Study of morphological and mechanical features of multinuclear and mononuclear SW480 cells by atomic force microscopy

Jinyun Liu^{1,2} | Yingmin Qu¹ | Guoliang Wang¹ | Xinyue Wang¹ |
Wenxiao Zhang¹ | Jingmei Li¹ | Zuobin Wang^{1,2} | Dayou Li^{1,2} | Jinlan Jiang³

¹International Research Centre for Nano Handling and Manufacturing of China, Changchun University of Science and Technology, Changchun, 130022, China
²Institute for Research in Applicable Computing, University of Bedfordshire, Luton, LU1 3JU, United Kingdom
³Scientific Research Centre of China-Japan Union Hospital, Jilin University, Changchun, 130033, China

Correspondence
Zuobin Wang International Research Centre for Nano Handling and Manufacturing of China, Changchun University of Science and Technology, Changchun, 130022, China.
Email: wangz@cust.edu.cn
Dayou Li, Institute for Research in Applicable Computing, University of Bedfordshire, Luton, LU1 3JU, United Kingdom.
Email: dayou.li@beds.ac.uk and
Jinlan Jiang, Scientific Research Centre of China-Japan Union Hospital, Jilin University, Changchun, 130033, China.
Email: jiangjinlan@jlu.edu.cn

Funding information
EU FP7, Grant/Award Number: BioRA No.612641; China-EU H2020, Grant/Award Number: FabSurfWAR Nos.2016YFE0112100 and 644971; EU H2020, Grant/Award Number: MNR4SCell No.734174; International Science and Technology Cooperation Program of China, Grant/Award Number: 2012DFA11070; Jilin Provincial Science and Technology Program, Grant/Award Number: 20140414009GH, 20140622009JC and 20160623002TC
Review Editor: Prof. Alberto Diaspro

Abstract

This article studies the morphological and mechanical features of multinuclear and mononuclear SW480 colon cancer cells by atomic force microscopy to understand their drug-resistance. The SW480 cells were incubated with the fullereneol concentrations of 1 mg/ml and 2 mg/ml. Morphological and mechanical features including the height, length, width, roughness, adhesion force and Young's modulus of three multinuclear cell groups and three mononuclear cell groups were imaged and analyzed. It was observed that the features of multinuclear cancer cells and mononuclear cancer cells were significantly different after the treatment with fullereneol. The experiment results indicated that the mononuclear SW480 cells were more sensitive to fullereneol than the multinuclear SW480 cells, and the multinuclear SW480 cells exhibited a stronger drug-resistance than the mononuclear SW480 cells. This work provides a guideline for the treatments of multinuclear and mononuclear cancer cells with drugs.

KEYWORDS

atomic force microscope, mechanical features, mononuclear cells, morphological features, multinuclear cells

1 | INTRODUCTION

A tumor cell, often contains more nuclei (Lu & Kang, 2009; Sheehy, Wakonig-Vaartaja, Winn, & Clarkson, 1974; Zhu & Friedland, 2006). The fusion of plasmodia (Beno et al., 2005; Ming, Dong, Zhong, Grevelding, & Jiang, 2006; Walter, Hoffmann, Ebeling, Haas, & Marwan, 2013), closed mitosis (Guffei et al., 2010; S. Kim et al., 2005) and

cell division (Cool et al., 1992; Kuroda & Furuyama, 1963) can lead to the coexistence of different nuclei and formation of multinuclear cells (Papadimitriou, Sforsina, & Papaalias, 1973). Studies have shown that multinuclear cells commonly exist in tumors and they are a key character for the diagnosis of cancer (Knecht et al., 2009; Knecht, Sawan, Lichtensztejn, Lichtensztejn, & Mai, 2010; W. Zhang, Lin, Ramoth, Fan, & Fidler, 2011).

Cancer treatment has been an important issue and great efforts have been devoted to the diagnosis and therapy of cancers (Babahosseini, Carmichael, Strobl, Mahmoodi, & Agah, 2015; Byun et al., 2013). Because of the nanoscale resolution of atomic force microscope (AFM) in the imaging, manipulation and measurement of biological objects and structures (Lazar et al., 2013; Nawaz et al., 2012; Sokolov, 2007; Xu et al., 2012; Zhang et al., 2013; Zhang, Aslan, Besenbacher, & Dong, 2014), it has been used to investigate morphological and mechanical features of living cells (Heu, Berquand, Elie-Caille, & Nicod, 2012; Li, Mansoor, Tan, & Lim, 2006; Liu, Song, Qu, Wang, & Wang, 2015; Sikora & Iwan, 2012). Thus, AFM can be a proper tool for the manipulation and measurement of cancer cells. Studies about the changes of morphological and mechanical features of cancer cells after the treatment with anti-cancer drugs were taken. Gaspar et al. (Gaspar, Freire, Pacheco, Barata, & Castanho, 2015) studied the effect of HNP-1 on PC-3 cells and MOLT-4 cells by AFM, and the results showed that the height decrease was caused by the HNP-1 treatment. The roughness of the cell membrane is related the cell motility, and it can be used to characterize the state of cells (Antonio, Lasalvia, Perna, & Capozzi, 2012; Girasole et al., 2007). Kim et al. (Kim et al., 2012) found by AFM that the roughness of HeLa cells was increased significantly after the treatment with paclitaxel. Pelillo et al. (Pelillo et al., 2016) investigated the adhesion and morphology features of colorectal cancer cells (HCT-116) to study their metastatic behaviors affected by ruthenium drug NAMI-A. Hayashi et al. (Hayashi & Iwata, 2015) and Ansardamavandi et al. (Ansardamavandi, Tafazzoli-Shadpour, Omidvar, & Jahanzad, 2016) evaluated the stiffness changes of cancer cells to understand the pathophysiology. Efremov et al. (Efremov et al., 2014) characterized the cell area, focal adhesion and Young's modulus distributions of tumor cells and normal cells with AFM to study their transformational changes. Hence, morphology and mechanics are significant features for the investigation of cancer cells (Cio & Gautrot, 2016; Wirtz, Konstantopoulos, & Searson, 2011). However, little work has been carried out to investigate the difference of drug-resistance between multinuclear cancer cells and mononuclear cancer cells after the treatment with anti-cancer drugs.

