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Observation of capillary flow in human skin during tissue compression using CCD video-microscopy

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Abstract: Recent technological advances of the CCD video-camera have made microscopes more compact and greatly improved their sensitivity. We newly designed a compact capillaroscopy which was composed with a CCD video-probe equipped a contact-type objective lens and illuminator. In the present study, we evaluated usefulness of the instrument for a bed-side human capillaroscopy to observe the capillary flow in various dermal regions. The influences of tissue compression on the dermal capillary blood flow were also investigated to confirm the utility for clinical applications. Our capillaroscopy visualized the nutritional capillary blood flow in almost all parts of skin surface. Our observations showed that a level of vertical stress similar to arterial pressure was required to stop the capillary flow. From these demonstrations the present CCD video-probe based capillaroscopy would be useful for clinical applications as a bed-side human capillaroscopy.

I. INTRODUCTION

Intravital microscopy allows real time observation of living systems, especially, human capillaroscopy is the only noninvasive method available that directly visualizes the nutritional dermal capillaries [1, 2]. Although clinical application of human capillaroscopy requires a compact microscope system, no such system has been developed. The reason for this is mainly due to the fact that a conventional optical stereotype microscope is generally used for capillaroscopy. Such sophisticates cause problems when applying capillaroscopy to various parts of the target tissue; it can be applied only to a limited region such as a finger nail-fold [3]. The recent rapid progress of CCD camera technique makes capillaroscopy easy to apply, even in clinical use, having the advantage of being compact and highly sensitive. Especially, CCD video-probe unified with an illuminator and an objective lens provided a new concept of an intravital microscopy because of its size and easy handling. In the present study, we describe a CCD video-probe equipped with a contact-type objective lens and illuminator, and evaluated it as a compact capillaroscopy for clinical applications to investigate the influences of tissue compression to the nutritional capillary blood flow in human skin.

II. MATERIALS AND METHODS

The study was approved by Shibaura Institute of Technology ethics committee and all of subjects gave written informed consent.

Capillaroscopy

Our capillaroscopy has been designed to visualize the dynamic responses of dermal capillary flow being compressed at the skin surface transcutaneously. The diagrammatic representation of placement of the system assembly is shown in Fig. 1. The system is composed of a CCD video-probe (VH-5910: Keyence, Japan) with a contact-type objective lens (VH-501: Keyence, Japan), a coaxial epi-illumination connected to a 100-W halogen lamp via fiber optics. The feature of this CCD video-probe is that the skin surface is illuminated at the angle of approximately 45 degrees for an optimal view, and this optical arrangement makes it possible to avoid disturbing reflections from the skin surface. The size and weight of the CCD video-probe are 135 mm long x 28 mm in diameter, and 600 g. In addition, in order to make the skin transparent and to further minimize the reflections from the skin surface, a mineral oil (heavy white oil: Sigma, St Louis, USA) has to be applied to the skin. The CCD probe was attached to a one-way movable actuator with a force transducer (9E01-L35: NEC, Japan) to measure the normal stresses loaded to the skin. The tip of an objective lens is fitted with a cover glass of 4 mm in diameter and vertically compresses the tissue surface, in which the highest resolutions of a moving distance and a loaded weight are 1 μm and 1 mg, respectively. The loading weight was calculated to the vertical stress against the skin surface of 4 mm in diameter, and was represented as pressure (mmHg). The microcirculatory images are displayed on a 15-in TV monitor and are also recorded on a VCR for off-line analysis. The final magnification of the system is approximately x 500 on the monitor.

Procedure

To examine the influences of tissue compression to microcirculation, the vertical stress to collapse the microvessels and to stop the dermal capillary blood flow were measured in three healthy male adult subjects. The dermal capillary flow in the nail-fold and at the middle part of dorsal finger, and anterior side of forearm was observed using the capillaroscopy. The vertical stress was gradually loaded to the skin surface using linear actuator during monitoring the dermal capillary flow, and the vertical stress when capillary flow was completely stopped was determined as the critical value of compression. In the experiments with the nail-fold in finger, the arterial blood pressure measurements in the same finger were carried out using the cuff-occlusion methods [4]. The arterial pressure of finger was determined by the cuff pressure when the dermal capillary flow in the nail-fold was stopped by occluding the finger at proximal site.

