Quantitative Study of the Effect of Nanoparticles on the Mechanical Properties of Colon Cancer Cells

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Abstract: Magnetic nanoparticles (MNPs) are often used as drug-carrying particles for targeted therapy of tumors. Studying their effects on cell activity and mechanical properties is of great significance for the targeted treatment of tumors. In this paper, we used a combination of atomic force microscopy (AFM) and fluorescent labeling to study the mechanical properties of cells after the endocytosis of MNPs. Colon cancer cells SW480 were selected to co-culture with MNPs with a particle size of 50 nm, and the cell viability was measured and systematically analyzed under different conditions. The results showed that the safe dose of MNPs to colon cancer cells SW480 was 50 μg/mL, and when the amount exceeded 50 μg/mL, the cell viability decreased significantly. Increase the concentration of MNPs step by step within the safe dose of 0-50 μg/mL. Through the analysis of a large number of data measured by AFM, the results show that the mechanical properties of cells change significantly with the increase of MNPs concentration. In this paper, the experimental results are analyzed by comparing concentration gradients. The concentrations are set to 0, 30, and 50 μg/mL, respectively, to verify the influence of MNPs on the mechanical properties of cells.

1 INTRODUCTION

With the development of nanotechnology, nanomedicine continues to mature. Magnetic nanoparticles have recently been widely used in cancer-targeted therapy and real-time imaging technology because of their excellent biocompatibility and superparamagnetic properties. Therefore, it is of great significance to study the influence of nanoparticles on cell mechanical properties.

Nanoparticles are emerging as effective agents for diagnostic and therapeutic applications (Tregubov AA, 2018; V.R. Cherkasov, 2020). The action mechanism of nano ferric oxide particles in cells confirmed that nanoparticles would act on lysosomes, mitochondria, and other organelles after being absorbed by cells, causing an autophagy reaction (Zhang, 2016). Composited targeted MNPs with (cetuximab), an excellent MRI damaging contrast agent. The mediated MFH and NIR hyperthermia could target and induce apoptosis of gliomas U25l cells in vitro and significantly target gliomas transplanted in nude mice in vivo (Li, 2011). Literature (Xing, 2012) has confirmed that the cells could phagocytize many nanoparticles into the cytoplasm and impact liver cancer cells under the action of the external alternating magnetic field.

Reference (Jin, 2010) has made stem cell nanoprobes (MNPs mESCs) for labeling mouse embryonic stem cells, which were injected into tumor-bearing mice through the tail vein. It was confirmed that the prepared MNPs had good fluorescence and magnetic response properties and good biological activity against mESCs in the 50 μg/mL concentration range. The surface properties of nanoparticles, such as particle size, charge, shape, and surface modification, will affect neurotoxicity differently (Liang, 2022). Magnetic nanoparticles have many applications in cell manipulation; For example, they can be used as carriers to deliver targeted drugs and act as nuclear magnetic resonance (NMR) contrast agents (Guo, 2011; Ruan, 2013). Magnetic hollow nanoparticles have excellent magnetic properties and are lightweight. In addition, they also have excellent biocompatibility. There is a cavity inside, which is often used for drug transportation (Wang, 2022). Red blood cells are studied as a means of conveyance of nano drug delivery system, that is, red blood cell hitchhiking technology. It is verified that red blood cell hitchhiking can also be very effective for nanoparticle delivery and tumor treatment, for treating invasive and small cell types of cancer and other lung diseases (Zelepukin IV, 2019).

According to the above analysis, current research mainly focuses on the phagocytosis of nanoparticles and the use of nanoparticles as intermediaries for drug delivery. However, there are few studies on the effect of nanoparticles on the mechanical properties of cells. In this work, the impact of different concentrations of magnetic nanoparticles on the mechanical properties of SW480 cells was studied by atomic force microscopy (AFM). Firstly, the cells were cocultured with varying concentrations of MNPs with a particle size of 50 nm. The fluorescence experiment proved that the nanoparticles

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successfully entered the cells and their distribution in the cells. Then, the cell images and mechanical data were obtained by AFM and quantified. Finally, the effect of MNPs concentration on cell mechanical properties (surface adhesion and Young's modulus) and activity was analyzed.

