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Distinct Patterns of Cell Motion inside a Micro-Channel under Different Osmotic Conditions

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***Abstract*— The effect of osmotic condition on a living cell inside a micro-channel is firstly studied in this work. By utilizing a high-speed camera, we observed distinct patterns of cell motion under different osmotic conditions, which are established by saline with different concentrations of sodium chloride (NaCl). The cell motions are tracked by a computer, and are presented by the coordinates of location and time (x-t chart). The motions of cells under hypotonic condition (NaCl% $< 0.9\%$) are convex curves on the chart while the ones under isotonic and hypertonic conditions (NaCl% $\geq 0.9\%$) are concave curves. Since saline is widely used in both medical practices and cell-related researches, our results point out two important facts: 1) Cells are sensitive to the percentage of NaCl. One percent difference in overall concentration makes dramatic changes in cell characteristics, such as cell stiffness. 2) The micro-channel method can clearly tell the difference between hypotonic, isotonic and hypertonic conditions according to the pattern of cell motion. Interpretations of the phenomena from different perspectives are also discussed in this paper.**

I. INTRODUCTION

Buffer solutions are widely used in biological researches. This raises a question: How is an experiment influenced by the conditions of the used buffer solution? An influenced result could lead to a misinterpretation or even a false diagnosis when it comes to medical practices. Therefore, we attempt to understand the relationship between solution conditions and cells. An experimental study on osmotic condition and cell behavior in a micro-channel is presented in this paper.

Mechanical properties of a human cell are often used as an index for disease diagnosis. It is reported that certain diseases cause the change of cell properties. For example, Malaria correlates with red blood cell (RBC) stiffening and cytoadherence, and Sickle cell anemia causes RBC stiffening and increased viscosity [1]. The stiffness of RBC is one of popular indices in practice. There are different methods for evaluating cell properties, such as atomic force microscopy [2], micro-channel method [3], optical tweezers [4] and etc. Buffer solution, usually normal saline, is needed for measuring in all these methods. The osmotic condition of saline is directed affected by the concentration of sodium chloride (NaCl), and could be changed due to the evaporation of water from the solution and other reasons. Micro-channel method is selected here because the inside of micro-channel is enclosed space, so the evaporation of water from saline is minimized comparing to other methods. Moreover, the capability of high-speed evaluation is a great advantage for statistical analysis.

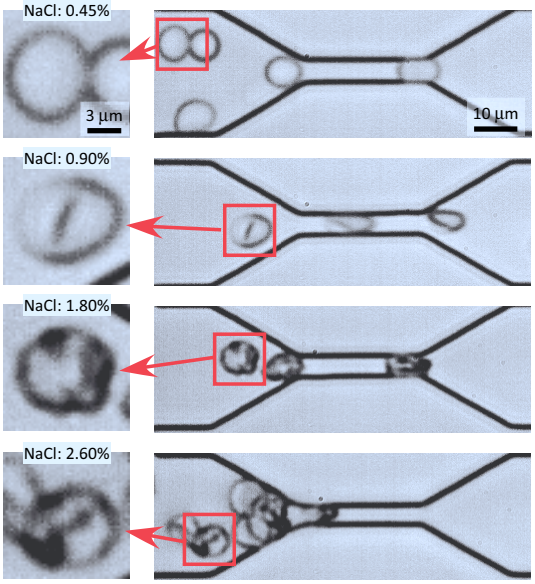

Fig. 1. The snapshots of cells passing through micro-channels in salines with different osmotic conditions, which are established by different concentrations of NaCl.

The relationship between osmotic condition and cell properties is studied by using optical tweezers by Sun *et. al* [4]. They found that the shear modulus of RBC increases with elevated osmolality. However, certain chemicals are needed for attaching the manipulable micro-beads onto the cells in optical tweezers method. There is a chance that the chemicals might affect the cell properties. On the other hand, micro-channel method has an advantage that it doesn't require any assistance from additional chemicals. To the best knowledge of the authors, this is the first work on the effect of osmotic condition on RBCs by using micro-channel method.

Four different salines with different osmotic conditions are used in the experiment. The osmotic condition is established by different concentrations of NaCl. From the results, we found that RBCs are sensitive to the change of osmotic condition, and 0.45% changes of overall NaCl concentration resulted in a dramatically different results. Furthermore, the patterns of cell motion inside a micro-channel is observed with different osmotic conditions.

