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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-

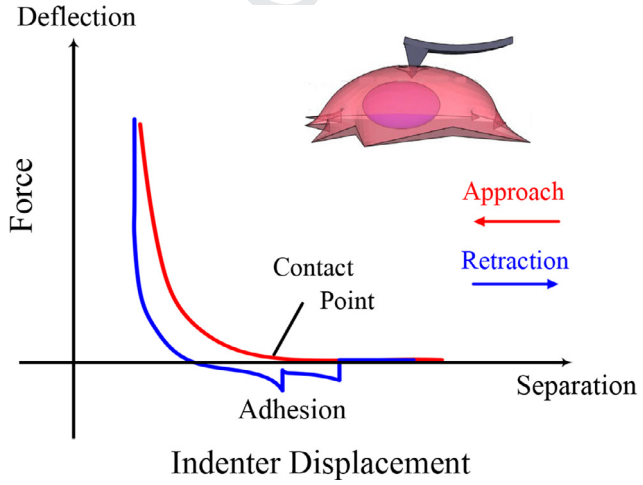


**Fig. 2.** A schematic illustration of a single cell indentation and a force-displacement curve obtained from a single cell.

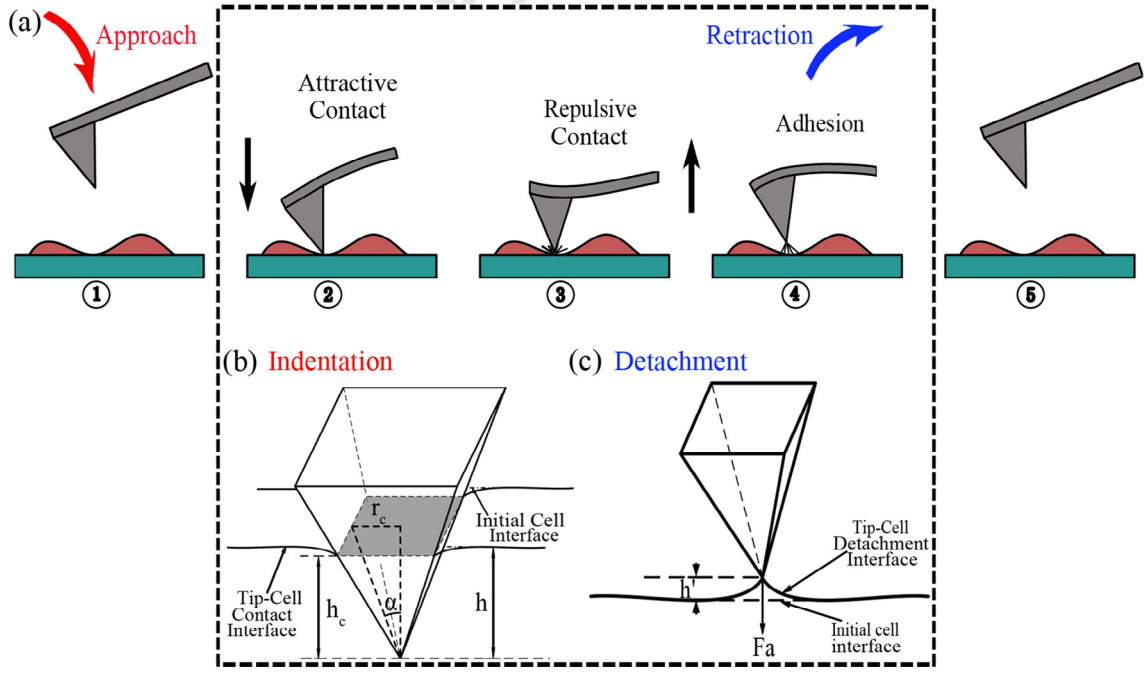


**Fig. 1.** (a) Schematic diagram of the tip movement during the approach and retraction processes of one cell for the measurement by AFM. (b) Diagram of indentation. (c) Diagram of detachment.