2 METHODS

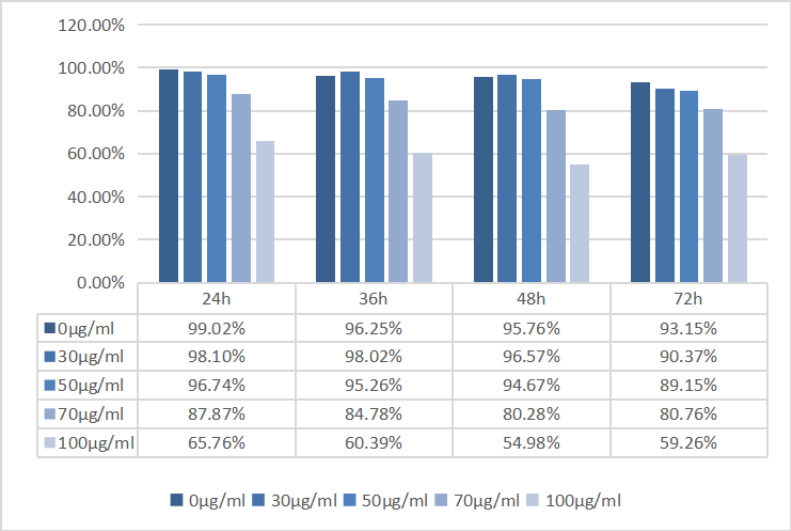
The magnetic nanoparticles used in this work are nanoparticles with a particle size of 50 nm, which have properties unique to nanomaterials, such as small particle size, huge specific surface, high coupling capacity, magnetic responsiveness, and superparamagnetic (Ito A, 2005; Ma, 2003).

The cell used in this paper is Colon Cancer Cell (SW480). First, the cells were resuscitated in a sterile environment. The medium was RPMI1640 containing 10% fetal bovine serum, which was placed in a 37 ℃ 5% cell incubator for routine culture. After more than three generations of cell passage, the cells reached a stable physiological state. The experimental equipment includes an atomic force microscope (cspm5500), scanning electron microscope (quant-250feb, USA), magnetic microscope (JPK instruments, Germany), and fluorescence microscope. Fluorescence microscope. Based on the optical lever, the atomic force microscope detects the ultrastructure of the sample surface by using the nuclear force between the probe tip and the sample and obtains the mechanical property data of the cell by processing the cell image.

3 RESULTS

It is shown that nanoparticles are absorbed by cells, enter lysosomes through endosomes and destroy lysosomes, increase autophagic vesicles by damaging mitochondria, endoplasmic reticulum, and Golgi apparatus, and prevent the standard binding of lysosomes and autophagic vesicles from accumulating autophagic vesicles, thus continuously increasing the level of cellular autophagy and finally triggering cell death. Autophagy is an egoless protection mechanism of cells, but excessive autophagy will cause apoptosis. In this paper, the safe concentration range of MNPs on cells was investigated by MTT experiments, and the experimental procedure was as follows [1].

The experimental results are shown in Fig.1. When the concentration of MNPs was lower than 50 μg/mL, the cell survival rate was about 95%, and MNPs had no significant effect on cells, and the trend of decreasing the survival rate of cells was not marked with the increase of culture time; however, when the concentration of MNPs was above 50 μg/mL, MNPs showed apparent toxicity to cells. The cell survival rate gradually decreased with the increase in culture time. It was inferred that the upper limit of the safe dose of MNPs to SW480 was 50 μg/mL.

