Scanning electron microscopy of the developing chick anterior corneal epithelium

By PHILLIP R. WAGGONER

From the Department of Anatomy, Wayne State University School of Medicine, Detroit

SUMMARY

The anterior corneal epithelium of the developing chick was observed with the scanning electron microscope at various stages of development. In the earlier stages, up to about 15 days of incubation, the cells are characterized by regular polygonal outlines and a proliferation of microvilli on the surface. The microvilli then begin to coalesce and flatten so that the surface is rather smooth by about 19 days of incubation. Just prior to hatching, however, the cells begin to round up and once again become covered by microvilli. The cells then lift off the surface and expose the underlying cells. After hatching, the surface cells lose the synchrony of development that characterized the embryo and are found in various stages of senescence. The cells eventually lose their regular polygonal outlines and the corneal surface takes on a patchwork appearance.

INTRODUCTION

In relatively recent years many morphological studies of the developing cornea have been carried out using the light microscope (Rones, 1932; Meyer & O'Rahilly, 1959; Nuttall, 1976a, b), transmission electron microscope (Miyashita, 1964; Brini, Porte & Stoeckel, 1966; Hay & Revel, 1969; Pei & Rhodin, 1971) and the scanning electron microscope (Nelson & Revel, 1975; Bard, Hay & Meller, 1975). From these studies and many other previous ones, there has evolved a rather detailed description of the morphological events encompassed by corneal development. Even so, there is a dearth of information on the development of the surface layer of the anterior corneal epithelium -- the layer that is frequently referred to as periderm or epitrichium of the embryonic ectoderm.

However, the adult anterior corneal epithelium as viewed with the scanning electron microscope has been described by several authors (Blumcke & Morgenroth, 1967; Hoffman, 1972; Pfister, 1973; Harding, Bagchi, Weinsieder & Peters, 1974). These studies have dealt with adult mammalian and fish corneas and in all cases the authors have described the corneal surface cells as exhibiting

1 Author's address: Department of Anatomy, Wayne State University School of Medicine, Detroit, Michigan, U.S.A.
a more or less regular pattern of microprojections either in the form of microvilli or microplicae. Other authors have dealt with the alterations of the surface topography of the anterior corneal epithelium produced by alkali burns (Henriquez, Pihlaja & Dohlman, 1976; Pfister & Burstein, 1976) or ophthalmic drugs (Mitsui, Takashima, Fujimoto & Kashiyama, 1976; Pfister & Burstein, 1976) but, to date, no one has used the scanning electron microscope to study the development of the anterior corneal epithelium. The present investigation describes the development of the chick anterior corneal epithelium as viewed with the scanning electron microscope.

**MATERIALS AND METHODS**

Fertile chicken eggs were incubated at 38 °C and 85% relative humidity in a circulating air incubator. The eyes of chick embryos were excised at daily intervals from 5 days of incubation through hatching. In addition, the eyes were excised from chicks at 1, 5 and 10 days after hatching and from adult chickens. The entire eye of the younger embryos was processed but only the anterior segment of the eye of the older embryos and post-hatched chickens was processed.

The tissue was fixed either in 2.5% glutaraldehyde (in 0.1 M cacodylic buffer or 0.1 M Sorensen’s phosphate buffer, pH 7.2) for 2 h with postfixation in 1% osmium tetroxide for 1 h or in a mixture of 2.5% glutaraldehyde (0.1 M cacodylic buffer, pH 7.2) and 1% osmium tetroxide according to the method of Hirsch & Fedorko (1968). After fixation, the tissues were washed in buffer and dehydrated through a series of ethanols. Dehydrated tissues were critical point dried using CO₂ or Freon 13 (in which case it was first run through a series of Freon 113) as the transitional fluid. The preferred procedure was fixation according to Hirsch & Fedorko (1968) and critical point drying with CO₂.

**FIGURES 1–6**

Fig. 1. The chick anterior corneal surface after 5 days of incubation. Note the regular polygonal shape of the cells and the microvilli and marginal folds of plasma membrane. ×3500.

Fig. 2. Anterior corneal surface of the chick after 5 days of incubation. ×10000.

