Effect of Different Trypsin Concentrations on SMCC-7721 Cell Adhesion

Jin Yan1,2, Liguo Tian1,2, Chenchen Xie1,2, Yan Liu1,2, Zhenccheng Lu1,2, and Zuobin Wang1,2,3
1International Research Centre for Nano Handling and Manufacturing of China, Changchun University of Science and Technology, Changchun 130022, China
2Ministry of Education Key Laboratory for Cross-Scale Micro and Nano Manufacturing, Changchun University of Science and Technology, Changchun 130022, China
3IRAC & JR3CN, University of Bedfordshire, Luton LU1 3U, UK
Email: [627423023, 904522208, 472728243]@qq.com, 5514tianliguo@163.com, liuyannnnnn@126.com, wangz@cust.edu.cn

Abstract—In this work, living human liver cancer cells (SMCC-7721) were exposed to the trypsin solution at the concentrations of 1.5 mg/ml, 2.0 mg/ml, 2.5 mg/ml, 3.0 mg/ml, 3.5 mg/ml and 4.0 mg/ml, respectively. After 2 min of dosing time, the physical and mechanical properties of the cells were detected by Atomic Force Microscope (AFM). With the increase of the trypsin concentration, the adhesion force between the cell and the probe gently decreased. The maximum lateral deflection voltage variation of the probe, which reflected the maximum external force that kept the cells attached to the substrate, was significantly reduced. This work indicates that we may change the cell morphology and regulate the mechanical properties of cells by controlling the concentration of trypsin solution to treat cells, which has important implications for bioengineering and cell manipulation.

Index Terms—atomic force microscope, cell adhesion, trypsin treatment, SMCC-7721 cell

I. INTRODUCTION

Trypsin, a member of the proteases of mixed nucleophile, is a serine protease, which exists in the digestive system as a digestive enzyme [1], [2]. Clinically, trypsin is mainly used in the treatment of open infected wounds, which can purify the wound, accelerate the shedding of the degenerated and necrotic tissue, promote the formation of granulation tissue, and accelerate the healing of the wound [3], [4]. Cell adhesion is closely associated with the dynamic regulation of tissue cohesion and adhesion processes, and it is also related to the formation of tissues, growth and variation of tissue cells. Attached cells create internal tension via the actin which produces cell-substrate adhesion force [5]. The dynamic process of cell adhesion will facilitate tissue reconstruction [6]. The migration of cells is the result of de-adhesion process. Thus, the adhesion between the cells and substrate is a necessary condition for cell survival [5]. Trypsin is known to be used in the cell culture to achieve the separation of cells from the substrate and other cells. There has not been much work reported on the quantitative analysis of the effect of trypsin on cell adhesion properties. In this work, we put forward that cell adhesion properties can be regulated by controlling the concentration of trypsin, which is of great significance in bioengineering and cell manipulation, and provides support for its application in the biological field.

II. MATERIALS AND METHODS

A. Cell Culture

In the experiment, SMCC-7721 cells were seeded on the glass coverslips (18 mm×18 mm), placed in the petri dishes (38 mm in diameter). RPMI-1640 with 10% Fetal Bovine Serum (FBS) was added as the cell culture medium. Both the media and the FBS were purchased from the Sigma Chemical Company. After that, the cells were placed in an incubator at 37 °C and 5% CO2 for 48 h cell culture. Then removed the liquid from the petri dishes and gently rinsed the glass coverslips with the phosphate-buffered saline (PBS, ×1, Invitrogen, USA) to remove the residual cell metabolites and the dead cells on the sample surface.

B. Preparation of Trypsin Treatment Solution

The trypsin powder (Thermo Fisher, USA) was dissolved to 4.0 mg/ml using phosphate-buffered saline (PBS, ×1, Invitrogen, USA) as the solvent. Then the trypsin solution was filtered by the 0.22 μm filter and diluted to the concentrations of 1.5 mg/ml, 2.0 mg/ml, 2.5 mg/ml, 3.0 mg/ml, 3.5 mg/ml, and 4.0 mg/ml, respectively, as required. Before the trypsin treatment, the thermostatic water bath was heated to 37 °C to heat the trypsin solution for 3 min, so that the trypsin was at the most suitable activity [2].

C. Sample Preparation

After the cells were rinsed with the PBS, 1 ml of trypsin solution of different concentrations was added to the 6 petri dishes containing the same number of cells (1.0×10⁴ cells per coverslip), respectively. After 2 min, the solution was sucked out with a pipette. 2 ml of PBS was added to each sample, carefully and gently rinsed for
at least 30 s, repeated 3 times to remove the residual trypsin solution on the cell surface. Then 2 ml cell culture solution was added for AFM detection. We named the trypsin-treated cells as the experimental groups. The cells without the trypsin treatment were added with 2 ml of the cell culture medium as the control group.

