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Silver Nanomaterials Applied in the Field of Diagnosis and Treatment

Jiaming Cui

Wuhan University of Technology, 207 Xiongchu Avenue Hongshan District, Wuhan, Hubei Province, China, 430070

heyueqi777@whut.edu.cn

Abstract. Nanomaterials have great application prospect in biological medicine due to their strong permeability, repair and regeneration. In particular, nano silver has excellent antibacterial ability and no toxic effect, which makes it widely used. The specific content of this paper introduces a method of using silver nanoparticles to generate silver ions, and then make it react with hydrogen peroxide, through the colorimetric device to react with different color changes corresponding to different concentrations, through this biosensor to determine the concentration of such as cholesterol lactic acid and other substances.And when detecting miRNA,AuNPs were further modified by complementing the DNA (cDNA) of target mirnas. AgNPs could not be attracted to negatively charged DNA due to electrostatic repulsion by immobilized modified gold nanoparticles on the electrode surface. After analyzing the peak of silver stripping, miRNA can be accurately detected.Furthermore, chitosan coated silver nanoparticles were designed, and polyethylene glycol was used as a stabilizer. Finally, its good blood compatibility and antibacterial ability, as well as its cytotoxicity to cervical cancer cells were demonstrated by characterization.Finally, the accumulation and changes of reactive oxygen species (ROS) and reactive nitrogen (RNS) induced by silver nano are introduced, and it is confirmed by measurement that they are important factors in apoptosis of human pancreatic ductal adenocarcinoma.

Keywords: Silver nanoparticles, Biosensor, Nanotechnology

1. Introduction

As a nanoscale precious metal material, silver nanoparticles have many physical properties that underlie their widespread application. It mainly includes surface effect, small size effect and macroscopic quantum tunneling effect. Compared to the variable color of AuNPs of different particle sizes, silver nanoparticles (AgNPs) are generally yellowish and do not change abruptly in color with changes in particle size. In addition, zero-valent silver on the surface of AgNPs is particularly susceptible to oxidation into Ag^+ by hydrogen peroxide, etc., and is therefore more involved in color rendering detection chemicals. For the reporting of silver nanoparticles in the cancer treatment, the potential molecular mechanism of the cell killing effect of nanosilver on cancer cells, such as silver nanoparticle can inhibit enzyme activity and regulating signaling pathways; The metastasis of cancer cells can be blocked by the ability of silver nanoparticles to inhibit blood vessels.

