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Technical Report

Scanning Electron Microscopic Observation of Human Skin Replica

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ABSTRACT : The skin is the largest organ of the integument system whose surface is closely related with many physiological and pathological conditions. Various methods are used to understand the structural and functional status of human skin. We would like to present usefulness of scanning electron microscopic (SEM) observation of skin replica and its significance of training module for a novice.

The silicon replicas from several regions of the body (hand, finger, forearm, lip, and face) were casted by applying Exafine® mixture. The positive replicas were prepared by applying EPON 812 mixture on negative silicon replicas. Some of the negative silicon replicas were cut with a razor blade and surface profiles were observed. The negative and positive replicas were coated with platinum and were observed under the scanning electron microscope.

We could investigate the detailed structures of the human skin surface without any physical damage to the subject. The positive replicas depicted real surface structure of the human skin vividly. The cross sectional view of the negative silicon replicas provided surface profile clearly.

The scanning electron microscopic observation of the human skin replicas would be useful to study skin surface structures and to evaluate medical and esthetical applications. (유연승, 정예지, 엄창섭: 사람피부 모사판의 주사전자현미경적 관찰)

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The skin is a covering of the body. It is the largest organ of the human body that takes up to 16 percent of the weight and acts as a primary defense against external hostiles and excessive water loss. The outer layer of skin is called epidermis which is composed of five layers called stratum corneum, stratum lucidum, stratum glanulosum, stratum spinosum, and stratum basale. Its other functions are insulation, temperature regulation, sensation, prevention of excessive water loss, and the production of vitamin D. The role of skin as aesthetics and communication is also stressed in modern society. Under those layers there are dermis and hypodermis (Junqueira & Carneiro, 2005).

The surface of the skin, or epidermal surface, shows a net-like structure. This structure is the irregular and roughly shaped polygons, most often resembling a square. The edges of the polygons define location of furrows or micro lines (Mark, 1983). As the skin texture becomes rough, the wrinkles tend to deepen and the surface mesh-like structure changes its arrangement or disappears (Gilchrest, 1996).

Some noninvasive methods such as magnifier, Wood's lamp, and skin scope have been used for routine evaluation skin status *in vivo*. Although a skin biopsy provides detailed information on the skin, it is an invasive method that needs serious consideration of a dermatologist. Various topographic analyses have been performed to understand the status of skin surface (Fischer et al., 1999; Lagadre et al., 2005). The negative or positive replicas of skin surface were analyzed by profilometry (mechanical, optical, laser, transmission, or interference). Objective assessment with profilometry was applied to various physiological, pathological and clinical studies (Choi and Oh, 1997; Son et al., 2005; Dessy et al., 2007)

In this study, we present the usefulness of scanning electron microscopic (SEM) observation of the skin replica and its significance as a training module for a novice.

Four people voluntarily provided replicas. The purpose and procedures of the study were fully explained to the participants. The skin surface for replica was swapped with alcohol sponge. After completely dried, the mixture of silicone mixture (Exafine®, GC Co, Japan) was spread over the region of interest and waited for 3~5 minutes to solidify, and the cast was re-

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moved carefully from the skin surface. The replicas were collected from forearm, back of hand, finger pad, dorsal surface of finger, outside of eye and upper lip.

The negative silicon replicas were used as molds to produce positive replicas. The Epon mixture (Epon 812: 48.5 mL, DDSA: 18.5 mL, NMA: 33.0 mL, and DMP-30: 1.5 mL) was poured onto the negative replicas and put into an oven of 60°C for 3 days. Some of the negative silicon replicas were cut with a razor blade vertically across the horizontal furrows of the hand and finger. The cut specimens were attached on a stub with carbon tape, and platinum-coated with an ion coater (IB-5 ion coater, Eiko Co., Japan). The replicas were investigated under SEM (S-4700S, Hitachi, Japan). To understand the three dimensional structure of the skin more vividly, three different tilting angles (0°, 5°, and 10°) were used for observation. Two stereo-paired images were selected and anaglyphic stereo-image was constructed by Anamaker® program (Takashi Sekitani, http://www.stereoeye.jp).

The negative silicon replica of the dorsum of hand has a mirror image of the original skin surface. It revealed that the skin surface was composed of large polygons containing small polygons inside (Fig. 1A). The positive Epon replica showed a real surface structure of the hand, which is at the same orientation of the skin surface (Fig. 1B). The sectional plane along the major horizontal skin furrow of the hand silicon replica revealed profiles of the furrows reminiscent of a mountain