D. MTT Assay

In this work, MTT colorimetric assay was used to determine the mitochondrial activity of SMCC-7721 cells, treated with trypsin at different concentrations for 2 min. The cells were seeded in 96-well plates (1.0×10^4 cells/well), and we set up 10 duplicate wells per group. After culturing in the 37 °C incubator containing 5% CO₂ for 24 h, the medium was discarded. 30 μl of different concentrations of the trypsin solution were added to the 6 experimental groups. After 2 min, carefully sucked the solution out of the wells, then added 200 μl of medium and 20 μl of MTT solution (5 mg/ml) to each group. After incubating for 4 h, blue-violet crystalline formazan was formed and deposited in the living cells. Then removed the medium and added the dimethyl sulfoxide solution (DMSO, 150 μl/well) to the wells. A shaker was used to shake the 96-well plate for 10 min to help the crystals fully dissolve. The optical density (OD) of each well was obtained with the microplate reader at the wavelength of 498 nm.

E. AFM Probe Lateral Deflection Voltage Measurements

In this work, the probe was purchased from the Budget Sensor, ContAl-G. The standard parameters were given by the company. The force constant ranged from 0.07 N/m to 0.4 N/m. The cantilever length was 450±10 μm. The mean width was 50±5 μm. The thickness was 2±1 μm. The tip height was 17±2 μm. Fig. 1 shows a schematic diagram of measuring the probe lateral deflection voltage of the single cell. Fig. 2 shows the measurement process.

![AFM Probe Schematic Diagram](image)

Figure 1. (A) The beam deflection method was used to record the optical signal, while the measurement was in progress. (B) The optical image before the measurement. (C) The optical image after the measurement in which the target cell was behind the probe.

III. RESULTS AND DISCUSSION

In this work, we used an AFM to detect the maximum probe lateral force deflection voltage variation on the experimental groups and the control group, 20 single cells were selected from each group for the measurement, as shown in Fig. 2a and Fig. 2b. Point P (P0, P1, P2, P3, P4, P5, P6) in Fig. 2a represents the maximum lateral deflection voltage variation of the probe that the cell can withstand when it adheres to the substrate (at this time, the probe receives the maximum deflection force). When the voltage variation is more than P, the cell is detached from the substrate. It was found that as the concentration of trypsin was increased, the maximum lateral deflection voltage of the probe was gradually decreased. Fig. 2b shows the P voltage values of the experimental groups and the control group. The SMCC-7721 cell was -809.5±78.72 mV, and the trypsin treated cells were -645.75±63.67 mV for 1.5 mg/ml, -504.16±43.52 mV for 2.0 mg/ml, -335.91±39.05 mV for 2.5 mg/ml, -241.41±33.42 mV for 3.0 mg/ml, -181.58±49.27 mV for 3.5 mg/ml and -136±30.16 mV for 4.0 mg/ml. It was indicated that under the same treatment time, the trypsin concentration effectively affected the adhesion between the cells and the substrates.

![AFM Probe Lateral Deflection Voltage Variation](image)

Figure 2. (A) (B) The maximum lateral deflection voltage variation of the SMCC-7721 cells grown on the glass. (C) The cell average spreading area. The results are given as the mean and standard deviation (±).

Many factors may affect the measurement result, including the cell incubation time, the target cell shape, the probe sliding direction, the thermal noise and the noise in the working environment. The measurement tasks were repeated with the protocol described above for the 6 test groups and the control group. In the experiment, so as to obtain reliable data, 20 single cells were selected from each group for the measurement, and the same probe was used to determine the changes of their maximum probe lateral deflection voltage variation which were caused by the trypsin solution at different concentrations, and the cells in the same group were prepared in the same batch.

