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A Simple Advanced Method for Studying the Morphology of a Single Isolated Human Corneocyte by Scanning Electron Microscopy: Identification of Anterior, Posterior, and Lateral Surfaces of a Corneocyte

Dongsik Bang, and Sungnack Lee*

Department of Dermatology, Yonsei University College of Medicine, Seoul,
Ajou University School of Medicine, Suwon, Korea*

A simple advanced technique for morphological evaluation of an isolated human corneocyte is described in this work. This technique may improve the process of specimen preparation for scanning electron microscopy. A detailed morphological profile of an isolated human corneocyte can be obtained from any direction viewed. It is suggested that this technique will be of particular value for studying the detailed morphological characteristics of corneocytes from various anatomical sites.

Key words : Corneocyte, Morphology, Scanning electron microscopy.

INTRODUCTION

The corneocytes are dead cells that comprise outermost layer of human skin. These cells have important functions, such as preventing water loss from the body surface and providing a barrier against external physiochemical stimuli.

There have been many studies of the morphological, biochemical, physical, and immunological aspects of corneocytes; their true character is now being revealed to some degree[1-5].

Although there have been many attempts since 1939 to determine the morphological aspects of corneocytes, no significant advances have been made. Difficulties in obtaining and examining corneocytes that have retained their natural form have been encountered over the years, and solutions to a suitable method of isolation have been limited. A diversified structural analysis of corneocytes was not possible until the 1970's, when electron microscopy was introduced as a tool for morphological research on the stratum corneum[6,7]. Even today it remains difficult to obtain corneocytes without damaging their original architecture, or to delineate the differences in cell morphology and structural composition within the stratum corneum from various anatomical sites.

The aim of this study was to develop a simple method to observe corneocytes that retain their natural forms within the limits of possibility. In addition, we have attempted to reveal their morphology within a three-dimensional structure.

Reprint requests to : Dongsik Bang, Department of Dermatology, Yonsei University College of Medicine, C.P.O. Box 8044, Seoul 120-752, Korea. Tel (82-2) 361-5734 / Fax (82-2) 393-9157

MATERIALS AND METHODS

Obtaining Corneocytes for Examination

To obtain the corneocytes, the abdomminal skin from 10 healthy men, aged 23-24 years and with no skin problems, was collected as follows: Using a teflon scrubber, a 3.8 cm^2 area of skin was scrubbed for 1 min with a detergent following the technique of McGinley et al[8]. The well-scrubbed corneocytes were collected in a glass cylinder containing 1 ml of 0.075 M phosphate buffer (pH 7.9) combined with 0.1% Triton X-100 (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

The washed fluid was withdrawn by a pipette and transferred to a glass test tube. The scales in the test tube were separated into individual cells by mechanical shaking for 30 sec. To avoid overlapping of the corneocytes to be examined by scanning electron microscopy, 9 ml of 0.075 M phosphate buffer (pH 7.9) was added to the scales in the test tube. This dilution was arrived at by repeated trials and by comparing the appearance of the corneocytes by scanning them under the electron microscope at various dilutions (data not shown). After shaking 10 ml of diluted solution for 30 sec, 1 ml was pooled in a 10 ml syringe. This syringe was then connected to a pop-top membrane holder (Nucleopore Corporation, Pleasanton, USA) containing a polycarbonate membrane with a 0.4 µm pore. The filtered corneocytes were collected on the polycarbonate membrane. Withdrawing the syringe from the pop-top membrane holder, the membrane holder was reconnected to another syringe containing a 3% solution of glutaraldehyde in a phosphate buffer. Then this syringe with 2 or 3 drops of the solution was passed through the pop-top membrane. Without passing more drops through the membrane, the corneocytes were left untouched for 2 h, at which time they became fixed. The fixed corneocytes were then washed with a phosphate buffer solution. The corneocytes were postfixed in a 1% osmium tetroxide in water for 2 h, and were passed through a graded ethanol series to conclude the dehydration process. This method was repeated several times for collecting of the corneocytes. Drying of the polycarbonate membrane, which was removed from the pop-top membrane holder, was accomplished by using a HCP-2 Critical Point Dryer (Hitachi, Japan). After drying, the membrane was mounted on a stub and uniformly coated with gold of about 400 Å thickness in 1B-3 Ion Coater (Eiko, Japan) to ensure good electrical conductivity. Then it was observed under a S-450 scanning electron microscope (Hitachi, Japan). A lateral view of the polycarbonate membrane was obtained by tilting the stub at angles ranging between 80°-90°. The accelerating voltage of the scanning electron microscope was 15 kV.