The rest of this paper is organized as follows. We firstly explain the experimental setup, procedure and results in Section II. The discussions on the experimental results are in Section III. Finally, this paper is concluded in Section IV.

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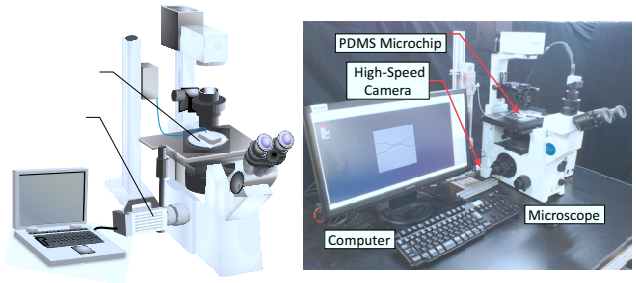

(a) The diagram of the experimental system (b) The photo of the experimental system

Fig. 2. The experimental system is constructed by a PDMS microchip, a microscope, a high-speed camera and a computer.

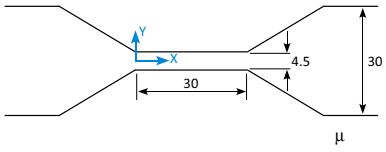

Fig. 3. The dimensions of the micro-channel are $4.5\mu m$ in width, $30\mu m$ in length and $5\mu m$ in depth. The blue arrows indicate the coordinate system for RBC position, and its origin (0, 0) is at the entrance of channel.

II. EXPERIMENT

A. Experimental Setup

The experimental system is constructed by a microscope (Olympus, IX71), a microchip, a high-speed camera (Photron, FASTCAM MH4-10K) and a computer as shown in Fig. 2(a) and (b). The high-speed camera is set to capture the images at the rate of 5000 frames per second (fps). The spatial resolution of captured images is 0.24 μm/pixel. While the diameter of RBCs is ranged from 6 to 10 μm and the time for passing through a micro-channel is from 2 *ms* to 45 *ms*, the experimental system is sufficient of observing and recording the cell motion inside a micro-channel. Micro-channels are fabricated inside a Polydimethylsiloxane (PDMS) microchip, and the dimensions of the channel are $4.5\mu m$ in width, $30\mu m$ in length and $5\mu m$ in depth as shown in Fig. 3. The defined coordinate of RBCs is originated at the entrance of the micro-channel, as indicated by blue arrows in the figure. Salines with four different concentrations of NaCl were prepared, and the concentrations are 0.45%, 0.9%, 1.8% and 2.6%. Since 0.9% is normal saline, the osmotic condition is called *isotonic*. The concentration lower than 0.9% is called *hypotonic* while the higher ones are called *hypertonic*. The blood is obtained from a healthy donor in a certified hospital 30 minutes before the experiment.

B. Experimental Procedure

The step-by-step procedure of experiment is described as follows:

1) The raw blood is mixed with four different salines at the blood-saline ration of 1:100 in room temperature. The mixtures were placed on a rotator for 20 minutes for the RBCs to fully react with the four osmotic conditions.
2) The blood-saline mixture is injected into the micro-channel, and the flow inside the channel is established by given an initial pressure from the inlet of the channel.
3) The flow rate inside the micro-channel is controlled by the pressure difference between the inlet and outlet of the microchip. When the pressure difference is increased, the flow rate inside the channel increases, and vice versa. The pressure is kept the same for all tests at 30 $cm \cdot H_2O$, approximately 2.9 kPa.
4) The image frames of cell passing through the micro-channel inside the microchip are recorded through the microscope by the high-speed camera, and the cell motion inside the channel is tracked by a computer using Matlab afterward.

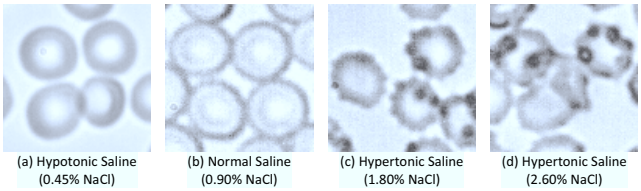

Fig. 4. The images of RBCs under different osmotic conditions. The concentrations of NaCl are (a) 0.45%, (b) 0.9%, (c) 1.8% and (d) 2.6%

