Scanning Electron Microscopic Observation of the Dermal Side of the Epidermis in Developing Chick Embryos

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Summary: The dermal side of the chick embryonic skin was observed by scanning electron microscopy (SEM) after the removal of dermal components. Epidermis free of dermis was prepared as follows: Tissues were fixed, hydrolyzed with hydrochloric acid (HCl), digested with collagenase or trypsin, and processed for SEM observation. The epidermis from the anterior tarsometatarsus and the foot pad was examined. The base of the tarsometatarsal epidermis of a 13-day-old embryo was smooth except for the ridge demarcating each large overlapping scale. The base of each basal cell of 18- to 20-day-old embryos, bulged in hemispherical fashion on the dermal side. The undulation of the base of the epidermis (i.e. the formation of the dermal papillae) was not prominent in the tarsometatarsal epidermis. In the foot pad, small round depressions, each corresponding to a reticulate scale, were observed on the dermal side of the epidermis. The bottom of a depression was smooth in a 13-day-old embryo. After 18-20 days of incubation, the downgrowth of the epidermis occurred. Many pits corresponding to the dermal papillae were clearly seen. These observations show the morphological diversity of the epidermis in its dermal side during the development. In addition, HCl treatment followed by protease digestion is useful in the study of morphogenesis of the epidermis.

Chick embryonic skin has widely been used as a model in the study of morphogenesis. The developmental process of scale ridges and feathers has been studied extensively in ovo and in vitro. In tarsometatarsal skin, analysis has been focused mainly on the scale ridge formation and the keratinization. We studied the keratinization process in the tissue culture system with a chemically defined medium. Alpha-type keratinization was preferentially induced by hydrocortisone of a physiological concentration, and was reversibly inhibited by DMSO.

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Another morphological change occurs in the developing epidermis, that is, the formation of the dermal papillae, which may serve as one of models for the analysis of morphogenesis. Three-dimensional images of the dermo-epidermal junction during the skin differentiation, however, have not been reported.

Scanning electron microscopy (SEM) has been used mainly in the observation of the free surface architecture such as the luminal surface of the digestive and respiratory tracts. Recently, Evan et al. introduced the procedure to remove the connective tissue elements by hydrolysis with hydrochloric acid (HCl) followed by collagenase digestion. The basal sides of epithelial tissues can be observed directly and three-dimensionally by SEM. Although the basal surface of the epidermis had been previously observed by SEM only in special cases such as bullous pemphigoid, the application of Evan's method made it possible to study the basal side of the epidermis by SEM in normal skin as well.

In this paper, we report the SEM images of the basal surface of the developing chick embryonic epidermis. The morphological changes are compared in the skin from tarsometatarsus and foot pad region during in ovo development.

**Materials and Methods**

Fertile eggs from commercial White Leghorn stock were incubated at 38°C. The embryos were obtained after 13, 15, 18, and 20 days of incubation. Skin was removed from the anterior tarsometatarsus and the foot pad regions. The skin was fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4 or in Karnovsky's fixative at half-strength. After the specimens were washed in phosphate buffered saline (PBS), the connective tissue components were removed according to the procedure of Evan et al. with modification. That is, fixed tissues were incubated with 8N HCl at 60°C for 50-60 min. After being cooled, the tissues were washed with 0.1 M phosphate buffer, pH 7.4 to remove the acid. The specimens were then incubated with collagenase (Millipore, CLS II, 1 mg/ml) in 0.1 M phosphate buffer, pH 6.8 at 37°C for 4-6 hr. In some cases, the incubation with collagenase was omitted or replaced with an incubation with trypsin (Difco, 1: 250, 1 mg/ml) in PBS at 37°C for 30 min. The tissues were rinsed with PBS, osmicated, and dehydrated in graded alcohols. After passing through isoamylacetate, the tissues were dried by the critical point-drying method using liquid CO₂, coated with gold, and observed with a JSM-T200 scanning electron microscope. To confirm complete removal of the dermal components, some of the specimens prepared for SEM were embedded in Epon 812. Sections were cut and observed with light, or transmission electron microscopes.

