Quantification of cell viability and rapid screening anti-cancer drug utilizing nanomechanical fluctuation

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**A B S T R A C T**

Cancer is a serious threat to human health. Although numerous anti-cancer drugs are available clinically, many have shown toxic side effects due to poor tumor-selectivity, and reduced effectiveness due to cancers rapid development of resistance to treatment. The development of new highly efficient and practical methods to quantify cell viability and its change under drug treatment is thus of significant importance in both understanding of anti-cancer mechanism and anti-cancer drug screening. Here, we present an approach of utilizing a nanomechanical fluctuation based highly sensitive microcantilever sensor, which is capable of characterizing the viability of cells and quantitatively screening (within tens of minutes) their responses to a drug with the obvious advantages of a rapid, label-free, quantitative, noninvasive, real-time and in-situ assay. The microcantilever sensor operated in fluctuation mode was used in evaluating the paclitaxel effectiveness on breast cancer cell line MCF-7. This study demonstrated that the nanomechanical fluctuations of the microcantilever sensor are sensitive enough to detect the dynamic variation in cellular force which is provided by the cytoskeleton, using cell metabolism as its energy source, and the dynamic instability of microtubules plays an important role in the generation of the force. We propose that cell viability consists of two parts: biological viability and mechanical viability. Our experimental results suggest that paclitaxel has little effect on biological viability, but has a significant effect on mechanical viability. This new method provides a new concept and strategy for the evaluation of cell viability and the screening of anti-cancer drugs.

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1. Introduction

Chemotherapy is a common treatment method in cancer therapy. However, anti-cancer drugs used in clinical treatment have poor selectivity, toxic side effects, and often result in resistance. Therefore, the advancement in identifying new drugs with high selectivity and less negative side effects has become a significant and urgent issue which needs to be resolved. A large number of plant extracts and compounds are identified every year, thus the development of a new highly efficient and practical method to screen the pharmacological effects of these potential anti-cancer drugs is of major clinical importance.

Cell viability assays have been gaining attention due to their strategic importance in both fundamental science and clinical medicine. Various techniques, such as flow cytometry (FCM), direct immunofluorescence staining, clonogenic assays, colorimetry and microscopic imaging have been used to investigate cell viability and its change under drug treatment (Banasiak et al., 1999; Ilkow et al., 2015; Park et al., 2015; Zhang et al., 2015). However, most of the current approaches are either relatively expensive or unable to quantifiably evaluate cell viability and are only able to distinguish between live and dead cells. In addition, some of these methods require chemiluminescent, fluorescent, or radioactive labeling steps which often involve destruction of the physiological...
function of cells and the loss of certain important biological information on viable cells. Thus, label-free, real-time, noninvasive, inexpensive and fast methods to quantify cell viability and its change under drug treatment are essential in cancer management, especially in anti-cancer drug discovery and screening.

Several label-free technologies, including cell–substrate impedance (Giaever and Keese, 1984, 1991; Pradhan et al., 2014), optical waveguide lightmode spectroscopy (Li et al., 1994; Ramsden et al., 1995; Fang, 2007; Ramsden and Horvath, 2009), and quartz microbalance (Zhou et al., 2000; Marx et al., 2005), have been reported as a means for monitoring live cell status in a real-time and non-invasive manner. Among the emerging technologies, microcantilever sensing, an ultrasensitive sensor technology, has been attracting major attention due to its high sensitivity, label-free, array sensing, in-situ and real-time detection of biochemical interactions (Gruber et al., 2011; Huber et al., 2013; Huang et al., 2014; Ndieyira et al., 2014; Ricciardi et al., 2013a; Tamayo et al., 2013). The principle behind the microcantilever biochemical sensor operated in the stress mode is the transduction of biochemical interactions between receptors and analytes into a surface stress due to steric or conformational change (Bai et al., 2014b; Federici et al., 2010; Kosaka et al., 2014; Wu et al., 2013). The stress that is generated on one side of the microcantilever bends the cantilever. The deflection of the cantilever is measured through the principle of an optical lever using an optical laser. Cantilever sensors have been used to detect various biochemical molecules, such as proteins, lipids, nucleic acids, peptides, as well as small molecules and heavy metal ions (Bai et al., 2014a; Joo et al., 2012; Kang et al., 2011; Ricciardi et al., 2013b; Zhao et al., 2010, 2012). In addition, this technique has also been used for physical sensing, such as uncooled infrared imaging (Cheng et al., 2008; Duan et al., 2003; Shi et al., 2009).

