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Direct Measurement of Erythrocyte Deformability in Diabetes Mellitus with a Transparent Microchannel Capillary Model and High-Speed Video Camera System

Kosuke Tsukada,* Eiichi Sekizuka,† Chikara Oshio,‡ and Haruyuki Minamitani*

**Institute of Biomedical Engineering, Faculty of Science and Technology, Keio University, Kohoku-ku, Yokohama 223-8522, Japan; †Department of Internal Medicine and Clinical Research, National Saitama Hospital, Wako-shi, Saitama 351-0102, Japan; and ‡Oshio Hospital, Kanda, Chiyoda-ku, Tokyo 101-0063, Japan*

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To measure erythrocyte deformability *in vitro*, we made transparent microchannels on a crystal substrate as a capillary model. We observed axisymmetrically deformed erythrocytes and defined a deformation index directly from individual flowing erythrocytes. By appropriate choice of channel width and erythrocyte velocity, we could observe erythrocytes deforming to a parachute-like shape similar to that occurring in capillaries. The flowing erythrocytes magnified 200-fold through microscopy were recorded with an image-intensified high-speed video camera system. The sensitivity of deformability measurement was confirmed by comparing the deformation index in healthy controls with erythrocytes whose membranes were hardened by glutaraldehyde. We confirmed that the crystal microchannel system is a valuable tool for erythrocyte deformability measurement. Microangiopathy is a characteristic complication of diabetes mellitus. A decrease in erythrocyte deformability may be part of the cause of this complication. In order to identify the difference in erythrocyte deformability between control and diabetic erythrocytes, we measured erythrocyte deformability using transparent crystal microchannels and a high-speed video camera system. The deformability of diabetic erythrocytes was indeed measurably lower than that of erythrocytes in healthy controls. This result suggests that impaired deformability in diabetic erythrocytes can cause altered viscosity and increase the shear stress on the microvessel wall. © 2001 Academic Press

Key Words: **erythrocytes; deformability; microchannel; diabetes mellitus; HbA1c.**

INTRODUCTION

It is generally known that erythrocytes change into a parachute shape in precapillary arterioles, retain their shapes through the capillaries, and change back in postcapillary venules. The mean diameters of capillaries and erythrocytes are 5 and 8 μm, respectively. Therefore, erythrocytes that are biconcave-shaped in a no-flow state must change their shape substantially when moving through capillaries (Skalak and Branemark, 1969). Figure 1 shows flowing erythrocytes axisymmetrically deformed into a parachute configuration while moving through a capillary of a rat mesentery. This image was recorded by an image-intensified high-speed video camera system. This unique characteristic of an erythrocyte transforming into a parachute-like shape in capillaries is expected for high fluidity in microcirculation and for high effi-

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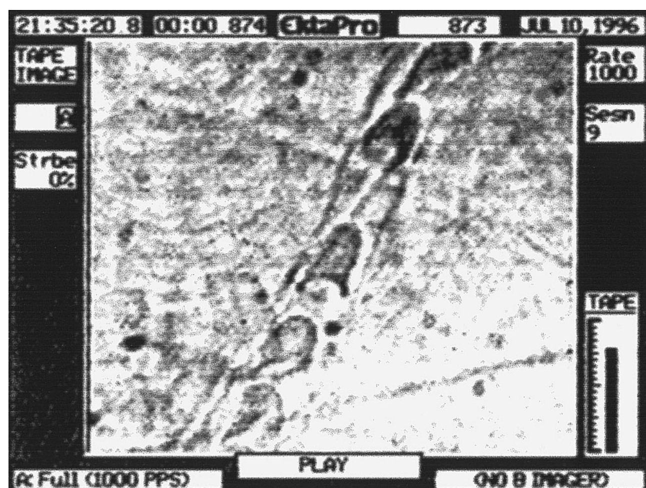

FIG. 1. Flowing erythrocytes axisymmetrically deformed in a parachute configuration in a capillary of rat mesentery. This image was recorded with a high-speed video camera and image-intensified imager.

ciency of oxygen diffusion to tissues by increasing the surface area against the endothelial cells.

Erythrocyte deformability refers to the ability of the erythrocyte to undergo a change in shape in response to a deforming force; it is a function of the geometry of the cell and the rheologic properties of the intracellular fluid and the erythrocyte membrane (Chien, 1987). A fundamental process in the supply of oxygen to tissues is the unloading of oxygen from erythrocytes in capillaries; thus oxygen change in tissues relies on blood flow in microcirculation. Erythrocyte deformability is therefore an important index of blood flow and oxygen transport in microcirculation. Reduced erythrocyte deformability could greatly disturb oxygen transport to tissues. It is argued that the reduction of erythrocyte deformability and hypoxia of tissue in some diseases are related (Parthasarathi and Lipowsky, 1999; Le Devehat *et al.,* 1991).

