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In-situ monitoring of breast cancer cell (MCF-7) growth and quantification of the cytotoxicity of anticancer drugs fluorouracil and cisplatin

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ABSTRACT

A wireless sensing device was developed for the *in-situ* monitoring of the growth of human breast cancer cells (MCF-7) and evaluation of the cytotoxicity of the anticancer drugs fluorouracil and cisplatin. The sensor is fabricated by coating a magnetoelastic ribbon-like sensor with a layer of polyurethane that protects the iron-rich sensor from oxidation and provides a cell-compatible surface. In response to a time-varying magnetic field, the magnetoelastic sensor longitudinally vibrates, emitting magnetic flux that can be remotely detected by a pick-up coil. No physical connections between the sensor and the detection system are required. The wireless property facilitates aseptic biological operation, especially in cell culture as illustrated in this work. The adhesion of cells on the sensor surface results in a decrease in the resonance amplitude, which is proportional to the cell concentration. A linear response was obtained in cell concentrations of 5×10^4 to 1×10^6 cells ml^{-1}, with a detection limit of 1.2×10^4 cells ml^{-1}. The adhesion strength of cells on the sensor is qualitatively evaluated by increasing the amplitude of the magnetic excitation field. And the cytotoxicity of the anticancer drugs fluorouracil and cisplatin is evaluated by the magnetoelastic biosensor. The cytostatic curve is related with the quantity of cytostatic drug. The lethal concentration (LC50) for cells incubated in the presence of drugs for 20 h is calculated to be 19.9 μM for fluorouracil and 13.1 μM for cisplatin.

1. Introduction

The degree of cell proliferation is a valuable parameter for estimation of the growth rate, metabolic state, and cytotoxicity effects of chemicals on cells (Andreescu et al., 2004). Many methods have been used to monitor cell viability and proliferation, such as fluorescent microscopy (Krtolica et al., 2002), flow cytometry (Cánovas et al., 2007), and quartz crystal microbalance (QCM) (Marx et al., 2003, 2007; Zhou et al., 2000; Fredriksson et al., 1998; Gryte et al., 1993). These techniques are valuable for the evaluation of chemical cytotoxicity effects and the assessment of environmental threats. Although each method has its own advantages, the described techniques require either electrical connections or line-of-sight telemetry. Furthermore, many of the techniques are time-consuming, and several of them require relatively expensive equipment.

Magnetoelastic sensor techniques have the unique characteristics of being able to wirelessly detect resonant frequency changes of a magnetoelastic foil in response to differences in physical parameters including stress, pressure, temperature, flow velocity, liquid viscosity, magnetic field, and mass loading (Grimes et al., 2002). When exposed to a time-varying AC magnetic field, magnetoelastic sensors oscillate at a fundamental frequency that is dependent on the chemical composition and the physical dimensions of the strip. A DC field is also used to offset the magnetic anisotropy of the strip by aligning the magnetic domains of the film to maximize its vibrational amplitude. The oscillation of the sensor creates a magnetic flux, which can then be detected remotely by a pick up coil. The signal can also be detected acoustically by the use of a microphone or by the modulation of backscattered light from a laser beam (Jain and Grimes, 2001). Magnetoelastic sensors behave similarly to surface acoustic wave (SAW) devices in that they respond to changes in physical parameters (Grimes et al., 2002). The detailed measurement principle has been described previously (Zeng et al., 2002, 2005, 2006). In recent years, this kind of biosensors has been developed for the determination of biological substances such as

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glucose (Cai et al., 2004), bacteria (Ruan et al., 2003; Pang et al., 2007), trypsin (Wu et al., 2006) and protein (Ruan et al., 2004). Magnetoelastic sensors have also been applied in blood coagulation monitoring (Puckett et al., 2003) and antigen immunoassay (Guntupalli et al., 2006).

