様　式　Ｃ－１９、Ｆ－１９、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

平成　２８　年　　６　月　　７　日現在

機関番号：　１４４０１
研究種目：　若手研究(B)
研究期間：　2014～2015
課題番号：　２６８２００８６

研究課題名（和文）Enhanced Cell Evaluation by Phase Decomposition

研究課題名（英文）Enhanced Cell Evaluation by Phase Decomposition

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交付決定額（研究期間全体）：（直接経費）　　2,900,000円

研究成果の概要（和文）：A new microfluidic system to evaluate pure cell stiffness is developed using phase decomposition method. Investigations on the diseases of diabetes and multiple myoloma had been performed. Significant difference, such as deformability distribution, between patients and normal people are found.

研究成果の概要（英文）：A new microfluidic system to evaluate pure cell stiffness is developed using phase decomposition method. Investigations on the diseases of diabetes and multiple myoloma had been performed. Significant difference, such as deformability distribution, between patients and normal people are found.

研究分野：　mechatronics

キーワード：　cell deformability　microfluidics　red blood cell　disease diagnosis

１．研究開始当初の背景

Biomechanical properties of human cells are important for disease diagnosis because it has been reported that certain diseases cause the change of cell deformability. For example, the patient suffered from Asthma is found to have stiffer airway smooth muscle cell than a healthy person [1]. Evaluating cell stiffness by a microchannel becomes popular with the advance of micro/nano technology [2]. Conventional methods evaluate cell stiffness by the time of a cell passing through a microchannel [3]. Fig.1 shows an example that a relatively stiffer red blood cell (RBC) from a patient takes longer time passing through a microchannel than a relatively softer RBC from a normal subject. Cell stiffness can be roughly determined by the passing time, but the time actually contains both information of cell stiffness and viscosity. This research project aims to improve the cell evaluation for pure cell stiffness.

２．研究の目的

The research goal is to correctly evaluate cell stiffness by removing cell viscosity effect, and also to enhance the evaluation environment. Fig. 2 shows a case where two different cells have the same passing time but totally different characteristics. Cell stiffness and viscosity are represented by the spring and damper, respectively. The proposed phase decomposition method can separate cell deformability from the spring and from the damper.

Fig. 3 shows the idea of phase decomposition. A mechanical model is used for cell behavior after entering a narrow channel. The red arrows indicate the contact force while the blue arrows indicate cell velocity. When a cell just entered the channel, both cell stiffness and viscosity against the deformation, and as a result, the contact force pushing channel wall reaches a peak value. After the cell gradually adapts to the deformation, the viscosity effect slowly disappears, and the cell velocity eventually reaches to a new equilibrium value. The pure cell stiffness can be evaluated if we only focus on cell motion in the equilibrium state instead of all passing period.

The red and blue curves in the lower part of Fig.3 show the expected contact force and cell velocity, respectively. Both the contact force and cell velocity eventually reach to constant values when the viscosity effect completely faded out. In other words, if we can decompose cell motion into non-constant velocity and constant velocity parts, we can evaluate pure cell stiffness from the dynamics of constant velocity part. Furthermore, it also becomes possible to determine the viscosity from the part of non-constant velocity.

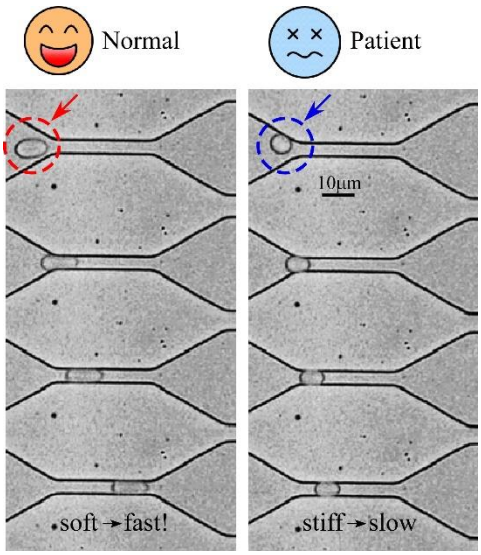

Fig.1 Conventional cell evaluation method.

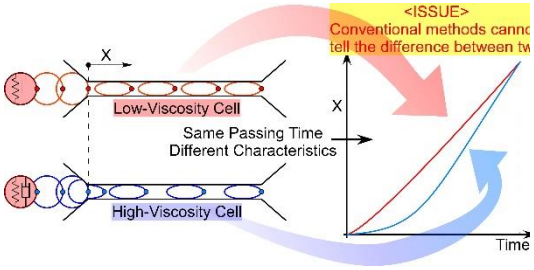

Fig.2 Phase decomposition for pure cell stiffness.