There have been many reports on erythrocyte deformability. The various studies have used different methods such as a rheoscope, a micropipette, a glass capillary, and the Nuclepore filter method. As a result, some problems in the quantitative analysis of deformability have arisen due to the quality of the filter material or to erythrocyte aggregation. In recent years, trials using a flow channel device made out of silicon by semiconductor processing techniques have been reported. In particular, a new technique using microchannels as a capillary model to measure blood rheology has been described. Cokelet *et al.* developed a microvascular flow system with circular cross sections by etching glass plates with mirror images of the vascular pattern (Cokelet *et al.,* 1993). Kikuchi *et al.* examined erythrocyte deformability using a silicon substrate on which 2600 microchannels were formed (Kikuchi *et al.,* 1992, 1994). Tracey *et al.* and Sutton *et al.* developed capillary models that used silicon bases and observed flowing cells (Tracey *et al.,* 1995; Sutton *et al.,* 1997). However, there have been no deformability measurements using a microchannel in which erythrocytes deform to a parachute shape in a similar manner to that seen *in vivo.* We developed transparent microchannel capillary models in which individual erythrocytes flow in microgrooves. These microchannels were used to observe erythrocyte deformations directly. We examined the ability of the microchannels to detect differences in erythrocyte deformability and investigated the deterioration of erythrocyte deformability in diabetes mellitus.

A number of studies have suggested that erythrocyte deformability decreases in diabetes mellitus, but no one has made a direct estimation of erythrocyte deformability from measurements of the shape of a single flowing erythrocyte. Several mechanisms have been proposed for the reduced erythrocyte deformability observed in diabetes. Some previous studies found impaired deformability to be related to glycemic control (increased HbA1c), a high level of intracellular sorbitol, and the stiffness of erythrocyte membranes, which refers to reduced spectrin phosphorylation or cross-linkage (Rice-Evans and Chapman, 1981), or an imbalance of the cholesterol/phospholipid ratio (Bryszewska *et al.,* 1986). It has been suggested that reduced erythrocyte deformability may contribute to microvascular complications in diabetic patients (Simpson, 1985). However, the response of blood flow to hyperglycosemia is still unknown in many processes; abnormalities in microvessels and blood rheology are expected to cause microvascular complications in diabetes.

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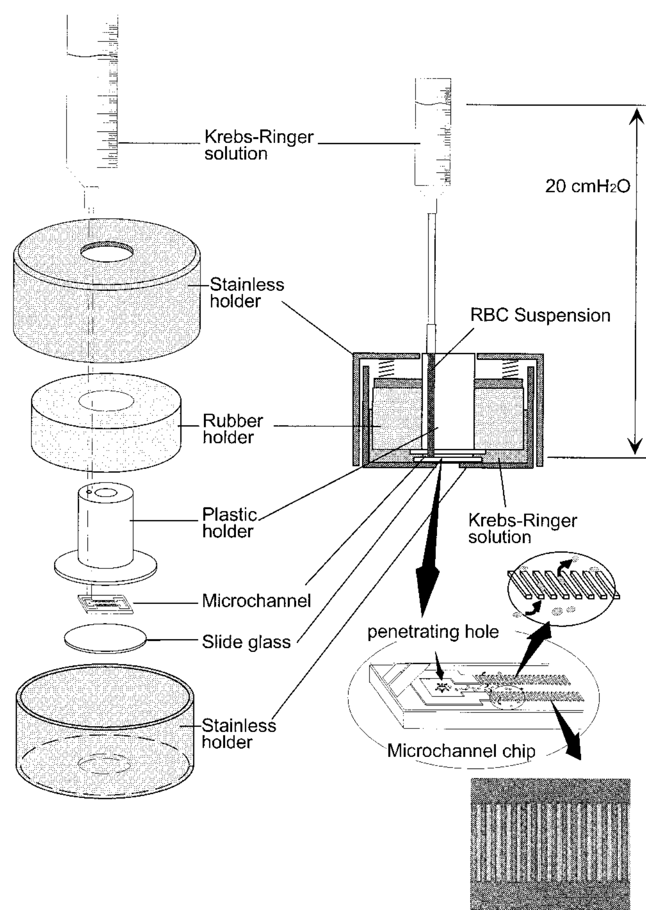

FIG. 2. Transparent crystal devices were used to observe flowing erythrocytes in microchannels. The crystal substrate was pressed against an optically flat cover glass in a holder and open grooves were closed without gaps. The holder consists of a glass plate, block, and cover cap. Each microchannel is 113 μm long, 9.1 μm wide, and 5.3 μm deep. All behaviors of erythrocytes passing through the grooves could be observed through the crystal substrate.