In this work, fullereneol ($C_{60}(OH)_x$) was used as an anticancer drug to treat SW480 colon cancer cells. The morphological and mechanical features of SW480 colon cancer cells, such as the height, length, width, roughness, adhesion force and Young's modulus, were investigated after the treatment with fullereneol to understand their drug-resistance. Different fullereneol concentrations of 1 and 2 mg/ml were used in the experiment. The details are described in the following sections.

2 | MATERIALS AND METHODS

2.1 | Culture of cells

Colon cancer cells SW480 (CBTCCAS, Shanghai, China) were maintained under 5% CO_2 at 37 °C. The cells were attached to 0.01 mg/ml Poly-L-Lysine (HyClone) treated glass bottom culture dishes (18 mm × 18 mm) and were cultured in the RPMI-1640 media (HyClone) including 10% fetal bovine serum (HyClone).

Fullereneol ($C_{60}(OH)_x$) (Baena, Gallego, & Valcárcel, 2002; Gao et al., 2011; Rade, Natasa, Biljana, Aleksandar, & Borut, 2008) has been used as antioxidants, anticancer, and antimicrobial drugs (Chaudhuri, Paraskar, Soni, Mashelkar, & Sengupta, 2009; Grebowski, Krokosz, & Puchala, 2013; Injac et al., 2008; Torres et al., 2010; Çavas, Çinkiliç, Vatan, & Yilmaz, 2014) due to the unique structures and properties. In this work, the fullereneol (Hengqiu Tech, China) concentrations of 1 and 2 mg/ml were used to treat SW480 cells for 24 hr, 48 hr, and 72 hr before the measurement experiment.

2.2 | Sample preparation

To study the morphological features of cells by AFM, cells were immersed in 2.5% glutaraldehyde for 15 min. The samples were then rinsed three times with phosphate buffer saline (PBS) and naturally dried up at the room temperature and humidity. The prepared samples were stored at 4 °C in the dark.

To investigate the mechanical features of living cells by AFM, the cell culture glass slides were washed three times by the PBS to sweep away the suspend cells, and then immersed in RPMI-1640 media to keep the living environment. All steps were taken in a super-clean bench.

2.3 | AFM

An Agilent 5500 AFM system was used for the measurement of morphology features (height, length, width and roughness) of SW480 cells in the air, at a temperature of 23 °C and a humidity of 50%. The tapping mode was used for imaging, the scanning range was varied from 20 μm × 20 μm to 90 μm × 90 μm , and the scanning speed was 0.2 line/s. The tip radius was 10 nm. The thickness of cantilever was 4 μm , and the width and length were 30 μm and 125 μm , respectively. The normal spring constant of cantilever was 40 N/m, and the resonance frequency was 300 kHz (Tap300, BudgetSensors). To analyze the changes of height, length, width and roughness of mononuclear cells and multinuclear cells after the treatment with fullereneol, and study their drug-resistance, 10–28 mononuclear cells and multinuclear cells were selected from each groups and measured by AFM. The height, length, width and roughness features of each group of cells were obtained by image processing software “Pico Image Expert 6.2” from Agilent (USA).

A JPK AFM system (NanoWizard®3, Germany) was used for the measurement of the mechanical features (adhesion force and Young's modulus) of SW480 living cells in the RPMI-1640 medium. The quantitative imaging (QI) mode was applied to obtain the force mapping images of the living cells and the loading speed is 200 $\mu m/s$. The scan rate was in the range of 0.5–0.8 Hz. The probe used in the experiments was MLCT (BRUKER). The tip radius was 20 nm. The thickness of cantilever was 0.55 μm , and the width and length were 20 and 225 μm , respectively. The resonance frequency of cantilever was 15 kHz and the spring constant was 0.03 N/m. For measuring of the adhesion force, at least 10 cells were detected in each of the groups and 15 force curves were recorded for each cell. The AFM force-displacement

curves were obtained from measuring the nucleus areas of cells (Cao, Sui, & Sun, 2013). Five cells were studied in each of the groups and 100 measurements were made for each cell to investigate the Young's moduli. The distributions of Young's moduli were obtained by Gaussian curve fitting. The probing positions were the nucleus areas of cells. The adhesion forces and Young's moduli were obtained by image processing software "JPKSPM Data Processing" from JPK Instruments AG (Germany).

2.4 | MTT assay

SW480 cells were planted (2×10^4) in a 96-well plate and incubated at 37°C for 48 hr. They were then treated with 1 mg/ml and 2 mg/ml of fullereneol and incubated for another 24 hr, 48 hr, and 72 hr. After that, 20 μ l of 5 mg/ml dimethyl thiazolyltetrazolium bromide (MTT) was added to each well. After the incubation at 37°C for 4 hr, all media were aspirated and each well was treated with the 150 μ l DMSO solution. Finally, the cell viability was measured using the microplate reader (Sanco, China) at an absorbance wavelength of 570 nm. Each treatment was repeated three times.