III. RESULTS

Figure 2 shows typical examples of observation of the dermal capillary flow in the nail-fold of the middle finger (A) and at the middle dorsal part of same finger (B), and the anterior side of the forearm (C). The observations showed the different morphological patterns of dermal capillaries between the nail-fold and middle part of the dorsal finger or the anterior side of the forearm. In most dermal areas, the nutritional capillary loops are located at a 90°angle to the skin surface, and only the tips of the capillary loops can be visualized. Capillary loops in the nail-fold become successively more parallel to the skin surface, and can be visualized in their full length as shown in Fig 2A. On the other hand, moving in the direction of a proximal site, the nutritional capillary loops gradually rise to become perpendicular to the skin surface, and finally only the apex of the capillary loops can be observed as points in the papillae. The angle of the nutritional capillary loop appears to depend on the thickness of dermal tissue. In the video image of forearm, some microvascular network of small arterioles and venules can vaguely be seen in the deeper portion.

We tried to determine the critical value of tissue compression, in which capillary flow was completely stopped by loading vertical stress to the skin surface. Video images of the responses of capillary flow to increases in loaded stress were shown in Fig. 3. These video images were captured in the nail-fold of the middle finger. All nutritional capillary loops run parallel to the skin surface, and can clearly be visualized in their full length. In the control state before compression, the smooth flow of the erythrocyte column is clearly seen in all capillary loops (Fig. 3A). By loading the vertical stress at 40 mmHg, a value which is considered to be comparable or slightly higher than capillary pressure under normal physiological conditions, the erythrocytes in all capillaries were still flowing (Fig. 3B). The erythrocytes flow in capillaries was finally restricted when the load of vertical stress increased to 80 mmHg (Fig. 3C). Even under such conditions, capillaries did not collapse and the erythrocytes kept their shape in the blood vessels.

The critical values of tissue compression to stop the dermal capillary flow in the nail-fold of the middle finger, the middle part of dorsal finger, and anterior side of forearm

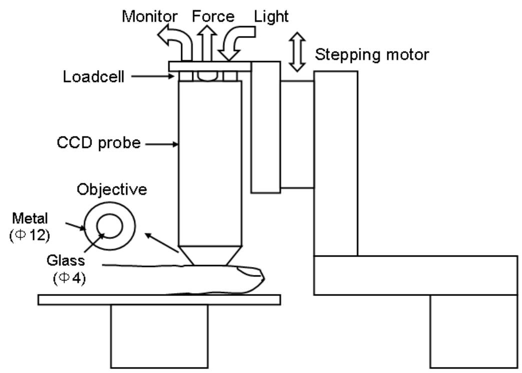

Fig.1 The diagrammatic representation of the system assembly. The capillaroscopy is composed of a CCD video-probe and a contact-type objective lens with a coaxial epi-illumination. The CCD probe was attached to a one-way movable actuator with a force transducer to visualize the dynamic responses of the dermal capillary flow being compressed at the skin surface.

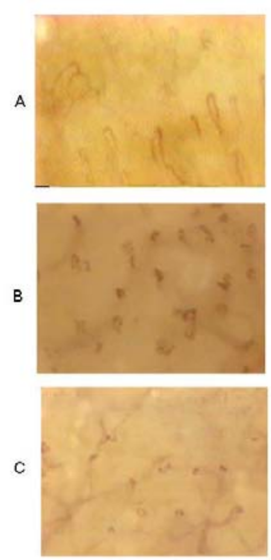

Fig.2 Video images to observe the dermal capillary flow in the nail-fold of the middle finger (A) and at the middle dorsal part of the same finger (B), and the anterior side of the forearm (C). The black bar along the side of the image represents 100 μm.

skin are shown in Fig. 4. It has been clearly demonstrated that the critical values to stop the capillary flow were about the same levels in all parts of the skin surface. Also, in this figure, the arterial blood pressure of the same middle finger, measured by the cuff-occlusion method, is indicated in the shaded bar of the graph. The critical values of the skin compression to stop the capillary flow were approximately equivalent to the arterial blood pressure rather than capillary pressure.