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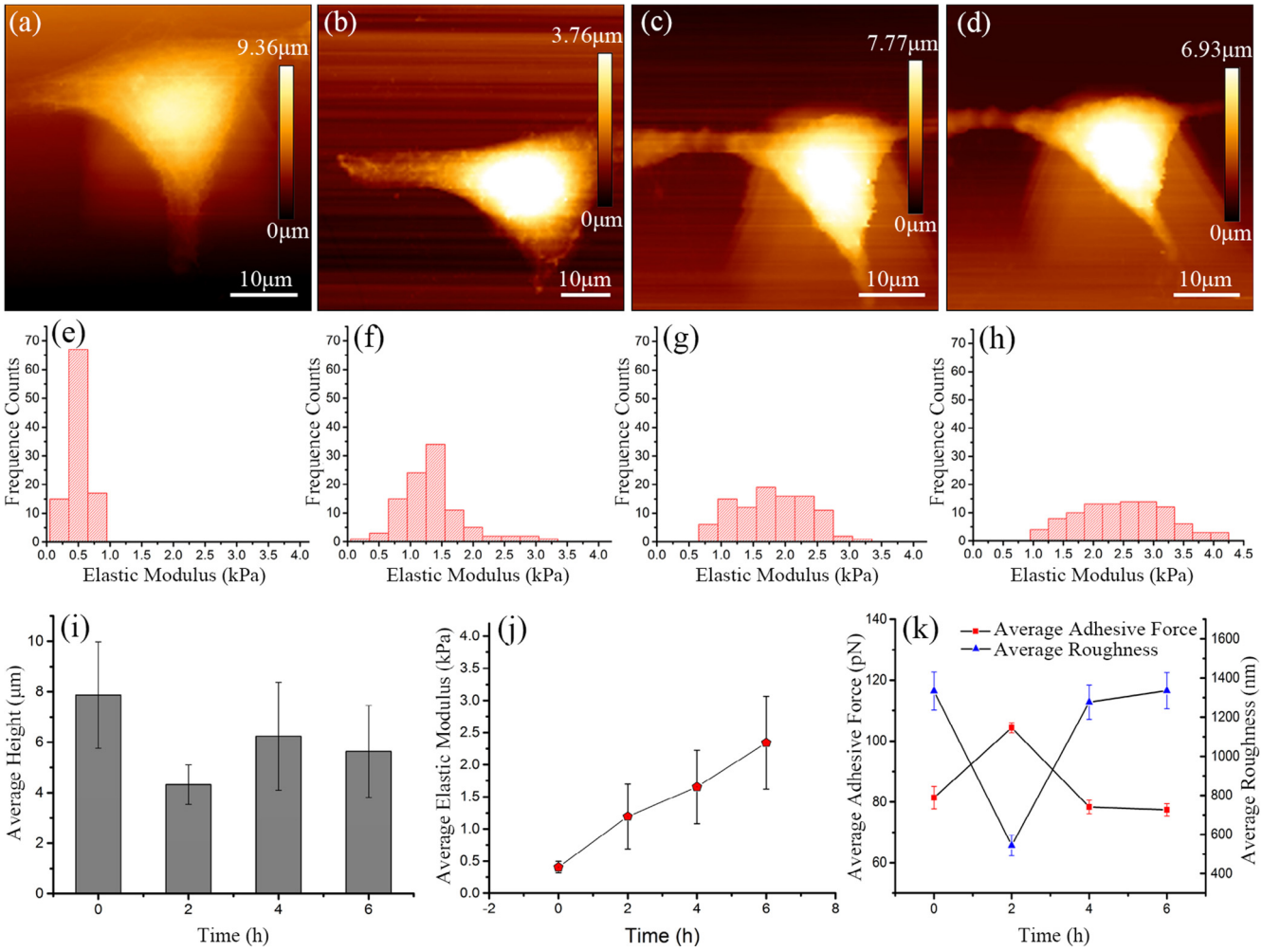


