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Biomechanical measurement and analysis of colchicine-induced effects on cells by nanoindentation using an atomic force microscope

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ABSTRACT

Colchicine is a drug commonly used for the treatment of gout, however, patients may sometimes encounter side-effects induced by taking colchicine, such as nausea, vomiting, diarrhea and kidney failure. In this regard, it is imperative to investigate the mechanism effects of colchicine on biological cells. In this paper, we present a method for the detection of mechanical properties of nephrocytes (VERO cells), hepatocytes (HL-7702 cells) and hepatoma cells (SMCC-7721 cells) in culture by atomic force microscope (AFM) to analyze the 0.1 μg/mL colchicine-induced effects on the nanoscale for two, four and six hours. Compared to the corresponding control cells, the biomechanical properties of the VERO and SMCC-7721 cells changed significantly and the HL-7702 cells did not considerably change after the treatment when considering the same time period. Based on biomechanical property analyses, the colchicine solution made the VERO and SMCC-7721 cells harder. We conclude that it is possible to reduce the division rate of the VERO cells and inhibit the metastasis of the SMCC-7721 cells. The method described here can be applied to study biomechanics of many other types of cells with different drugs. Therefore, this work provides an accurate and rapid method for drug screening and mechanical analysis of cells in medical research.

1. Introduction

Gout is a disorder of purine metabolism and affects people with an upward trend worldwide (Richette and Bardin, 2010). Colchicine has been used over a century for the treatment of acute gout and in prophylaxis (Deveaux et al., 2004; Stern et al., 1997), but it has some potential side-effects such as severe kidney failure, gastroenteritis, fluid loss, electrolyte disturbance (low Na^+, K^+, Ca^{2+} and Mg^{2+}), hypotension and hypovolemic shock (Kicka et al., 2010; Putterman et al., 1992; Wollersen et al., 2009). If a dose of colchicine exceeds 0.8 mg/kg, it can lead to multiple organ failures (Alaygut et al., 2016; Bismuth et al., 1986; Kupper et al., 2010). Although colchicine has been used for the treatment of gout, its mechanism of action has not been clearly defined on the nanoscale (Fordham et al., 1981; Roberts et al., 1987; Sauder et al., 2016). Thus, many efforts have been made to find out the mechanism of action. As the significant expressions of biological functions and characteristics, biomechanical properties have been widely investigated on the nanoscale in medicine. Sato et al. (1980) and Cross et al. (2008) reported that the metastasis of cancer cells was influenced by the deformability of cells, and the deformability was related to the cell stiffness.

In recent years, biophysical techniques such as magnetic twisting cytometry, micropipette aspiration, atomic force microscopy (AFM) and optical tweezers have been developed to measure biomechanical properties of cells. Among them, AFM has its advantages of ultra-high resolution, high reliability and multi-dimensional information detection (Biswas et al., 2014), and it is promising for many potential applications in biomedicine. Cell elasticity and deformability have been recognized as markers for cellular phenotypic events related to the alterations in cytoarchitecture and adhesion during malignant transformation (Bercoff et al., 2003; Cross et al., 2007; Discher et al., 2005; Guck et al., 2005; Suresh, 2007; Suresh et al., 2005). A number of approaches have been made by AFM indentation. Hayashi and Iwata (2015) measured the stiffness of Hela cells and End1/E6E7 cells, and found

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that cancer cells were softer than normal cells. Nikkhah et al. (2011) reported that nonmalignant breast epithelial cells had significant higher Young's moduli than their malignant counterparts. Dokukin et al. (2016) reported that the force signature of the pericellular brush layer of Guinea pig fibroblast cells was significantly changed after a treatment with hyaluronidase. Kasas et al. (2013) investigated the nanomechanical properties (Young's modulus, deformability and adhesion) of biological samples (mammalian cells, plant cells, yeast cells, bacteria and viruses).

Although some side effects of colchicine have been studied, there is still lack of evidence showing the damage level of cells, the function time and action mechanism of colchicine, and there is no work reported for the study of colchicine-induced effects based on the analysis of biomechanical properties of living cells on the nanoscale. In this work, nephrocytes (VERO cells), hepatocytes (HL-7702 cells) and hepatoma cells (SMCC-7721 cells) were used to study the side effects and the anti-cancer effects of colchicine. Biomechanical properties of VERO, HL-7702 and SMCC-7721 cells were detected and analyzed before and after the treatment with the colchicine solution by AFM for two, four and six hours. The changes in biomechanical properties of single cells were observed on the nanoscale. The results of the cell profile and biomechanical properties showed the mechanistic changes of cell stiffness, deformability and cytoadherence. This work provides an accurate and rapid method for drug screening and mechanical analysis of cells in medical research.