2.5 | Statistical analysis

Statistical analysis was performed by SPSS 18.0 software. One-way analysis of variance (ANOVA) was used to evaluate the data distribution. Data were shown as Mean $\pm$ SD. The value of $p < 0.05$ was considered significant and $p < .01$ was considered highly significant.

3 | RESULTS AND DISCUSSIONS

Figure 1a–c show the optical images of SW480 cells of the control group and the two groups incubated with the fullereneol concentrations of 1 and 2 mg/ml for 48 hr. The MTT assay was used to evaluate the growth inhibitory effects of fullereneol and the results of cell viability distributions of SW480 cells were treated with two concentrations of fullereneol for 24 hr, 48 hr, and 72 hr as shown in Figure 1d. It can be observed clearly from Figure 1a–c that with the increase of the concentration of fullereneol, the number and the length of cells are declined. From Figure 1d, it can be seen that the viabilities of SW480 cells after the treatment with 2 mg/ml fullereneol are smaller than those of the cells treated with 1 mg/ml fullereneol.

It is known that the morphology property change is a good way to evaluate the drug-resistance of cancer cells (Cai, Gao, Cai, Wu, & Deng, 2009). In this work, morphological images of SW480 cells were obtained by AFM. Figure 2a–c show the AFM morphological images of control group, 1 and 2 mg/ml fullereneol treatment for 48 hr groups of SW480 cells. It has been found that the shapes of SW480 cells untreated with fullereneol are normal, but the shapes of cells treated with the fullereneol have been changed. The color bar indicates the height of the cells. Figure 2d is the 3D structure of mononuclear SW480 cell. Figure 2e,f show the 3D structures of multinuclear cells. It can be clearly seen that there are two nuclei in Figure 2e and three nuclei in Figure 2f. From the 3D structures of cells, many nano/micro scale characters of cells can be obtained (Labernadie, Thibault, Vieu, Maridonneau-Parini, & Charrière, 2010).

FIGURE 1 Optical images and viabilities of SW480 cells after the treatment with two concentrations of fullereneol. (a) Control group, (b) treatment with 1 mg/ml fullereneol, (c) treatment with 2 mg/ml fullereneol, and (d) distributions of SW480 cell viabilities ($p < 0.05$, when compared with the control group) [Color figure can be viewed at wileyonlinelibrary.com]

FIGURE 2 AFM images of mononuclear and multinuclear SW480 cells incubated with fullereneol for 48 hr. The (a-c) are the SW480 cells of control group, treated with 1 mg/ml and 2 mg/ml fullereneol, respectively. The image resolution is 512×512 pixels. (d) is the 3D structure of mononuclear SW480 cell. (e) and (f) are the 3D structures of multinuclear SW480 cells [Color figure can be viewed at wileyonlinelibrary.com]

Figure 3 shows the distributions of height, length, width and roughness of mononuclear and multinuclear SW480 cells in the control group and the two groups treated with fullereneol for 48 hr. Figure 3a is

the mononuclear SW480 cell and (B) is the two-nuclear SW480 cell. From the optical images, the number of nuclei can be seen clearly and they are labeled by arrows. The height distributions of the mononuclear

FIGURE 3 Effects of fullereneol on the morphological features of SW480 cells. (a) and (b) are the optical images of mononuclear and multinuclear SW480 cells, respectively. (c) and (d) are the height and length distributions of mononuclear and multinuclear SW480 cells. (e) and (f) are the width and roughness distributions of mononuclear and multinuclear SW480 cells. $p < 0.05$, when compared with the control group [Color figure can be viewed at wileyonlinelibrary.com]

and multinuclear SW480 cells are shown in Figure 3c. The height of mononuclear cells in the control group is $2.16 \pm 0.29 \mu\text{m}$, and after 2 mg/ml fullereneol treatment it is increased to $3.32 \pm 0.15 \mu\text{m}$. The height of multinuclear cells in the control group is $1.74 \pm 0.20 \mu\text{m}$ and it is changed to $2.47 \pm 0.38 \mu\text{m}$ after 2 mg/ml fullereneol treatment. The length distributions of mononuclear and multinuclear SW480 cells are shown in Figure 3d. The length of mononuclear cells in the control group is $41.78 \pm 1.90 \mu\text{m}$, and after 2 mg/ml fullereneol treatment it is decreased to $10.13 \pm 1.02 \mu\text{m}$. The height of multinuclear cells in the control group is $46.82 \pm 3.68 \mu\text{m}$ and it is changed to $30.32 \pm 6.36 \mu\text{m}$ after 2 mg/ml fullereneol treatment. The width distributions of mononuclear and multinuclear SW480 cells are shown in Figure 3e. The width of mononuclear cells untreated with fullereneol is $10.36 \pm 1.39 \mu\text{m}$, and after 2 mg/ml fullereneol treatment it is decreased to $8.31 \pm 1.03 \mu\text{m}$. For the multinuclear SW480 cells, the width in the control group is $10.78 \pm 2.64 \mu\text{m}$, and after 2 mg/ml fullereneol treatment it is reduced to $9.27 \pm 1.03 \mu\text{m}$. The roughness distributions of mononuclear and multinuclear SW480 cells are shown in Figure 3f. The roughness of mononuclear cells untreated with fullereneol is $0.42 \pm 0.10 \mu\text{m}$, and after 2 mg/ml fullereneol treatment it is increased to $0.74 \pm 0.07 \mu\text{m}$. For the multinuclear SW480 cells, the roughness in the control group is $0.30 \pm 0.02 \mu\text{m}$, and after 2 mg/ml fullereneol treatment it is increased to $0.49 \pm 0.15 \mu\text{m}$. $p < 0.05$.