In addition, through the detection of deflection or resonance frequency shift of the cantilever due to specific binding, cantilever sensors have also been used to detect the presence of bacteria and cells (Kuan et al., 2012; Mader et al., 2012; Park et al., 2008; Sharma and Mutharasan, 2013; Wang et al., 2014). However, as a highly sensitive sensor, cantilevers have not yet been used in the quantification of cell viability, especially in quantifying the viability of cancer cells, which plays an important role in anti-cancer drug screening. When we used a cantilever sensor to detect the presence of cancer cells, we found that the deflection and resonance frequency shift of the cantilever was detected as expected, and additionally, found that the presence of cancer cells on the cantilever produced a larger fluctuation of the microcantilever (Fig. 1), which was also been found in the presence of normal mammalian cells and bacteria (Kasas et al., 2015; Longo et al., 2013). The energy released from the metabolism of cells may drive the fluctuations of the cantilever. Therefore, in the present study, we developed a methodology to monitor the viability of cancer cells in a label-free and real-time manner using the fluctuations of a microcantilever. The amplitude of the fluctuation reflects the viability of the cancer cells, and thus we quantified the viability of cancer cells by calculating the variance of the deflection curves.

![Fig. 1. Schematic representation of the fluctuation mode measurement set-up. (A) Real-time nanomechanical deflection of the microcantilever with MCF-7 cells adhered to it. Illustration: optical micrograph of a microcantilever, on which several adherent cells can be observed. (B) Corresponding results for the naked microcantilever without cells. Illustration: optical micrograph of a microcantilever. (C) Schematic representation of the microcantilever sensor experimental setup. (D) Depiction of nanomechanical fluctuation caused by adherent cells on the microcantilever surface.](image-url)
Based on this feature, we exploited the microcantilever sensor in a fluctuation mode to characterize the viability change in breast cancer cells (MCF-7) subjected to various doses of an anti-cancer drug, and thus anti-cancer drug screening can be realized in a rapid and quantitative manner.

2. Material and methods

2.1. Materials, reagents and apparatus

(3-aminopropyl) triethoxysilane (APTES), phosphate buffered saline (PBS, pH 7.5), paclitaxel, all analytical grade, were purchased from Sigma-Aldrich (St. Louis, MO, USA). 96-well polystyrene microplates and cell culture plates were supplied by NEST Biotechnology Co. Ltd (Wuxi, China). A microplate reader (Multi- skanMK3) was obtained from Thermo (Vantaa, Finland).

2.2. Preparation of MCF-7 cells

The Michigan Cancer Foundation-7 (MCF-7, Keygen Biotech. Corporation, Nanjing, China) cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) (Gibco, Grand Island, NY, USA) with 10% fetal bovine serum (FBS) (Hyclone, Thermo Fisher Scientific Inc., UK), 100 U L⁻¹ penicillin G, 100 U L⁻¹ streptomycin (Hyclone, Thermo Fisher Scientific Inc., UK), and incubated at 37 °C in a 5% CO₂ humidified atmosphere. The culture medium was changed every 2 days. The cells collected at approximately 80% confluence were washed with PBS and detached from the culture flasks by the addition of 0.2 ml cm⁻² 0.25% trypsin (Sigma-Aldrich, Taufkirchen, Germany) for 5 min at 37 °C. The cells were then centrifuged at 80g for 5 min and resuspended in 1 × 10⁵/ml DMEM with 10% FBS. The resuspended cells were then ready for further use.