**Fig. 3.** AFM images of the living VERO cells: (a) Image of an untreated cell. (c–d) Images of a VERO cell exposed to the colchicine solution for two, four and six hours. (e) Distribution of frequencies of the elastic modulus of the VERO control cell. (f–h) Distributions of frequencies of the elastic modulus of the VERO cell exposed to the colchicine solution for two, four and six hours. (i) Statistical analysis of the average height. (j) Diagram of the average elastic modulus during the experimental time period. (k) Graphs of the average adhesive forces and roughness values for the living VERO cells before and after the treatment with the colchicine solution for two, four and six hours.

electric stage was used to perform the scan and indentation tasks. The indentation process for the measurement of elastic modulus is shown in Fig. 1(b). The elastic force was calculated according to the half-cone angle $\alpha$ of the pyramidal tip, the tip-cell contact radius $r_c$ and the tip-cell contact height h. The detachment of the indentation process is shown in Fig. 1(c). The adhesion force $F_a$, which is the maximal force required to detach the tip from the sample, can be directly measured. The Hertz's theory (Hertz, 1881) provides a solution for the elastic indentation with a spherical or conical indenter. In the experiment, the contact area was defined by the AFM tip geometrical shape (Liu et al., 2015; Sirghi et al., 2008). The force F is a function of the spring constant k and the deformation $\delta$. This relationship is described by the Hooke's law

$$F = k \cdot \delta \tag{1}$$

The elastic force of an ideal regular square pyramidal AFM tip model can be expressed as (Rico et al., 2005, 2007)

$$F_e = \frac{E \tan \alpha}{\sqrt{2}(1 - V^2)} h^2 \tag{2}$$

where $F_e$ is the indentation force, E is the Young's modulus, V is the Poisson's ratio of the sample, $\alpha$ is the half-cone angle of a pyramid tip (Fig. 1(b)). It can be seen that $F_e$ is a function of the Young's modulus, half-cone angle and indentation depth. For living cells, V has a value of 0.5 (Kirmizis and Logothetidis, 2010). It is reported that the surface tension for soft cells can significantly affect the indentation response (Ding et al., 2015, 2017). The indenter tip-cell contact interface shows different processes (Fig. 2).

The adhesion component term can be given as (Sirghi et al., 2008)

$$F_a = -\frac{32 \cdot \tan \alpha}{\pi^2 \cdot \cos \alpha} \cdot \Delta\gamma \cdot h \tag{3}$$

where $\Delta\gamma$ denotes the work of adhesion. The relationship between the elastic modulus and the spring constant can be expressed as

$$k = E\frac{A}{L} \tag{4}$$

where A is the cross sectional area, and L is the sample length. Based on the Hooke' law, the height of the raised AFM probe as shown in Fig. 1(c), $h'$ can be calculated by the following equation

$$h' = F_a \frac{L}{EA} \tag{5}$$

### 2.3. Statistical analysis

A non-conductive square pyramidal silicon nitride probe (spring constant = 0.057 N/m) was calibrated and used to carry out the measurements. The radius of the square pyramidal probe