C. Experimental Results

1) The shape of RBCs: The images of cells in salines with NaCl concentration of 0.45%, 0.9%, 1.8% and 2.6% are shown in Fig. 4(a), (b), (c) and (d), respectively. According to [5], the shape of a RBC in a healthy body is a biconcave disk with a flattened center, and it is similar to the ones in the normal saline(0.9% NaCl) shown in Fig. 4(b). When cells are placed in hypotonic saline (0.45% NaCl), water osmoses from the saline into the cells due to the osmotic pressure. As a result, the cells are "filled up" with water, and become spherical as shown in Fig. 4(a). On the other hand, when the RBCs are put in the hypertonic salines (1.8% or 2.6% NaCl), the water inside the cells osmoses out. Hence, the cells become flat, and wrinkles on the edge are appeared as shown in Fig. 4(c) and (d).

2) The passing time of RBCs: Figure 5 shows the distribution of passing time. The distribution of cells under hypotonic, isotonic and hypertonic conditions are in blue, green and red lines, respectively. Since the RBCs in the saline with 2.6% NaCl is stuck at the entrance of the channel as shown in Fig. 1, there is only the results from the other three conditions. The passing time of RBCs in isotonic saline are all within the range 4 ± 1.5 [ms], while the time of the ones in hypotonic and hypertonic salines are more widely spread, and the value goes up to 40.8 [ms]. The passing time of a cell through a micro-channel is utilized as the index of cell stiffness in conventional methods, such as [6], [3]. A stiff cell takes longer time to pass through a channel, and a compliant one takes less time. it is on the ground that the resisting force, which is between the cell and the channel along the direction against cell motion, is positively correlates to the stiffness of the cell [7]. According to this, the experimental results indicate that the cell stiffness is uniform in the isotonic condition, but becomes divergent in hypotonic

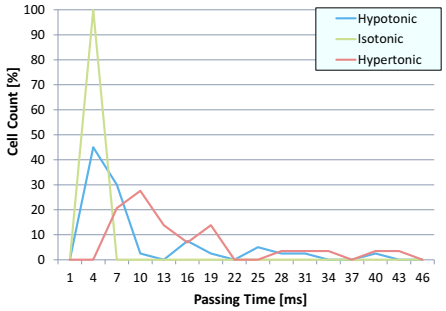

Fig. 5. The distribution of passing time in hypotonic, isotonic and hypertonic conditions.

and hypertonic conditions. Furthermore, the value of stiffness is significantly increased when the concentration of NaCl is changed, either increased or decreased.

3) The Motion profile of RBCs: The motions of cells inside the micro-channel are tracked frame-by-frame using a computer, and are presented in Figs. 6(a), (b) and (c) for hypotonic, isotonic and hypertonic conditions, respectively. There is no motion profile of cells under the saline of 2.6% NaCl because the cells were stuck at the entrance of the channel. The horizontal axis in the figures indicate the elapsed time from cell entering the channel, and the vertical axis is the distance between the instantaneous location of cell and the entrance of the channel. Each mark on the chart represents a time-location point tracked from a captured frame. Figures. 6(a), (b) and (c) are plotted in the same scale for the convenience of comparison. A series of images of a selected motion profile in each condition is shown on the right of the charts. The marks of red crosses and green triangles represent the transient phase and equilibrium phase as defined in [3], respectively.

Interestingly, the motion profiles are totally different from one to another. The motion profile of transient phase are convex curves under hypotonic condition, and are concave curves under hypertonic and isotonic conditions. The slopes of equilibrium phase are greater in hypertonic and isotonic conditions than hypotonic condition, and it shows that the cells move slower inside the channel in hypotonic condition. For the RBCs in isotonic condition, they pass through the channel fast and smoothly, and the equilibrium state is reached within 3 μm from the entrance of the channel for all RBCs.