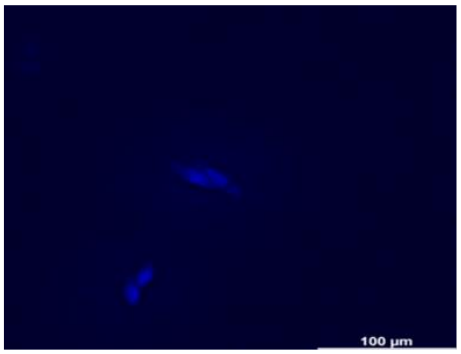


	24h	36h	48h	72h
0μg/ml	99.02%	96.25%	95.76%	93.15%
30μg/ml	98.10%	98.02%	96.57%	90.37%
50μg/ml	96.74%	95.26%	94.67%	89.15%
70μg/ml	87.87%	84.78%	80.28%	80.76%
100μg/ml	65.76%	60.39%	54.98%	59.26%

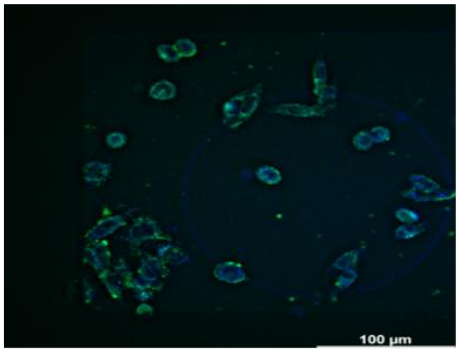
Fig.1 MTT assay to characterize the effect of MNPs on the survival rate of SW480 cells

To observe the distribution of MNPs in cells, we modified the surface of MNPs with FITC fluorescein, and the FITC fluorescein-modified nanoparticles (FMNPs) appeared green under the excitation of blue light. mL and FMNPs concentration of 50 μg/mL. The three groups of cells were cultured for 24 h, and SW480 was stained with 4', 6-diamidino-2-phenylindole (DAPI) stain, which could bind to the AT base pairs of the double-stranded DNA minor groove through the intact cell membrane. The nuclei were shown to fluoresce blue under UV excitation using a fluorescence microscope. The distribution of FMNPs in cells could be obtained by superimposing the channels of DAPI and FITC under a fluorescence microscope by adjusting the two fluorescence channels as well as the focal length, as shown in Fig.2, Fig.3, and Fig.4. The experimental results showed that FMNPs were successfully taken up by the cells and distributed in the cytoplasm without entering the nucleus. The number of particles ejected into the compartment increased with the increased FMNPs concentration. The cells in Fig.2(a) did not add FMNPs, but only the blue cell outline can be seen. When 30μg/mL FMNPs were added to the solution, the fluorescent particles with green light can be seen in Fig.2(b). Most of the fluorescent particles are distributed at the edge of the cell, and a few surround the nuclear area. Scattered fluorescent particles can be seen in the

solution. When 50μg/mL FMNPs were added to the dissolution, it can be seen that a large number of green fluorescent nanoparticles were distributed inside the cell, as shown in Fig.2(c).



(a)



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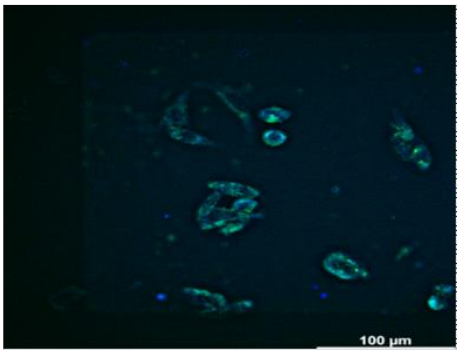


(c)

Fig.2 MNPs were co-cultured with cells. (a) Fluorescence imaging of cells in the FMNPs concentration of 0 μg/mL culture group. (b) Fluorescence imaging of cells in the FMNPs attention of 30 μg/mL culture group. (c). Fluorescence imaging of cells in the FMNPs attention of 50 μg/mL culture group

It has been demonstrated that the amount of MNPs ingested by SW480 increases with the increase of MNPs concentration through SW480 MNPs ingestion experiments and MTT experiments in Chapter 3, and the safe dose of MNPs for SW480 is 50 μg/mL, so in this work, the effect of MNPs concentration on mechanical properties of cells was studied in the range of 0-50. The mechanical properties included adhesion force, roughness, and Young's modulus.

