The photothermal effect of polypyrrole modified gold nanoparticles on SKOV-3 cells using SEM and AFM

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Abstract. Nanotechnology and its application are widely used in the field of life, human exploration of life science has entered the nano level, which is of great significance for exploring the essence of cellular life. This paper explores the changes in the mechanical characteristics of tumor cells after the photothermal effect of nanomaterials. The experiments used AFM and SEM to measure and observe SKOV-3 cells before and after the treatment. It was found that the cell height, morphology, cell adhesion, and Young's modulus had significantly changed. In the PPy-GNPs+Laser group, the adhesion force value was 3.57±1.25 nN, and Young’s modulus was 27.4±2.47 kPa. From the data, a 53.9% increase in Yong’s modulus of laser test PPy-GNPs as compared with the control group. It shows that the photothermal effect of nanoparticles has an important effect on the changes of cell ultrastructure, which has important significance for explaining the changes of cell physiological functions from the perspective of cell mechanics.

1. Introduction
Since 1986, atomic force microscopy (AFM) has been rapidly applied in various fields, especially promoting the development of biotechnology. AFM can perform real-time high-resolution imaging of biological samples in a near-physiological environment [1]. With the continuous research of AFM application technology by humans, AFM can be used to study the mechanical properties of single cells, the adhesion between cells and substrates, and the interaction between molecules and other physical properties [2]. Therefore, it is widely used in many fields such as disease diagnosis, new drug development, and material biocompatibility evaluation. In 2003, Chen Yong and his team from Jinan University applied atomic force microscopy to test the surface of tumor cell membranes such as gastric cancer, lung cancer, and liver cancer in morphology [3]. Yu Miao of Harbin Institute of Technology used AFM's nano-processing system to perform micro-cutting and indentation experiments on the surface of cancer cells and obtained physical properties such as layering balance force, volume and internal pressure inside the cell [4]. Stewart et al. of the Federal Institute of Technology in Zurich used a cantilever without a needle tip to indent cells [5]. This method not only prevents the cells from sliding but also improves the detection method of the physical properties of the cells. Li and his team from Peking University applied the calcium-binding protein S100A8/A9 complex on the surface of human cervical squamous carcinoma Caski cells to study their effects [6]. By using atomic force microscopy to perform high-resolution imaging of living cells and to observe
the cell surface structure, it was found that this effect is mainly achieved through the redistribution of actin. They also used RNAi technology and blocking agents to silence genes in the RhoC pathway, and the ability of cervical tumor cells to be evaluated by AFM detection of cell mechanical properties. The Young's modulus can quantitatively describe the invasion of cervical tumor cells. Young's modulus can quantitatively describe the change of cervical tumor cell invasiveness, provide a new method for the clinical judgment of cervical tumor cell invasiveness [7-10].

Photothermal therapy generally uses the properties of photothermal conversion materials to convert light energy into heat energy [11]. The photochemical conversion material gathered around the tumor cells is irradiated by laser (mostly near-infrared light), which makes the cells unable to withstand high temperatures and killed. Photothermal therapy has the characteristics of non-invasive, direct, and accurate [12-14]. It reacts with oxygen under irradiation of a certain range of wavelengths, and the generated oxide macro-molecules will destroy the cells, causing the tumor cells to become smaller, causing inflammation locally and causing the immune response [15]. Although this method is still in the primary research stage, with the rapid development of nanotechnology in medicine, we firmly believe that photothermal therapy combined with nanotechnology will usher in their new opportunities in tumor treatment [16].

In this paper, polypyrrole modified gold nanoparticles were used to synthesize composite nanomaterials (PPy-GNPs). The particles were co-cultured with SKOV-3 cells, and the photothermal conversion effect of PPy-GNPs was realized under the irradiation of 808 nm laser. The ultrastructural changes of the treated cells were detected by SEM and AFM was utilized to reveal the mechanical characteristics of the cells.