In this work, for the first time, a wireless magnetoelastic sensor was developed for the *in situ* monitoring of cancer cell (MCF-7) growth. Our sensor is comprised of a ribbon-like magnetoelastic free-standing thick film interfaced with a layer of chemical or biochemical sensing film (Cai and Grimes, 2001). A magnetic field impulse is used to impart energy into the ribbon-like magnetoelastic sensor, causing it to vibrate at a characteristic resonance frequency that shifts linearly in response to small mass loading or viscosity change. No physical connections between the sensor and the detection system are required for signal telemetry, nor does the sensor require any internal power source such as a battery or fuel cell. These properties make the sensor ideally suited for *in situ* and *in vivo* bioassays, particularly those analyses requiring aseptic operation. The adhesion of cells on the sensor surface resulted in changes in both the resonance frequency and amplitude. The growth curves clearly demonstrate cell growth and death, enabling rapid quantification of the cytotoxicity of chemicals, in this work fluorouracil and cisplatin, on the cells.

2. Materials and methods

2.1. Materials

Human breast cancer cells (MCF-7) were obtained from the Cell Bank of Xiangya Central Experiment Laboratory of Central South University (Changsha, China). Fluorouracil and cisplatin were purchased from the Hunan Provincial Institute for Drug Control (Changsha, China). Phosphate-buffered saline (PBS) solution (pH 7.4, 136.7 mM NaCl + 2.7 mM KCl + 8.1 mM Na_2HPO_4 + 1.5 mM KH_2PO_4) was used. Fluorouracil and cisplatin stock solutions were prepared with ultrapure water and sterilized using 0.22-μm filters (Whatman) and stored at 4 °C. Prior to use, the stock drug solutions were diluted by PBS to desired concentrations. All other chemicals were of reagent grade.

A magnetoelastic ribbon of Metglas alloy 2826MB, of composition $Fe_{40}Ni_{40}P_{14}B_6$ was donated for the investigators use by Honeywell Corporation (Morristown, NJ). The sensor was cut from the continuous ribbon in 13 mm length. The resonance frequency of an uncoated sensor in air was approximately 170 kHz. The magnetoelastic sensors were ultrasonically cleaned in Micro-Cleaning solution, followed by a water and acetone rinse, and then dried in a stream of nitrogen. On both sides of the sensor, a layer of Bayhydrol 110 (Bayer Corporation, Pittsburgh, PA) was applied by dip coating. The polyurethane-coated sensors were dried in air and then annealed at 150 °C for 2 h to form a robust membrane that protected the iron-rich sensor from oxidation and offered a cell-compatible surface.

2.2. Cell culture and detection

MCF-7 was routinely cultured in 25-cm^2 cell culture flasks at 37 °C in 5% CO_2 in RPMI-1640 medium (Gibco Corporation, New York) in which were added 10% newborn calf serum, 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin prior to use. After the cancer cells were cultured for 2–3 days, they were harvested through trypsinization with 0.25% trypsin–0.02% EDTA. Upon proper dilution with fresh culture medium, a cell suspension was obtained. The density of cells was determined with a hematocytometer (Shanghai, China).