It can be seen from the AFM measurement results that the morphological features of height, length, width and roughness between the multinuclear SW480 cells and mononuclear SW480 cells are significantly different after the treatment with fullereneol. The changes of mononuclear SW480 cells are larger than the multinuclear cells. It can be concluded that the drug-resistance of multinuclear cells are stronger than that of mononuclear SW480 cells.

The mechanical properties such as the adhesion force and elasticity of cells can be used to reflect the cell viability (Cai et al., 2009; Jin et al., 2011). In this work, the adhesion force and Young's modulus of multinuclear and mononuclear cancer cells were measured to investigate their drug-resistance after the fullereneol treatment. The Hooke's law and Hertz model were used to measure the distributions of adhesion force and Young's modulus, respectively (Butt, Cappella, & Kappl, 2005; Zhao, Srinivasan, Burgess, & Huey, 2011). The adhesion force of tip-cell can be calculated by the deflection of the cantilever, and it can be expressed by the Hooke's law

$$F = k \times \Delta L \quad (1)$$

where k is the spring constant of the cantilever ($k = 0.03 \text{ N/m}$). ΔL is the deflection of the cantilever. The original Hertz theory is an approximation for the contact of the very shallow indentation of two spheres in contact. For a pyramidal tip, the Hertz model can be modified as (Rico et al., 2005)

$$F = \frac{1}{\sqrt{2}} \frac{E}{1-\nu^2} \delta^2 \tan \alpha \quad (2)$$

where F is the force. E is the Young's modulus of the sample. ν is the Poisson's ratio ($\nu = 0.5$). δ is the indentation depth calculated from the force-displacement curve. In this work, δ is in the range of 350–600 nm. α is the angle of the pyramidal tip ($\alpha = 15^\circ$).

Figure 4 shows the adhesion force images of mononuclear and multinuclear SW480 cells after the treatment with 1 mg/ml fullereneol for 48 hr. The color bar indicates the adhesion force of the cells. It can be seen that the adhesion forces between mononuclear cells and multinuclear cells are different even though they are both SW480 cells. The optical microscope images in Figure 4b,d show that the multinuclear cells are normal but the mononuclear cells are in apoptosis.

Figure 5 shows the force-displacement curves of living SW480 cells after the treatment with fullereneol for 48 hr. Figure 5b is the enlarged image of the square area in Figure 5a. The distance between the lowest position and the base line is the adhesion force of tip-cell. It can be seen that the adhesion forces between the mononuclear and the multinuclear SW480 cells are different. It can be found that the adhesion forces are declined with the increase of the concentration of fullereneol and the adhesion forces of multinuclear cells are larger than those of mononuclear cells after the treatment with fullereneol with the same concentration.

Figure 6 shows the adhesion force distributions of multinuclear and mononuclear SW480 cells in the control group and the two groups treated with the two concentrations of fullereneol for 24 hr, 48 hr, and 72 hr. For the control groups untreated with fullereneol, the adhesion forces of mononuclear cells and multinuclear cells after incubation for 24 hr are $49.4 \pm 2.5 \text{ pN}$ and $50.3 \pm 2.4 \text{ pN}$, respectively. The values are almost the same. After incubation for 48 hr, the adhesion force of mononuclear cells is $44.9 \pm 4.2 \text{ pN}$ and the adhesion force of multinuclear cells is $49.0 \pm 2.4 \text{ pN}$. After 72 hr incubation, the adhesion force of mononuclear cells is $42.7 \pm 3.2 \text{ pN}$ and the adhesion force of multinuclear cells is $47.6 \pm 1.9 \text{ pN}$. It can be seen that the adhesion force differences of multinuclear and mononuclear cells untreated with fullereneol are insignificant in the same time periods.

Figure 6a shows the SW480 cells treated with fullereneol for 24 hr, the adhesion forces of mononuclear cells and multinuclear cells after treatment with 1 mg/ml of fullereneol are $42.2 \pm 2.9 \text{ pN}$ and $48.7 \pm 1.5 \text{ pN}$, respectively. For the SW480 cells treated with 2 mg/ml of fullereneol for 24 hr, the adhesion forces of mononuclear cells and multinuclear cells are $33.9 \pm 3.5 \text{ pN}$ and $48.5 \pm 3.5 \text{ pN}$. Figure 6b presents the SW480 cells treated with fullereneol for 48 hr, the adhesion forces of mononuclear cells and multinuclear cells treated with 1 mg/ml of fullereneol are $30.6 \pm 3.6 \text{ pN}$ and $43.7 \pm 2.0 \text{ pN}$. For the SW480 cells treated with 2 mg/ml of fullereneol for 48 hr, the adhesion forces of mononuclear cells and multinuclear cells are $29.7 \pm 4.0 \text{ pN}$ and $42.4 \pm 1.1 \text{ pN}$. Figure 6c shows the SW480 cells treated with fullereneol for 72 hr, the adhesion forces of mononuclear cells and multinuclear cells treated with 1 mg/ml of fullereneol are $28.5 \pm 3.6 \text{ pN}$ and $39.4 \pm 3.0 \text{ pN}$. For the SW480 cells treated with 2 mg/ml of fullereneol for 72 hr, the adhesion forces of mononuclear cells and multinuclear cells are $26.6 \pm 3.0 \text{ pN}$ and $36.3 \pm 3.6 \text{ pN}$, respectively.

Significantly higher adhesion forces of multinuclear cells are observed than the mononuclear cells after the treatment with 1 or 2 mg/ml fullereneol for the same time periods. It can be concluded that after the treatment with fullereneol, the multinuclear SW480 cancer cells exhibit significant drug-resistance than the mononuclear cells.