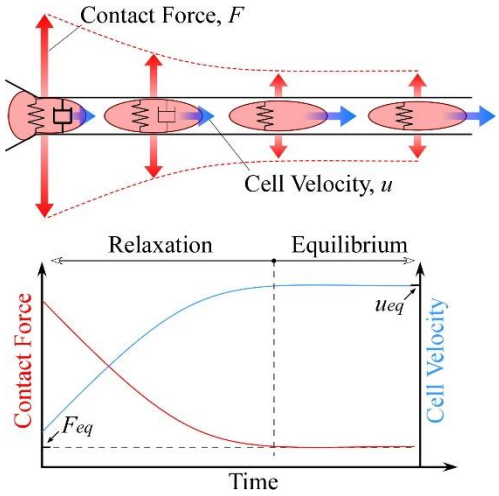

Fig.3 Mechanical model for cell and the idea.

３．研究の方法

Fig.4 shows an example of a RBC passing through a microchannel and 3 key points to improve the evaluation of cell deformability. The 3 key points are “phase decomposition”, “cell flow-in position” and “fluid pressure” as indicated. The first one is the main focus of this work, and the latter two play critical roles to improve the sensing environment. The methods to achieving these three points are explained one-by-one as follows:

(1) Phase Decomposition: The actual decomposing method is achieved based on cell motion. Fig.5 shows an example of tracked cell motion in the plot of position v.s. time. The cell positions are determined using image processing techniques from the raw image recorded by a high-speed camera. The cell is in constant velocity motion if the motion profile on x-t plot is linear, and vice versa. Based on the linearity of the motion profiles, each profile and be separated into deformation phase (red) and equilibrium phase (green) as the ones shown in Fig.5.

(2) Cell Flow-in Position: It is found that cell flow-in position significantly affect the cell overall motion through the channel. If a cell is coming along channel edge instead of central line, cell usually asymmetrically deforms, and would result in wider variation of evaluation data. Fig.6 shows the method to align the cell along central line prior to the test channel. The method is simply to place an additional channel in front of test channel, so the cells are spontaneously aligned due to fluid dynamics and cell deformation. The experimental results in Fig.6 shows cell trajectories (yellow dots) and cell velocities (arrows), and they support that the function of alignment while keeping the same velocity profiles across the microchannel.

(3) Fluid Pressure Sensing: It is important to know the pressure inside the channel for the evaluation. Since there is no such a micro-scale pressure sensor, an on-chip pressure sensor as shown in Fig.7 is developed. The working principle is based on the deformation of PDMS, which is a common material for fabricating microchannel. When pressure increases, the deformation chamber inflates and pre-filled color fluid flows into the sensing chamber. Thus, the pressure can be determined by the color intensity in the sensing chamber. The intensity increases with pressure and vice versa.

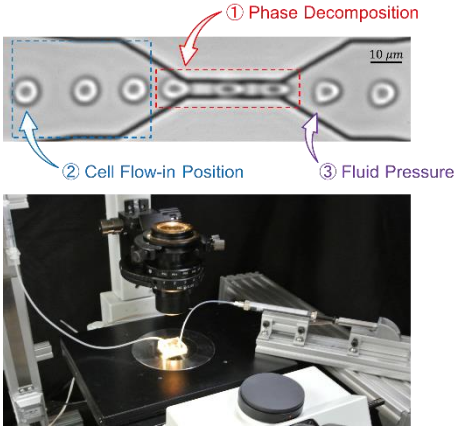

Fig.4 Three ways to enhance cell evaluation.

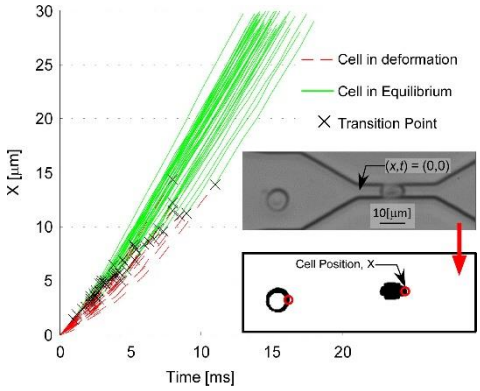

Fig.5 Cell tracking and phase decomposition.