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Lactic acid detection and cholesterol detection. In this paper, a biosensor using colorimetric fluorescence method is introduced to detect two substances. The principle of the two substances is basically the same. For the detection of cholesterol, Li Yong nano silver position dual-function probe, which is not only used as the chemical reaction probe of intermediate hydrogen peroxide, but also as the signal probe to display the detection result, firstly, cholesterol oxidase is used to oxidize cholesterol to generate hydrogen dioxide. Then the generated hydrogen dioxide will oxidize silver nanoparticles into silver ions, silver ions due to different color changes to reflect the concentration of hydrogen dioxide and then confirm the concentration of cholesterol. This type of biosensor has high efficiency, specificity and other excellent performance characteristics. miRNA detection. Accumulated evidence shows that miRNA expression is closely related to many diseases, including cancer. Therefore, Early diagnosis of the disease can confirm the presence of mirnas due to their specificity. In this paper, the electrochemical transmitter of silver nanoparticles interacting with modified gold nanoparticles is proposed to detect miRNA. Gold nanoparticles with citric acid cap were first prepared. Gold nanoparticles were then modified with DNA (cDNA) of complementary target mirnas. Gold nanoparticles could be recognized by DSNS after binding to mirnas. After the gold nanoparticles were fixed on the electrode surface, AgNPs are blocked by negatively charged DNA strands due to electrostatic repulsion. After analyzing the peak of silver stripping, miRNA can be accurately detected. In this paper, the health problem of bacterial infection led to the synthesis of silver nanoparticles using PEG as stabilizer and chitosan graft polymerization as reducing agent. The nitrogen treated chitosan was combined with acrylamide monomer in an inert atmosphere. Then, after dissolution of the modified chitosan, polyethylene glycol was added to prepare CTS-G-PAAM /PEG/Ag Nps. After diverse representation, it was confirmed that it had excellent blood compatibility. In the antibacterial test, after different kinds of Gram-positive bacteria, negative bacteria treatment, found that the synthesis of nano-silver can quickly through the cell wall directly on bacteria, has a good antibacterial ability. In the cytotoxicity experiment, cervical cancer cells were selected as the experimental material, and half of the cells died during the experiment, which was considered as the cytotoxicity of synthesizing silver nanoparticles. Pancreatic cancer is one of the common malignant tumors in the digestive tract, and is known as the "king of cancer" in the tumor field. Successful treatment of pancreatic cancer is a challenge in oncology today. There is currently no definitive treatment for the complex, obscure causes of pancreatic cancer. Oxidation, nitro oxidation is considered to be one of the causes of the death of pancreatic ductal adenocarcinoma cells.The accumulation and change of reactive oxygen species (ROS) and reactive nitrogen species (RNS) induced by AgNPs were introduced. To evaluate the cytotoxicity of nitro oxidation and oxidative stress on human pancreatic cancer cells and pancreatic ductal adenocarcinoma cells, the levels of nitric oxide and nitrogen dioxide were measured at different concentrations, which increased with nitric oxide synthase. And the interference of antioxidant enzymes, glutathione peroxidase and so on was measured. And we've also shown features at the cellular level after cell damage. Finally, oxidation, nitro oxidation and AGNPS mediated destruction of human pancreatic ductal adenocarcinoma cells were determined.

2. Preparation methods of AG NPS

2.1. Physical methods

There are ball milling, crushing and grinding and other mechanical methods, arc discharge method, laser ablation method, steam condensation method, plasma method, soft landing method, etc. This kind of method can obtain pure nanoparticles without chemical additives, and the principle of physical method is relatively simple, which is mainly used for large-scale industrial preparation with low requirements on particle size and morphology. For example, Xu *et al.* used mechanical method to ball mill silver powder at -196℃ to prepare spherical AgNPs with small particle size [1]. The silver nanoparticles prepared by physical method have uniform distribution and high purity, but due to the lack of stabilizer, they will face difficulties, especially agglomeration.

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2.2. Chemical method

At present, chemical method is the most commonly used method for the preparation of nano-silver materials, which is more convenient to operate and easier to control the size and shape of particles, so it is the most widely used method. Its main principle is to produce silver atoms by chemical reduction, pyrolysis, photolysis, electrolysis and other methods of high-priced metal ions (such as chloroauric acid and silver nitrate), and then condense into silver nanoparticles. Reductants such as sodium borohydride and sodium citrate are commonly used to promote the chemical synthesis of silver nanoparticles [2,3] chemical methods can accelerate the preparation process of silver nanoparticles with the help of external energy also such as acoustic chemical methods. In the use of photoreduction method, chemical method is the most commonly used preparation method of nano-silver materials, it is through the chemical reaction to reduce Ag^{+}, so that it forms nano particles. Zou *et al.* [4] synthesized AgNWs with diameters ranging from 35 nm to 120 nm and lengths of 50mm by using photochemical reduction and seed-mediated methods. They found that the molar ratio of PVP/$AgNO_3$, the volume of silver particles and other factors had a great influence on the preparation of AgNWs.

