Studying on the Elasticity of White Blood Cell in Vitro by Optical Tweezers

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***Abstract*—The elasticity of white blood cell membrane which can give an expression to the activity of white blood cell is an important physiological target. The attention to the study of the inherent mechanism for white blood cell membrane elasticity is increasing. This paper reports experiments to measure the white blood cell membrane elastic modulus incorporating acousto-optic deflector(AOD) scanning optical tweezers system.**

Keywords—white blood cell; elastic modulus; optical tweezers

I. INTRODUCTION

Leukemia is a Hematopoietic stem cell disease, a highly heterogenous tumor of the hematopoietic and lymphoid tissues with more acute leukemia than chronic leukemia[1]. Leukemia is the sixth leading cause of death of malignant tumors of all ages , and ranks first among children and those under 35 years of age, posing a serious threat to human life and health[2]. Early detection, early diagnosis and early treatment are therefore important measures to improve the survival rate of acute leukemia patients. At present, the conventional diagnostic methods of acute leukemia include morphology, karyotype and immunological diagnostic methods. Especially in recent years, immunological diagnosis based on the changes of leukemic cell immune phenotype has been widely used in clinical examination[3~6]. A number of electrochemical methods, quartz crystal microbalance methods, flow cytometry, optical methods and so on based on nucleic acid fitness are developing rapidly. The development of high reliability, high sensitivity and high throughput detection capability, simple operation and low cost blood tumor cell detection technology are still urgent problems to be solved. Leukemia cells are formed when the tumor proliferates in the bone marrow or other hematopoietic tissues and then enters the peripheral blood. In general, blood cells in the peripheral blood of patients with leukemia undergo qualitative and quantitative changes, for single-celled detection, it becomes necessary. Optical tweezers can capture and manipulate particles from tens of nanometers to tens of micrometers. They can also act as tiny probes to measure the force of piconewton(pN), so the optical tweezers can be used to study the mechanical behavior of biological cells, then the basic rules of cell life activity were revealed from the single-cell level. This report studies the characteristics of the life activity of single white blood cells on the basis of optical tweezers.

II. EXPERIMENT METHODS

A. Isolating white blood cells

Take a 50 ml centrifuge tube, add a plasma sample and same amount of separation fluid to the centrifuge tube, and carefully take blood sample and add it to the liquid surface of the separator with a straw, and centrifuge for 25 minutes. The second layer of ring-like milky white layer was absorbed by a suction tube, the cells were resuscitated with 5 ml PBS solution after centrifugation, repeated washing, centrifugation, and discarding the supematant.

B. Experiment setup

The AOD scanning optical tweezers system (Tweez250si) was purchased from Aresis Company. The key components consist of a 5000mW Nd:YAG laser source (1064 nm) connected to a 60 × water immersion inverted microscope (Nikon Eclipse Ti), highly sensitive CMOS camera (PL-B74 IU) and AOD, as shown in Fig. 1. Profiting from the AOD, the optical trap position and strength can be arbitrarily controlled just by cursor based on computer soft (Trap positioning resolution is < 0.001 nm). The experimental images are captured by CMOS camera and recorded by the computer in real time. The recorded data are subsequently analyzed by image analysis software (TweezForce) ships with the system. Handily we can calibrate optical trap stiffness by thermal noise analysis [7], then figure out the real-time force throughout the experiment.

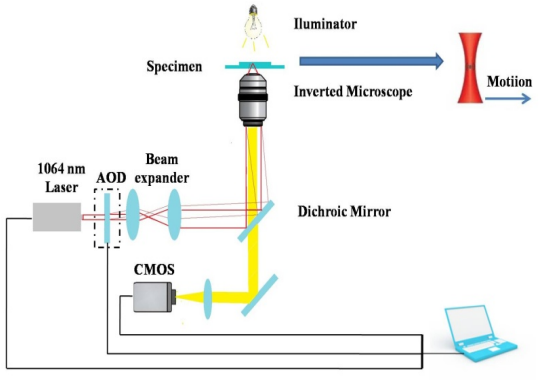

Fig. 1. The schematic diagram of AOD scanning optical tweezers system. A continuous wave laser beam emitted by Nd:YAG laser pass through the

AOD controlled by computer to achieve beam shifting. Then the beam is expanded by beam expander before it is transmitted into the microscope. The expanded beam is coupled into the optical pathway of the microscope by a Dichroic Mirror after which it is tightly focused into the sample chamber using a water immersion objective lens with Numerical Aperture (NA) of 1.0. The experimental images are captured by CMOS camera

III. Result

Fig. 2 shows a map of the changes in white blood cells that use three different laser powers to stretch the white blood cells in 4-degree environments for one day, figure 2b~2d is the maximum deformation length that white blood cells can reach when they are stretched by a laser power of 0.5w, 0.7w and 0.9w, by comparing the changes in the maximum deformation length achieved by the white blood cells in the diagram, it is found that as the laser power increases, so does the optical trap force increase, which causes the length of white blood cells to be increased.