FIGURE 4 Adhesion force images of mononuclear and multinuclear SW480 cells after the treatment with 1 mg/ml fullereneol for 48 hr. (a) is the adhesion force image of a mononuclear SW480 cell. The image resolution is 64×64 pixels. (c) is the adhesion force image of a multinuclear SW480 cell. The image resolution is 64×45 pixels. (b) and (d) are the detected cells captured by optical microscope [Color figure can be viewed at [wileyonlinelibrary.com](#)]

Figure 7 shows the distributions of Young's moduli of multinuclear and mononuclear SW480 cancer cells after treatment with fullereneol for 24 hr, 48h and 72 hr. The Young's moduli are calculated from 5 cells in each of groups and 100 measurements were made for each cell, as shown in Table 1. $p < 0.01$, when compared with the control group. Figure 7a shows the distributions of Young's moduli of SW480 cells exposed to fullereneol for 24 hr. For the multinuclear cells, the Young's

moduli are distributed between 150 and 400 Pa. The Mean $\pm$ SD modulus of the control group is 295.0 ± 112.1 Pa, and those of the SW480 cells treated with 1 mg/ml and 2 mg/ml fullereneol are 297.7 ± 104.3 Pa and 302.5 ± 109.8 Pa, respectively. For the mononuclear cells, the Young's moduli of control group and the group treated with 1 mg/ml fullereneol SW480 cells are distributed between 150 and 400 Pa, and there are insignificant difference between their Mean $\pm$ SD moduli,

FIGURE 5 (a) shows the force-displacement curves of living SW480 cells in culture. (b) shows the enlarged image of the square area of (a) [Color figure can be viewed at [wileyonlinelibrary.com](#)]

FIGURE 6 Adhesion force distributions of multinuclear and mononuclear SW480 cells treated with fullerene. $p < 0.05$, when compared with the control group [Color figure can be viewed at wileyonlinelibrary.com]

311.8 ± 142.3 Pa and 310.3 ± 113.8 Pa, respectively. The Young's moduli of mononuclear SW480 cells treated with 2 mg/ml fullerene are distributed between 200 and 500 Pa and the Mean $\pm$ SD modulus is 357.8 ± 111.8 Pa. It can be observed that after 24 hr treatment with fullerene, the Young's moduli distributions of multinuclear cells and their Mean $\pm$ SD values were not significantly changed. However, there is a significant difference in Young's moduli of the mononuclear cells treated with 2 mg/ml fullerene group.

Figure 7b shows the distributions of Young's moduli of SW480 cells exposed to the fullerene for 48h. The Young's moduli of the multinuclear cells and mononuclear cells of control groups are distributed

FIGURE 7 Distributions of the Young's moduli of multinuclear and mononuclear SW480 cancer cells treated with fullerene. $p < 0.01$, when compared with the control group [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Young's modulus (Pa) of SW480 cancer cells treated with fullerene

Group		Control Group	1 mg/ml	2 mg/ml
24 hr	Multi	295.0 $\pm$ 112.1	297.7 $\pm$ 104.3	302.5 $\pm$ 109.8
	Mono	311.8 $\pm$ 142.3	310.3 $\pm$ 113.8	357.8 $\pm$ 111.8
48 hr	Multi	375.8 $\pm$ 93.6	528.4 $\pm$ 130.0	536.3 $\pm$ 125.1
	Mono	395.9 $\pm$ 113.5	538.5 $\pm$ 137.1	724.7 $\pm$ 207.1
72 hr	Multi	202.5 $\pm$ 80.2	301.1 $\pm$ 101.8	302.0 $\pm$ 95.8
	Mono	235.5 $\pm$ 83.0	316.8 $\pm$ 152.2	377.2 $\pm$ 226.5

between 250 and 500 Pa and their Mean $\pm$ SD moduli are 375.8 ± 93.6 Pa and 395.9 ± 113.5 Pa, respectively. The Young's moduli of the multinuclear cells treated with 1 mg/ml and 2 mg/ml fullereneol are distributed between 350 and 650 Pa and their Mean $\pm$ SD values are 528.4 ± 130.0 Pa and 536.3 ± 125.1 Pa, respectively. The Young's moduli among the groups of multinuclear cells treated with fullereneol showed a small variation in their distributions and Mean $\pm$ SD values. For mononuclear cells, the Young's moduli of the SW480 cells treated with 1 mg/ml fullereneol are distributed between 350 and 650 Pa and the Mean $\pm$ SD value is 538.5 ± 137.1 Pa. The Young's moduli of the SW480 cells after 2 mg/ml fullereneol treatment are distributed between 500 and 850 Pa and the Mean $\pm$ SD value is significantly increased to 724.7 ± 207.1 Pa. It can be stated that the Young's moduli of the mononuclear cells are significantly different from the multinuclear cells groups.

Figure 7c shows the distributions of Young's moduli of SW480 cells treated with fullereneol for 72 hr. The Young's moduli of the control group of the multinuclear cells and mononuclear cells are distributed between 100 and 350 Pa, and their Mean $\pm$ SD values are 202.5 ± 80.2 Pa and 235.5 ± 83.0 Pa, respectively. For the groups treated with 1 mg/ml fullereneol, the Young's moduli of multinuclear cells and mononuclear cells are distributed between 150 and 400 Pa, and their Mean $\pm$ SD values are 301.1 ± 101.8 Pa and 316.8 ± 152.2 Pa, respectively. For the groups treated with 2 mg/ml fullereneol, the Young's moduli of multinuclear cells and mononuclear cells are distributed between 150–450 Pa and 150–400 Pa, respectively and the Mean $\pm$ SD value increased from 302.0 ± 95.8 Pa to 377.2 ± 226.5 . It can be observed that after the treatment with fullereneol, there is a significant change of the Young's moduli of mononuclear cells groups.

