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Short communication

In vitro study on the individual and synergistic cytotoxicity of adriamycin and selenium nanoparticles against Bel7402 cells with a quartz crystal microbalance

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ABSTRACT

Selenium nanoparticles (Se NPs) were prepared based on the reduction of selenious acid (H_2SeO_3), by employing sodium alginate (SA) as a template. The real-time monitoring of the drug-inducing apoptosis process of human hepatic cancer cells Bel7402 was performed with the quartz crystal microbalance (QCM) measurement. The anti-tumor effect of adriamycin (ADM) used in combination with Se NPs was investigated. It is found that both drugs were able to inhibit cell proliferation in a dose-dependent way and the combined treatment with ADM and Se NPs was more effective in inhibiting cell growth than each of the two drugs alone. The cytotoxic effects of drug combination were evaluated with the modified Bürgi formula (Jin equation) based the Δf_0 responses. The grades gradually changed from apparent synergism to simple addition with the drug-treatment time increasing but the drug combination with lower concentrations still exhibited synergism after 24 h, suggesting a potential application in cancer therapy.

1. Introduction

Chemotherapy is one of the widely used methods in cancer therapy. Adriamycin (ADM) is an effective clinical anti-tumor drug, whose interaction with DNA in cells can result in the dissociation of the DNA bi-helix and the ultimate apoptosis of tumor cells. It has been used in the treatment of a number of cancers for decades (Taatjes et al., 1996). But ADM may be harmful to some normal cells and its cardiotoxicity, a prominent side effect of ADM chemotherapy, has been well known (Kalyanaraman et al., 2002; Komagata and Sakai, 2003).

Selenium, as one of essential trace elements in the human body, plays an important role in nourishment and medicine (Xu and Huang, 1994). It has been found that selenium can prevent free radicals from damaging cells and tissues *in vivo* (WJO working group, 1987) and selenium supplementation can reduce the cancer mortality and incidence (Combs et al., 1997). Recently, selenium nanoparticles (Se NPs) are attracting more and more attention due to some unique electrical, optical, mechanical, chemical and biologic properties. Se NPs have high biological activities and low toxicity (Zhang et al., 2001) and many methods have been exploited for their synthesis (Smith and Cheatham, 1980; Mees et al., 1995; Gates et al., 2000; Abdelouas et al., 2000; Nandhakumar et al., 2001; Gao et al., 2002; Zhang et al., 2004; Xia, 2007).

Tumor cells growing in the presence of a single anti-cancer drug may become resistant to a wide range of structural dissimilar drugs, which is known as multidrug resistance (MDR). The resistance development of tumor cells to chemotherapeutic drugs is a major obstacle in the treatment of human cancer (Muller et al., 1994). Many doctors chose drug combination in actual therapies in order to intensify therapeutic effect, avoid MDR phenomenon and reduce drug toxicity. A great deal of reports on synergistic effect induced by drug combination has been presented (Toma et al., 1997; Naruse et al., 2007; Wan et al., 2008). Some optical methods including microscopy observation, methyl thiazolyl tetrazolium (MTT) colorimetry and flow cytometry (FCM) are usually used in the *in vitro* investigation on the cytotoxicity of drug. Undoubtedly the accuracy of assays using the existing methods is satisfactory. However, some drawbacks in analysis, e.g., multistep operation and inapplicability for real-time or continuous monitoring, exist at the same time.

Since its liquid-purpose application in the very beginning of 1980s, the quartz crystal microbalance (QCM) has been widely applied in chemical and biological fields. It not only can provide information about mass loading but also can reveal the physicochemical properties including elastic moduli, density and viscosity near the electrode. The investigated objects in biological researches

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included proteins, enzymes, antibody/antigen, nucleic acids, bacteria and cells (Janshoff et al., 2000; Marx, 2003). Due to its satisfactory performance, e.g., high sensitivity, facile operation and dynamic monitoring, QCM has been successfully employed to monitor living cell attachment and incubation (Redepenning et al., 1993; Wegener et al., 2000; Lord et al., 2008).

It is expected that the cytotoxic effect of a combined application of ADM and Se NPs on tumor cells should be different with that of an individual drug. To the best of our knowledge, to date there are no reports on application of QCM for study on the synergistic effect of drug combination. In this study, Se NPs were synthesized in an aqueous solution by using sodium alginate as a soft template. The anti-tumor effects of ADM and Se NPs on hepatic cancer cells Bel7402 were investigated with the QCM measurement, respectively. The research on the synergistic cytotoxicity of the drug combination was performed.

2. Materials and methods

2.1. Chemicals and instruments

Human hepatic cancer cells Bel7402 was obtained from XiangYa Central Laboratory of Central South University, China. A pH 7.4 phosphate buffer solution (PBS, 1.56 g L^{-1} $Na_2HPO_4{\cdot}H_2O$ + 0.20 g L^{-1} KH_2PO_4 + 8.00 g L^{-1} NaCl + 0.20 g L^{-1} KCl) and a growth medium, RPMI1640 with 10% fetal bovine serum (CS), were used for cell culture. Doxorubicin hydrochloride was purchased from Shenzhen Main Luck Pharmaceuticals Inc., China. Other chemicals were of analytical reagent grade.