In order to quantify the cell spreading, we selected 5 areas of the same size (600 μm×600 μm) in each group of samples to evaluate the average cell spreading area using Image J software (Bethesda, Maryland, USA), as shown in Fig. 2c. The average spreading area of the control
group was 779.13±60.74 μm². In the experimental groups, as the concentration of trypsin increased, it showed a downward trend as a whole. Compared with the control group, the average cell spreading area of the experimental groups with the trypsin concentration in the range of 2.5 mg/ml to 4.0 mg/ml was significantly decreased, which was 518.2±47.80 μm² for 2.5 mg/ml, 459.06±37.74 μm² for 3.0 mg/ml, 391.86±14.67 μm² for 3.5 mg/ml and 361.36±27.79 μm² for 4.0 mg/ml (732.52±35.19 μm² for 1.5 mg/ml and 671.64±41.07 μm² for 2.0 mg/ml). As the trypsin hydrolyzes the protein between the substrate and the cells, the cell adhesion is reduced. The pressure in the liquid causes the cytoskeleton to contract, and the cells appear approximately round. Therefore, the average spreading area of cells is reduced. The decrease in the cell average spreading area means that the number of the adhesion sites for the cells bonded to the substrates is reduced. It weakens the cell-substrate adhesion, making it easier for the cells to detach from the substrate under the action of external force, which is consistent with the decrease of the maximum lateral deflection voltage variation of the probe in the experiment.

In the experiment, the QI mode AFM (AFM; JPK, Germany) was used for the cell morphological imaging and the cell average surface adhesion force measurement. Fig. 3 shows the comparison of AFM imaging before and after the treatment of living SMCC-7721 cells with the trypsin solution at the concentration of 2.5 mg/ml. The lamellipodium on the edge of the cells cultured 48 h can be obviously observed in Fig. 4a, and the shape of the cells was polygonal. After the cells were treated with the trypsin solution (2.5 mg/ml) for 2 min as shown in Fig. 4b, the cell morphology contracted significantly, and the lamellipodium attached on the substrate was damaged, which could not be seen clearly. The shape of the cells also changed into round.

![Image](image_url)

Figure 3. (a) The topographic image of control cells in RPMI-1640 media. (b) Topographic image of SMCC-7721 cells treated with 2.5 mg/ml trypsin solution for 2 min. (scanning range: 60 μm×60 μm)

We selected 10 cells from each group and examined the average adhesion force of them, as shown in Fig. 4. The cells with no trypsin treatment had the maximum average surface adhesion force of 3.05±0.98 nN. As the concentration of the trypsin solution increased, the average cell surface adhesion force showed a downward trend. (2.81±0.56 nN for 1.5 mg/ml, 2.53±0.45 nN for 2.0 mg/ml, 2.63±0.53 nN for 2.5 mg/ml, 2.35±0.68 nN for 3.0 mg/ml, 2.20±0.53 nN for 3.5 mg/ml and 2.11±0.46 nN for 4.0 mg/ml) The change of the cell adhesion has an important influence on the maintenance of the cell morphology and the production of mechanical signal [7].

It implies that the different concentrations of trypsin solution have the potential applications in regulating cell surface adhesion.

![Image](image_url)

Figure 4. Adhesion force between the cell surface and the probe. The results are given as the mean and standard deviation (±).

We selected a 600×600 μm² area at the center of the substrate for cell counting. Fig. 5a shows the counting result. The number of cells in the control group was 226±37, and the number of the cells in the experimental groups were 1.5 mg/ml for 176±30, 2.0 mg/ml for 161±27, 2.5 mg/ml for 128±31, 3.0 mg/ml for 74±33, 3.5 mg/ml for 65±22 and 4.0 mg/ml for 49±15. It proves that the appropriate concentration of trypsin can regulate the number of the substrate cell binding.

![Image](image_url)

Figure 5. (a) The average number of the bonded cells in the experimental groups and the control group. (b) MTT assays. After 48 h culture, the cells were treated with the different concentrations of the trypsin solution at 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 mg/ml for 2 min for the MTT assay. The results are represented as the mean and standard deviation (±).

In order to reveal the effect of different concentrations of trypsin on cell viability, we performed the MTT colorimetric assay on each group of cells. Fig. 5b shows the percentage of the cell activity in the experimental groups compared with the control group. After the data analysis, it was obvious that the cell activity decreased gradually with the increase of the trypsin concentration under the same dosing time. The cell viability of the experimental groups with the trypsin concentration ranging from 1.5 mg/ml to 2.5 mg/ml reached 90.86%±14.29%, 85.02%±10.21% and 81.72%±6.54% of the control cells, respectively. When the trypsin concentration in the experimental groups was greater than 2.5 mg/ml, the cell activity was relatively lower than the others (77.88%±10.86% for 3.0 mg/ml, 71.27%±9.41% for 3.5 mg/ml, and 66.82%±6.74% for 4.0 mg/ml). Excessive trypsin hydrolysis of cellular proteins can
cause damage to the cells [8]. Thus, the higher the concentration of the trypsin acting on the cells, the more obvious the effect on the cell viability will be.