3.1 Effect of MNPs on cell adhesion

Cell adhesion is the ability of cells to adhere to each other or the matrix. Cell adhesion is expressed as the process of cell interaction through adhesion molecules on the surface. Cell adhesion is one of the criteria for evaluating the risk of cancer in the study of tumor cells. Currently, many methods have been established in studying cell-matrix interaction forces, most of which are based on AFM. AFM can measure the adhesion force of individual cells under physiological conditions.

The experimental procedure is as follows: After the MNPs with concentrations of 0 μg/mL, 30 μg/mL, and 50 μg/mL were co-cultured with cells for 24 h, and then AFM was used to obtain cell images and related mechanical parameters in QI mode. AFM received the image and mechanical properties of the cell surface in a physiological state through the contact between the probe tip and the surface of the cell membrane. The scanning resolution was 256×256, the setpoint was 1.2nN, and the scanned area was 100×100 μm. The mechanical parameters of the cell margins and the vicinity of the nucleus were extracted for analysis, and the average of the 50 cells in each group was taken as the final result. When the concentration of MNPs was 0 μg/mL, the average adhesion force near the nucleus was 2.054nN, and the average adhesion force at the edge of the cell was 2.376nN; when the concentration of MNPs increased to 30 μg/mL, the average adhesion force near the nucleus decreased to 1.899nN and the adhesion force at the edge of the cell was 2.012nN; when the concentration of MNPs was 50 μg/mL, the average adhesion force near the nucleus decreased to 1.899nN and the adhesion force at the edge of the cell was 2.012nN. The dotted line in the figure shows that within the safe dose range, cell surface adhesion generally decreases with the increase of MNPs concentration. The experimental results of cell adhesion are demonstrated in Fig.3.

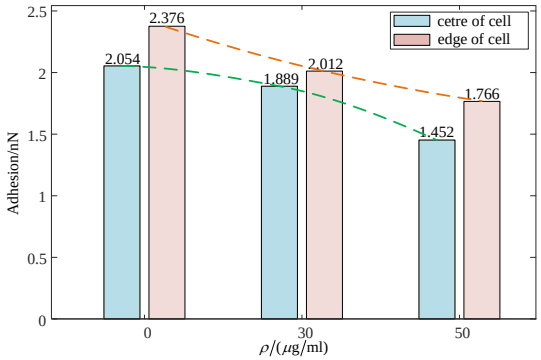
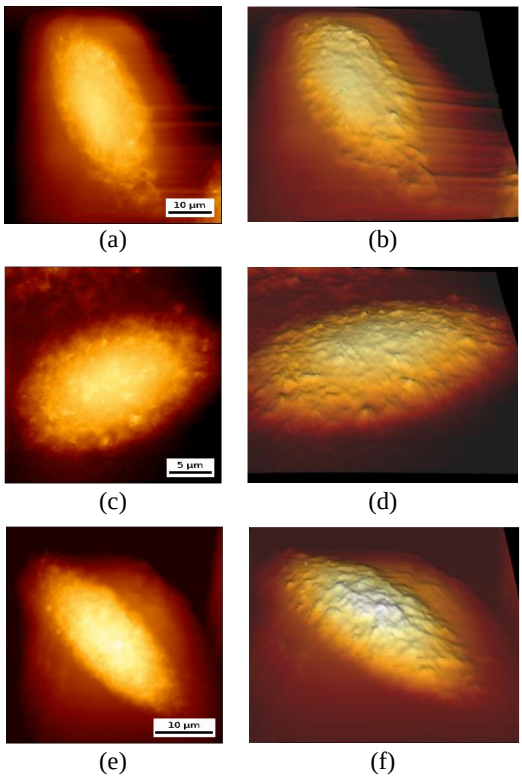