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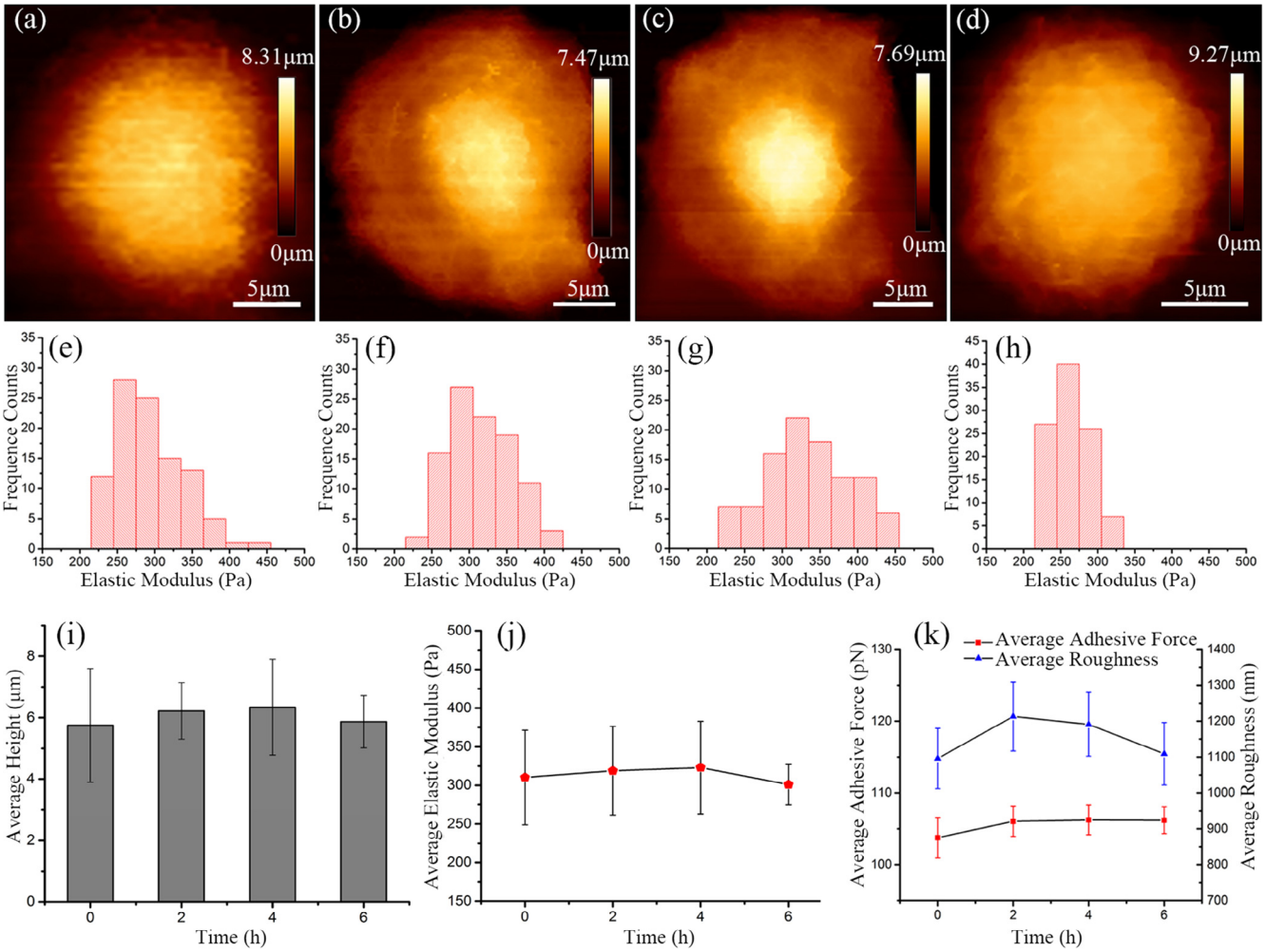


**Fig. 4.** AFM images of the living HL-7702 cells: (a) Image of an HL-7702 cell. (b–d) Images of HL-7702 cells exposed to the colchicine solution for two, four and six hours. (e–h) Distributions of frequencies of the elastic modulus of the HL-7702 cell before and after exposed to the colchicine solution for two, four and six hours. (i) Statistical analysis of the average height. (j) Diagram of the average elastic modulus during the experimental time period. (k) Graphs of the average adhesive forces and roughness values for the living HL-7702 cells before and after the treatment with the colchicine solution for two, four and six hours.

tip was 20 nm. The typical pattern of indentation-force curve is illustrated in Fig. 2, which presents the indentation and detachment models. The tip-surface adhesive force is characterized as a short-range force (Zhu et al., 2016), and thus it will not take effect until the intimate contact between the tip and cell forms. In this regard, the adhesive force is likely to be present in the detachment stage (as shown in the blue[1] straight line in Fig. 2) rather than the indentation stage (as shown in the red dotted line in Fig. 2).