MATERIALS AND METHODS

Transparent Microchannels Used for Measuring Erythrocyte Deformability

Transparent microchannels on a crystal substrate and the holder are illustrated in Fig. 2. We used silicon processing techniques to construct microgrooves on a crystal glass wafer because silicon devices do not have the optical clarity to allow direct observation of flowing erythrocytes. The microchannels were commercially fabricated by Hitachi Haramachi Co., Ltd., using a dry etching method applied to a crystal glass base plate. This method enabled us to make a miniature reproduction of the required pattern. We could obtain grooves, the length and width of which could be changed by altering the design of the etching mask if necessary. The crystal substrate was pressed against an optically flat cover glass in a holder and open grooves were closed with no gaps. The microchannels shown in Fig. 2 were 113 μm long, 9.1 μm wide, and 5.3 μm deep. Pressure of 20 cm H_2O allowed erythrocytes to flow from a hole penetrating each channel. Erythrocytes flowed at a different velocity in each channel; those in channels closer to the hole flowed faster. It is convenient and effective that erythrocyte deformability could be analyzed at various velocities simultaneously. Another advantage is that the microchannels can be used repeatedly, unlike the filter method. Moreover, the flow behavior of erythrocytes passing through grooves can be observed under a microscope.

Recording Images of Flowing Erythrocytes

Microchannels in a target area were observed under a microscope. Magnified images ($\times$200) were recorded with an image-intensified high-speed video camera system (Kodak CR2000), which can process 1000 frames/s. In general, under a high-power field of the microscope, erythrocytes flow so swiftly that only a shaded belt can be seen. With a standard video system processing 30 frames/s, it is difficult to detect an individual erythrocyte image in each frame. However, the high-speed video system, processing 1000 frames/s, allows us to record clear images of individual erythrocytes (Ishikawa *et al.,* 1998). The images recorded on high-speed videotape were reproduced at a lower speed, 1/33 of the original recording speed, and then recorded on conventional videotapes. Based on the sampling times of the two tapes, one frame (1/1000 s) of the high-speed videotape corresponds to one frame (1/30 s) of the conventional videotape. The images from the video were digitized in each frame and processed using image-processing software (Tsukada *et al.,* 2000). A schematic diagram of the measurement system with a high-speed video camera is shown in Fig. 3.

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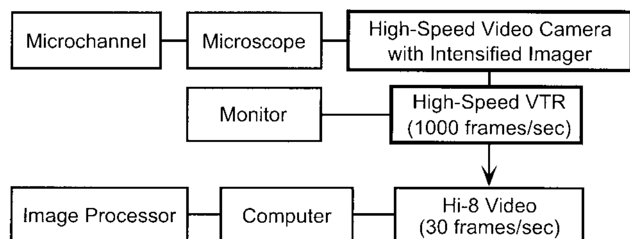

FIG. 3. Schematic diagram of the measurement system with a high-speed video camera that can record 1000 frames/s. Erythrocyte velocity is measured by off-line processing, and the deformation index is calculated automatically with an image processor.

Using the high-speed video system, we observed erythrocytes flowing in microchannels, as shown in Fig. 4. In the microchannels, erythrocytes deformed into different shapes depending on the velocity, pressure, and vessel diameter. In this study, we observed axisymmetrically deformed erythrocytes in a "parachute configuration" in order to estimate their deformability. We defined the deformation index (DI) for estimating erythrocyte deformability by Eq. (1). Here, d is the diameter of the erythrocyte deformed in the parachute configuration and l is the erythrocyte length as seen in Fig. 5. From recorded images, we selected only erythrocytes deformed in a parachute configuration; then DI was calculated automatically by a personal computer with an image processor. During the analysis, independent erythrocytes that were separate from erythrocyte clusters were selected to avoid cell–cell interactions.

$$\text{Deformation index (DI)} = l/d \qquad (1)$$

Blood Sample

Fresh heparinized venous blood was obtained from healthy adults. Plasma and packed erythrocytes were separated by centrifugation at 2500 rpm for 5 min. The erythrocytes were divided into two groups for the preparation of control erythrocytes and erythrocytes having their deformability reduced by glutaraldehyde (Simchon *et al.*, 1987). In the latter group, erythrocytes were hardened by incubation in a very dilute solution of glutaraldehyde (0.025 and 0.1%) in phosphate-buffered saline (pH 7.4) at room temperature for 30 min. The technique of hardening the membrane of erythrocytes with glutaraldehyde is often used for organ perfusate and deformability studies.