IV. DISCUSSION

Direct monitoring of the dermal capillary blood flow using capillaroscopy provides a unique noninvasive method of investigating dynamic capillary function in both a healthy and a diseased state. However, this technique has not become widely used in clinical fields compared with Laser Doppler flowmetry and thermography, since no simple device which permits easy access to clinical applications exists [2]. In the present study, we demonstrated that a recent commercialized CCD video-probe, which is affordable and compact, can be utilized for human capillaroscopy. The major advantage of this capillaroscopy is its high utility for handling, which is achieved by combining an illuminator with an objective lens. This all-in-one design makes the capillaroscopy more compact and facilitates easy observation of various regions of the human skin. The CCD capillaroscopy which we developed can visualize the nutritional capillary blood flow in almost all parts of the skin surface that are responsible for the maintenance of tissue viability.

The CCD video-probe was attached to a linear actuator with a force transducer, and then the responses to the dermal capillary flow, by loading the vertical stress to the skin surface, were investigated. The vertical stress values to stop the dermal capillary flow were much higher than capillary pressure under physiological conditions [5] in all parts of skin i.e. the finger nail-fold, the middle part of dorsal finger, and anterior side of forearm. It was considered that the skin capillaries were not collapsed by tissue compression from external stress, and, it appears that the capillary flow would be stopped by collapsing the arterioles located in the preceding capillaries. Consequently, all erythrocytes remained in the capillaries keeping their shape when a vertical stress of 80 mmHg was loaded to the skin nail-fold. Furthermore, these results suggest that vertical stress to skin tissue is not a serious risk factor of growth of pressure ulcers, but other mechanical forces like shear stress also might affect their progress [6].

Our results revealed an additional benefit of applying the CCD video-probe to human capillaroscopy. Since there was little influence on dermal capillary flow by loading vertical stress of less than 40mmHg, the microcirculatory observation can be performed to keep the CCD video-probe in contact with the skin surface. Such handling of a contact-type CCD-probe might be useful to stabilize the motion of both the probe and the subject. In summary, a newly designed compact capillaroscopy utilizing a

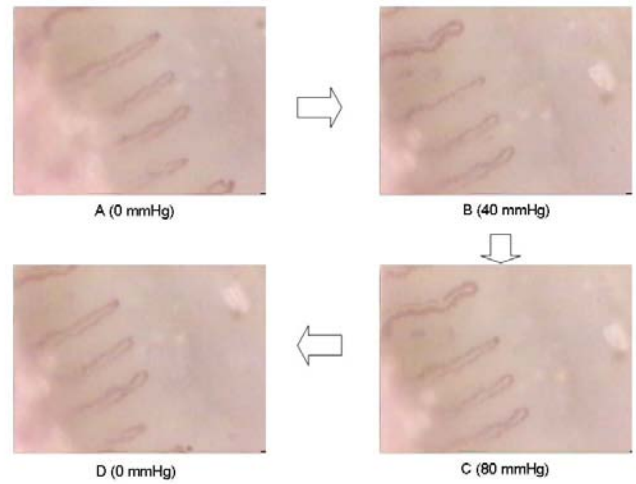

Fig.3 Video images of the responses of the dermal capillary flow to increases in loaded stress. These video images were captured in the nail-fold of the middle finger. In the control state before compression (A), by loading the vertical stress at 40 mmHg (B), by loading vertical stress at 80 mmHg (C), during reactive hyperemia after releasing vertical stress (D).

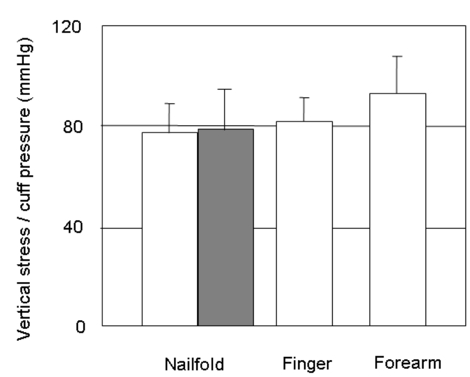

Fig.4 The critical values of tissue compression to stop the dermal capillary flow in the finger nail-fold, the middle part of the dorsal finger, and the anterior side of the forearm skin. The arterial blood pressure of finger measured by the cuff-occlusion method is indicated in the shaded bar of the graph.

commercially available CCD video-probe has been evaluated for clinical applications to investigate the influences of tissue compression on the nutritional capillary blood flow in human skin. Our capillaroscopy visualized the nutritional capillary blood flow in almost all parts of the skin surface as a result of its compactness. From these demonstrations, the present CCD video-probe based capillaroscopy would be useful for clinical applications as a bed-side human capillaroscopy.

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