2.3. Preparation of the sensor

Commercial V-shaped silicon nitride microcantilevers (Model: DNP-10, Bruker, Camarillo, CA, USA) with dimensions of 0.6 × 40 × 200 μm³ and a spring constant of 0.06 N m⁻¹ were used. The real-time deflection of the microcantilever was measured by monitoring the deflection of a laser beam that was reflected from the microcantilever. Therefore, in order to reflect the laser beam, one side of the cantilever was coated with a 40 nm layer of gold deposited by e-beam evaporation. The cantilever was immersed in washing solution (“piranha dip”: H₂O₂/H₂SO₄=1:3, 200 μl) for 10 min before use, and then washed four times with deionized water and dried under a gentle stream of nitrogen gas. To promote cell adhesion to the silicon nitride surface, the microcantilever was treated with 3-aminopropyltriethoxysilane (APTES), a commonly used coupling agent for the modification of substrates to increase cell adhesion for biological implants and in lab-on-a-chip applications. The microcantilever was immersed in 200 μl of 10% APTES solution in deionized water for 30 min at room temperature, and then washed four times with deionized water and dried under a gentle stream of nitrogen gas; the modified microcantilevers were then ready to use.

2.4. Cell adhesion and culture on the microcantilever

The modified microcantilevers were placed carefully in a 24-well plate, and then the resuspended cells were added to the same 24-well plate. The cells settled down due to gravity and some fell onto the microcantilever. The cells with the microcantilevers were cultured in DMEM with 10% FBS, 100 U L⁻¹ penicillin G, 100 U L⁻¹ streptomycin, and incubated at 37 °C in a 5% CO₂ humidified atmosphere for 4 h until the cells adhered to the microcantilever (illustration in Fig. 1A). Typically, the microcantilevers, with a uniform coverage of cells greater than 85% and without cells covering the apex (reflection area) which was used to reflect the laser beam, was selected for use in each experiment. Different new microcantilevers were used in each experiment.

2.5. Detection of microcantilever fluctuation

The experimental setup used for microcantilever detection was a home-built microcantilever sensing system with 1 nm determination sensitivity of the microcantilever deflection as described in our previous studies (Nan et al., 2012; Tan et al., 2010; Wu et al., 2013, 2014; Xue et al., 2011; Zhao et al., 2010), which was equipped with a laser, a position sensitive detector (PSD) and a liquid cell. There was a microcantilever holder and a capacity of 0.6 ml in the cell and an inlet and outlet for solution exchange in the edge of the cell. The solution was exchanged using a high-precision peristaltic pump which controlled the flow rate in the range of 0.4–40 ml h⁻¹ and kept the flow rate constant for an indefinite period of time.

The cell-adhered microcantilever was mounted in the liquid cell using a polystyrene screw, and the liquid cell was then covered using a glass sheet which was sealed with a soft gasket and three screws. The inlet and outlet of the liquid cell were connected to the injection module and liquid waste discharge module of the microcantilever sensing system, respectively. Before being fixed in the microcantilever sensing system, the liquid cell was filled with the solution and air bubbles were removed. The temperature of the liquid cell was controlled using a high-precision temperature control system (Beijing Nni Science and Technology Co., Ltd. Beijing, China) and maintained at 310.00 ± 0.01 K, and the temperature of the room was maintained at 301 ± 1 K. An optical lever system was used for measurement. PBS was used as medium in each measurement.

2.6. The effects of paclitaxel on biological viability

The Cell Counting Kit-8 (CCK-8) was used to estimate the biological viability of the cells. WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2, 4-disulfophenyl)-2H-tetrazolium, monosodium salt], commercially known as the Cell Counting Kit-8 (Dojin Laboratories, Japan) provides a sensitive colorimetric assay to determine the number of viable cells. The same number of cells was placed in the 96-well plate and exposed to solutions with/ without paclitaxel for 4 h at 37 °C. The 0.01 ml assay reagent was added into each well. After 1.5 h, the 96-well plate was analyzed using an enzyme-linked immunosorbent assay. The assay reagent was a tetrazolium compound, which can be reduced by live cells resulting in a colored formazan product. The quantity of formazan product measured at 450 nm is directly proportional to the number of viable cells. This experiment was repeated in quadruplicate wells.