For deformability analyses of diabetic erythrocytes, fresh blood was drawn from both healthy and diabetic

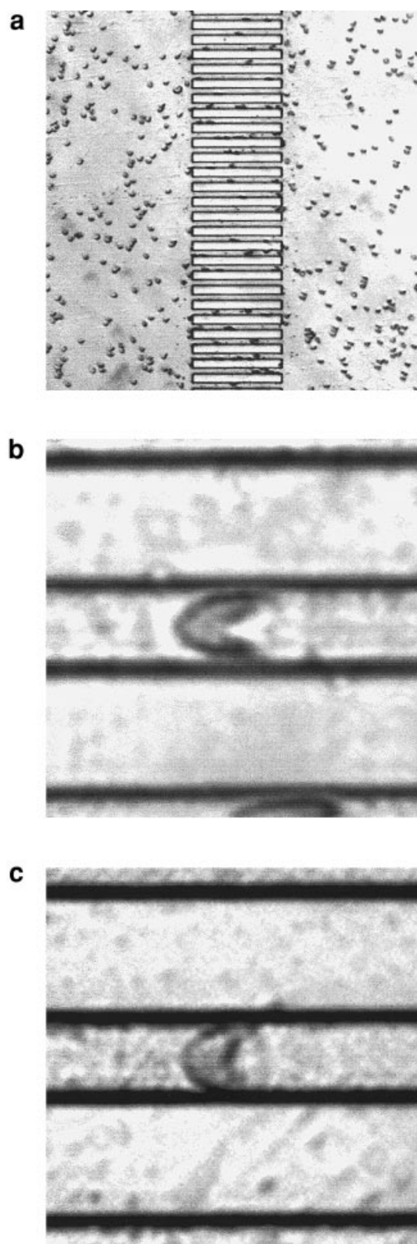

FIG. 4. Low- and high- magnification images of flowing erythrocytes in the microchannel recorded with a high-speed camera system. Microchannel is 9.1 μm wide, 113 μm long, and 5.3 μm deep. Erythrocytes went through the groove without any change in shape. The velocities in (b) and (c) are 2.13 and 1.07 mm/s, respectively.

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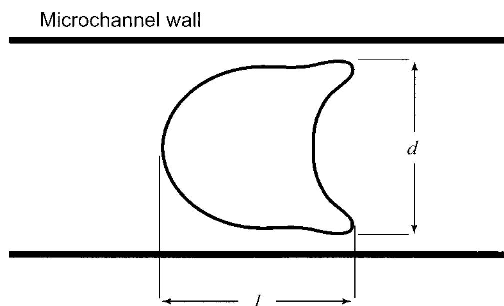

FIG. 5. Axisymmetrically deformed erythrocytes in a "parachute configuration" were used to estimate the deformability. *d* is the diameter of erythrocytes deformed in the parachute configuration and *l* is the erythrocyte length. These parameters were used to calculate the deformation index.

patients using heparin as an anticoagulant. There were 24 type-2 (non-insulin-dependent diabetes mellitus) diabetic patients, whose average age was 63.2 years. Whole blood was diluted with a physiological buffer solution to make erythrocytes flow individually in the microgrooves.

Values are expressed as the means ± standard deviations. *P* values less than 0.05 were not considered statistically significant.

RESULTS

Erythrocyte Velocity versus DI Curve in Control and Nondeformable Erythrocytes

First, we examined the relationship between erythrocyte velocity and *DI* using normal and hardened erythrocytes with a 9.1-μm-wide microchannel. The membranes of erythrocytes were hardened in a dilute solution of glutaraldehyde (0.025 and 0.1%). Figure 6 shows that as erythrocyte deformability decreased, DI did not change greatly, even when the velocity increased. There are differences in the relevance of velocity and DI. These differences represent the deterioration of erythrocyte deformability. Curve fitting with an exponential function was determined by Eq. (2), where v was erythrocyte velocity, and a and b were curve fitting parameters. DI at velocity zero (0.32) was calculated using the aspect ratio of an erythrocyte in the normal state, i.e., thickness (2.4 μm) divided by diameter (7.5 μm). In the case of decreased erythrocyte deformability, DI was less than for normal erythrocytes as the velocity became much higher.

$$\mathrm{DI} = 0.32 + a(1 - \exp(-v/b)) \quad (2)$$

Comparison with the Glass Capillary Model

To consider whether microchannels are effective as a capillary model, we compared their experimental results with ones obtained using a glass capillary tube (diameter: 9.3 μm). Figure 7 is an image of erythrocytes that passed through a glass capillary tube model, recorded with a high-speed camera. We determined the relationship between erythrocyte velocity and DI for both a glass capillary tube and microchannels. Figure 8 plots DI from capillary tube experiments against DI from microchannel experiments at equivalent velocities.