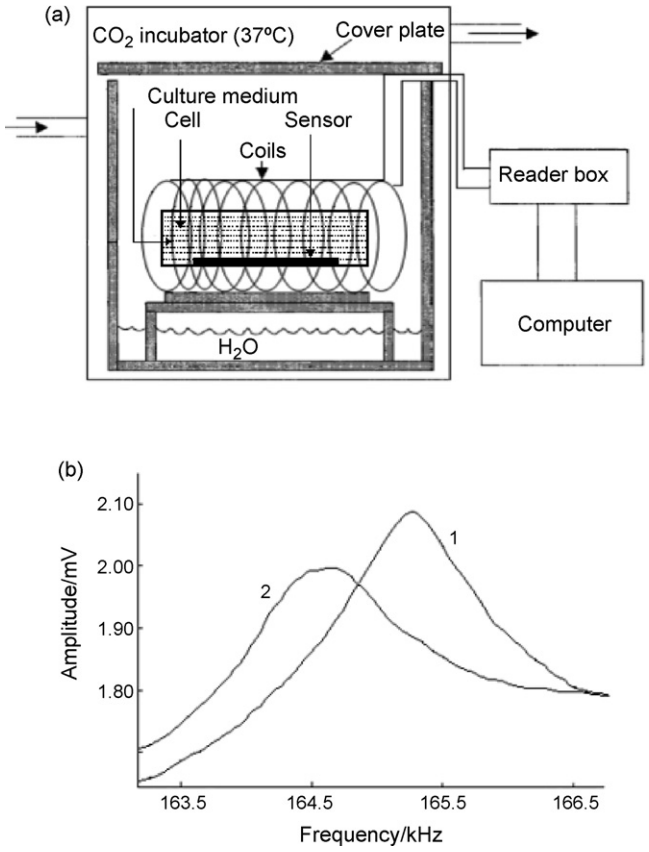

Fig. 1. (a) Schematically showing the test setup. The magnetoelastic sensor is placed in a vial. The tube-like vial is then inserted into a small coil which was connected to a 'sensor-reader' box. (b) The measured amplitude spectrum at the beginning (1) and end (2) of an experiment lasted for 24 h with an inoculation density of 1.0×10^5 cells ml^{-1}.

Bayhydrol 110-coated magnetoelastic sensors were sterilized at 160 °C for 120 min prior to use. The polyurethane-coated sensor was placed in a small vial containing 1 ml culture medium. The tube-like vial was then inserted into a small coil which was connected to a 'sensor-reader' box, see Fig. 1(a), used for signal telemetry. Fig. 1(b) shows the response profiles measured at the beginning and end of an experiment with an inoculation density of 1.0×10^5 cells ml^{-1}, which lasted for 24 h. After the incubation, both the resonance frequency and the resonance amplitude decrease. A microprocessor-based frequency counting technique was used to detect the resonance frequency and amplitude of the magnetoelastic sensors (Zeng et al., 2002).

The cell staining was performed with Ho.33342 (Sigma) and propidium iodide (Sigma), and the fluorescence microscopy images were taken with a fluorescence microscope (DMI4000B, Leica, Germany). The morphologies of cells during the culture course on polyurethane-coated glass surfaces were also observed using the microscope.

3. Results and discussion

3.1. Sensor responses and cell growth curves

Fig. 2(a) shows the MCF-7 cell growth traces. The sensor was firstly incubated in a blank culture medium without adding cells in a CO_2 incubator (37 °C, 5% CO_2) for several hours to detect any possible nonspecific adsorptions, which were not observed. A certain amount of cells were then added (indicated by an arrow) in the

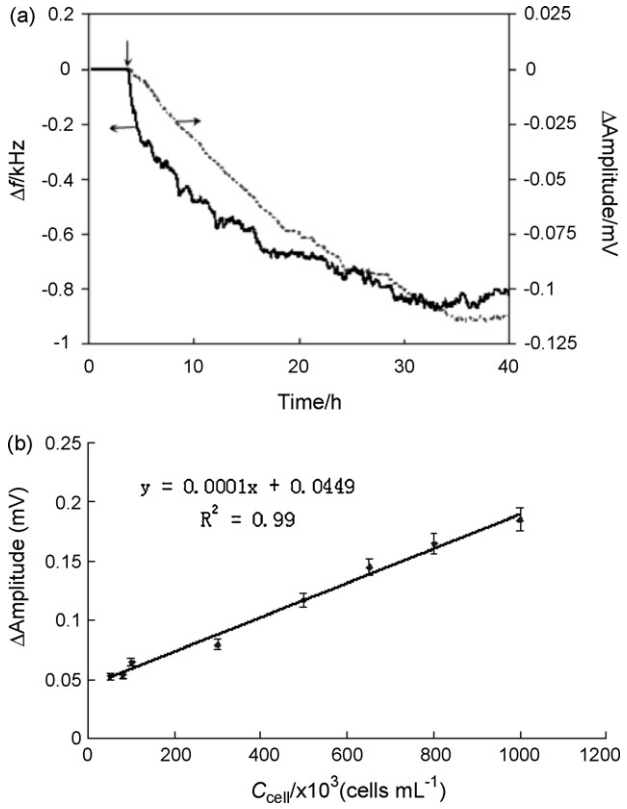

Fig. 2. (a) Time-dependent cell adhesion monitored by magnetoelastic sensor: changes of resonance frequency (solid line) and resonance amplitude (dotted line). The vertical arrowhead indicates the addition of MCF-7 cells with a cell density of 10^5 cells ml^{-1}. (b) The calibration curve of the resonance amplitude response recorded at 15 h after cell inoculation vs. cell density ranged from 5.0×10^4 to 1.0×10^6 cells ml^{-1} (data presented are mean ± S.D., $n = 3$).