2. The measurement of AFM
AFM uses the theoretical basis of the interaction between atoms as its microscopic imaging principle. Atomic force microscopy not only can scan and image samples, but also detect physical quantities [17]. In force measurement, the force generated when the probe interacts with the sample is detected by an atomic force microscope to measure the mechanical properties of the sample. One of the cantilevers is fixed, and the other is a scanning probe. The sample is placed on a micro-displacement platform composed of piezoelectric ceramics, and the piezoelectric ceramic scanner is controlled by a control system. When a laser beam is irradiated on the microcantilever at the top of the probe tip, the laser light reflects on the cantilever beam, and the reflected light illuminates the photodetector. The deformation amount of the micro-cantilever is detected by the photodetector, and the height fluctuation of the sample surface can be pushed back to push out the sample surface morphology (Figure 1).

![Figure 1. The experimental setup of an atomic force microscope [18].](image-url)
When the probe is far away from the sample, the cantilever beam is in free without deflection, the force between the needle tip and the sample surface is too small to deflect the cantilever (Figure 2A). As the probe gradually approaches the sample, the distance between the probe and the sample becomes smaller and smaller, reaching the range of attractive action. At this time, the cantilever is subjected to attractive action. When the distance reaches a certain point, the attractive force reaches the maximum value, overcoming the elastic deformation of the cantilever. At this time, the cantilever reaches the maximum deflection and the needle tip quickly jumps into the sample surface. When the probe continues to feed into the sample, the probe and sample enter the repulsive force, and the cantilever begins to deflect upward. During the withdrawal of the probe, as the distance between the needle tip and the sample increases, the repulsive force gradually decreases. When reaching a certain distance, it appears attractive. As the distance increases, the attractive force gradually increases in a short distance, and the upward deflection of the cantilever becomes smaller and smaller. With the needle withdrawal, the distance between the tip of the needle and the sample becomes farther and farther, and the attractive force gradually disappears. Due to the adhesion between the needle tip and the sample, the adhesion causes the cantilever to continue to deflect downward. When the probe continues to move away from the sample and the distance between them is far enough, the adhesion is overcome, the tip of the needle separates from the surface of the sample again, and the cantilever returns to its free state [19](Figure 2B). According to Hooke's law, the height of the sample can be calculated at this time, and Young's modulus of cells can be measured according to the deflection of the cantilever [20].

Figure 2. The probe of Atomic force microscope (a) and the principle of atomic force microscopy (b).

3. Cell mechanics analysis by AFM

AFM analysis of cell nanoparticle interaction. AFM measurements including Agilent Technologies 5500 Scanning Probe Microscope (SPM, Agilent Technologies Company, USA) and JPK (NanoWizard®3, JPK Instruments, Germany) were applied. For the AFM test, a Tap300Al-G AFM probe was utilized with the tapping mode below the resonant frequency (Typically 100-400 kHz) under ambient conditions at room temperature. For the JPK AFM test, a pyramidal shaped MLCT probe (spring constant, 0.07 N/m) was utilized with quantitative imaging (QI) mode. The adhesion forces were induced by the interaction between the tip and the cell membrane [21]. The Hertz model was used to calculate the elastic parameters of cells in this work. After treated with nanoparticles for 48 hours, and laser illumination for 300s (25 W/cm², λ=808 nm, Xi’an Leize BOT808-2000D). The irradiation environment temperature is 22.4℃. The living cells in the culture medium were measured by AFM to analyze the cell surface morphology, height, adhesion force, and Young's modulus parameters. All the experiments were repeated more than 3 times in parallel. Under the same
experimental conditions, without interfering with each other. Some results are shown in figure 3 and figure 4.

**Figure 3.** The image of the SKOV-3 cell (control group) measured by AFM (A), Cell height (B), adhesion force (C), and Young's modulus (D).

**Figure 4.** The image of the SKOV-3 cell (PPy-GNPs+ laser group) measured by AFM (A), Cell height (B), adhesion force (C), and Young's modulus (D).