Fig.3 Effect of MNPs on cell adhesion

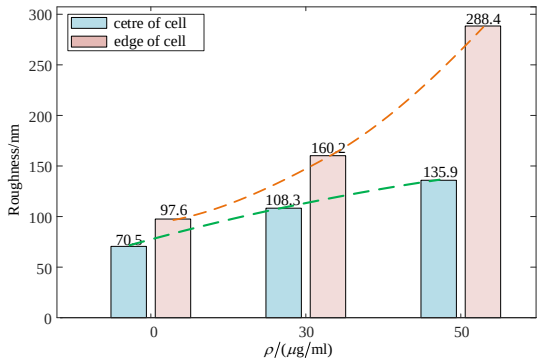
3.2 Effect of MNPs on cell roughness

To characterize the effect of MNPs concentration on cell surface roughness, we established the 3D imaging of SW480 cells at different MNPs concentrations, and the AFM images are shown in Fig.4, and three groups of cells were scanned, in which the SW480 cell image at MNPs concentration of 0 μg/mL was characterized in Fig.4(a), and Fig.4(b) showed the 3D image of the cell in Fig.4(a).

Fig.4(c) characterized the SW480 cell image at an MNPs concentration of 30 μg/mL, and Fig.4(d) showed the three-dimensional image of the cell in Fig.4(c). The image of the SW480 cell at the concentration of 50 μg/mL of MNPs was characterized in Fig.4(e), and the 3D image of the cell in Fig.4(e) was shown in Fig.4(f). It can be visualized by the 3D images that the number of bumps on the cell surface increases significantly, and the cell membrane becomes rougher as the concentration of MNPs increases. In order to observe the effect of increasing MNP concentration on the cell roughness more intuitively, the three groups of cell images in Fig.4 were analyzed by data processing point by point. The results of the data analysis are shown in Fig.4(g). When the concentration of MNPs was 0 μg/mL, the roughness near the nucleus was 70.5 nm, and the value of cell edge roughness was 97.6 nm; when the concentration of MNPs was 30 μg/mL, the roughness near the nucleus. When the attention of MNPs was 30 μg/mL, the roughness near the nucleus was 108.3 nm, and the roughness at the cell edge was 160.2 nm; when the concentration of MNPs was 0 μg/mL, the roughness near the nucleus was 135.9 nm, and the roughness at the cell edge was 288.4nm, which was greater than the growth trend of roughness near the nucleus. The dotted line in Fig.4(g) indicates the roughness change of cells.



(a) (b) (c) (d) (e) (f)



(g)

Fig. 4 AFM images of SW480 cells. (a) The image of SW480 cell at MNPs concentration of 0 μg/mL. (b) The three-dimensional image of the cell is in fig.(a). (c) The image of SW480 cell at MNPs concentration of 30 μg/mL. (d) The three-dimensional image of the cell is in fig.(c). (e)The image of SW480 cell at MNPs concentration of 50 μg/mL. (f) The three-dimensional image of the cell in fig.(e). (g) Effect of MNPs concentration on cell surface roughness.

3.3 Effect of MNPs on Young's modulus of SW480

The physiological environment in which cells live and their physiological activities are highly complex, and some of the physiological actions of cells can cause reorganization of the cytoskeleton. Changes in cellular tissue structure can be characterized by changes in the mechanical properties of cells. Young's modulus, as one of the basic mechanical properties of cells, is measured by the following methods: AFM, optical tweezers, and AFM is currently the most commonly used method for measuring Young's modulus. This paper used AFM to measure Young's modulus of cells. Through a large number of experiments, the conclusions were shown in Fig.5, which showed that when the concentration of MNPs was 0 μg/mL, Young's modulus near the nucleus was 0.942 kPa, and Young's modulus at the cell edge was 1.147 KPa; When the concentration of MNPs was 30 μg/mL, Young's modulus near the nucleus was 1.145 kPa, and Young's modulus at the cell edge was 1.145 kPa. When the concentration of MNPs was 30 μg/mL, Young's modulus near the nucleus was 1.145 kPa, and Young's modulus at the cell edge was 0.738 kPa; when the concentration of MNPs was 50 μg/mL, Young's modulus near the nucleus was 1.459 kPa, and Young's modulus at the cell edge was 0.682 kPa; it can be seen that with the increase of the concentration of MNPs, Young's modulus around the nucleus and the cell edge showed a gradual decrease, while Young's modulus at the cell edge showed a gradual decline. Young's modulus at the edge of the cell showed a gradual increase. The dotted line in Fig.5 shows the changing trend of Young's modulus of cells.