The QCM sensor consisted of a thin AT-cut quartz crystal wafer with one gold electrode (6-mm diameter) on each side. The 9-MHz crystal with Au electrodes was mounted between two biocompatible silicon O-rings to allow only one side of the electrode to be exposed to the liquid. The reaction chamber above the crystal was held with a 1 mL chlorinated polyethylene centrifugal tube. The device, being covered, was placed in a humidified CO_2 incubator controlled at 5% CO_2 and 37 °C to prevent evaporation from the culture holder. The quartz crystal electrodes were wire-extended to a research QCM (Maxtek Inc., USA) to achieve simultaneous recording of f_0 and R_1. The cell-modality observation was performed with an inverted optical microscope (OLYMPUS CKX41, Japan). The ultrasonic treatment in the nanoparticle-synthesis process was achieved by using an ultrasonicator (KQ3200, 120 W, 40 kHz, Kunshan China). The sizes of the Se nanoparticles were characterized ex situ by transmission electron microscope (TEM, JEOL-1230, Japan).

2.2. Preparation of Se NPs

After the appropriate amounts of sodium alginate (SA) were mixed with selenious acid solution, the ascorbic acid solution was added into the mixtures to initiate the reaction. In reaction solution, the concentrations of SA, selenious acid and ascorbic acid were 0.2%, 1×10^{-3} mol L^{-1} and 4×10^{-3} mol L^{-1}, respectively. The reaction lasted 2 h via ultrasonic treatment. Then the Se particles were centrifuged, followed by washing with water several times to remove any surplus reactants or any physically adsorbed SA molecules from the surface of the particles. Finally, the required red Se particles were obtained. These particles were suspended in PBS (pH 7.4) by ultrasonication for the following experiments.

2.3. Cell culture and measurement procedures

After being sterilized with 75% ethanol under UV light for 0.5 h, the QCM culture-chamber was washed thrice with pH 7.4 phosphate buffer solution. Then 700 μL of the growth-medium was added and the entire culture-chamber was put into the incubator. The density of resuspended cells after trypsinization was determined with a hematocytometer. Some cells were transferred to a centrifugal tube followed by dilution with growth medium until 1 mL of a suspension containing approximately 4×10^5 cells was obtained. When the QCM readout became steady, 100 μL of the cell-suspending solution was added evenly onto QCM Au electrode. The Δf_0 and ΔR_1 responses were simultaneously monitored up to two days. The drug was introduced at 24 h when the cells were in their growth phases. The crystal regeneration was achieved by dealing with trypsin for 24 h, followed by washing with chromic-sulphuric acid and water in sequence for several times. After cleaning in this way, the QCM electrode could be used repeatedly with good recovery of its initial f_0 and R_1 values.

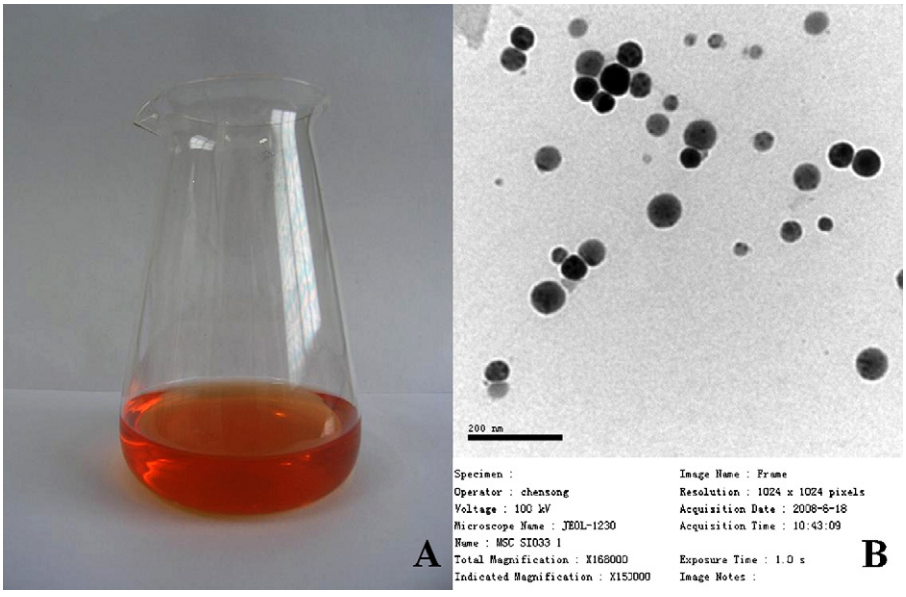

Fig. 1. (A) Photograph of the Se NPs solution obtained via 2 h ultrasonic treatment. (B) TEM images of Se NPs. The bar represents 200 nm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)