Obtaining Stratum Corneum for Examination

Stratum corneum was obtained according to a procedure described previously[9]. Briefly, to obtain a stratum corneum for examination, a sheet of stratum corneum (3-6 cells thick) was taken from the abdomens of the 10 subjects using the skin surface biopsy method designed by Marks and Dawber[10]. A drop of the cyanoacrylate adhesive permabond (Staident Products Ltd., Staines, Middx., England) was placed on the area of skin to be studied. A clean glass slide was firmly placed over the drop, and was removed after 20-30 sec. The glass slides with the skin surface biopsies which were cut to the required size, were then mounted on the scanning electron microscope stubs.

RESULTS

Morphology of Corneocyte Attached to the Polycarbonate Membrane

Corneocytes from the abdomen had a generally polygonal shape, showing a clear trabecular network (Fig. 1). In a lateral view obrained by tilting the stub at an angle of 80°, a trabecular network of small pits and fine wrinklings was clearly observed on the cell surfaces (Fig. 2).

Morphology of the Posterior Surface of the Stratum Corneum from Skin Surface Biopsies

The posterior surface of the stratum corneum taken from the abdomen showed divisions of square-shaped folding lines, and many corneocytes had ill defined cell borders. However, at a higher magnification, a smooth, flat posterior surface was observed on the major parts of the

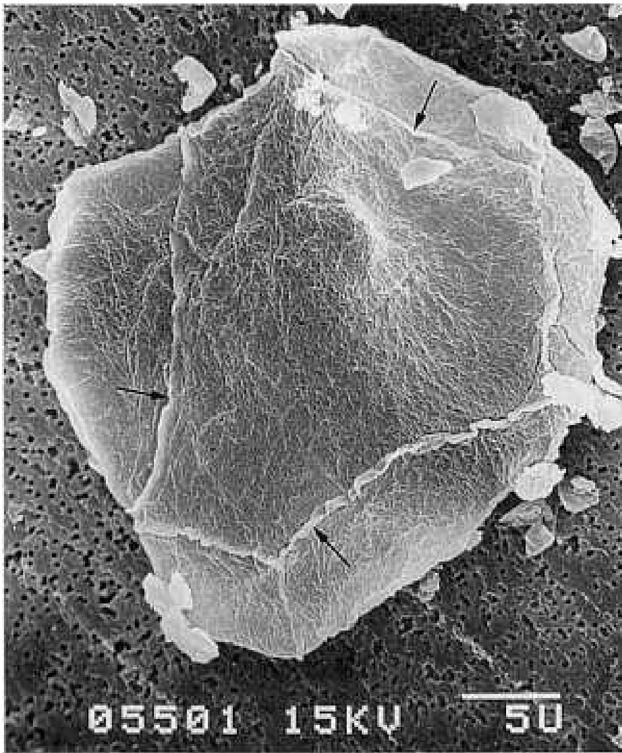

Fig. 1. Scanning electron micrograph of a corneocyte from the abdomen, showing polygonal shape and a clear trabecular network (arrows).

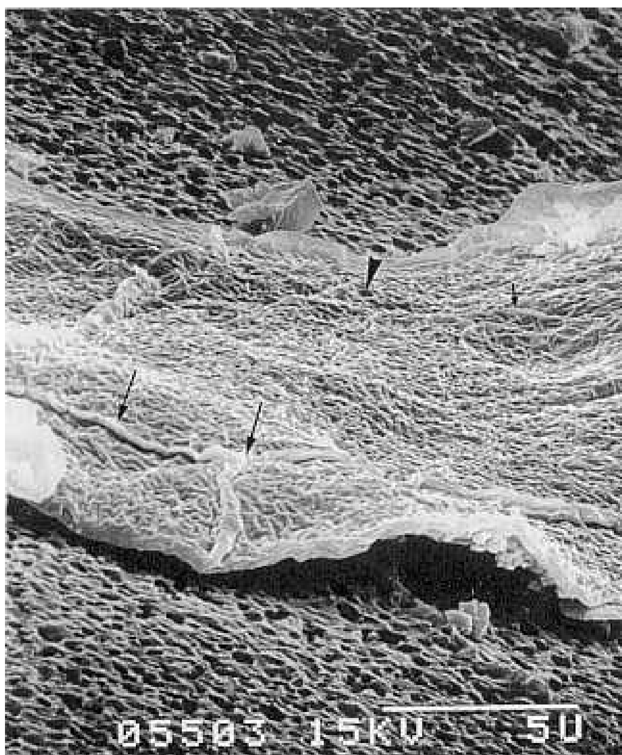

Fig. 2. Scanning electron micrograph of a laterally viewed corneocyte from the abdomen, showing small pits (arrowhead), fine wrinklings (short arrow), and a clear trabecular network (long arrows).