medium and the resonance frequency and amplitude were measured. During the cell culture, both the resonance frequency and resonance amplitude gradually decreased in the first 30 h, and then increased slightly as shown in Fig. 2(a). The decreases in frequency and amplitude were due to the adhesion of cells on the sensor surface. And the slight increase in resonance frequency and amplitude after 1 day's incubation appears due to the detachment of dead cells. To confirm this hypothesis, after being cultured for 1, 2 and 3 days, respectively, the cells adhered to the sensor surface were stained with Ho.33342 and propidium iodide, and the corresponding fluorescence microscopy images are shown in Fig. 3. It can be observed that MCF-7 cells adhered and proliferated on the sensor surface. The increasing number of red-colored cells with the incubation time indicates that the viability of cells decreased gradually, which was caused by the depletion of nutrition in the small tube-like glass vial. So, the slight increase in resonance frequency and amplitude after 35 h incubation shown in Fig. 2(a) is due to the loose attachment of cells on the sensor surface.

The adhesion strength of the cells was qualitatively evaluated by applying different excitation field amplitudes, controlled through the drive voltage, to the sensor to try and shake off the cells. The measurement electronics can continuously tune the excitation voltage from 0.5 to 3.5 V (Zeng et al., 2005), passed through a 2 Ω solenoid comprised of 200 turns over 2 cm, with an 8 mm diameter. A high excitation field amplitude can shake off loosely attached cells. After the cells being cultured for at least 24 h, an excitation voltage of 1.2–2.7 V, increased step-wise, was applied to the sensor (vertical orientation) for 10 min, and then the sensor was measured at a (standard) excitation voltage of 0.66 V. The results showed that after each 'shaking' of the sensor with high amplitude fields the sensor returned to its original resonance frequency and amplitude, indicating that a tightly adhered cell film was formed.

The adhesion of cells results in more sensitive responses in amplitude with a higher signal-to-noise ratio than frequency. Fig. 1(b) plots the measured amplitude at the beginning and end of an experiment with an inoculation density of 1.0×10^5 cells ml^{-1}. The resonance frequency decreases from 165.2 kHz to 164.6 kHz, a decrease of 0.36%; the maximum amplitude decreases from 2.099512 mV to 2.028225 mV during the experiment, a 3.40% change. Specifically, the sensor's response is expressed as the maximum amplitude change, obtained by dividing the maximum amplitude (amplitude at resonance frequency) by the initial maximum amplitude (resonance amplitude at the beginning of the experiment). It is expected that a higher frequency sensitivity can be achieved by improving the surface affinity to cells. In this work