Collect the mechanical characteristic parameters of the cells and calculate the average value of the samples to avoid errors caused by individual differences. Figure 5 and figure 6 show the statistical distribution of the cell height, adhesion force, and Young's modulus of SKOV-3 cells. Compared with the control group, the height of SKOV-3 cells becomes higher, the adhesion force is reduced, and Young's modulus increases, after being subjected to the nano-photothermal effect.
The selected statistical sections all included the nuclear and perinuclear areas of cells and at least 150 points were selected in each area for statistical analysis. The data were analyzed by origin 8. The average height of SKOV-3 cells was 2.67±0.64 μm and finally reached 3.05±0.53 μm in the PPy-GNPs+Laser group. The adhesion force values of control SKOV-3 cells were 4.14±1.18 nN, and Young’s modulus was 17.8±1.14 kPa. In the PPy-GNPs+Laser group, the adhesion force value was 3.57±1.25 nN, and Young’s modulus was 27.4±2.47 kPa. From the data, the adhesion force has a small difference, however, a 53.9% increase in PPy-GNPs with laser testing of Young’s modulus.

4. Cell morphology analysis by SEM
To investigate the micro-morphology of single cells, a Quanta FEG-250 SEM (FEI, Massachusetts, USA) was utilized. It offers high resolution for sizing and counting microscale cell. It is considered as a standard method to quantify cell structure. SKOV-3 cells used in this measurement should be treated through the following processes. The cells were placed on circular coverslips for 24 h incubation and treated with GNPs or PPy-GNPs in NIR laser irradiation, then fixed with 4 % glutaraldehyde buffer for 12 hours at 4°C after washing the cell. The samples were further washed with PBS for three times and dried in sterile conditions. Next, 10-20 μl reduced osmium solution was added and the samples were kept at 4°C for 30 min. After rinsing with phosphate buffer, the samples underwent a dehydration process by increasing the concentration of ethanol and then immersed into tertiary butyl alcohol for 12 hours [22]. Finally, the resulting coverslips were dried in vacuum and metalized with a thin gold layer of about 1.5 nm for SEM imaging.

SEM was utilized to investigate the morphology changes of SKOV-3 cells, as shown in figure 7. The characteristics of the cells varied not only in size and shape but also in membrane structure. In the control group, the cells had attached well with substrates and covered large areas (Figure 7a). There are more intricate “cell tentacles” on the cell membrane surfaces, which contributed to the formation of reticulated fissures between the protuberances in laser irradiation (Figure 7b). But, in GNPs and PPy-GNPs groups, the cells are shrinking, and the cell tentacles are becoming less than in the control group (Figure 7c and Figure 7e). What is more, PPy-GNPs make the cell protuberances. This is most probably because the surface charge of PPy-GNPs affects the adherent growth of cells, but this effect cannot cause cell apoptosis or death. After laser irradiation, cells in the GNPs and PPy-GNPs groups showed varying degrees of morphological changes, wrinkles, invagination, atrophy, aberration, vesicles, suspension, and cell damage. The surface fragility of the cells increased, especially in the PPy-GNPs group (Figure 7d and Figure 7f). To reveal the structure of the cell morphology, the experiment set the scale of the image to 10 microns, so only a single cell can be carried on the image. The cells show in the picture are not specific or representative, just one of the cells after the experiment treatment.

Figure 7. SEM images of SKOV-3 cell treated with different conditions for 48 h
a: Control Group; b: Laser irradiation group; c: GNPs group; d: GNPs with laser irradiation group;
e: PPy-GNPs group; f: PPy-GNPs with laser irradiation group.
5. Conclusions
In recent years, the development of nanotechnology has greatly promoted the application of nanomedicine in cancer diagnosis and treatment [23, 24]. Photothermal conversion nanomaterials are non-invasive, direct, and accurate, so they are used as photosensitizers in photothermal therapy. Although it is still in the preliminary stage of clinical research, it has been proven to have high anticancer efficacy in laboratory and clinical research. The results of some clinical experiments show that due to the diversity, complexity, and heterogeneity of tumors, photothermal therapy still cannot effectively prevent tumor recurrence and metastasis. Now, the research has gradually shifted from single photothermal therapy to combination therapy. Multi-mode combination therapy based on photothermal therapy can have a synergistic effect on tumor elimination, overcome hypoxia in deep tumors, and break the limitation of local drug administration. Although the clinical application of photothermal therapy needs further research, it provides a new opportunity for the clinical treatment of cancer. With the rapid development of the application of nanotechnology in the medical field, we are convinced that this photothermal treatment based on photothermal conversion nanomaterials will make clinical photothermal treatment more refined, functional, and intelligent. It will certainly be a new opportunity in the clinical treatment of cancer.

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