3. AGNPS for biological detection

3.1. Detection of lactate

When carcinogenesis occurs, the production of lactic acid can prove the activity of glucose in cancer cells, and thus reflect the life activity of cancer cells. This phenomenon, known as the Warburg effect, can be observed even in the presence of oxygen in normally functioning mitochondria. In addition, The presence of lactic acid can therefore be tested to detect early cancers. For efficient, simple detection of lactic acid, hence the birth of lactic acid biosensors.Using silver nanoparticles with excellent electronic and optical properties, also can be oxidized by hydrogen peroxide, colorimetric biosensors are made by combining with natural enzymes. At the same time, carbon dot (CD), carbon nanotubes, graphene and other carbon-based materials have the advantages of chemical stability, biocompatibility, low toxicity and so on, thus a biosensor of carbon dots coated with silver nanoparticles has been proposed as the background. Loo et al. [5] synthesized a nanocomposite composed of Ag NPs and CDs (Ag-CDS) to detect lactic acid by using the advantages of the above two substances. Ag-cds was used to react with lactic acid by lactate oxidase (LOx) to generate hydrogen peroxide, which then decomposed Ag NPs to sense lactic acid. Figure 1(a) illustrates the whole process of the chain reaction between the silver nanoparticle coated carbon dot biosensor and lactic acid, in which hydrogen dioxide is used as an intermediate. Then the obtained Ag-CDS sensor is analyzed. As a colorimetric sensor, the Ag-CD sensor can measure lactate within a few minutes according to the color change on the strip paper precisely. And Figure 1(b) depicts the relationship between fluorescence intensity and lactate concentration.

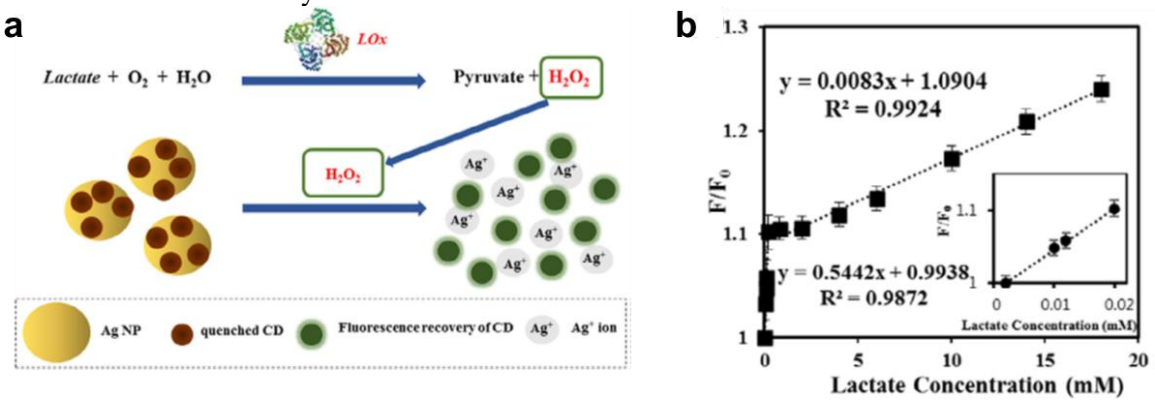

Figure 1. (a) Sensing process of lactic acid biosensor. (b) Relative fluorescence intensity of Ag containing LOx in the presence of lactic acid at different concentrations[5].

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3.2. Detection of cholesterol