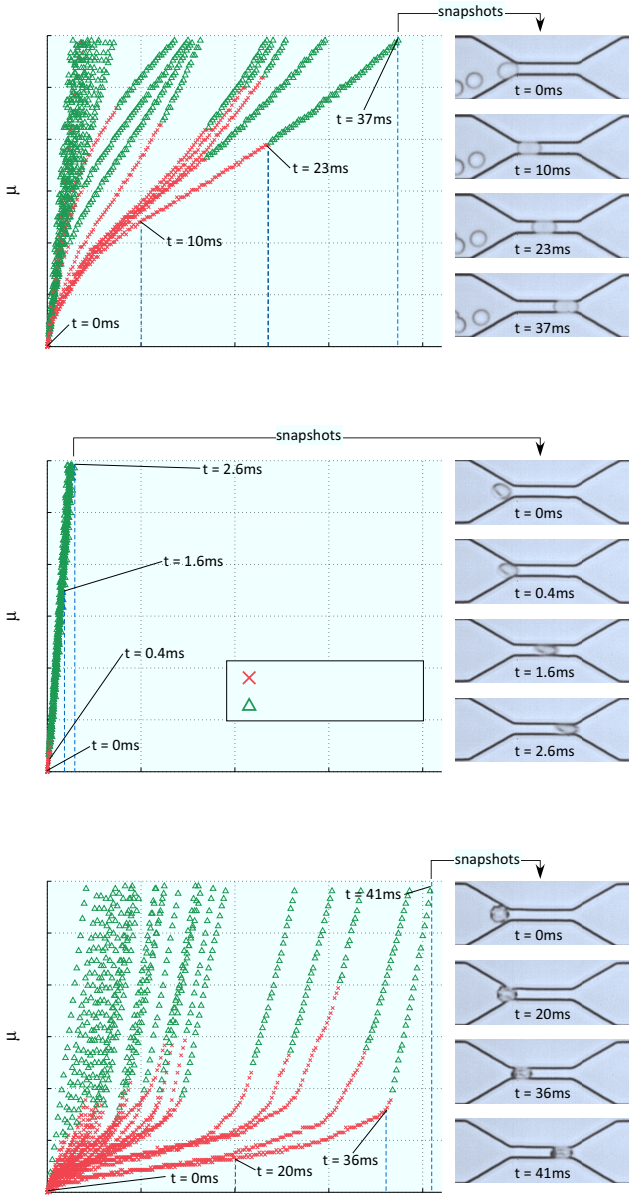

Fig. 6. The motion profiles of RBCs passing through a micro-channel in (a) hypotonic condition (NaCl 0.45%), (b) isotonic condition (NaCl 0.9%) and (a) hypertonic condition (NaCl 1.8%).

III. DISCUSSION

A. Collapse of RBCs

Some of RBCs were collapsed while passing through a micro-channel under the hypotonic condition. Figure 7 is an example of a collapsing RBC inside a micro-channel. This phenomenon can be explained by the changing of internal pressure in hypotonic condition. The internal pressure is increased due to osmosis, and it stretches the membrane like a water balloon. Thus the membrane becomes thinner and fragile. In addition, a higher internal pressure results in a greater contact force on membrane against the channel wall while moving inside a channel. Finally, these two effects lead to the collapsing of RBCs.

B. The Patterns of Cell Motion

According to the experimental results of cell motion presented in Section II, the patterns of cells moving inside a micro-channel are summarized in Fig. 8. The patterns for the RBCs in hypotonic, isotonic and hypertonic conditions are plotted in the red dotted, green solid and blue dashed line, respectively. All three patterns eventually concludes to a equilibrium state, where the cell moves in a constant velocity as the linear segments shown in the figure. The final equilibrium velocities of isotonic and hypertonic condition is

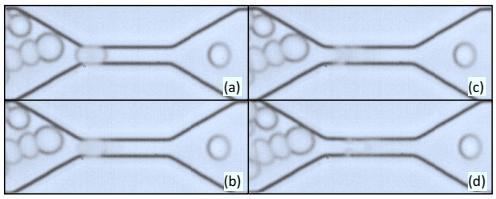

Fig. 7. The cell under hypotonic condition collapsed while passing through the micro-channel

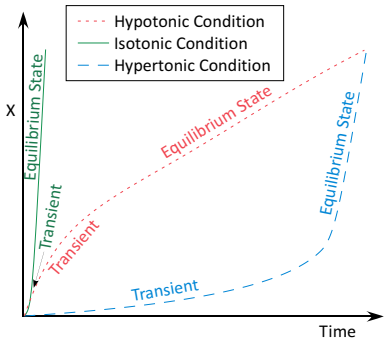

Fig. 8. The patterns of cell motion inside a micro-channel under different osmotic conditions are summarized. The patterns of cells in hypotonic, isotonic and hypertonic conditions are in the red dotted line, green solid line and blue dashed line, respectively.

relatively higher than the one of hypotonic condition. This is believed due to greater internal pressure, which generates a greater resisting force against cell motion from the contact of RBCs and channel walls in hypotonic condition.