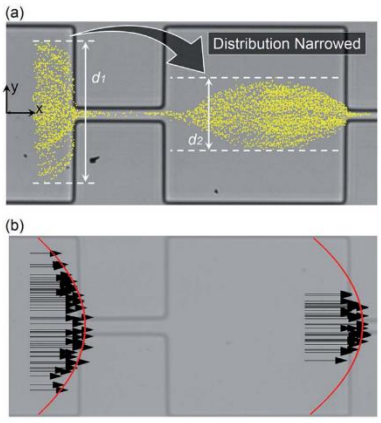

Fig.6 Cell alignment for flow-in position

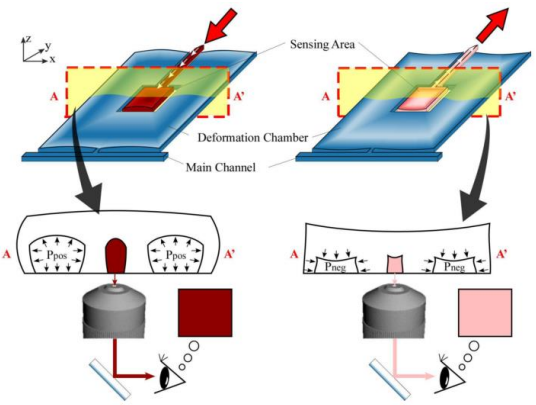

Fig.7 pressure sensing for flow monitoring.

４．研究成果

The phase decomposition and two environment control methods above are applied for evaluating cell deformability between healthy subjects and patients. A dimensionless deformability index (DI) is defined as:

$$\mathrm{DI} = \frac{D_c \lambda u_{eq}}{w^2 u_f}$$

where D_c, λ, u_{eq}, w and u_f are the undeformed cell diameter, deformed cell length, equilibrium velocity, channel width and flow velocity, respectively. The parameters are also illustrated in the modeling shown in Fig. 8.

Fig.9 shows the distribution of DI for a tested healthy subject (blue) and a diabetic patient (red). The greater DI indicate a better cell deformability (soft). The distribution between the patient and the normal subject are well distinguishable. The patient not only has lower cell deformability but also wider distribution in terms of DI range. The p value of 0.004 is obtained from a student T-test between two data, and it shows the difference between is statistically significant.

Fig.10 demonstrates another results with RBCs from a patient suffered from multiple myeloma (MM). In this test, we additionally measured the RBCs by an atomic force microscope (AFM), a gold standard method for cell stiffness evaluation. The results on the top and bottom of Fig.10 are by AFM and the proposed phase decomposition method, respectively while the left and right are the results of the patient and a normal subject. Although the average cell stiffness in AFM results seems to be similar, the distribution range of the patient is much wider than the range of the normal subject. The same results were obtained using the phase decomposition method as shown in the lower part of Fig.10 where x and y axes of the plots are cell size and cell velocity. It shows that for the same size of RBC, the velocity range of the patient is much wider than the range of the normal subject.

In summary, the phase decomposition method has been successfully developed as well as been cross checked with AFM method. The method has been applied for medical studies on the diseases of diabetes and MM. Significant difference between patients and normal people are found.

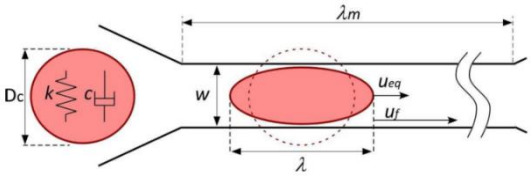

Fig.8 The parameters for deformability index.

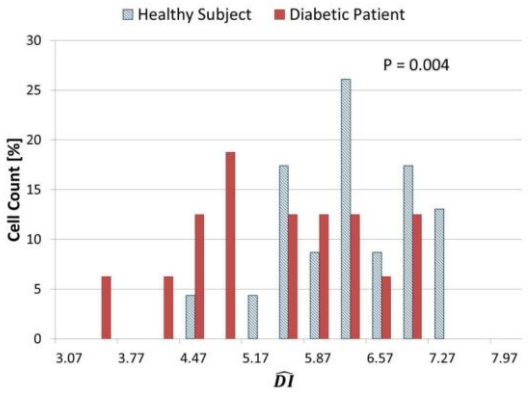

Fig.9 The results of normal v.s. diabetic patient.

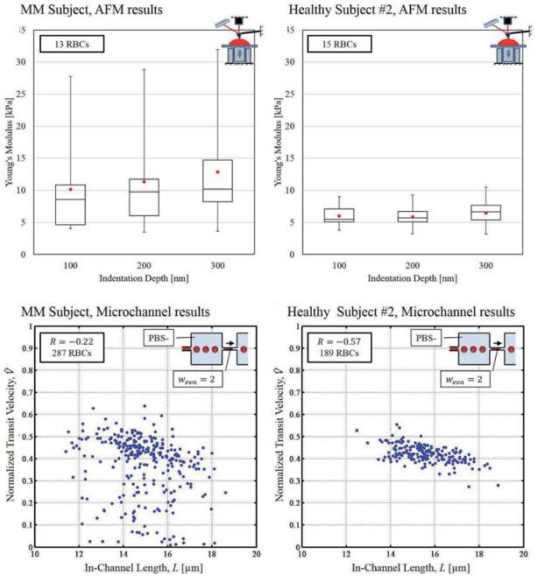

Fig. 10 The results of normal v.s. MM patient

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〔その他〕
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６．研究組織

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