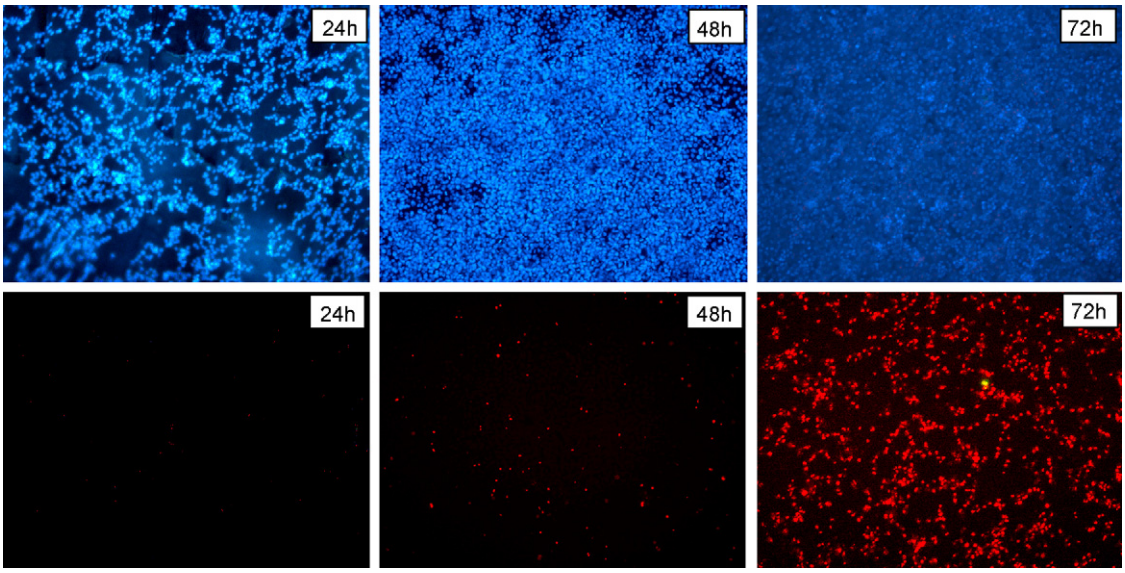

Fig. 3. Fluorescence microscopy images of Ho.33342 and propidium iodide-stained MCF-7 cells cultured on the magnetoelastic sensor recorded under ultraviolet (top) and green light irradiation (bottom), respectively.

the resonance amplitude was therefore used to characterize the cell proliferation.

MCF-7 cells with various concentrations were inoculated and the resonance amplitude was recorded as a function of time (every determination interval is 60 s). Fig. 2(b) shows the calibration curve of the resonance amplitude response recorded at 15 h after cell inoculation vs. initial cell density. From Fig. 2(b), it can be found that the resonance amplitude response was linearly related to the initial density of MCF-7 cells in the range of 5×10^4 to 1×10^6 cells ml^{-1}, with a detection limit of 1.2×10^4 cells ml^{-1}.

3.2. Evaluation of anti-cancer drug cytotoxicity

Two anti-cancer drugs, fluorouracil and cisplatin, were chosen as the model chemicals to test the ability of the sensor system for evaluation of cytotoxicity. These two chemicals have different inhibition mechanisms (Fujimoto, 2006). Fluorouracil was selected as the representative of cell cycle-specific antineoplastic drugs, and cisplatin was selected as the representative of cell cycle non-specific antineoplastic drugs. Fig. 4(a) shows the typical time course of the resonance amplitude responses of the sensor incubated in the presence of different concentrations of fluorouracil. The response profiles of the sensor in the presence of cisplatin are shown in Fig. 4(b). The addition of the anti-cancer drugs obviously inhibited the cell proliferation as shown in Fig. 4. Each experiment was repeated for three times to test the reproducibility of the inhibition of the anti-cancer drugs. The resonance amplitude responses of the sensors recorded at 15 h versus the concentration of drugs are shown in the insets of Fig. 4. The small error bars indicate the satisfactory reproducibility of the sensor.

Table 1 lists the inhibition efficiency F_i which is defined as

$$F_i\ (\%) = \frac{\Delta A_0 - \Delta A}{\Delta A_0} \times 100$$

where ΔA_0 and ΔA are the shifts in the resonance amplitude of the magnetoelastic sensors without and with addition of drugs, respectively.

Addition of 10.0 μM of fluorouracil or cisplatin inhibited the cell proliferation by ~30%, and 30 μM of fluorouracil could kill 60% the cells after 16 h, while 20 μM of cisplatin could kill 67% of the cells during the same period. The increase of the concentration of fluorouracil from 30 μM to 50 μM did not result in significantly increased efficiency, showing that the dosage of 30 μM appears to reach the maximum effect for MCF-7, and overdose cannot improve the curative effect. The inhibition mechanisms of these two chemicals, however, were different as shown in the response profiles. Fluorouracil worked on the cells instantly, while cisplatin worked on the cells slowly. The short-term inhibition efficiency of fluorouracil was higher than cisplatin, but the long-term inhibition efficiency was the same. Moreover, lethal concentration (LC50, the concentration of a drug that reaches 50% inhibition efficiency) for cells incubated in the presence of drugs for 20 h was calculated to be 19.9 μM for fluorouracil and 13.1 μM for cisplatin.