**Results**

1. Tarsometatarsal epidermis of 13-15 days in ovo

The anterior tarsometatarsal skin is made up of large overlapping scales (scutate scales) in the chick. Fig. 1 is the survey view of the dermal side of the epidermis after 13 days of incubation. The ridges divide the basal surface of the epidermis into many hexagonal areas. These ridges correspond to the furrows separating each scales on the outer surface. This paper deals with the epidermis demarcated by these ridges. The basal surface of the hexagonal area bordered by these ridges was smooth at this stage of development. The basal cells, the intermediate cells, and the superficial peridermal cells were clearly dis-
cerned in the cross-sectioned view of the epidermis (Fig. 2). The shape and the arrangement of the epidermal cells were well preserved after removal of the cellular and extracellular dermal components. The basal surface of the epidermal basal cells was successfully exposed as shown in Fig 2. The dermal side of each basal cell was easily discerned when the basement membrane was enzymatically digested after HCl treatment (Fig. 3). The base of the basal cell was round to polygonal in shape, and varied in size. We did not see the regular arrangement of basal cells often seen in covering cells, such as the corneal endothelium. Thin cellular processes were seen in the intercellular gap between the basal cells. After 15 days of incubation, the basal surface of the epidermis demarcated by the scale ridges remained smooth.

2. Tarsometatarsal epidermis of 18-20 days in ovo

After 18-20 days of incubation, keratinization of the subperidermal and epidermal cells occurs. Fig. 4 shows the cross section of the epidermis of a 20-day-old embryo. The flat disk-shaped keratinized cells were formed in the middle of the epidermis. The periderm and subperiderm were stacked over these keratinized cells. Epidermal downgrowth occurred to some extent so that the basal surface of the epidermis became undulated. The characteristic epidermal peg structure (i.e. the compensatory dermal papillae), however, was not observed. The size and shape of the base of the basal cells varied (Fig. 5). The base of each cell bulged in hemispherical fashion on the dermal side.

3. Foot pad epidermis of 13-15 days in ovo

Small non-overlapping scales (reticulate scales) are formed in chick's foot pads. When the dermal side of the foot pad epidermis was observed, many round depressions, each corresponding to a scale, were seen (Fig. 6). The bottom of each depression was smooth and similar to that of the tarsometatarsal epidermis of a 13-day-old embryo (Fig. 7).

4. Foot pad epidermis of 18-20 days in ovo

After 18 days of incubation in ovo the characteristic papillary structure was formed in the round depression of each reticulate scale in the foot pad. The epidermal cell cord protruded to the dermal side forming a reticular framework (Figs. 8 and 9). The pits in the framework corresponding to the dermal papillae were formed. Each reticulate scale had 10 to 30 dermal papillae. In the ridges corresponding to the furrows on the outer surface of the scale, the characteristic papillary structure was not formed.

Discussion

In this study we clearly showed the three-dimensional images of the epidermal downgrowth (the formation of the papillary structure) in the dermo-epidermal junction during in ovo development by SEM. The epidermal cell cords protruded to form a reticular framework of the epidermis in the foot pad. This characteristic papillary structure, on the other hand, was not formed in the tarsometatarsal skin.

The tarsometatarsal skin differs from the foot pad skin in various aspects during embryonic development. Large overlapping scales (scutate scales) are formed in the tarsometatarsus5,10, whereas small non-overlapping scales (reticulate scales) are formed in the foot pad11. The epidermal cells in the outer surface of the scale in the tarsometatarsus elaborate both alpha- and beta-keratins5,10. The epidermal cells in the foot pad, on
The regional differences in the papillary structure of the dermo-epidermal junction shown in this paper may be attributed to the functional differences of the skin. Since the foot pad supports the body weight, its skin is exposed to various kinds of mechanical forces. The reticular downgrowth of the epidermis forms a peg into the foot pad dermis, which may play a role in the mechanical support of the epidermis. The dermal papillae are often the route for the blood capillaries and nerves as well. The round gaps between the epidermal protrusions may correspond to the dermal papillae for vascularization and innervation. In the overlapping scales of the anterior tarsometatarsal skin, on the other hand, the thick keratinized layer with beta-keratin (scale-type keratin) and the dermal papillae protruding into the overlapping region may play a role in mechanical support.