Based on these results, it can be concluded that there are significant differences in Young's moduli of multinuclear cells compared with the mononuclear cells after treatment with fullereneol, which is responsible for the variation in their drug-resistance. Furthermore, the Young's moduli of mononuclear cells are significantly higher than those of multinuclear cells, and they were increased with the increase of the fullereneol concentration. However, the Young's moduli of multinuclear cells showed an insignificant change.

Fullereneol influenced the conformation and function of membranes of cells and deformed the protein in the binding residues (Grebowski et al., 2013; Qiao, Roberts, Mount, Klaine, & Ke, 2007). Thus, the skeleton and structure of cells were changed (Rungaldier, Oberwagner, Salzer, Csaszar, & Prohaska, 2013) and led to the alterations of mechanical properties (Ansardamavandi et al., 2016).

4 | CONCLUSION

The work reported in this article shows that there are significant differences in the morphological and mechanical features between the mononuclear and multinuclear SW480 cells after the treatment with fullereneol. The distributions of the height, length, width and roughness of the multinuclear SW480 cells untreated with fullereneol and treated with two concentrations of fullereneol are similar, but for the

mononuclear SW480 cells, their distributions are clearly different. The adhesion force of mononuclear SW480 cells is significantly less than that of the multinuclear cells after the treatment with fullereneol for the same time period. The variation of Young's modulus of mononuclear SW480 cells is significantly larger than the multinuclear SW480 cells after exposure to the fullereneol for the same time period. The AFM results indicated that the mononuclear SW480 cells were more sensitive to fullereneol than the multinuclear SW480 cells, and the multinuclear SW480 cells exhibited a stronger drug-resistance than the mononuclear SW480 cells. This work provides a guideline for the treatments of multinuclear and mononuclear cancer cells with drugs.

ACKNOWLEDGMENTS

This work was supported by EU FP7 (BioRA No.612641), China-EU H2020 (FabSurfWAR Nos.2016YFE0112100 and 644971), EU H2020 (MNR4SCell No.734174), International Science and Technology Cooperation Program of China (No.2012DFA11070), and Jilin Provincial Science and Technology Program (Nos.20140414009GH, 20140622009JC and 20160623002TC).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ORCID

Zuobin Wang <http://orcid.org/0000-0002-7168-0918>

REFERENCES

- Ansardamavandi, A., Tafazzoli-Shadpour, M., Omidvar, R., & Jahanzad, I. (2016). Quantification of effects of cancer on elastic properties of breast tissue by atomic force microscopy. *Journal of the Mechanical Behavior of Biomedical Materials*, 60, 234–242.
- Antonio, P. D., Lasalvia, M., Perna, G., & Capozzi, V. (2012). Scale-independent roughness value of cell membranes studied by means of AFM technique. *Biochimica Et Biophysica Acta-Biomembranes*, 1818, 3141–3148.
- Babahosseini, H., Carmichael, B., Strobl, J. S., Mahmoodi, S. N., & Agah, M. (2015). Sub-cellular force microscopy in single normal and cancer cells. *Biochemical and Biophysical Research Communications*, 463, 587–592.
- Baena, J. R., Gallego, M., & Valcárcel, M. (2002). Fullerenes in the analytical sciences. *Trends in Analytical Chemistry*, 21, 187–198.
- Beno, M., Hurbankova, M., Dusinska, M., Cerna, S., Volkovova, K., Staruchova, M., ... Kyrtopoulos, S. A. (2005). Multinucleate cells (MNC) as sensitive semiquantitative biomarkers of the toxic effect after experimental fibrous dust and cigarette smoke inhalation by rats. *Experimental and Toxicologic Pathology*, 57, 77–87.
- Butt, H. J., Cappella, B., & Kappl, M. (2005). Force measurements with the atomic force microscope: Technique, interpretation and applications. *Surface Science Reports*, 59, 1–152.
- Byun, S., Son, S., Amodei, D., Cermak, N., Shaw, J., Kang, J. H., ... Manalis, S. R. (2013). Characterizing deformability and surface friction of cancer cells. *Proceedings of the National Academy of Sciences of the United States of America*, 11, 7580–7585.