2. Theoretical methods

2.1. AFM nanomanipulation

The cell elastic modulus, indentation force and surface roughness of VERO, HL-7702 and SMCC-7721 cells were determined using the quantitative imaging mode of the AFM system (NanoWizard® 3 NanoOptics BioScience AFM System, JPK Instruments AG, Germany). A schematic diagram of the indentation experiment process is shown in Fig. 1(a). As the probe approaches (shown in Fig. 1(a)-①) within a few tens of nanometers, it goes into a regime of an attractive van der Waals force. The probe is weakly attracted toward the sample surface and as it approaches closer to the sample (shown in Fig. 1(a)-②), it enters in the repulsive realm of Lennard-Jones potential, where the probe is strongly repelled from the surface (shown in Fig. 1(a)-③). As the cantilever is retracted from the sample, the tip remains in contact with the surface due to interaction forces (shown in Fig. 1(a)-④), and the cantilever is deflected downwards. At some point of retraction, the force required to disrupt the adhesion is reached. Then the tip leaves the surface (shown in Fig. 1(a)-⑤).

2.2. Biomechanical measurements

During the indentation process, we ensured that the AFM probe remained immobile when the sample stage scanned. The piezo-

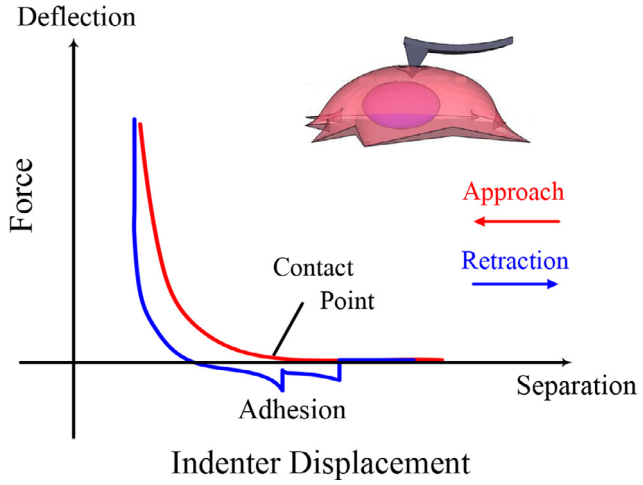

Fig. 2. A schematic illustration of a single cell indentation and a force-displacement curve obtained from a single cell.

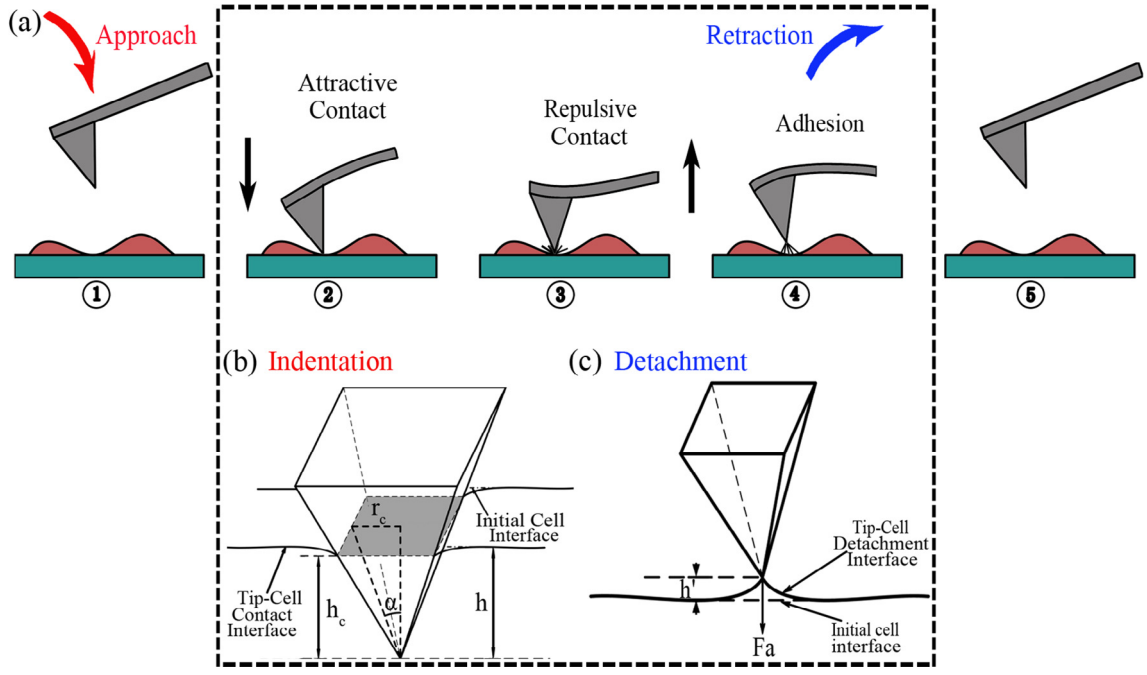

Fig. 1. (a) Schematic diagram of the tip movement during the approach and retraction processes of one cell for the measurement by AFM. (b) Diagram of indentation. (c) Diagram of detachment.

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