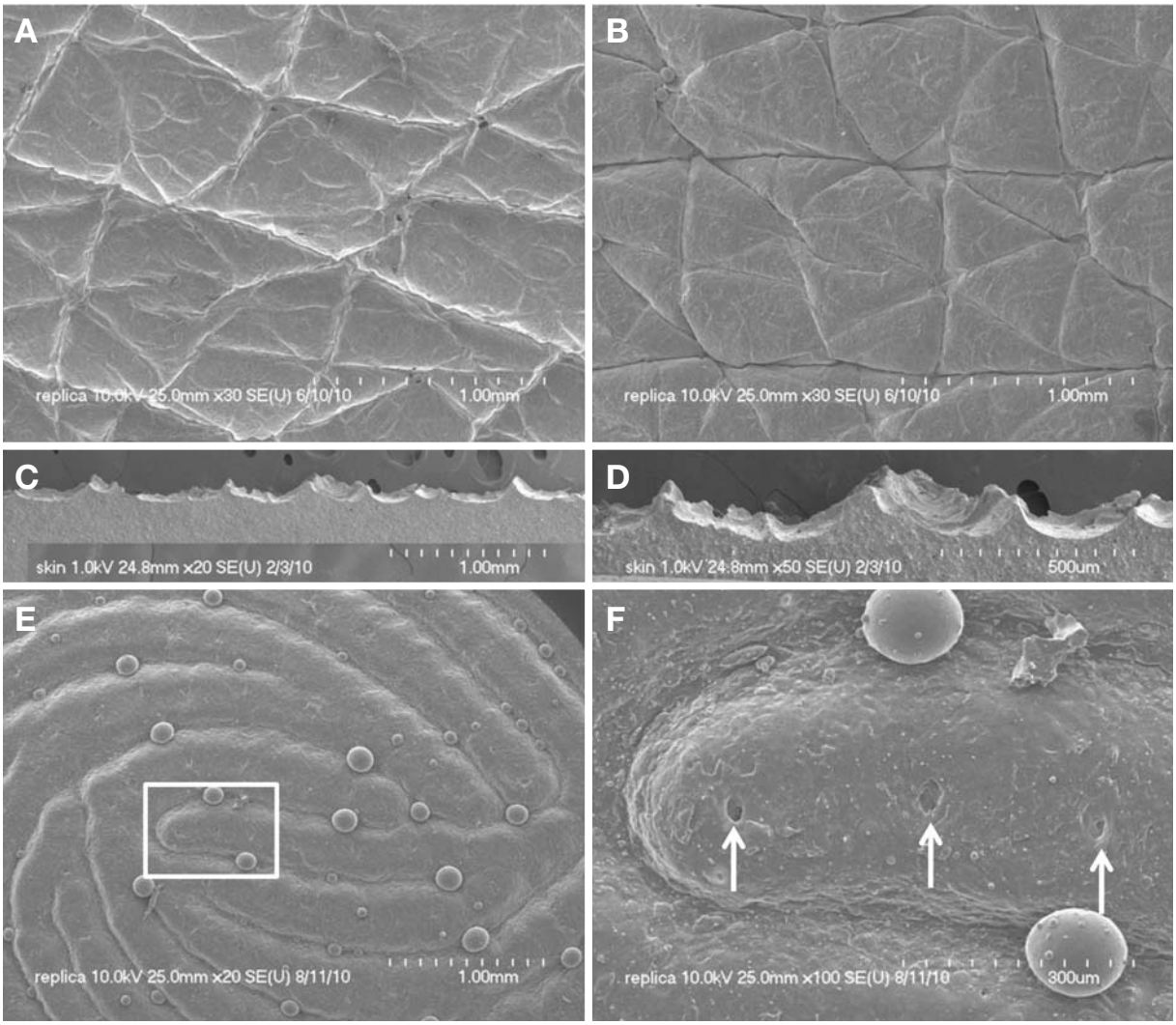

Fig. 1. Scanning electron microscopic image of silicon replica (A) prepared from dorsum of hand, which is a negative cast of superficial skin, the positive replica (B) can be produced by applying epoxy resin on the negative replica. The cross sectional view of a negative silicon replica reveals detailed profiles of the finger surface (C, D). The positive replica of finger print shows typical ridge and furrow of the print (E), the sweat pores (arrow) are observed on the ridge of the finger print (F: inset of E).