Fig. 3. Anterior corneal surface of the chick after 9 days of incubation. Note that there is an increase in the number of microvilli and a decrease in cell diameter when compared to the situation after 5 days of incubation (Fig. 1). ×3500.

Fig. 4. Surface of chick anterior corneal epithelium after 9 days of incubation. ×10000.

Fig. 5. Superficial layer of chick corneal epithelium after 15 days of incubation. Note the greater number of microvilli than was observed at 9 days of incubation (Fig. 3). ×3500.

Fig. 6. Cell surface from chick anterior corneal epithelium after 15 days of incubation. Notice that the microvilli appear to be interconnected. ×10000.
The dried specimens were mounted on aluminium stubs, coated with gold in a Hummer sputtering system and viewed either in a Philips 500 or an ETEC autoscan scanning electron microscope.

RESULTS

The surface cells of the chick anterior corneal epithelium, after 5 days of incubation, were not unlike the cells of the remainder of the embryonic ectoderm. They were polygonal in outline and exhibited scattered short microvilli and cell boundaries demarcated by discontinuous folds of the plasma membrane (Figs. 1, 2). For the next 4 days of incubation there was no detectable changes in the cell surfaces except for an increase in the number of microvilli and a slight decrease in the cell diameter (Figs. 3, 4).

From 9 days of incubation to about 14 days of incubation there was an ever increasing number of microvilli found on the surface cells of the chick anterior corneal epithelium. After 15 days of incubation the microvilli had covered the cell surfaces so that the cells had taken on a shaggy appearance (Fig. 5). With closer examination it was found that the microvilli had begun to change. It appeared as if interconnexions had formed between adjacent microvilli or else the individual microvilli had simply become more tortuous (Fig. 6). The number of microvilli and/or interconnexions or tortuosity continued to increase for the next few days until the surface began to take on a sieve-like appearance (Figs. 7, 8). It was not unusual during that time span (14–17 days of incubation) to see cells in the final stages (telophase) of mitosis (Fig. 9). The cell surfaces after 19 days of incubation became rather smooth with only occasional microvilli extending above the sieve-like surface (Fig. 10). Just prior to hatching (20 days of incubation) that monotonous landscape was broken by the

Figures 7-12

Fig. 7. Cell surface of chick anterior corneal epithelium after 16 days of incubation. The number of microvilli has continued to increase and they continue to coalesce with one another. \( \times 10000 \).

Fig. 8. Anterior corneal cell surface after 17 days of incubation. There appears to be a continued coalescence of microvilli. \( \times 10000 \).

Fig. 9. A late telophase figure observed on the chick anterior corneal surface after 15 days of incubation. \( \times 3500 \).

Fig. 10. The surface of the chick anterior corneal epithelium after 19 days of incubation. Notice the sieve-like appearance and that only occasional microvilli extend above the surface. \( \times 20000 \).

Fig. 11. The chick anterior corneal surface after 20 days of incubation. There are cells with a variety of shapes and textures scattered over the surface. \( \times 400 \).

Fig. 12. Higher magnification of part of the field seen in Fig. 11. Some of the cells appear wrinkled (W) and are surrounded by 'stress furrows' while others are more rounded (R) and are sitting in little depressions. The latter cells are covered with microprojections. At other locations there are slight depressions (D) where second layer cells can be seen. \( \times 1000 \).
SEM of chick anterior corneal epithelium

appearance of a plethora of polymorphic cells over the entire corneal surface (Figs. 11, 12). Some of these cells were flattened and wrinkled while others were rounded and covered with microvilli. In addition, depressions were observed exposing second layer cells. The wrinkled cells were frequently surrounded by radiating furrows and attachments to surrounding cells were observed (Figs. 13, 14). The rounded cells that were covered by microvilli were frequently seen to be sitting in slight depressions (Figs. 13, 15 and 16). The second layer cells found in depressions had surface morphologies similar to that observed at 17 days of incubation (Figs. 13, 14).