### 2.4. Cell culture

VERO cells were isolated from kidney epithelial cells extracted from an adult African green monkey. The cells were established as a cell line by Yasumura et al. in 1962 (Sun et al., 2011; Yasunura and Kawakita, 1963). HL-7702 cells were human hepatocytes, and SMCC-7721 cells were human hepatoma cells. In the experiment, the VERO, HL-7702 and SMCC-7721 cells were grown in the 1640 culture solution containing 10% of fetal bovine serum (FBS).

The VERO, HL-7702 and SMCC-7721 cells were cultured with 5% of $CO_2$ at 37 °C. The cells were plated on glass coverslips (18 × 18 $mm^2$). The cell density was $1.0 \times 10^5$ cells/mL (1 mL per coverslip). The cells were cultured in 38-mm plastic dishes for 24 h at 37 °C.

### 2.5. Preparation of colchicine solution

A colchicine dose of 0.5 mg per pill was used in the experiment. The concentration of colchicine solution was 45 g/L at 20 °C. One pill was dissolved in 1-mL deionized water and the solution was then filtered. According to the drug concentration and the blood proportion of one person weight (7–8%) (Alberts et al., 2002), the solution was diluted 50 times with deionized water.

### 2.6. Sample preparation

The cells were plated on glass coverslips in plastic petri culture dishes for 24 h. Phosphate buffered saline (PBS) was then used to wash the dishes and remove the cells that were dead and did not adhere.

The control cells were measured by AFM indentation in culture. Then 2-mL culture solution and the 20-μL colchicine solution were added to each culture dish. The concentration of 0.1 μg/mL colchicine solution was used in the experiment and it complied with the usual prescribed dose. The cells were cultured in the colchicine solution in an incubator with the control of temperature and $CO_2$

[1] For interpretation of color in Fig. 2, the reader is referred to the web version of this article.

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concentration for two, four, and six hours. After incubation, the exposed cells were measured using an AFM.

## 3. Results

In the experiments, the mechanical properties of VERO, HL-7702 and SMCC-7721 cells were obtained using the quantitative imaging mode of an AFM. Ten measurement cells were selected for each of the VERO, HL-7702 and SMCC-7721 cells, and ten points were measured for each of the cells. The measurement was repeated three times. Biomechanical properties such as the elastic modulus, adhesion and surface roughness of the cells were obtained by the data analysis.

The morphology of one VERO cell after 24 h culture is shown in Fig. 3(a). The morphology of the VERO cell after the treatment with the colchicine solution for two, four and six hours are presented respectively in Fig. 3(b)–(d). Fig. 3(e)–(h) shows the distribution of the elastic modulus of the living VERO cell before and after the treatment with the colchicine solution for two, four and six hours. According to the analysis of the measurement, the average height (as shown in Fig. 3(i)), the average elastic modulus (as shown in Fig. 3(j)), the average adhesive force and the average roughness (as shown in Fig. 3(k)) of the VERO cells had been obtained before and after the treatment. We found that the average height of VERO cells was decreased after two hours of the treatment compared with the control cells. This further increased after four hours compared with two hours of the treatment but it only slightly changed after six hours of the treatment. The elastic modulus was obviously enhanced within six hours. For the VERO cells, the average adhesive force increased but the average roughness decreased after two hours of the treatment with the colchicine solution. After four hours of the treatment, the adhesive force was reduced but the roughness was enhanced. Compared with four hours of the treatment, the average adhesive force was lower at six hours.