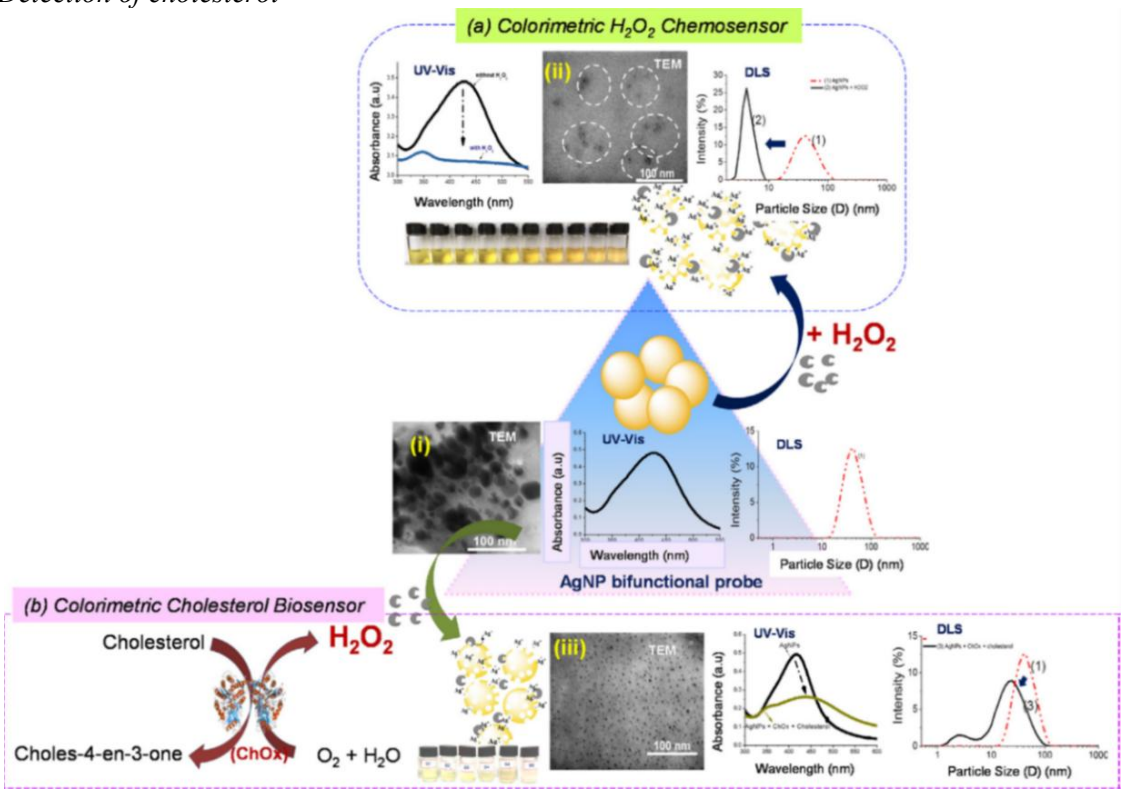

Figure 2. A probe of AgNPs used in sensing (a) hydrogen peroxide sensor and (b) cholesterol biosensor working principles. TEM images of (I, II, and III) AgNP solutions inserted[6].

Cholesterol plays an important raw for the synthesis of sex hormone, bile acid, vitamin D and other physiologically active substances. However, clogging and narrowing heart arteries, especially in men, also increases the risk of vascular-related strokes due to cholesterol abnormalities. Therefore, it is very important to determine the cholesterol content in the body in the right way for the early diagnosis of these diseases. Conventional biosensors for cholesterol determination use cholesterol oxidase enzyme to react with cholesterol to produce hydrogen dioxide. The cholesterol content was determined by electrochemical method through the reaction of Horseradish peroxidase (HRP) with hydrogen peroxide. Tran *et al.* [6] used silver nanoparticles to replace HRP, and used chemical capture probes identified by hydrogen peroxide and signal probes displaying detection results to determine cholesterol content. The working principle of this colorimetric sensor is based on the oxidation reduction reaction between silver ions and hydrogen dioxide. First, the device can break down the cholesterol by coming silver nanoparticles with cholesterol oxidase enzyme and the resulting hydrogen dioxide then reacts with silver nanoparticles, which act as a bifocal probe. Hydrogen dioxide oxidizes silver nanoparticles as follows: 2AgO+H2O2=2Ag++2OH-, the whole experimental reaction process is shown in Figure 2. The transformation of silver nanoparticles into silver ions producing color changes by color developing device (1-2). Besides, the discoloration is caused by the oxidation and etching of the silver by hydrogen peroxide, which changes its shape and size. So it is a chain reaction in the experiment. Hydrogen dioxide is used as an intermediate. And TEM imaging was used to determine the etching degree of silver nanoparticles by hydrogen dioxide. This experiment emphasizes colorimetry that is a method to determine the content of components to be measured by comparing or measuring the color depth of colored substance solution. This cholesterol biosensor is likely to be used clinically as a measure of cholesterol levels in the body for its high sensitivity and specificity.