After hatching, the anterior epithelial surface took on a quilted appearance with cell surfaces showing various textures and degrees of brightness. The cell textures ranged from definite microvillous surfaces to rather smooth surfaces. The cells with the surface projections appeared much lighter in the scanning scope than the cells with the smooth surfaces (Fig. 17). This was in stark contrast to the embryonic stages when all the surface cells had, in general, the same appearance as if they were synchronized in their development. The adult chicken corneal epithelial surface cells not only showed a lack of synchrony in development but they no longer had regular polygonal outlines. The irregular cell outlines and the short irregular microvilli on the lighter cells gave the adult corneal surface a patchwork appearance (Fig. 18).

DISCUSSION

The significance of the increasing number of microvilli on the developing chick cornea is not apparent, though other authors (Pedler, 1962; Ehlers, 1965; Lemp, Holly, Iwata & Dohlman, 1970) have suggested that in the adult of other species the microvilli may serve to anchor the tear film. This, most assuredly,

Figures 13-18

Fig. 13. Part of the field seen in the previous two figures. A wrinkled cell and three round cells can be seen as well as two second layer cells. × 2000.

Fig. 14. The wrinkled cell seen in the three previous micrographs. Note the stress furrows surrounding the cell and the points of attachment at the periphery of the cell. × 3500.

Fig. 15. Three round cells seen in Fig. 13. They are sitting in slight depressions and on close observation points of attachment can be seen along the edge of the center cell. × 3500.

Fig. 16. A round cell sitting on the surface of the chick anterior corneal epithelium after 20 days of incubation. × 7000.

Fig. 17. Anterior corneal surface of the chicken 10 days after hatching. The cells are still polygonal in shape with various surface textures and degrees of brightness which gives the corneal surface a quilted appearance. × 1000.

Fig. 18. Adult chicken anterior corneal epithelium. The cells exhibit various surface textures and degrees of brightness but have lost their regular polygonal shapes which gives the surface a patchwork appearance. × 1000.
is not the case in the chick embryo. However, one must wonder if the microvilli in the chick embryo are functioning in the dehydration and acquisition of transparency of the chick cornea that has been found to occur in the chick between incubation days 14 and 19 (Coulombre & Coulombre, 1958).

The telophase figures that were observed between days 14 and 17 probably represented the terminal division for these peridermal cells since Nuttall (1976a) has found that the synthetic index of these cells decline from day 14 after having reached a peak of about 9.5%.

The reason for the microvilli being obscured as the chick nears hatching is not entirely clear but other authors (Pfister, 1973; Harding et al. 1974) have pointed out that some cells of the adult cornea in other species appear to be covered by a coating material when observed with the scanning electron microscope. This has most often been ascribed to a mucus or mucous-like coating of the cells. Pfister (1973) reported that acetylcysteine partially removed the coating material from mammalian cornea but a pilot study by this author found that acetylcysteine had no effect on the appearance of the chick embryo cornea in the scanning electron microscope. Pei & Rhodin (1971) reported that surface cells of the developing mouse cornea become smoother preparatory to desquamation and it is very likely that a similar thing is occurring with the chick cornea.

The appearance of the polymorphic cells on the anterior corneal epithelium just prior to hatching has been interpreted as a sloughing or desquamating process. The more flattened wrinkled cells with the stress lines around them represent the first stages of rounding up and lifting off. As the cells round up they simultaneously become covered with microvilli and are eventually lost from the surface and thus expose the second layer cells found in the bottom of the depressions. Whether this method of desquamation is unique to the embryo or is also found in the adult remains to be determined. This method of desquamation is very different from that described by Pfister (1973) for the adult mammalian cornea. He found that exfoliation was accomplished by the development of a full-thickness hole through the center of the cell followed by the retraction of the edges of the hole toward the periphery of the cell.

The different degrees of brightness and the variability of the surface texture that was observed after hatching has been reported for other adult species (Hoffman, 1972; Pfister, 1973; Harding et al. 1974). This phenomenon has been attributed to the number and/or length of microvilli and the presence or absence of coating material. Harding et al. (1974) suggest that the variability of surface cells in the fish corneal epithelium could be the result of various states of differentiation or senescence and a variable affinity for a mucus coating. The present data suggest that the different surface textures of chicken anterior corneal epithelial cells is due to actual changes in the cell membrane as a reflection of the state of differentiation or senescence but it neither supports nor refutes the idea of a differential adhesion of a mucous coating.
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REFERENCES


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