Fig. 4(a)–(d) shows the AFM images of the living HL-7702 cells exposed to the colchicine solution under the same conditions and for the same time periods as for the VERO cells. The corresponding distribution of the elastic modulus is shown in Fig. 4(e)–(h). Based on the analysis of the measurements, the average height (as shown in Fig. 4(i)), the average elastic modulus (as shown in Fig. 4(j)), the

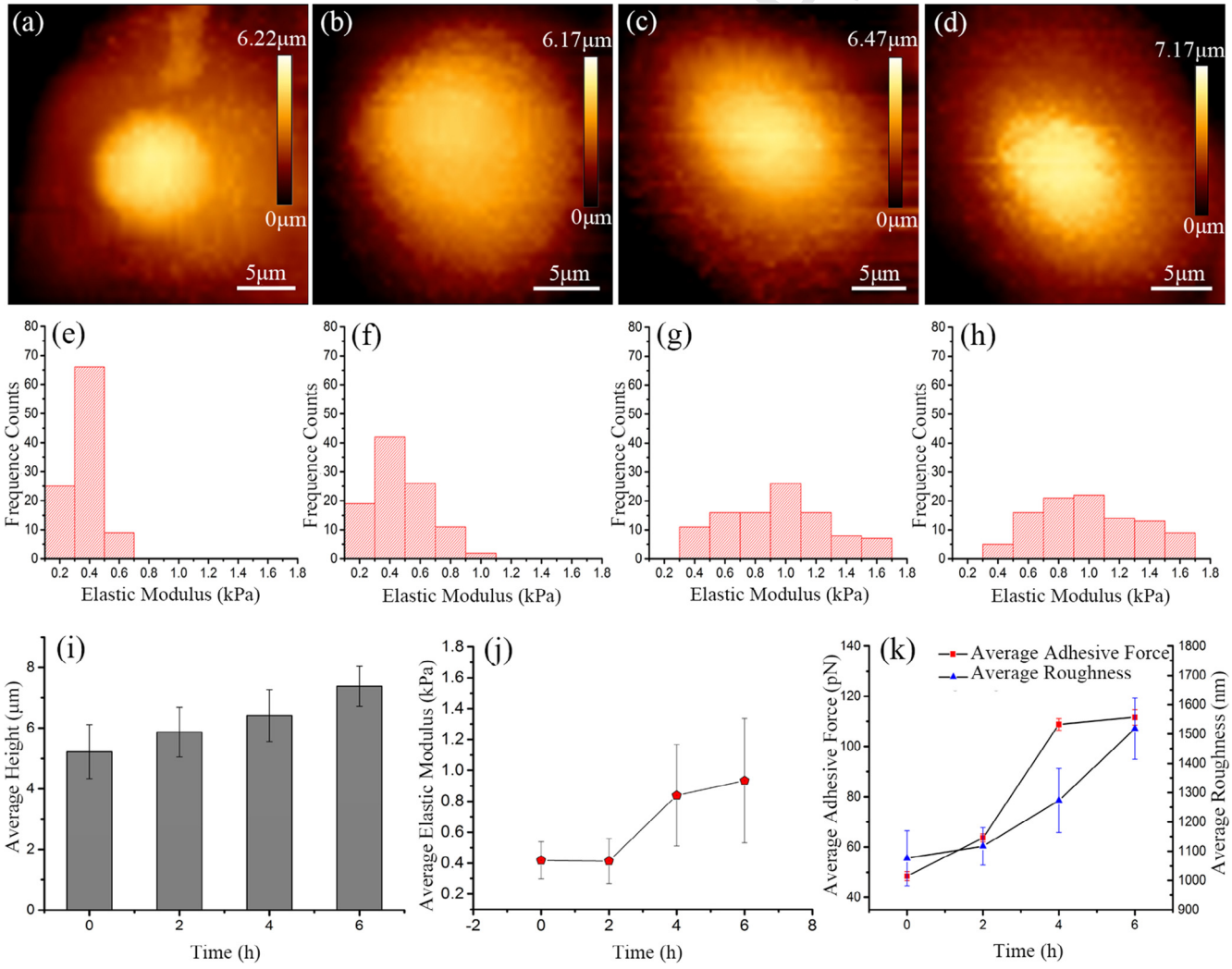


**Fig. 5.** AFM images of the living SMCC-7721 cells: (a) Image of a SMCC-7721 cell. (b–d) Images of SMCC-7721 cells exposed to the colchicine solution for two, four and six hours. (e–h) Distributions of frequencies of the elastic modulus of the SMCC-7721 cell before and after exposed to the colchicine solution for two, four and six hours. (i) Statistical analysis of the average height. (j) Diagram of the average elastic modulus during the experimental time period. (k) Graphs of the average adhesive forces and roughness values for the living SMCC-7721 cells before and after the treatment with the colchicine solution for two, four and six hours.

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**Table 1**
Biomechanical properties of VERO, HL-7702 and SMCC-7721 cells treated with the colchicine solution.

| Cell | Action time (h) | Average height (μm) | Average elastic modulus (kPa) | Average adhesive force (pN) | Average roughness (nm) |
|---|---|---|---|---|---|
| VERO | Control | 7.87 ± 2.11 | 0.41 ± 0.09 | 81.38 ± 3.65 | 1333 ± 97 |