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3.3. Detection of MIRNA

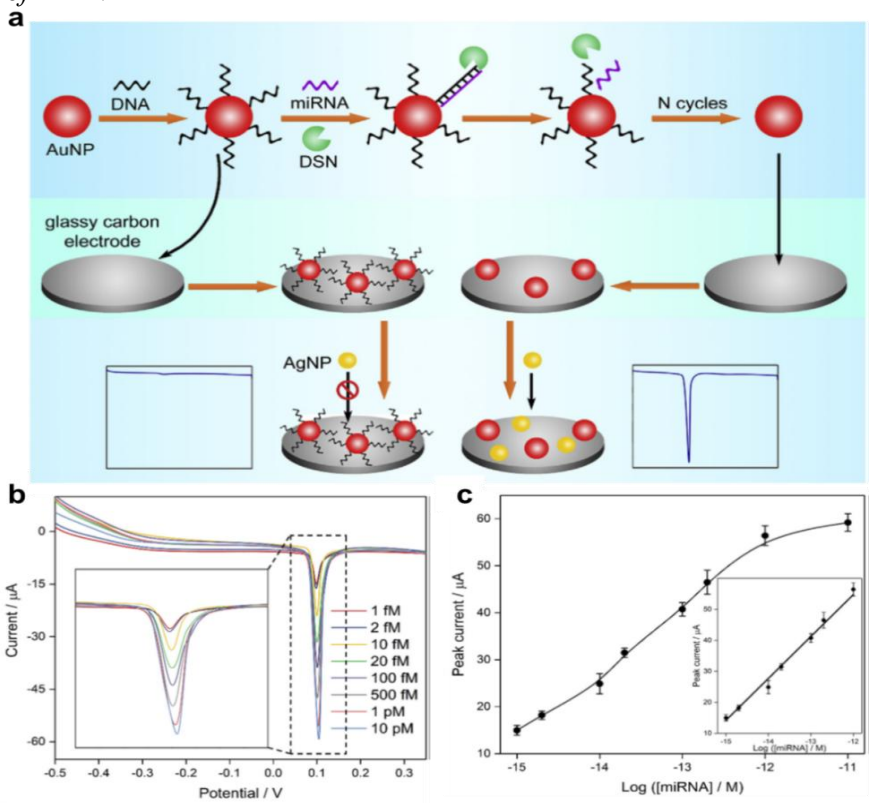

Figure 3. (a) Schemetic of biosensors. (b)Scanning voltammetry of miRNAs at different concentrations. (c) The miRNA concentration change corresponds to the LSV peak current[7].

MiRNAs are small non-coding Rnas used for gene regulation, and accumulated evidence suggests that miRNA expression is closely related to many diseases, including cancer. Therefore, Early diagnosis of the disease can confirm the presence of mirnas due to their specificity. Based on this Wang *et al*. [7] proposed an electrochemical biotransmitter based on a convenient interaction between DNA-modified gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs) to detect specific detection mirnas. AgNPs are widely used in the field of electrochemistry Firstly, AuNPs and AgNPs with citric acid cap were prepared. AuNPs further modify AuNPs by complementing DNA (cDNA) of target mirnas. DSNS can recognize DNA/RNA duplex forms due to the influence of miRNA. Many DNA strands in AuNPs can be removed by this enzyme. Figure 3(a) depicts the process in which modified gold nanoparticles can be recognized by DSNS after binding to miRNA, and in addition, AgNPs can be further loaded by placing them at an interval from AuNPs after being immobilized on the electrode surface. AgNPs are sequestered by negatively charged DNA due to electrostatic repulsion, in the case where gold nanoparticles are attached to the surface of the electrode. AgNPs cannot be located at the electrode interface, and the electrochemical response is turned off. Due to the specific binding of miRNA to DNA on the surface of AuNPs, AgNPs could not be repulsed by gold nanoparticles immobilized on the electrode surface in advance. In the quantification of miRNA, Figure 3(b) shows the corresponding LSV peak change when miRNA is quantified. And Figure 3(c) shows the change of peak current during miRNA quantification. Target miRNA levels can be determined by analyzing the increased electrochemical reactions. This method has high sensitivity and selectivity.