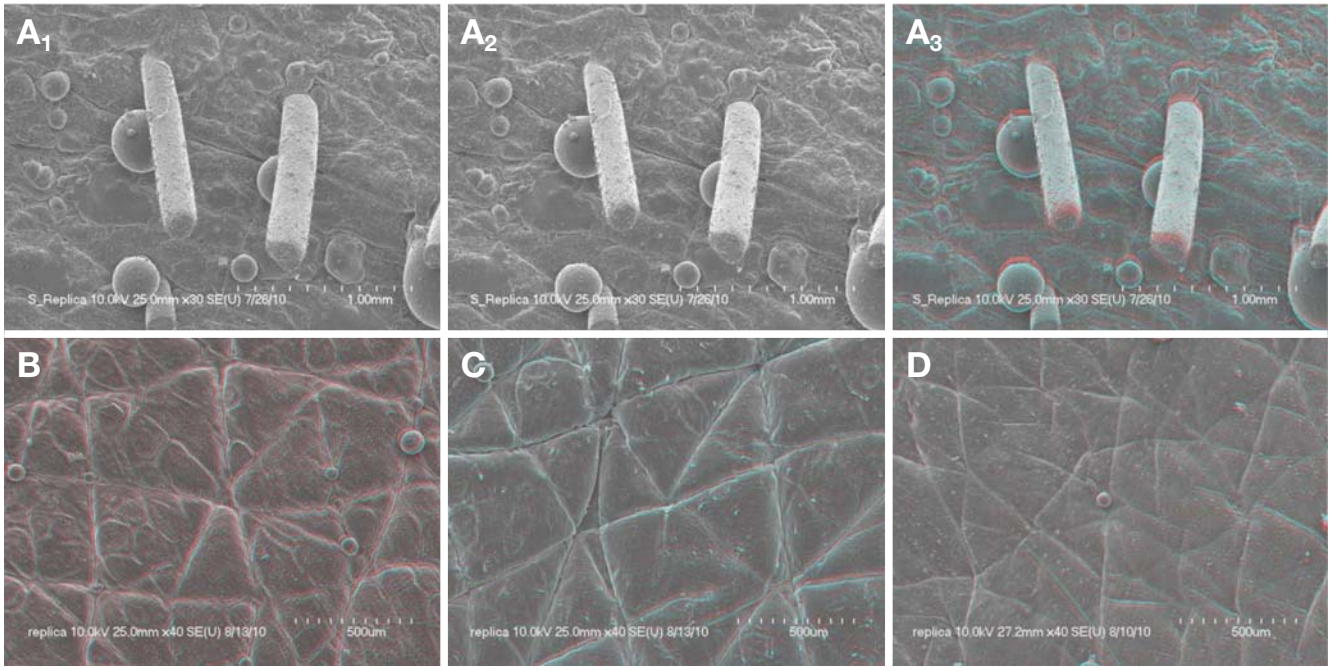

Fig. 2. Stereo pair images (A_1: 0˚ tilted, A_2: 5˚ tilted) of positive replica of upper lip show three dimensional configuration of hair and their underlying skin. Anaglyphic image was constructed with a pair of 5˚-tilted micrographs and stereoscopic features of hair are observed under red-blue glasses (A_3). Anaglyphic images of forearm (B), dorsal surface of finger (C) and outside of eye (D) show different microrelief according to the anatomical location.

chain. The top of a mountain in the replica falls on is the bottom line of a furrow and overall shape of the mountain reflects the dimension of the skin furrow (Fig. 1C, D). The positive replica obtained at the pad of a fingertip showed fingerprint pattern clearly, with smooth ridges and shallow grooves (Fig. 1E). At the higher magnification, the openings of sweat pores were clearly demonstrated on the ridge (Fig. 1F).

The positive replica image from upper lip shows hair shafts and their ground surface structure (Fig. 2A). Stereo pair images revealed three dimensional configuration of the skin surface (Fig. $2A_{1, 2}$). Anaglyphic images constructed from stereo-pair of different tilting angle and stereoscopic features of the skin can be observed under red-blue glasses (Fig. $2A_3$). Anaglyphic images from forearm, dorsum of hand and outside the eye show characteristic surface structures according to their anatomical locations.

The skin silicon replica has been used for the evaluation of many physiological and pathological conditions (Choi et al., 1997; Son et al., 2005; Zou et al., 2005). The profiles of skin replica provide an objective assessment of clinical interventions such as Botulinum toxin injection (Grove et al., 1991; Dessy et al., 2007) or application of anti-wrinkle compounds (Rbino et al., 2005; Kawada et al., 2008). In this study, we showed usefulness of comparative observation of the negative and positive replicas providing complementary image of the skin surfaces. The positive replica provides imitation of the real skin surface very closely without any physical damage to the skin.

Especially, the method observing the vertically cut silicon replicas would be highly useful for the analysis of the surface of the skin furrows. The cross sectional view of the silicon replica turned out to be a unique approach demonstrating an exact profile of the skin surface under the SEM. Thanks to the high resolution image of the skin profile, we could analyze exact dimension of the skin furrow, which would be important parameter to understand skin surface texture properties.

There are some advantages of using a replica, although it might lose some original details and make artifacts during preparations. The replica is an inorganic compound, which does not affected by temperature, moisture, and biochemical changes such as rotting happening in the biological samples. Also the replica can be cut in any direction as needed, can be observed at any time after collection without damage to the subject. And replica does not require fixation step, which is an essentially required step for biological sample preparation for SEM, a novice can learn scanning electron microscopy with

easy. Therefore, SEM study with replica system could be used as an education module for beginners to have a concept of microscopic system.

The stereo-paired images analyses provide 3 dimensional perception, which has been used by many previous investigators (Kim et al., 2007; Hortola, 2009). Although experts can perceive 3 dimensional configuration from stereo-paired images without any additional glasses, the beginner requires stereoscopic glasses such as red-blue glasses, polarized glasses. Anaglyphic image is one of the useful methods to present 3 dimensional images, because it contains information from stereo paired images on the single picture appears on the paper or the computer monitor.

In conclusion, this SEM research of skin replica will provide an objective way to evaluate the human skin surface status related with various skin related applications. Due to the low level of handling procedure, replica system with SEM could be used as an education module for students to have a concept on microscopy.

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