerous cell's more permeable membrane.

Our hypothesis is supported by the data collected as the MTT assay's results indicated that the cell viability decreased in both cell lines and even more in cancerous cell lines. Additionally, the MNPs could be synthesized with an agent that might be compatible with telomerase and target telomerase more specifically to reduce cell viability. The effects of MNPs can be increased even more by applying a rotating magnetic field.

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