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4. AGNPS for treatment of disease

4.1. Treatment of cancer

Cervical cancer ranks third in the incidence of malignant tumors among women worldwide, second only to lung cancer and breast cancer, and more and more young women are suffering from cervical cancer. Tharani *et al.* [8] used different essays to evaluate the anticancer effect of chitosan coated silver nanoparticles on cervical cancer cell lines. For synthesis of CEAM-AgNp, the synthesis of silver nanoparticles (Ag+~Ag0) was adjusted with sodium hydroxide at pH 9.0. This indicates that the silver nanoparticles were reduced and silver ions were obtained after 24 hours of incubation. Silver nanoparticles were synthesized by coating with chitosan by ion gel method. After synthesis, the bioactivity is ensured in several ways so that it can be tested on cancer cells. To investigate the anticancer activity of CEAMAgNp through EMT pathway by various experimental methods. Most importantly, the observed results demonstrate that CEAMAgNp has very potent anticancer activity under in vitro conditions, sustained intercellular drug retention and enhanced anti-proliferation capacity (Figure 4).

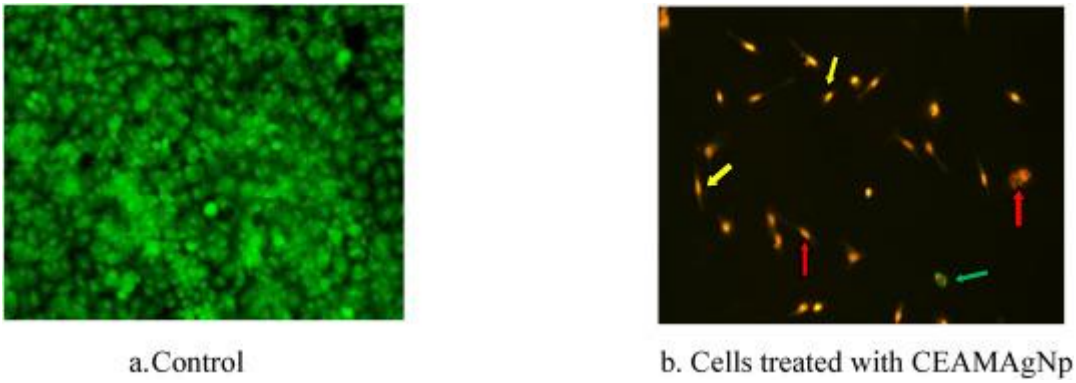

Figure 4. Different assay plots for apoptosis[8].

Pancreatic cancer is one of the common malignant tumors of digestive tract, and has been known as the "King of cancer" in the tumor field. The five-year survival rate after diagnosis is about 10%, making it one of the worst malignancies, according to the Lancet. Ductal adenocarcinoma of the pancreas is the main tumor type of pancreatic cancer, accounting for 80%~90% of pancreatic cancer should be ductal adenocarcinoma. There is currently no definitive treatment for the complex, obscure causes of pancreatic cancer.

Studies have shown that changes in oxidative stress may be a factor in cancer cell death. Oxidation, nitro oxidation is considered to be one of the causes of the death of pancreatic ductal adenocarcinoma cells.Ewelina et al. [9] found that AgNPs can induce the accumulation and change of reactive oxygen (ROS) and reactive nitrogen (RNS), and their changes are closely related to the death of human pancreatic ductal adenocarcinoma cells. They treated human pancreatic cancer cells and human pancreatic ductal cells with 2.6 or 18 nm AgNPs and then measured the production of intracellular ROS levels and nitric oxide levels by flow cytometry. The changes of mitochondrial membrane potential were analyzed by Muse 1.4 analysis software. After AgNPsc treatment, endogenous ROS levels increased in both types of cells (see Figure 5a). Especially at the concentrations of 25 and 50 μg/mL, the effect of 18 nm AgNPs on ROS production was four times that of the control value.