IV. CONCLUSIONS

This work shows a better understanding of the effect of different concentrations of trypsin on the living SMCC-7721 cell adhesion via the AFM. With the increase of the trypsin concentration, the proteolysis is enhanced, and the adhesion strength between the cells and the substrate is weakened. The cells are easier to detach from the substrate, so that the maximum deflection voltage variation of the probe is significantly reduced. The contraction of the cytoskeleton, which causes by the liquid tension, changes the shape of cells from the polygonal to circular. Trypsin acts on the cell surface, weaken the protein structure, and also reduces the cell adhesion (including the cell adhesion between the probe and the cell surface, and the cell adhesion between the cell and the substrate). The adhesion of cells is crucial for the cell transfer, shape maintenance and signal generation. It is also the basis of the physiological and pathological processes of wound healing, recombination of neurons and cancer metastasis. Thus, the method to regulate the cell adhesion is significant in cell biology, medicine, biological materials, tissue engineering and regeneration.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Jin Yan conducted the research, analyzed the data and wrote the manuscript. Liguo Tian wrote the AFM mechanical testing program. Chenchen Xie, Yan Liu, and Zhengcheng Lu cultured the cells for the experiment. Zuobin Wang proofed and gave the comment on the manuscript. All authors had approved the final version.

ACKNOWLEDGMENT

This work was supported by National Key R&D Program of China (No. 2017YFE0112100), EU H2020 Program (MNR4SCell No.734174), Jilin Provincial Science and Technology Program (Nos.20180414002GH, 20180414081GH, 20180520203JH, 20190702002GH and 20200901011SF), and “111” Project of China (D17017).

REFERENCES

[1] N. D. Rawlings and A. J. Barrett, “Families of serine peptidases,” Methods Enzymol., vol. 244, pp. 19-61, 1994.
[2] W. Kühne, “Über das Trypsin (Enzym des Pankreas),” FERS Lett., vol. 1, pp. 194-198, 1976.
[3] G. I. Cetrulo, “Use of trypsin intravenously in a gunshot wound,” Am. Med. Assoc., vol. 152, pp. 605-606, 1953.
[4] S. N. Carson, C. Wiggins, K. Overall, and J. Herbert, “Using a castor oil-balsam of Peru-trypsin ointment to assist in healing skin graft donor sites,” Ostomy Wound Manage, vol. 49, pp. 60-64, 2003.
[5] Y. S. Chu, W. A. Thomas, O. Eder, F. Pincet, E. Perez, J. P. Thierry, et al., “Force measurements in E-cadherin-mediated cell doublets reveal rapid adhesion strengthened by actin cytoskeleton remodeling through Rac and Cdc42,” J. Cell Biol., vol. 167, pp. 1183-1194, 2004.
[6] C. E. Orsello, D. A. Laufenburger, and D. A. Hammer, “Molecular properties in cell adhesion: A physical and engineering perspective,” Trends Biotechnol., vol. 19, pp. 310-316, 2001.
[7] Y. Hou, Z. Wang, D. Li, R. Qiu, Y. Li, and J. Jiang, “Cellular shear adhesion force measurement and simultaneous imaging by atomic force microscope,” J. Med. Biol. Eng., vol. 37, pp. 102-111, 2017.
[8] C. Xiong, et al., “Trypsin-catalyzed deltamethrin degradation,” PLoS One, vol. 9, article e89517, 2014.

Jin Yan is a PhD student in International Research Centre for Nano Handling and Manufacturing of China, Changchun University of Science and Technology. Her current research interest is in nanomanipulation techniques and applications in biomedicine.

Liguo Tian is an engineer at International Research Centre for Nano Handling and Manufacturing of China, Changchun University of Science and Technology. His current research interest in control science and image processing.

Chenchen Xie is a PhD student at International Research Centre for Nano Handling and Manufacturing of China, Changchun University of Science and Technology. His current research interest is in the biological application of electrospinning techniques.

Yan Liu is a postdoctoral researcher at International Research Centre for Nano Handling and Manufacturing of China, Changchun University of Science and Technology. She obtained her PhD degree in 2020 from Changchun University of Science and Technology. Her research interest is in atomic force microscopy and biological applications.

Zhengcheng Lu is a PhD student in Institute for Research in Applicable Computing, University of Bedfordshire, UK. Her research interest is in nanomanipulation for single cancer cells.
Zuibin Wang is professor and director of International Research Centre for Nano Handling and Manufacturing of China, Changchun University of Science and Technology. He is also a visiting professor with the University of Bedfordshire since 2009. He received his PhD degree from the University of Warwick in 1997. His current research interests include nano manipulation, manufacturing and measurement. He is a founding chair of the IEEE International Conference on Manipulation, Manufacturing and Measurement on the Nanoscale (IEEE 3M-NANO).