2.7. Statistic analysis

Statistical difference among groups was analysed using the unpaired Student’s t-test. P values of < 0.05 were considered as significant.

3. Results and discussion

3.1. Effect of cell viability on nanomechanical fluctuation

The experimental setup used to detect microcantilever fluctuations was a home-built microcantilever sensing system which
measured the deflection of the microcantilever tip in the nanometer scale (Nan et al., 2012; Tan et al., 2010; Wu et al., 2013, 2014; Xue et al., 2011; Zhao et al., 2010) and is described in detail in the Section 2. When the cells (MCF-7) had adhered to the microcantilever in the incubator (micrograph in Fig. 1A), the microcantilever was mounted in the liquid cell and the deflection of the microcantilever was measured in real-time (Fig. 1C). The mechanical movements driven by the metabolic energy of the cell produced a larger fluctuation of the cantilever (Figs. 1A and 2A), while the microcantilever fluctuated very little for the naked microcantilever in PBS without cells (Fig. 1B). To determine if the measured signal was because of adherent cells on the microcantilever rather than cellular debris floating in the liquid, the microcantilever (Fig. S1B) treated using the same procedure as the microcantilever chip in A, of which the silicon nitride substrate still had roughly the same number of cells as the microcantilever chip (Fig. 1A) in A, but without cells on the sensor surface. (C) Corresponding results for the microcantilever with dead cells adhered to it. (D) Corresponding result for the experiment involving starved cells. (E) Corresponding variance of the fluctuation curve in A–D. Data represent mean ± standard deviation (S.D.) of four repeated measurements (*P < 0.05, **P < 0.01, by t-test). NS, not significant.
than those caused by MCF-7 cells (variance dropped to very low values (3.85 and 2.26 nm², respectively). To confirm that the significant large fluctuation of the microcantilever was generated by the cells adhered to the surface of the microcantilever. When the cells died due to long exposure (20 h) in PBS without culture medium, the fluctuation decayed (Fig. 2C) compared with that produced by live cells and was almost the same magnitude as the microcantilever without cells. These findings indicated that the fluctuations may be related to cell viability.

The variance of the fluctuation curve was calculated to quantify the magnitude of this fluctuation (Fig. 2E). Each point in Fig. 2E represents the average for four determinations and the error bars represent the standard deviation of data. One reason for this deviation may be due to the little difference of cell number on microcantilever. For the microcantilever with live cells adhered to it (Figs. 1A and 2A), the variance was approximately 16.1 nm². After cell death and in the microcantilever without cells, the variance dropped to very low values (3.85 and 2.26 nm², respectively). To confirm that the fluctuation was related to cell viability only, the fluctuation caused by the starved cells whose viability was between live and dead cells was measured. Following immersion in PBS without culture medium for about 9 h, the cells were in a state of starvation, and the biological viability of the cells decreased, however, they were not dead and still had some biological viability (details of the biological viability estimated by the Cell Counting Kit-8 (CCK-8) are provided in Supporting information Section 2 and Fig. S2). The fluctuation (Fig. 2D, variance=9.79) caused by the starved cells was between that of live (variance=16.1) and dead cells (variance=3.85). These results again suggest that the fluctuations were dependent on cell viability. Thus, the microcantilever fluctuation can be used to characterize the viability of cells in a real-time and label-free manner, and detection of cell viability can be achieved by calculating the variance of the fluctuation curve. To verify that this technique is applicable to other cells, we tested another type of cell (human umbilical vein endothelial cells, HUVECs) using the procedure described above for MCF-7 cells (the microcantilever with HUVECs adhered to it is shown in Fig. S3). The cells had high viability initially (0.7 h) and generated larger fluctuations (variance=10.6 nm²) of the microcantilever than that generated by the cells whose viability decreased due to immersion in PBS without culture medium for approximately 7 h (variance=5.1 nm², Fig. 3). The change in fluctuation caused by MCF-7 cells and HUVECs showed the same tendency, confirming that the signal was associated with cell viability. In addition, the microcantilever fluctuations (variance=10.6 nm²) produced by HUVECs were significantly less than those caused by MCF-7 cells (variance=16.1 nm²), suggesting that MCF-7 cells produced stronger signal demonstrated higher viability than HUVECs. On the basis of these findings, microcantilevers may be used to measure the change in cancer cell viability following drug treatments, and thus anti-cancer drug screening may be realized.