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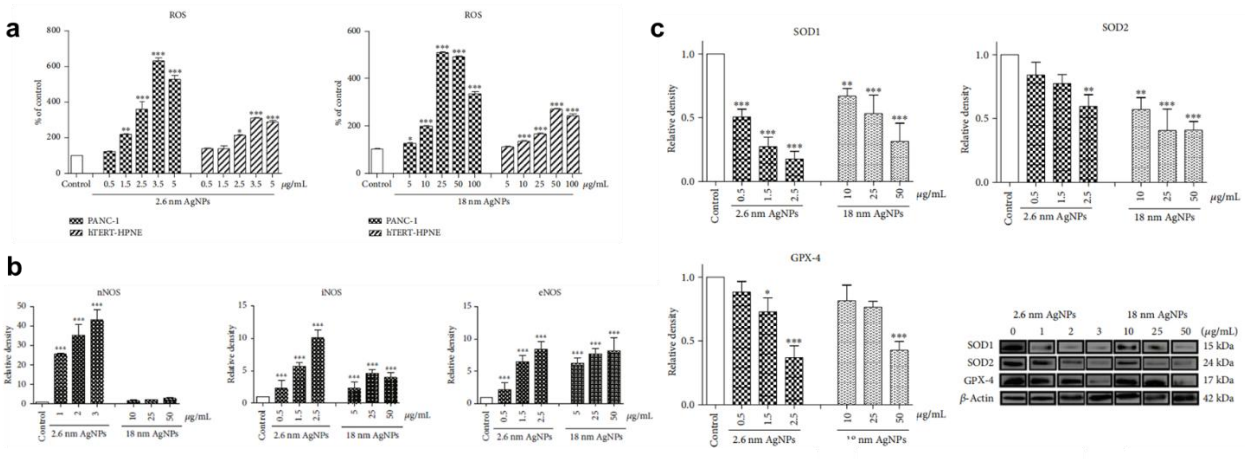

Figure 5. (a) Changes of ROS levels in PANC-1 and HTERt-HPNE cells. (b)Changes in NOS protein after AgNPs treatment. (c) Antioxidant enzyme protein content in PANC-1 cells[9].

After treatment, the concentration of NO was significantly increased. It was also noted that both 2.6nm AgNPs and 18 nm AgNPs produced growth in eNOS and iNOS levels (Figure 5(b)), and 2.6nm AgNPs also induced significant increases in nNOS protein levels. At the protein level, SOD and GPX-4 were significantly reduced in cytoplasm and mitochondria (Figure 5(c)). In fact, abnormal production or metabolism of NO increases the expression of iNOS, eNOS, etc., is strongly associated to the cell proliferation and abnormal expression of cancer cells.Moreover, we found that the reduction of SOD2 protein and mRNA levels was also caused by the same concentration of AgNPs. The manganese dismutase SOD2 is known to be a major antioxidant enzyme in the mitochondrial matrix. The interference of SOD1, SOD3 and other antioxidant systems was also detected. These results indicate that the hypothesis of pancreatic ductal adenocarcinoma cell death is strongly associated to oxidation, nitro oxidation.There is great potential for future clinical application.

4.2. Antibiotic therapy

Bacterial infections are one of the major health problems facing the human race. Visceral infection, drop in blood pressure, even shock could have caused by it.And it causes up to 700,000 deaths per year in recent years. However, due to the existence of dense biofilm of bacteria, the cells are isolated from the outside world and the invasion of bacteria by drugs is reduced. These factors make the treatment of bacterial infection often in trouble. Therefore, some biomaterials with antibacterial properties have come into people's view. Chitosan is considered to be an excellent biomaterial combined with nanomaterials due to its excellent biocompatibility, non-toxicity and degradability. In order to improve the antibacterial ability of chitosan, in addition to pH control, the binding of nanoparticles is considered. But nanoparticles are unstable in the binding process. Based on the above considerations, Banerjee et al. [10] designed a silver nanoparticle modified with polyethylene glycol as a stabilizer and acrylamide as a reducing agent to treat bacterial infections to a certain extent.