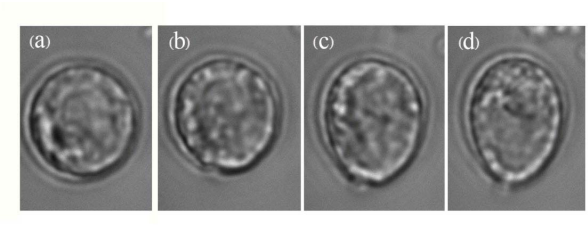

Fig. 2. Microscopy manipulation of leukocytes by using different-sized laser traps: (a) original cell; (b) cell pulled with 0.5W laser power; (c) cell pulled with 0.7W laser power; (d) cell stretched with 0.9W laser power

In the experiment, five white blood cells of different sizes were stretched with optical tweezers, and a relative elongation-tension dispersion diagram was drawn, as shown in Fig. 3, the slope of the fitting line corresponding to the white blood cell size of 8.16μm, 6.12μm, 6.55μm, 6.79μm and 5.81μm are obtained, which are 0.10158, 0.081, 0.0799, 0.1091, 0.1315, the relation formula between slope K and shear elastic modulus[8]:

$$H = \sqrt{\frac{1}{125 \cdot d \cdot B \cdot k^3}} \quad (1)$$

In which, d represents diameter of white blood cells, the elastic modulus of B=2x10 ^-19N.m. The shear elastic modulus are 6.84μN/m, 11.1μN/m, 10.95μN/m, 6.75μN/m and 5.5μN/m, respectively, the average shear modulus H=8.228±2.608μN/m of white blood cells preserved in 4-degree for one day were calculated.

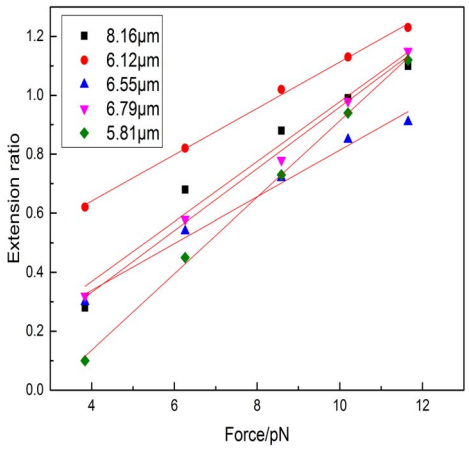

Fig. 3. Extension ratio-Force relation curves of leukocytes and fitting lines (in vitro time: 1 day)

Fig. 4 shows the observed white blood cells were kept in the 4-degree refrigerator for one day, two days, four days, six days and eight days under the optical tweezers, which shows an increase in the length of time that is kept in the 4 degree refrigerator, the cell membranes of the white blood cells gradually become mangled, preserving for one or two days to keep the cell membrane smooth and intact, but after three days of preservation the cell membrane begins to fold and bulge and the cell membrane becomes roughened until it is preserved for 8 days, the cell membrane breaks, the cell fluid overflows, and the cytoplasm changes dramatically. As the number of days of preservation increases, the contrast of color becomes more and more obvious.

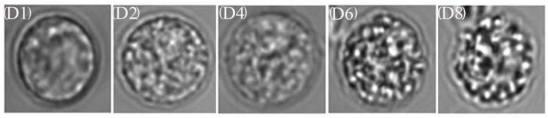

Fig. 4. White blood cell changes in different days at 4 degree environment: (D1) cell stored at 4 degree environment for one day; (D2) cell stored for two days at 4 degree environment; (D4) cell stored for four days at 4 degree environment; (D6) cell stored for six days at 4 degree environment; (D8) cell stored for eight days at 4 degree environment

IV. Conclusion

In this experiment, the membrane elasticity of leukocytes in vitro was studied based on the technique of optical tweezing micromanipulation. The shear elastic modulus of the leukocyte membrane was measured as H=8.228±2.608μN/m, in vitro preservation, it was found that as the retention time was prolonged, the cell membrane became more and more damaged and the cell fluid inside it was overflowed. Cell membrane elasticity is an important physiological index of all cells. The technique of leukocyte membrane elastic optical tweezers is very important for study of the life-activity characteristics and other clinical applications of leukocytes.

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REFERENCES

[1] Siegel R, Ma J, Zou Z, et al. Cancer statistics, 2014.[J]. Ca A Cancer Journal for Clinicians, 2015, 65(1):5.

[2] Ward E, Desantis C, Anthony Robbins MD PhD, et al. Childhood and adolescent cancer statistics, 2014[J]. Ca A Cancer Journal for Clinicians, 2014, 64(2):83-103.

[3] Rawstron A C, Fazi C, Agathangelidis A, et al. A complementary role of multiparameter flow cytometry and high-throughput sequencing for minimal residual disease detection in chronic lymphocytic leukemia: an European Research Initiative on CLL study[J]. Leukemia, 2016, 30(4):929-936.

[4] Knoechel B, Roderick J E, Williamson K E, et al. An epigenetic mechanism of resistance to targeted therapy in T cell acute lymphoblastic leukemia.[J]. Nature Genetics, 2014, 46(4):364-70.

[5] Sarma A, Hazarika M, Das D, et al. Expression of aberrant CD markers in acute leukemia: a study of 100 cases with immunophenotyping by multiparameter flowcytometry.[J]. Cancer Biomarkers, 2015, 15(4):501.

[6] Karawajew L, Dworzak M, Ratei R, et al. Minimal residual disease analysis by eight-color flow cytometry in relapsed childhood acute lymphoblastic leukemia.[J]. Haematologica, 2015, 100(7):935-44.

[7] Florin E L, Pralle A, Ehk S, et al. Photonic force microscope calibration by thermal noise analysis[J]. Applied Physics A, 1998, 66(1):S75-S78.

[8] Hamasaki N, Yamamoto M. Red blood cell function and blood storage[J]. Vox Sanguinis, 2000, 79(4):191-7.