Deformability Analysis of Diabetic Erythrocytes

The difference in erythrocyte deformability between control and diabetic patients is shown in Fig. 9. The HbA1c value of this diabetic blood sample was 9.1%. A difference in DI at high velocity is evident and this indicates a decrease in deformability. At high velocities, diabetic erythrocytes had less deformability than

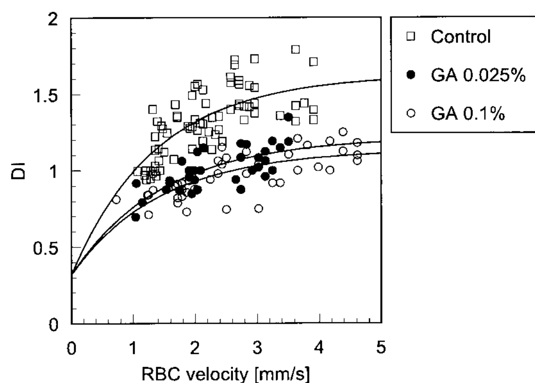

FIG. 6. Relationship between erythrocyte velocity and deformation index for healthy controls and for erythrocytes whose membranes were hardened by glutaraldehyde (GA). Curve fitting was applied with an increasing exponential function using formula (1). Correlation coefficients were $r = 0.722$ in normal and $r = 0.723$ (0.025%) and 0.686 (0.1%) in GA, respectively.

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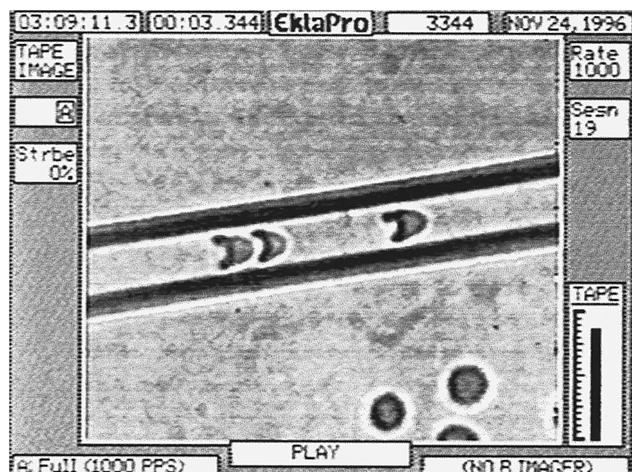

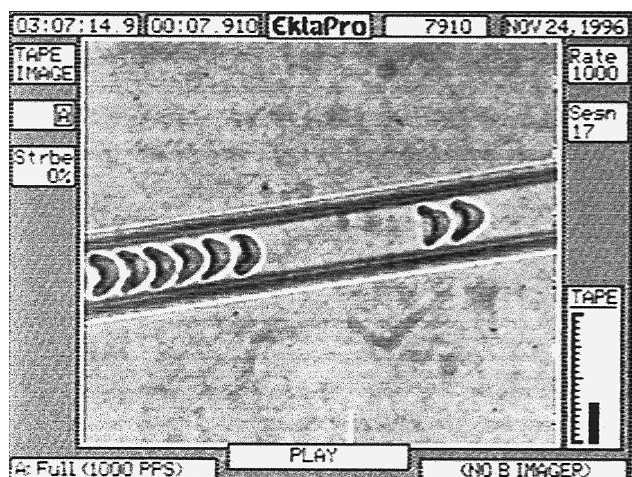

FIG. 7. Image of erythrocytes passing through a glass capillary tube model at different velocities. This was used for comparison with the microchannel.

the controls. These results indicate that when the erythrocyte velocity is high, DI represents the difference in deformability. So we calculated DI at erythrocyte velocity of 5 mm/s for each blood sample from the relationship between erythrocyte velocity and DI by curve fitting. Figure 10 shows a negative correlation ($r = -0.486$; $P < 0.05$) between HbA1c and DI at erythrocyte velocity of 5 mm/s in diabetes.