- Cai, X., Gao, S., Cai, J., Wu, Y., & Deng, H. (2009). Artesunate induced morphological and mechanical changes of Jurkat cell studied by AFM. *Scanning*, 31, 83–89.
- Cao, G., Sui, J., & Sun, S. (2013). Evaluating the nucleus effect on the dynamic indentation behavior of cells. *Biomechanics and Modeling in Mechanobiology*, 12, 55–66.
- Çavas, T., Çinkılıç, N., Vatan, Ö., & Yılmaz, D. (2014). Effects of fullerene nanoparticles on acetamiprid induced cytotoxicity and genotoxicity in cultured human lung fibroblasts. *Pesticide Biochemistry and Physiology*, 114, 1–7.
- Chaudhuri, P., Paraskar, A., Soni, S., Mashelkar, R. A., & Sengupta, S. (2009). Fullerene-cytotoxic conjugates for cancer chemotherapy. *ACS Nano*, 3, 2505–2514.
- Cio, S. D., & Gautrot, J. E. (2016). Cell sensing of physical properties at the nanoscale: Mechanisms and control of cell adhesion and phenotype. *Acta Biomaterialia*, 30, 26–48.
- Cool, D. E., Andreassen, P. R., Tonks, N. K., Krebs, E. G., Fischer, E. H., & Margolis, R. L. (1992). Cytokinetic failure and asynchronous nuclear division in BHK cells overexpressing a truncated protein-tyrosine-phosphatase. *Proceedings of the National Academy of Sciences of the United States of America*, 89, 5422–5426.
- Efremov, Y. M., Lomakina, M. E., Bagrov, D. V., Makhnovskiy, P. I., Alexandrova, A. Y., Kirpichnikov, M. P., & Shaitan, K. V. (2014). Mechanical properties of fibroblasts depend on level of cancer transformation. *Biochimica Et Biophysica Acta-Biomembranes-Molecular Cell Research*, 1843, 1013–1019.
- Gao, J., Wang, Y., Folta, K. M., Krishna, V., Bai, W., Indeglia, P., ... Moudgil, B. (2011). Polyhydroxy fullerenes (fullerols or fullereneols): beneficial effects on growth and lifespan in diverse biological models. *Plos One*, 6, e19976.
- Gaspar, D., Freire, J. M., Pacheco, T. R., Barata, J. T., & Castanho, M. A. (2015). Apoptotic human neutrophil peptide-1 anti-tumor activity revealed by cellular biomechanics. *Biochimica Et Biophysica Acta-Biomembranes-Molecular Cell Research*, 1853, 308–316.
- Girasole, M., Cricenti, A., Generosi, R., Longo, G., Pompeo, G., Cotesta, S., & CongiuCastellano, A. (2007). Different membrane modifications revealed by atomic force/lateral force microscopy after doping of human pancreatic cells with Cd, Zn, or Pb. *Microscopy Research and Technique*, 70, 912–917.
- Grabowski, J., Krokosz, A., & Puchala, M. (2013). Fullerene $C_{60}(OH)_{36}$ could associate to band 3 protein of human erythrocyte membranes. *Biochimica Et Biophysica Acta-Biomembranes*, 1828, 2007–2014.
- Guffei, A., Sarkar, R., Klewes, L., Righolt, C., Knecht, H., & Mai, S. (2010). Dynamic chromosomal rearrangements in Hodgkin's lymphoma are due to ongoing three-dimensional nuclear remodeling and breakage-bridge-fusion cycles. *Haematological the Hematology Journal*, 95, 030171.
- Hayashi, K., & Iwata, M. (2015). Stiffness of cancer cells measured with an AFM indentation method. *Journal of the Mechanical Behavior of Biomedical Materials*, 45, 105–111.
- Heu, C., Berquand, A., Elie-Caille, C., & Nicod, L. (2012). Glyphosate-induced stiffening of HaCaT keratinocytes, a peak force tapping study on living cells. *Journal of Structural Biology*, 178, 1–7.
- Injac, R., Perse, M., Obermajer, N., Djordjevic-Milic, V., Prijatelj, M., Djordjevic, A., ... Strukelj, B. (2008). Potential hepatoprotective effects of fullerene $C_{60}(OH)_{24}$ in doxorubicin-induced hepatotoxicity in rats with mammary carcinomas. *Biomaterials*, 29, 3451–3460.
- Jin, H., Zhong, X., Wang, Z., Huang, X., Ye, H., Ma, S., ... Cai, J. (2011). Sonodynamic effects of hematoporphyrin monomethyl ether on CNE-2 cells detected by atomic force microscopy. *Journal of Cellular Biochemistry*, 112, 169–178.
- Kim, S., Kim, R., Ryu, J., Jho, E., Song, K., Jang, S., & Kee, S. (2005). Multinuclear giant cell formation is enhanced by down-regulation of Wnt signaling in gastric cancer cell line. *Experimental Cell Research*, 308, 18–28.
- Kim, Y. S., Kim, K. S., Cho, C. H., Yoon, K. S., Park, H. K., & Jung, M. H. (2012). Quantitative analysis with atomic force microscopy of cisplatin-induced morphological changes in HeLa and Ishikawa cells. *Journal of Nippon Medical School*, 79, 320–326.
- Knecht, H., Sawan, B., Lichtensztejn, D., Lemieux, B., Wellinger, R. J., & Mai, S. (2009). The 3D nuclear organization of telomeres marks the transition from Hodgkin to Reed-Sternberg cells. *Leukemia*, 23, 565–573.
- Knecht, H., Sawan, B., Lichtensztejn, Z., Lichtensztejn, D., & Mai, S. (2010). 3D Telomere FISH defines LMP1-expressing Reed-Sternberg cells as end-stage cells with telomere-poor 'ghost' nuclei and very short telomeres. *Laboratory Investigation*, 90, 611–619.
- Kuroda, Y., & Furuyama, J. (1963). Physiological and biochemical studies of effects of mitomycin C on strain HeLa cells in cell culture. *Cancer Research*, 23, 682–687.
- Labernadie, A., Thibault, C., Vieu, C., Maridonneau-Parini, I., & Charrière, G. M. (2010). Dynamics of podosome stiffness revealed by atomic force microscopy. *Proceedings of the National Academy of Sciences of the United States of America*, 107, 21016–21021.
- Lazar, P., Zhang, S., Safárová, K., Li, Q., Froning, J. P., Granatier, J., ... Otyepka, M. (2013). Quantification of the interaction forces between metals and graphene by quantum chemical calculations and dynamic force measurements under ambient conditions. *ACS Nano*, 7, 1646–1651.
- Li, A., Mansoor, A. H., Tan, K. S. W., & Lim, C. T. (2006). Observations on the internal and surface morphology of malaria infected blood cells using optical and atomic force microscopy. *Journal of Microbiological Methods*, 66, 434–439.
- Liu, X., Song, Z., Qu, Y., Wang, G., & Wang, Z. (2015). Mechanical properties study of SW480 cells based on AFM. *Cell Biology International*, 39, 972–977.
- Lu, X., & Kang, Y. (2009). Cell Fusion as a Hidden Force in Tumor Progression. *Cancer Research*, 69, 8536–8539.
- Ming, Z., Dong, H., Zhong, Q., Grevelding, C. G., & Jiang, M. (2006). The effect of a mutagen (N-methyl-N-nitro-N-nitrosoguanidine) on cultured cells from adult *Schistosoma japonicum*. *Parasitology Research*, 98, 430–437.
- Nawaz, S., Sánchez, P., Bodensiek, K., Li, S., Simons, M., & Schaap, I. A. T. (2012). Cell visco-elasticity measured with AFM and optical trapping at sub-micrometer deformations. *Plos One*, 7, e45297.
- Papadimitriou, J. M., Sforsina, D., & Papaelis, L. (1973). Kinetics of multinucleate giant cell formation and their modification by various agents in foreign body reactions. *American Journal of Pathology*, 73, 349–364.
- Pelillo, C., Mollica, H., Eble, J. A., Grosche, J., Herzog, L., Codan, B., ... Bergamo, A. (2016). Inhibition of adhesion, migration and of $\alpha 5 \beta 1$ integrin in the HCT-116 colorectal cancer cells treated with the ruthenium drug NAMI-A. *Journal of Inorganic Biochemistry*, 160, 225–235.
- Qiao, R., Roberts, A. P., Mount, A. S., Klaine, S. J., & Ke, P. C. (2007). Translocation of C_{60} and its derivatives across a lipid bilayer. *Nano Letters*, 7, 614–619.
- Rade, I., Natasa, R., Biljana, G., Aleksandar, D., & Borut, S. (2008). Bioapplication and activity of fullerene $C_{60}(OH)_{24}$. *African Journal of Biotechnology*, 7, 4940–4950.
- Rico, F., Roca-Cusachs, P., Gavara, N., Farré, R., Rotger, M., & Navajas, D. (2005). Probing mechanical properties of living cells by atomic force microscopy with blunted pyramidal cantilever tips. *Physical Review E*, 72, 021914.
- Rungaldier, S., Oberwagner, W., Salzer, U., Csaszar, E., & Prohaska, R. (2013). Stomatin interacts with GLUT1/SLC2A1, band 3/SLC4A1,