| | 2 | 4.32 ± 0.78 | 1.19 ± 0.50 | 104.37 ± 1.68 | 544.3 ± 53 |
| | 4 | 6.24 ± 2.14 | 1.65 ± 0.57 | 78.33 ± 2.20 | 1277 ± 88 |
| | 6 | 5.64 ± 1.82 | 2.34 ± 0.72 | 77.41 ± 2.00 | 1335 ± 93 |
| HL-7702 | Control | 5.75 ± 1.85 | 0.30 ± 0.03 | 103.76 ± 2.84 | 1096 ± 84 |
| | 2 | 6.22 ± 0.92 | 0.32 ± 0.06 | 106.04 ± 2.10 | 1213 ± 96 |
| | 4 | 6.34 ± 1.56 | 0.31 ± 0.05 | 106.23 ± 2.06 | 1191 ± 89 |
| | 6 | 5.87 ± 0.85 | 0.31 ± 0.06 | 106.19 ± 1.86 | 1109 ± 87 |
| SMCC-7721 | Control | 5.22 ± 0.89 | 0.42 ± 0.12 | 48.44 ± 1.82 | 1076 ± 94 |
| | 2 | 5.87 ± 0.82 | 0.41 ± 0.15 | 63.65 ± 1.63 | 1117 ± 64 |
| | 4 | 6.41 ± 0.86 | 0.84 ± 0.33 | 108.78 ± 1.31 | 1273 ± 109 |
| | 6 | 7.38 ± 0.66 | 0.93 ± 0.40 | 111.62 ± 1.31 | 1518 ± 104 |

average adhesive force and roughness (as shown in Fig. 4(k)) had been obtained before and after the treatment. The results showed that they were almost unchanged before and after two, four and six hours of the treatment.

Fig. 5(a)–(d) shows the AFM images of the living SMCC-7721 cells before and after treated with the colchicine solution for two, four and six hours. Fig. 5(e)–(h) shows the corresponding distributions of the elastic modulus. The average height (as shown in Fig. 5(i)), the average elastic modulus (as shown in Fig. 5(j)), the average adhesive force and the average roughness values (as shown in Fig. 5(k)) were obtained after the data analysis. The results displayed that the average height, the average elastic modulus, the average adhesive force and the average roughness had gradual increase trends.