3.2. Nanomechanical quantitation of drug efficacy on cell viability

We investigated the characteristics of microcantilever fluctuation in the MCF-7 cell line treated with different concentrations of paclitaxel which can inhibit cell viability (Jordan et al., 1993) and is one of the most effective natural anticancer agents usually used in clinical practice for the medical treatment of ovarian, breast and non-small cell lung cancers (Murphy et al., 1993). When the cells had adhered to the microcantilever, paclitaxel was introduced and the cells were cultured for 4 h in an incubator. The microcantilever with drug-treated cells adhered to it was mounted in the microcantilever sensing system and the fluctuation was then measured. Fig. 4A–F shows a group of fluctuation curves reflecting the effect of different concentrations of paclitaxel on MCF-7 cells. The variances of the curves were calculated and shown in Fig. 4G, and each point represents the average for four determinations and the error bars represent the standard deviation of data. Live cells without drug treatment produced a larger fluctuation with a variance of 16.1 nm². After treatment with 0.75 μg ml⁻¹ paclitaxel, the variance of the fluctuation curve dropped to a low value (10.1 nm²). In other samples, with increasing concentrations of paclitaxel during cultivation (1.5, 3.0, 4.5 and 6 μg ml⁻¹), the variance was further reduced (Fig. 4B–G), suggesting that the microcantilever fluctuation is capable of quantifying cell viability and its change under drug treatment.

We next evaluated the effect of paclitaxel exposure on MCF-7 cells in the measurement process. When the cells had adhered to the microcantilever in the incubator without drug treatment, the microcantilever was mounted in the liquid cell of the sensing setup. The liquid cell was filled with PBS containing different concentrations of paclitaxel. When the cells had been exposed to paclitaxel for approximately 6 h, the measured fluctuation is shown in Fig. 5A–D. The variances of the fluctuation curves are calculated in Fig. 5E, and each point represents the average for four determinations and the error bars represent the standard deviation of data. Compared to the control (Fig. 5A), in the presence of paclitaxel the magnitude of the fluctuation and variance of the fluctuation curves decreased gradually with increasing concentrations of drug (1.5, 7.5 and 20 μg ml⁻¹, Fig. 5B–E).
Fig. 4. Nanomechanical quantitation of cell viability after treatment with different concentrations of drug. (A–F) Nanomechanical fluctuation of the microcantilever in the experiment involving MCF-7 cells after treatment with paclitaxel at the indicated concentrations. When MCF-7 cells had adhered to the microcantilever in the culture medium, paclitaxel was added and the cells were cultured for 4 h in the incubator. The microcantilever was then mounted in the liquid cell of the sensing set-up and the fluctuation was measured in PBS. (G) Corresponding variance of the fluctuation curve in A–F. Variance data error bars represent the deviation of the variance data for four measurements performed in similar conditions.
Live cancer cells with the best viability induced a larger fluctuation of the microcantilever. Treatment with paclitaxel at various concentrations caused a decrease in the sensor’s fluctuations. Depending on the drug treatment environment, the effects of the drug on cell viability, estimated by the microcantilever, were different. The effect on cells treated in the culture environment (sufficient medium and appropriate temperature and humidity, Fig. 4) was better than that in PBS without medium (Fig. 5). The different magnitude of the fluctuation indicated that the drug may be absorbed more easily by the cells in the culture medium than in PBS. Fluctuation of the microcantilever could distinguish cell viability in one environment from that in another environment.