- and aquaporin-1 in human erythrocyte membrane domains. *Biochimica Et Biophysica Acta-Biomembranes*, 1828, 956–966.
- Sheehy, P. F., Wakonig-Vaartaja, T., Winn, R., & Clarkson, B. D. (1974). Asynchronous DNA synthesis and asynchronous mitosis in multinuclear ovarian cancer cells. *Cancer Research*, 34, 991–996.
- Sikora, A., & Iwan, A. (2012). AFM study of the mechanical wear phenomena of the polyazomethine with thiophene rings: tapping mode, phase imaging mode and force spectroscopy. *High Performance Polymers*, 24, 218–228.
- Sokolov, I. (2007). Atomic force microscopy in cancer cell research. *Cancer Nanotechnology*, (chapter 1), 1–17. 2007 by American Scientific Publishers' Inc. ISBN: 1-58883-071-3. Edited by Hari Singh Nalwa and Thomas Webster.
- Torres, V. M., Srdjenovic, B., Jacevic, V., Simic, V. D., Djordjevic, A., & Simplicio, A. L. (2010). Fullerenol C₆₀(OH)₂₄ prevents doxorubicin-induced acute cardiotoxicity in rats. *Pharmacological Reports*, 62, 707–718.
- Walter, P., Hoffmann, X., Ebeling, B., Haas, M., & Marwan, W. (2013). Switch-like reprogramming of gene expression after fusion of multinucleate plasmoidal cells of two *Physarum polycephalum* sporulation mutants. *Biochemical and Biophysical Research Communications*, 435, 88–93.
- Wirtz, D., Konstantopoulos, K., & Searson, P. C. (2011). The physics of cancer: the role of physical interactions and mechanical forces in metastasis. *Nature Reviews. Cancer*, 11, 512–522.
- Xu, W., Mezencev, R., Kim, B., Wang, L., McDonald, J., & Sulchek, T. (2012). Cell stiffness is a biomarker of the metastatic potential of ovarian cancer cells. *Plos One*, 7, e46609.
- Zhang, S., Andreasen, M., Nielsen, J. T., Liu, L., Nielsen, E. H., Song, J., . . . Dong, M. (2013). Coexistence of ribbon and helical fibrils originating from hIAPP20–29 revealed by quantitative nanomechanical atomic force microscopy. *Proceedings of the National Academy of Sciences of the United States of America*, 110, 2798–2803.
- Zhang, S., Aslan, H., Besenbacher, F., & Dong, M. (2014). Quantitative biomolecular imaging by dynamic nanomechanical mapping. *Chemical Society Reviews*, 43, 7412–7429.
- Zhang, W., Lin, Q., Ramoth, A. J., Fan, D., & Fidler, I. J. (2011). Formation of solid tumors by a single multinucleated cancer cell. *Cancer*, 117, 4092–4099.
- Zhao, M., Srinivasan, C., Burgess, D. J., & Huey, B. D. (2011). Rate- and depth-dependent nanomechanical behavior of individual living Chinese hamster ovary cells probed by atomic force microscopy. *Journal of Materials Research*, 21, 1906–1912.
- Zhu, X. W., & Friedland, J. S. (2006). Multinucleate giant cells and the control of chemokine secretion in response to *Mycobacterium tuberculosis*. *Clinical Immunology*, 120, 10–20.