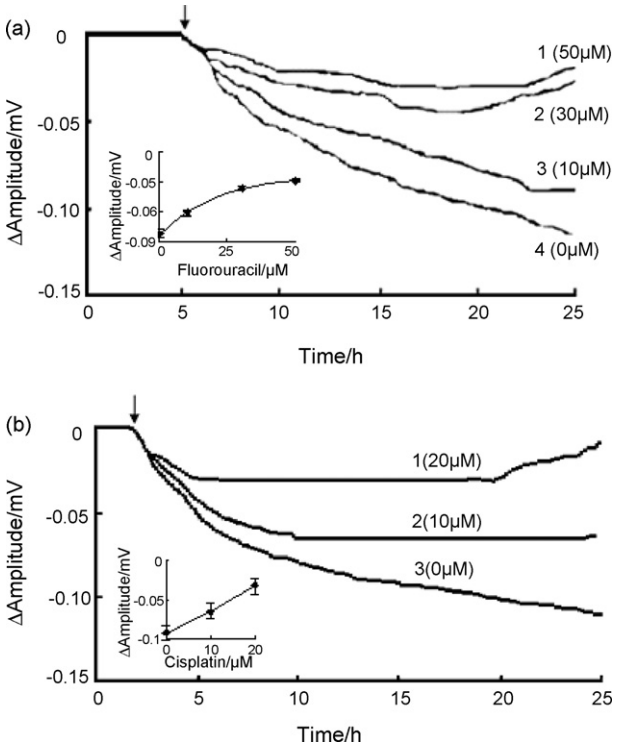

Fig. 4. Time courses of the resonance amplitude responses of the magnetoelastic sensors incubated in the presence of (a) fluorouracil and (b) cisplatin with different concentrations. MCF-7 cells with a cell density of 1.0×10^5 cells ml^{-1} were added at arrowhead, and the drugs were added at the same time. The insets show the resonance amplitude responses of the sensors recorded at 15 h vs. the concentration of drugs (data presented are mean ± S.D., $n = 3$).

An inverted phase contrast microscope was used to confirm the results. Fig. 5 shows the microscopy images of the cells cultured on the polyurethane film-coated glass surface during the culture course in the presence of 30.0 μM fluorouracil and 20.0 μM cisplatin, respectively. The results of the control experiment without addition of drugs are also shown in Fig. 5. In the absence of drugs, MCF-7 cells proliferate well on the polyurethane film since the cells number is visibly increasing and the morphology of cells is normal, indicating that polyurethane is a cell-compatible material of little cytotoxicity. The proliferation of cells was obviously inhibited in the presence of the drugs as compared with the results of control experiment. The cells gradually died and detached from the

Table 1
Inhibition efficiency[a] of anti-tumor drugs on MCF-7 (%)

Concentration (μM)	Time after drug addition (h)									
	2	4	6	8	10	12	14	16	18	20
Fluorouracil										
10.0	26.4	26.5	26.7	27.1	27.7	28.3	28.9	29.6	30.4	31.3
30.0	47.4	48.5	50.6	54.8	55.4	56.2	58.3	59.5	67.3	74.8
50.0	60.3	64.9	65.1	66.9	67.6	67.9	68.0	71.6	75.2	83.9
Cisplatin										
10.0	18.2	18.4	18.8	18.9	23.6	27.7	30.4	32.0	34.5	36.5
20.0	32.9	47.5	55.1	59.5	62.0	63.5	64.7	66.8	68.9	80.2

where ΔA_0 and ΔA are the shifts in the resonance amplitude of the magnetoelastic sensors without and with the addition of drugs, respectively.

[a] The inhibition efficiency is defined as $F_i\ (\%) = \frac{\Delta A_0 - \Delta A}{\Delta A_0} \times 100$.