Fig. 5. Nanomechanical quantitation of cell viability in the experiment involving MCF-7 cells exposed to paclitaxel. (A–D) Nanomechanical fluctuation of the microcantilever following paclitaxel exposure in MCF-7 cells in the measurement process (exposed to PBS with paclitaxel for 6 h). When the cells adhered to the microcantilever, the microcantilever was mounted in the sensing set-up and the fluctuations were measured in PBS containing paclitaxel at the indicated concentrations. Data recording started 6 h after the cells were exposed to paclitaxel in the liquid cell of the sensing set-up. (E) Corresponding variance of the fluctuation curve in A–D. Variance data error bars represent the deviation of the variance data for four measurements performed in similar conditions.
3.3. Mechanism of nanomechanical fluctuation caused by the cells

To understand the origin of the fluctuations, the morphology of adherent cells (MCF-7) was observed in-situ using conventional optical microscopy (Supporting information Video 1 and Video 2). We observed both live and dead cells which died due to lack of culture medium (Supporting information Video 1 and Video 2). It can be seen from the microscope videos that live cells moved continuously and dead cells were relatively motionless. The observed micromotion of adherent cells and the lack of motion in dead cells clearly indicated that the fluctuations were produced by active micromotion. In previous electrical method, the measured electrical impedance of the electrodes is observed when cells attach and spread on these electrodes, and the fluctuation in an electrical signal was also observed that are a direct measure of cell motion and spread on these electrodes, and the trical impedance of the electrodes is observed when cells attachcontinuously and dead cells were relatively motionless. The observed micromotion of adherent cells and the lack of motion in dead cells clearly indicated that the fluctuations were produced by active micromotion. In previous electrical method, the measured electrical impedance of the electrodes is observed when cells attach and spread on these electrodes, and the fluctuation in an electrical signal was also observed that are a direct measure of cell motion (Giaever and Keese, 1991). The decrease in the variance of fluctuations (Figs. 2 and 3) when the cells were starved due to lack of culture medium indicated that the energy needed to produce the microcantilever fluctuations (i.e., the micromotion) was from cell metabolism. Microtubules (MTs), are primary components and functional units in the cytoskeleton system, and are necessary for cell division, cell locomotion, cell polarization and vesicle transport (Watanabe et al., 2005). Microtubules are nucleated from their minus ends and the plus ends of MTs alternate between two phases of growth and shrinkage (a state termed ‘dynamic instability’) to explore intracellular spaces (Kirschner and Mitchison, 1986). This dynamic state of MTs is necessary and critical for cell locomotion (Watanabe et al., 2005). Paclitaxel is known to act on MTs by binding to MT polymer (Schiff et al., 1979), and low concentrations of paclitaxel have been reported to decrease the dynamic instability of MTs in vitro, with little effect on MT mass (Jordan et al., 1993; Derry et al., 1995), and diminish cell locomotion, and the rate of cell locomotion decreases with increasing concentration of paclitaxel (Liao et al., 1995). This is compatible with the results obtained in the present study regarding microcantilever fluctuation: following treatment with paclitaxel, fluctuation of the microcantilever also decreased with increasing concentrations of paclitaxel. Thus, our interpretation of the origin of these fluctuations is that the nanomechanical sensor (micromanometer) can transduce small movements which are driven by the force generated from the cytoskeleton, using cell metabolism as its energy source.