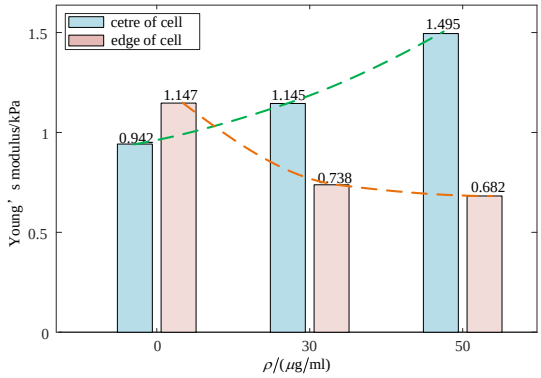


Fig. 5 Effect of MNPs on Young's modulus of SW480

In this experiment, two control groups were set up, the culture group with 0 μg/mL of MNPs and the cultural group with 50 μg/mL of MNPs, which were cured after 24 h co-culture, it could be seen in Fig.6(a) and Fig.6(b) that the surface of the cells in the regular SW480 experimental group was smooth. In contrast, the surface of SW480 with the addition of 50 μg/mL MNPs was rough and raised, as shown in Fig.6(c) and Fig.6(d), which could more clearly visualize the effect of MNPs on the surface of SW480. The impact of MNPs on the surface roughness of SW480 could be more clearly visualized. The variation of the roughness values in the cell's mechanical properties was also verified.

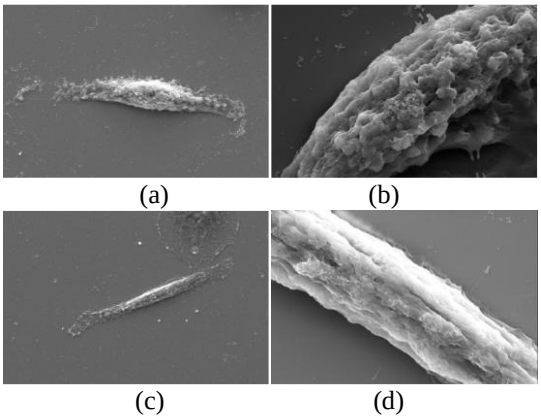


Fig.6 SEM images of MNPs on SW480. (a) The image of SW480 cell at MNPs concentration of 0 μg/mL. (b) Detail of the cell in fig.(a). (c) The image of SW480 cell at MNPs concentration of 50 μg/mL. (d) Detail of the cell in fig.(c)

4 DISCUSSION

In the past, the research methods for MNPs and cells were very traditional. In this paper, the technique of combining atomic force measurement and fluorescence observation was adopted, and innovation was made in experimental methods and accuracy. This paper used discriminant statistical methods in the data analysis process. At the same time, error analysis methods were used to ensure the reliability and accuracy of data. It has practical significance in providing new detection methods and data statistics for targeted drug delivery research of cancer. The limitation of this study is that while MNPs affect the mechanical properties of cancer cells, the growth of cells themselves will also have a slight change. We look forward to further exploring this mechanism in future expansion research. In future research, I will process the data in this paper through data processing algorithms to quickly determine the impact of MNPs on cells.

5 CONCLUSION

In this study, the effect of MNPs on the mechanical properties of cells was verified by a new method: the combination of a fluorescence microscope and AFM. After MNPs were swallowed into cells, the effect on organelles changed the mechanical properties of cells. The experimental results showed that when we differentiated the concentration gradient within the safety measurement and adjusted the concentration of MNPs, with the increase of MNPs concentration, the adhesion and surface roughness of SW480 cells increased, and the roughness of cell edges increased more than that near the nucleus. This is most intuitively verified in SEM imaging and AFM 3D images. The Young's modulus near the nucleus shows a downward trend, and Young's modulus at the cell edge shows a gradually increasing trend. The change of mechanical properties of cancer cells by magnetic nanoparticles is of great significance in tumor-targeted therapy. The experimental method proposed in this study also provides a new idea for the future cell detection system.

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