DISCUSSION

Erythrocyte deformability is a critical factor for blood rheology in microcirculation. We observed axi-

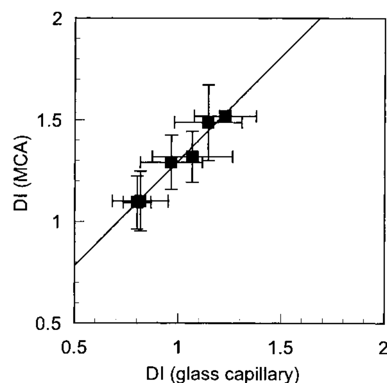

FIG. 8. Relationship between erythrocyte velocity and deformation index using a glass capillary tube and using a microchannel. Plots represent the average deformation index at 0.5 mm/s intervals. The correlation coefficient was $r = 0.984$.

symmetrically deformed erythrocytes by using a high-speed video system *in vivo* (Fig. 1) and *in vitro* (Fig. 4). As shown in Figs. 2 and 4, transparent microchannels on a crystal substrate are used as a capillary model. Using semiconductor technology, various types of microchannels have been produced experimentally during the past several years. However, to use a microchannel as the capillary vessel model, the width and height of the grooves must be about 8 μm, and the groove length should be sufficient to observe the shape of flowing erythrocytes. The groove cross-section is not circular because the groove is etched like the letter "U" and covered with a cover glass. However, comparing DI of a glass tube model with that of

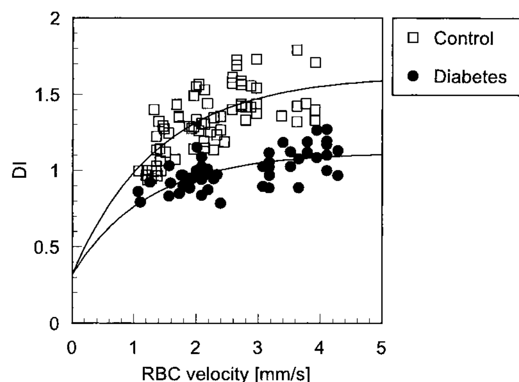

FIG. 9. Relationship between velocity and deformation index for normal and diabetic erythrocytes. The correlation coefficient was $r = 0.722$ in healthy controls and $r = 0.625$ in diabetic erythrocytes. The HbA1c value of this diabetic blood sample was 9.1%.

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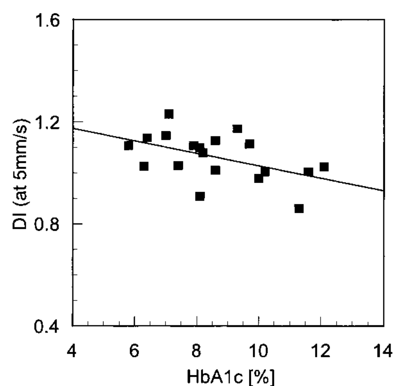

FIG. 10. Relationship between HbA1c and DI at erythrocyte velocity of 5 mm/s for each blood sample calculated from the relationship between erythrocyte velocity and DI by curve fitting. The results indicate that impaired deformability may be related to glycaemic control ($r = -0.486$, $P < 0.05$).

the microchannel system (Fig. 8), we see that DI did not depend on the sectional shape of the microchannel or glass capillary. We think that the reason the DI of the microchannel is higher is that the glass tube diameter is slightly larger than the microchannel width. The glass capillary method enables us to take the clear images (Fig. 7), but has some drawbacks: diameters are not constant because of the heating and drawing, and it is difficult to reuse and refresh the inside of the tube.

When blood flow is recorded on videotape under physiological conditions, it is difficult to observe an individual erythrocyte in each frame with a standard video system, which processes 30 frames/s. However, the high-speed video system, processing 1000 frames/s, can record clear images of individual erythrocytes. The clear images of deformed erythrocytes enable us to make a quantitative analysis of erythrocyte deformation. Because it enables us to observe the shape of individual flowing erythrocytes, the crystal microchannel is a valuable tool for measuring erythrocyte deformability. To evaluate erythrocyte deformability, we examined erythrocyte velocity and deformed shape. As shown in Figs. 6 and 9, DI increased with erythrocyte velocity. In Secomb's mathematical model, the dependence of erythrocyte deformation on velocity is such that with increasing erythrocyte velocity, the erythrocyte length l increases and the erythrocyte diameter in the axial direction d becomes shorter (Secomb *et al.,* 1986). In this study, DI reached a limiting value at erythrocyte velocity of about 5 mm/s. This shows that erythrocytes have a deformation limit, which becomes lower if the deformability deteriorates. Figure 6 shows that erythrocytes whose membranes were hardened by glutaraldehyde had reduced deformability. Therefore, the differences in erythrocyte deformability can be detected by DI when the erythrocyte velocity is 5 mm/s or higher in our microchannels.