On the other hand, The transient state are different for all three conditions. The transient state of RBCs in isotonic condition is immediately finished right after entering the channel, but it takes much longer time for the ones in hypotonic and hypertonic conditions. The patterns of cell motion in hypotonic and hypertonic conditions on x-t chart are convex and concave curves, respectively. For interpreting this observation, we postulate that the cell stiffness can be separated into two components, the membrane stiffness and the stiffness due to internal pressure, as shown in Fig. 9. The membrane stiffness is the combined effect of the stiffness from cell cytoskeleton formed by microtubules, micro filament and etc. The internal pressure inside a cell is assumed to be uniformly distributed. A latex rubber balloon filled with water is a good analogy for this model, while rubber and pressure of water inside act as the membrane and internal pressure, respectively. Based on this hypothesis, the concave-shaped pattern in hypertonic condition can be interpreted as the folding of membrane. Once the cell is folded to a size less than the cross-sectional area of the channel, the contact force suddenly dropped, and the cell rush through the rest of the channel. It is because that most of water osmosed out in hypertonic condition, and internal pressure becomes low. Oppositely, the internal pressure for RBCs in hypotonic condition is greater than other two conditions, and becomes even larger while squeezing into a micro-channel because of the compression of the cells. The convex curves in x-t chart show the period while the forces pushing forward

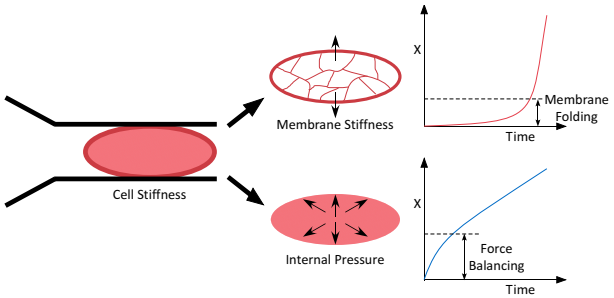

Fig. 9. The stiffness of a cell is affected by its membrane stiffness while being folded and the internal pressure of the cell.

and backward are trying to balance each other. The relations between the two components of stiffness and x-t chart are shown in the diagrams on the right of Fig. 9.

IV. CONCLUSIONS

We conclude following three main observations based on the experimental results presented in this paper:

1) RBCs are sensitive to osmotic condition. The cell characteristics are significantly changed with 0.45% change of NaCl in overall concentration.
2) The RBCs takes much longer time to pass through a micro-channel when they are in hypotonic or hypertonic conditions. It indicates that the cell stiffness is heavily increased in those conditions.
3) The cell motion inside a micro-channel are very different from hypotonic to hypertonic conditions. The two conditions can be distinguished by their patterns of motion profile.

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REFERENCES

[1] G. Y. H. Lee and C. T. Lim. Biomechanics approaches to studying human diseases. *Trends in Biotechnology*, 25(3):111–118, 2007.
[2] E. P. Wojcikiewicz, X. Zhang, and V. T. Moy. Force and compliance measurements on living cells using atomic force microscopy (afm). *Biological Procedure Online*, 6:1–9, 2004.
[3] C. D. Tsai, M. Kaneko, S. Sakuma, and F. Arai. Evaluation of cell impedance using a μ-channel. In *Proc. of the IEEE Engineering in Medicine & Biology Society, EMBC*, pages 5518–5521, San Diego, USA, August 2012.
[4] Y. Tan, D. Sun, J. Wang, and W. Huang. Mechanical characterization of human red blood cells under different osmotic conditions by robotic manipulation with optical tweezers. *IEEE Transactions on Biomedical Engineering*, 57(7):1816–1825, 2010.
[5] P. B. Canham. The minimum energy of bending as a possible explanation of the biconcave shape of the human red blood cell. *Journal of Theoretical Biology*, 26(1):61–76, 1970.
[6] A. Adamo, A. Sharei, L. Adamo, B. Lee, S. Mao, and K F. Jensen. Microfluidics-based assessment of cell deformability. *Analytical Chemistry*, 84:6438–6443, 2012.
[7] Y. Hirose, K. Tadakuma, M. Higashimori, T. Arai, M. Kaneko, R. Iitsuka, Y. Yamanishi, and F. Arai. A new stiffness evaluation toward high speed cell sorter. In *Proc. of the IEEE Int. Conf. on Robotics and Automation (ICRA)*, pages 4113–4118, Anchorage, USA, May 2010.