For a deeper insights of the origin of the fluctuation, the Cell Counting Kit-8 (CCK-8) which provides a sensitive colorimetric assay of the biological viability of cells was conducted. The absorbance values of the CCK-8 assay for the cells treated with (‘Medium+ Paclitaxel’) / without (‘Medium’) paclitaxel in medium under the same conditions (exposed to solutions with/without paclitaxel for 4 h) in Fig. 4A and C were 1.257 and 1.204, respectively (Fig. 6). The absorbance values using the CCK-8 assay for the cells treated with (‘PBS+ Paclitaxel’) / without (‘PBS’) paclitaxel in PBS under the same conditions (exposed to PBS with/without paclitaxel for 6 h) in Fig. 5A and C were 0.652 and 0.524, respectively (Fig. 6). Whether treated in medium or in PBS, the biological viability of cells measured by the CCK-8 was little changed after treatment with paclitaxel, suggesting that paclitaxel has little effect on the biological viability of cells. In fact, paclitaxel is known to act on MTs by binding to MT polymer (Schiff et al., 1979) and decreases the dynamic instability of MTs (Jordan et al., 1993; Derry et al., 1995). This decrease in the dynamic instability of MTs with little effect on the biological viability of cells after paclitaxel treatment was another indication that these fluctuations were closely correlated with the cytoskeleton (here is the dynamic instability of MTs) which is critical for the generation of cell force. Thus, we propose that cell viability consists of two parts: mechanical viability and biological viability. Following treatment with paclitaxel, the variance of the fluctuation curve estimated by the microcantilever significantly decreased (54%, 0 and 0.75 μg ml⁻¹ in Fig. 4C), and the absorbance value (Fig. 6) estimated by the CCK-8 assay was little changed (decreased by 4%, a comparison of the results of fluctuation and the CCK-8 is described in detail in Supporting information Section 4 and Fig. S4). The corresponding results involving the cells treated with PBS (0 and 7.5 μg ml⁻¹ in Fig. 5E) showed the same tendency (Fig. 6). These results indicated that paclitaxel had little effect on biological viability estimated by the biological method (CCK-8), but had a significant effect on mechanical viability estimated by the nanomechanical method (the microcantilever). This new mode based on nanomechanical fluctuation can not only determine the biological viability of cells (Fig. 2), but can also quantify mechanical viability in the pN range (force was roughly estimated in Supporting information Section 5 and Fig. S5), which cannot be determined by existing methods such as the CCK-8. This also indicates that the nanomechanical fluctuation mode of the microcantilever is more sensitive to the changes in cell viability induced by drug treatment. The effects of

Video S1. Video 1 Optical microscope analysis of the cells on the microcantilever. Supplementary material related to this article can be found online at 10.1016/j.bios.2015.09.024.

Video S2. Video 2. Optical microscope analysis of the cells on the microcantilever. Supplementary material related to this article can be found online at 10.1016/j.bios.2015.09.024.
drug treatment can be obtained using this new method, which cannot be obtained by existing methods. This new method provides a new concept and strategy for the evaluation and assay of cell viability and the screening of anti-cancer drugs. This will be particularly useful for anti-cancer drugs which cannot be screened out by existing methods.

4. Conclusions

In this study, we demonstrated the capability of a fluctuation-based nanomechanical sensor for monitoring the micromotions of live cells under various conditions. We proved that the microcantilever sensor is capable of quantifying cell viability by measuring nanoscale fluctuations of the microcantilever and calculating the variance of the fluctuation curve when cancer cells were exposed to different concentrations of anti-cancer drug. Compared with existing methods, this new mode based on fluctuations of the microcantilever has obvious advantages in cell viability monitoring and anti-cancer drug screening due to the rapid, label-free, quantitative, noninvasive, real-time and in-situ assay of cell viability within tens of minutes instead of days or weeks. This presents the successful use of nanomechanics for anti-cancer drug screening, namely that of characterizing the viability change of cancer cells (MCF-7) subjected to various doses of anti-cancer drug.

The experiments show that fluctuations of the microcantilever with cancer cells (MCF-7) adhered to it decreased with increasing concentrations of paclitaxel. The experiments demonstrate that the nanomechanical fluctuations are driven by the force generated from the cytoskeleton, using cell metabolism as an energy source, and the dynamic instability of microtubules plays an important role in this process. We propose that cell viability consists of two parts: biological viability and mechanical viability. The results show that paclitaxel has little effect on biological viability which can be estimated by existing biological methods such as the CCK-8, but has a significant effect on mechanical viability which was estimated by the nanomechanical method. The technique can not only determine the biological viability of cells, but can also quantify mechanical viability in the pN range, which cannot be determined by existing methods. This will be particularly useful for anti-cancer drugs which cannot be screened out by existing methods. One might consider the nanomechanical approach as being more direct than the electrical and optical methods. This new method provides a new concept and strategy for the evaluation and assay of cell viability and the screening of anti-cancer drugs.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2015.09.024.

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