There are some evidences that rheological factors such as deformability and viscosity may play an important role in the hemodynamics in microcirculation. However, it is not yet clear that reduced deformability of erythrocytes causes alterations in flow resistance in diabetes mellitus. Microangiopathy is one of the major complications in diabetes mellitus and the decrease in erythrocyte deformability could contribute to this complication. There have been many studies about the relationship between diabetes and reduced erythrocyte deformability. Nevertheless, it should be noted that there are problems concerning the methodologies used and the evaluation of erythrocyte deformability. We thought that it is important to measure the deformability directly from erythrocytes. In this study the relationship between erythrocyte velocity and DI was examined (Fig. 9). As erythrocyte velocity increased, the change in DI became larger. This revealed a limit to erythrocyte deformation due to deterioration from type-2 diabetes. A correlation between DI and HbA1c was recognized at erythrocyte velocity of 5 mm/s (Fig. 10). This indicates that impaired deformability may be related to glycemic control. In the course of diabetes, the conformation of the erythrocyte membrane and hemoglobin may be considerably altered (Watala *et al.,* 1992). It is thought that the erythrocyte membrane is glycosylated under hyperglycosemia. The alterations in membrane lipid–protein interactions together with the increased glycosylation may consequently imply altered viscoelastic properties of erythrocyte membranes (Watala *et al.,* 1992). This hardening of the membrane could increase the apparent viscosity in microvessels and disturb the blood flow in microcirculation. The influence of erythrocyte deformability on the functioning of an isolated organ was studied (Sumpio *et al.,* 1989), with emphasis

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on the importance of erythrocyte deformability in microcirculation.

MacRury *et al.* found a higher mean cell hemoglobin concentration in diabetic patients in association with a lower mean cell volume (MacRury *et al.,* 1990). Watala *et al.* reported that the intracellular viscosity of erythrocytes of diabetic patients was elevated (Watala *et al.,* 1996). The increase in hemoglobin concentration raises intracellular viscosity. Recently Fujita *et al.* found a significant correlation between the elevation of intracellular calcium concentration and reduced erythrocyte deformability in diabetic patients (Fujita *et al.,* 1999). One might think that this is one of the causes of deformability deterioration, but in our investigation there was no difference in intracellular hemoglobin concentration. Therefore, we think that the deterioration of deformability was caused by alterations in the erythrocyte membrane rather than by a change in intracellular viscosity.

McMillan *et al.* pointed out that the erythrocyte membrane itself may have altered its viscous properties in diabetes, and as the erythrocyte enters smaller and smaller vessels in microcirculation, its ability to deform becomes important (McMillan *et al.,* 1978). The blood vessel wall is always stressed by blood flow. It is important to examine the mechanical stress on vessel endothelial cells when the stress field changes because of reduced erythrocyte deformability. The thickness of the basement membrane and the disappearance of pericytes can be recognized in capillaries in the very early stages of diabetic retinopathy. The transparent microchannel could be used to test this hypothesis. We are trying to culture human endothelial cells on the ceiling of the microchannel. By flowing blood with different deformabilities over a collagen-coated cover glass, we will be able to examine the influence of deformability on endothelial cells. Endothelial cells could elongate or exfoliate due to the shear stress of blood flow. In addition, the influences of erythrocyte deformability on oxygen diffusion will be examined.

In summary, we have developed a transparent microchannel flow system made of crystal glass. The key point of this capillary model is that we can observe erythrocyte deforming to a parachute shape in a similar manner to that seen *in vivo* using a high-speed video camera. We were able to measure the flow velocity and DI directly from individual erythrocyte images. The DI limit correlated with HbA1c in glycemic controls. Reduced deformability may affect the viscosity and shear stress in microcirculation and the progress of microangiopathy.

REFERENCES

Bryszewska, M., Watala, C., and Torzecka, W. (1986). Changes in fluidity and composition of erythrocyte membranes and in composition of plasma lipids in type I diabetes. *Br. J. Haematol.* **62,** 111–116.

Chien, S. (1987). Red cell deformability and its relevance to blood flow. *Annu. Rev. Physiol.* **49,** 177–192.

Cokelet, G. R., Soave, R., Pugh, G., and Rathbun, L. (1993). Fabrication of in vitro microvascular blood flow systems by photolithography. *Microvasc. Res.* **46**, 394–400.