Fig. 3. Scanning electron micrograph of the posterior surface of the stratum corneum from the abdomen, showing a smooth, flat surface without any villous projections or pits. Bar=5 μm.

corneocytes. No villous projections or pits were seen (Fig. 3).

DISCUSSION

The functional anatomy of the corneocyte is still unclear despite its known physical, physiological and biochemical factors. To clarify this point, more detailed observations of the morphology of isolated corneocytes are necessary.

Several techniques have been developed over the past decades to study the morphology of corneocytes. As early as 1939, Wolf[11] described a technique of adhesive-tape stripping. Subsequently, the adhesive slide technique[12], the detergent scrub technique[8], and skin surface biopsy[10] were introduced for visualization and quantitation of corneocytes using light microscopy, scanning electron microscopy, or transmission electron microscopy. The bioassay detergent scrub technique according to McGinley et al[8] has been

commonly used to collect corneocytes in most studies for visualization and quantitation of corneocytes.

The original method of the detergent scrub is used widely even today. However Barton et al[13] reported in 1980 a new method to be used with the detergent scrub technique for studying the structural detail of isolated human corneocytes. In this method, a chamber was devised and built to hold the cells during processing and to facilitate the critical point drying process to minimize any drying artifacts. However, the only morphological difference observed in corneocytes prepared by this method was that they did not appear as flat or "curled up", revealing their irregular edges or a reduced number of large folds, compared to corneocytes smeared onto glass microscopic slides and then fixed by air drying. With this method, care had to be taken to use an optimal amount of suspension; too much resulted in large clumps of corneocytes on the filter, thus obscuring some detail. Use of the polycarbonate membranes gave better results than those obtained with glass fiber filters, although the transparency of the membranes made them less useful.

In our new method, we tried to overcome all difficulties associated with the technique according to Barton et al.[13] By immediately diluting the cell suspension with the proper amount of phosphate buffer, clumps or overlaps of corneocytes on the filter were largely prevented, and damage to the corneocyte membrane caused by use of Triton X-100 detergent in the scrub fluid was lessened. Making use of this process, it was possible for us to examine clearly an isolated corneocyte by scanning electron microscopy. By using the pop-top membrane holder, polycarbonate membrane was easy to handle despite its transparency. Instead of a specifically devised chamber, a regular 10 ml syringe was adequate for containing the corneocytes during fixation and dehydration. The scanning electron microscopic observation of corneocytes performed by this method showed ultrastructural features in detail including trabecular network, wrinkling, and small pits on the surface of the corneocyte. In addition, it was possible to examine the lateral aspect of corneocytes by tilting the stub to an angle of 80°.

We added a procedure for skin surface biopsy to our experiment in order to examine the posterior surface of corneocyte by scanning electron microscopy. We followed exactly the same technique previously described very well by Marks and Dawber[10] and Dawber et al[14]. Usually, the middle layer of stratum corneum (3-6 cells thick) was detached by this technique. At a higher magnification, it was possible to observe detailed ultrastructural characteristics of a corneocyte in an attached sheet of stratum corneum.

Corneocytes attached to the polycarbonate membrane by using the pop-top membrane holder can be placed on either their anterior surface or their posterior surface. If we can control the depth of the horny layer to be examined by the detergent scrub technique, in theory it may be possible to examine both the anterior and posterior surface of corneocytes at the same level of the horny layer. Of course, to determine whether the anterior or the posterior surface of the corneocyte is being viewed, it is necessary to perform repeated comparisions between photographs of corneocytes attached to polycarbonate membrane and photographs of the posterior surfaces of corneocytes obtained by skin surface biopsy.

In our present study, scanning electron microscopic examination of the corneocytes attached to the polycarbonate membrane, and of the posterior surface of stratum corneum from skin surface biopsies, made it possible for us to observe the anterior, posterior, and lateral surfaces. By this new advanced method using the polycarbonate membrane, it has become easier to observe clearly the morphology of corneocytes. Further studies are presently in progress to investigate the morphological characteristics of corneocytes from various anatomical sites of normal subjects and from patients with various dermatological disorders.

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