## 4. Discussion

There are growing evidences that many diseases are related to the changes of cell mechanical properties (Cross et al., 2007, 2008; Liu et al., 2014; Shojaei-Baghini et al., 2013). Therefore, researchers have paid more attention to biomechanical properties in recent years. In this paper, the biomechanical properties of VERO, HL-7702 and SMCC-7721 cells were measured by AFM during the same time periods before and after the treatment with the colchicine solution within six hours, in order to investigate the effects of colchicine on the nephrocytes, hepatocytes and hepatoma cells. Some extensive studies confirmed that colchicine had a destabilizing effect (Bhattacharya et al., 2016; Ravelli et al., 2004; Sivakumar, 2013) on microtubules in the cytoskeleton structure (Janke and Bulinski, 2011). In the experiment, we found that the images of VERO cells (see Fig. 3(a)–(d)), HL-7702 cells (see Fig. 4(a)–(d)) and SMCC-7721 cells (see Fig. 5(a)–(d)) changed significantly after treated with the colchicine solution within six hours. The corresponding average height (see Figs. 3(i), 4(i), 5(i) and Table 1), elastic modulus (see Fig. 3(j), 4(j), 5(j) and Table 1), roughness and the adhesive force (see Fig. 3(k), 4(k), 5(k) and Table 1) were varied with the different cells. The mechanical behavior of cells is related by cytoskeleton structure (Griffin et al., 2017; Titushkin and Cho, 2007). In this case, the cell cytoskeleton could be changed after the treatment with the colchicine solution within six hours. The results indicate that colchicine inhibits microtubule polymerization on the nanoscale.

The investigation of the changes of elasticity stiffness in biological cell progression helps to understand the individual differences between normal cells and cancer cells (Lekka and Laidler, 2009; Plodinec et al., 2012). In the experiment, compared with the control cells, the VERO cells showed a nearly sixfold increase of the elastic modulus after the treatment with the colchicine solution for six hours. Besides, the average cell height and roughness of the VERO cells were increased with the reduction of tip-cell surface adhesive force and vice versa. For the HL-7702 cells, the change of biomechanical properties was not obvious before and after the treatment. In contrast, the height, elastic modulus, adhesive force and average roughness of the SMCC-7721 cells were gradually increased after the treatment with the same colchicine solution within the same time period. Compared with the control cells, the SMCC-7721 cells showed a twofold increase of the elastic modulus after the treatment with the colchicine solution for six hours. Cell stiffness determines the deformability of cells (Cross et al., 2008; Sato et al., 1980). The deformation of the VERO and SMCC-7721 cells was more difficult after the treatment with the colchicine solution within six hours. Indeed, the average adhesive force of VERO cells was significantly enhanced after about two hours of the treatment. Then, it was obviously decreased after four hours of the treatment, and slightly decreased after six hours. Considering that the VERO cells failed to produce interferon (O'Driscoll et al., 2002; Rhim et al., 1969), we assumed that the colchicine solution stimulated the VERO cells after about two hours of the treatment but before the cells adapted to the colchicine solution after four hours of the treatment. For the SMCC-7721 cells, the increase in elastic modulus and the average adhesion fore was most pronounced after treated with the colchicine solution for four hours. It suggested that the colchicine solution worked on the SMCC-7721 cells after immersed for about four hours.

## 5. Conclusion

Compared to the HL-7702 cells, the stiffness of the VERO and SMCC-7721 cells gradually increased after the treatment with the colchicine solution within six hours. Correspondingly, the deformability of them was significantly decreased due to the biochemical alterations resulting from the colchicine solution within six hours. The greater the ability to deform cancer cells, the greater the ability to attack (Gavara and Chadwick, 2012; Li et al., 2012; Sokolov et al., 2013). Therefore, the colchicine solution had a side effect on the VERO cells within six hours and it could decrease their survival rate with the normal dose after more than six hours. However, no significant side effect was observed for the HL-7702 cells with the same concentration of colchicine within six hours. For the SMCC-7721 cells, the ability of cell invasion decreased with the decrease of its deformation ability, which reduced the ability of cell metastasis. The effect of the colchicine solution on the cells was observed after exposed for about four hours.

This work provides an accurate and rapid method for drug screening and mechanical analysis of cells in medical research. The results of this study will help to better understand human health and disease at cellular and molecular levels for disease prevention and diagnosis.



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## Conflict of interest statement

The authors have no conflicts of interest to declare.

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