Fujita, J., Tsuda, K., Takeda, T., Yu, L., Fujimoto, S., Kajikawa, M., Nishimura, M., Mizuno, N., Hamamoto, Y., Mukai, E., Adachi, T., and Seino, Y. (1999). Nisoldipine improves the impaired erythrocyte deformability correlating with elevated intracellular free calcium-ion concentration and poor glycaemic control in NIDDM. *Br. J. Clin. Pharmacol.* **47,** 499–506.

Ishikawa, M., Sekizuka, E., Shimizu, K., Yamaguchi, N., and Kawase, T. (1998). Measurement of RBC velocities in the rat pial arteries with an image-intensified high-speed video camera system. *Microvasc. Res.* **56,** 166–172.

Kikuchi, Y., Sato K., Ohki, H., and Kaneko, T. (1992). Optically accessible microchannels formed in a single-crystal silicon substrate for studies of blood rheology. *Microvasc. Res.* **44,** 226–240.

Kikuchi, Y., Da, Q., and Fujino, T. (1994). Variation in red blood cell deformability and possible consequences for oxygen transport to tissue. *Microvasc. Res.* **47,** 222–231.

Le Devehat, C., Vimeux, M., Bondoux, G., and Khodabandehlou, T. (1991). Effects of buflomedil on microvascular disorders in diabetic patients. *Blood Vessels* **28**(Suppl. 1), 38–43.

MacRury, S. M., Small, M., Anderson, J., MacCuish, A. C., and Lowe, G. D. (1990). Evaluation of red cell deformability by a filtration method in type 1 and type 2 diabetes mellitus with and without vascular complications. *Diabetes Res.* **13,** 61–65.

McMillan, D. E., Utterback, N. G., and La Puma, J. (1978). Reduced erythrocyte deformability in diabetes. *Diabetes* **27,** 895–901.

Parthasarathi, K., and Lipowsky, H. H. (1999). Capillary recruitment in response to tissue hypoxia and its dependence on red blood cell deformability. *Am. J. Physiol.* **277**(6 Pt. 2), H2145–H2157.

Rice-Evans, C., and Chapman, D. (1981). Red blood cell biomembrane structure and deformability. *Scand. J. Clin. Lab. Invest.* **44**(Suppl. 156), 99–110.

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Secomb, T. W., Skalak, R., Özkaya, N., and Gross, J. F. (1986). Flow of axisymmetric red blood cells in narrow capillaries. *J. Fluid Mech.* **163,** 405–423.

Simchon, S., Jan, K., and Chien, S. (1987). Influence of reduced red cell deformability on regional blood flow. *Am. J. Physiol.* **253** (*Heart Circ. Physiol.* 22), H898–H903.

Simpson, L. O. (1985). Intrinsic stiffening of red blood cells as the fundamental cause of diabetic nephropathy and microangiopathy. *Nephron* **39,** 344–351.

Skalak, R., and Branemark, P. I. (1969). Deformation of red blood cells in capillaries. *Science* **164,** 717–719.

Sumpio, B. E., Upchurch, G. R., and Johnson, G., Jr. (1989). The influence of perfusate viscosity, RBC deformability and drag on the function of an isolated perfused rat kidney. *J. Surg. Res.* **46,** 4–8.

Sutton, N., Tracey, M. C., Johnston, I. D., Greenaway, R. S., and Rampling, M. W. (1997). A novel instrument for studying the flow behaviour of erythrocytes through microchannels simulating human blood capillaries. *Microvasc. Res.* **53,** 272–281.

Tracey, M. C., Greenaway, R. S., Das, A., Kaye, P. H., and Barnes, A. J. (1995). A silicon micromachined device for use in blood cell deformability studies. *IEEE Trans. Biomed. Eng.* **42**(8), 751–761.

Tsukada, K., Minamitani, H., Sekizuka, E., and Oshio, C. (2000). Image correlation method for measuring blood flow velocity in microcirculation: correlation "window" simulation and in vivo image analysis. *Physiol. Meas.* **21,** 459–471.

Watala, C., Witas, H., Olszowska, L., and Piasecki, W. (1992). The association between erythrocyte internal viscosity, protein non-enzymatic glycosylation and erythrocyte membrane dynamic properties in juvenile diabetes mellitus. *Int. J. Exp. Pathol.* **73,** 655–663.

Watala, C., Golanski, J., Witas, H., Gurbiel, R., Gwozdzinski, K., and Trojanowski, Z. (1996). The effects of in vivo and in vitro non-enzymatic glycosylation andglycoxidation on physico-chemical properties of haemoglobin in control and diabetic patients. *Int. J. Biochem. Cell Biol.* **28,** 1393–1403.

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