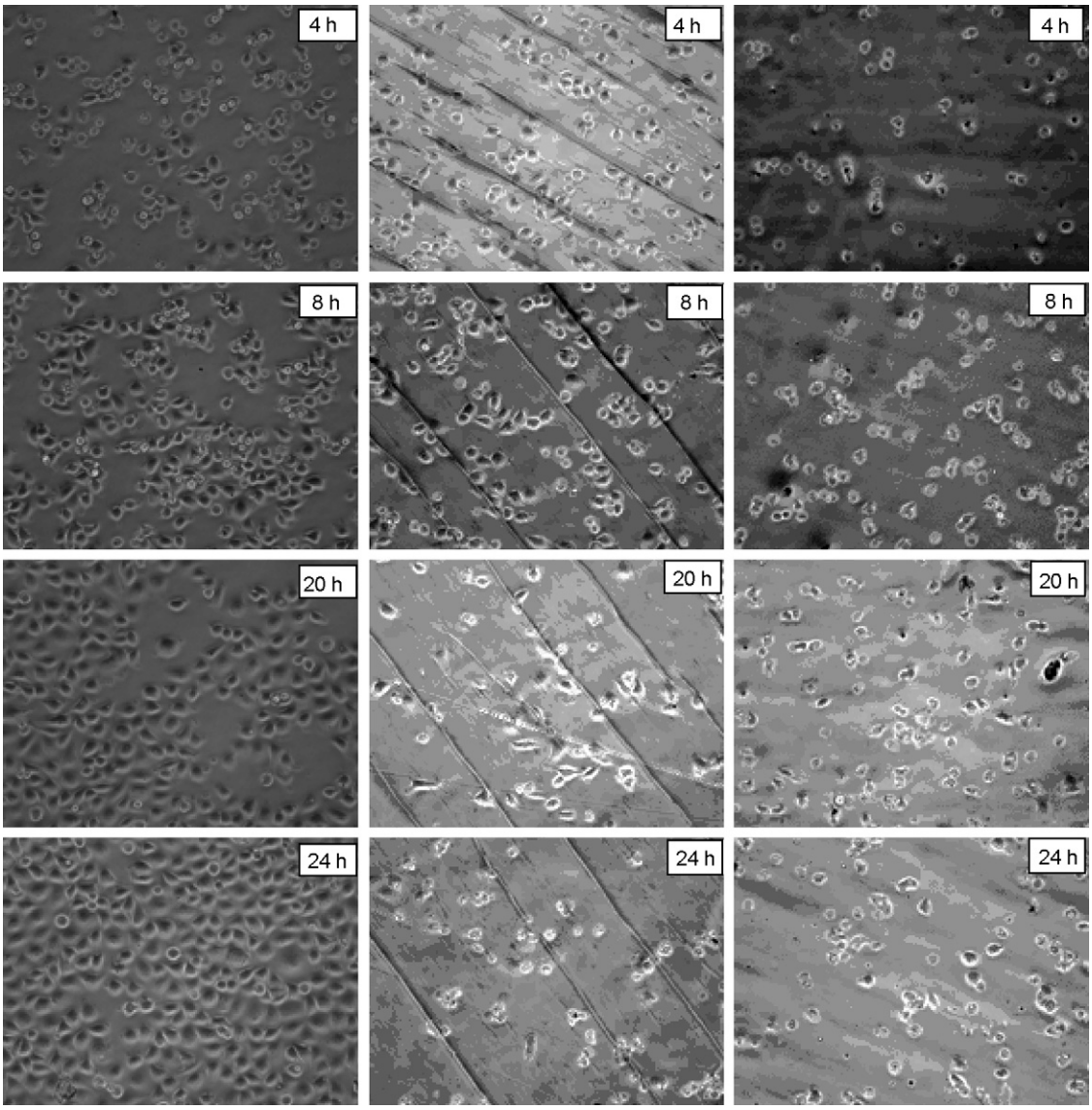

Fig. 5. Inverted phase contrast microscopy images of cells cultured on polyurethane film-coated glass during the culture course in the absence of drugs (left column), and in the presence of 30.0 μM fluorouracil (middle column), or 20.0 μM cisplatin (right column). Cells were inoculated at a density of 1.0×10^5 cells ml^{-1}, and fluorouracil or cisplatin was added at the same time.

substrate with time. The results given by microscopy images are consistent with those obtained by the sensor, indicating that the proposed sensor can be applied for the evaluation of the cytotoxicity of anti-cancer drugs.

4. Conclusions

In this work, a wireless magnetoelastic sensing device has been developed for the *in-situ* monitoring of MCF-7 cancer cell growth, and it has been successfully used for the evaluation of the cytotoxicity of anticancer drugs using fluorouracil and cisplatin as models. The proposed magnetoelastic sensing device can be readily and cost effectively used for rapid detection of MCF-7 cells, with a detection limit of 1.2×10^4 cells ml^{-1}, and continuous and real-time monitoring of the effects of chemicals on cells evaluating the utility of different medicines. No direct physical connections between the sensor and the detection system are required for signal telemetry, which greatly facilitates aseptic biological operation.

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