The shape of the base of each basal cell was well discerned after the digestion with collagenase or trypsin. Trypsin seems to be more effective than collagenase for removing the basal lamina, as we have previously reported in the unfixed tissues.

SEM observation has several advantages compared with conventional histological observation by light microscopy and transmission electron microscopy; the three-dimensional features can be seen easily, it saves time and labor compared with reconstruction by serial sections, and the relationship between the specific structures is easily determined, etc. The three-dimensional features of the base of the epidermis, however, had been difficult to observe by SEM. The basal surface can only be observed under the pathological conditions such as bullous pemphigoid. HCl hydrolysis followed by the protease digestion of the fixed skin serves as a good method to study the three-dimensional features directly by SEM. Observation of the cross sections of the specimens by light, and transmission electron microscopy, as well as by SEM, confirmed the complete removal of the dermal components. In addition, the arrangement of the epidermal cells were well preserved. The three-dimensional relationship between the epidermal downgrowth was easily distinguished by SEM in this study.

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References


Explanation of Figures

Plate I

Tarsometatarsal epidermis of a 13-day-old chick embryo.

Fig. 1. A survey view of the dermal side of the epidermis. The pentagonal ridge, which corresponds to the furrow on the outer surface of the skin, demarcates each large overlapping scale. The surface surrounded by the ridge is smooth. HCl treatment only. ×240.

Fig. 2. A cross section of the epidermis. The basal cells (B), the intermediate cells, and the superficial peridermal cells (P) are easily discerned. The integrity of the epidermis is well preserved. The cellular and the intercellular components of the dermis, on the other hand, are completely removed (bottom). The base of the basal cells is successfully exposed. HCl-trypsin treatment. ×4200.

Fig. 3. The basal surface of the epidermis. The surface is smooth and the base of each basal cell is easily discerned. The size and shape of the basal cell vary. Fine processes connect each cell. HCl-trypsin treatment. ×2400.
Plate II

Tarsometatarsal epidermis of a 20-day-old chick embryo.

Fig. 4. A cross section of the epidermis. The keratinized layer is detached from the underlying epidermis during the preparation of the specimen so that the flattened keratinized cells (K) are seen. The lower right (B) is the base of the epidermis exposed by HCl treatment. Note the irregular bulge of the basal cells. P. superficial periderm. ×590.

Fig. 5. The basal surface of the epidermis. The base of each basal cell is round to oval in shape and bulged to the dermal side. HCl-collagenase treatment. ×1,800.
Plate III

Foot pad epidermis of a 13-day-old chick embryo.

Fig. 6. A survey view of the dermal side of the epidermis. Many round depressions, each corresponding to a reticulate scale, are seen. HCl-collagenase treatment. ×260.

Fig. 7. An enlargement of a scale indicated by the rectangle in Fig. 6. The bottom of the depression is smooth, whereas each basal cell bulges to some extent in the ridge. HCl-collagenase treatment. ×1,100.
Plate IV

Foot pad epidermis of a 18-day-old chick embryo.

Fig. 8. A survey view of the dermal side of the epidermis. Six reticulate scales are seen. The ridge demarcating each scale is rather smooth. Many epidermal protrusions are prominent in the bottom of each scale. HCl-collagenase treatment. ×240.

Fig. 9. An enlargement of the bottom of a scale indicated by the rectangle in Fig. 8. Pits (*) which correspond to the dermal papillae are seen. HCl-collagenase treatment. ×890.