The chitosan treated with nitrogen was combined with acrylamide monomer in an inert atmosphere. Then, on the basis of the original addition of polyethylene glycol to participate in the binding of stability after It was characterized and analyzed to determine the different colors, to determine the degree of synthesis of the final product. This experiment also determined whether PEG was involved in the synthesis of AgNPs. In the antibacterial experiment of prepared silver nanoparticles, selecting three Gram-positive bacteria and three Gram-negative bacteria. Figure 6a shows that silver nanoparticles have antibacterial effect on the above bacteria. The cytotoxicity of polymerized silver nanoparticles was determined by using cervical cancer cells. Significant changes in the viability of cervical cancer cells were observed with different concentrations of polymerized Ag NPs (Figure 6b). The results showed that at the concentration of 40 g/mL, CTS-G-PAAM PEG AgNps caused the Hela

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cells to die completely. The CTS-G-PAAM PEG cap AgNps have potential applications in the medical field, such as antimicrobial and anticancer materials.

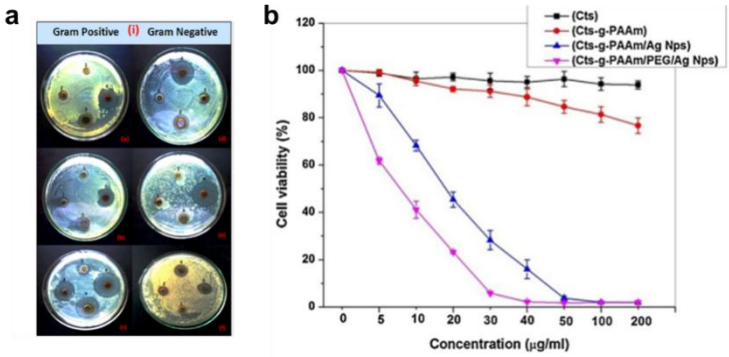

Figure 6. (a) Cultured status. (b) Effect of each comparison on cell viability[10].

5. Conclusion

By sorting out the applications of silver nanoparticles for substance detection and disease treatment, the wide application of silver nanoparticles in biomedicine was confirmed in this paper. A biosensor for the detection of cholesterol and lactic acid by colorimetric fluorescence method is introduced. The substance to be measured is oxidized by oxidase to produce intermediate hydrogen dioxide, which reacts with silver nanoparticles to produce silver ions. The concentration of the substance is determined by colorimetric device. By fixing the modified gold nanoparticles on the electrode surface, AgNPs cannot be attracted to negatively charged DNA due to electrostatic repulsion. After analyzing the peak of silver stripping, miRNA can be accurately detected. Furthermore, a chitosan coated silver nanoparticle was designed, and PEG was used as a stabilizer. Through different characterization methods, its excellent blood compatibility and antibacterial ability, and cytotoxicity to cervical cancer cells were confirmed. Finally, the accumulation and changes of reactive oxygen species (ROS) and reactive nitrogen (RNS) induced by silver nanoparticles were introduced, which could destroy cancer cells. Furthermore, the levels of nitric oxide and nitric oxide were increased at different concentrations after silver nanoparticles treatment. The destruction of pancreatic ductal adenocarcinoma cells by oxidation and nitro oxidation caused by silver nanoparticles was determined by different characterization methods. The above introduction, summarizes the nano silver antibacterial ability, excellent cell toxicity, resistant and do not wait for a characteristic, as well as in the nanometer material in the material field, authorities in the field of biomedical applications, landmark great contribution to the development of science and technology, believe that will be developed more silver nanoparticles in the field of biomedical applications.

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