Differences Between Connective Tissue-Epithelial Junctions in Human Skin and the Anagen Hair Follicle

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Although the ultrastructure of the dermal-epidermal junction has been well characterized, little is known about the junctions between the dermal papilla and the surrounding epithelial cells of the hair bulb, or between the connective tissues and the epithelial cells on the outside of the hair follicle. Because the dermal papilla plays a major role in controlling the hair follicle, we also examined the ultrastructure of the potentially important dermal papilla-epithelial junction in normal scalp anagen follicles. The dermal-epidermal junction in skin was a trilaminar basement membrane characterized by the anchoring points of hemidesmosomes and tonofilaments in keratinocytes. In the hair follicle, the junction that separated the dermal papilla and epithelial cells was a trilaminar basement membrane, but relatively few putative anchoring points were seen. These were similar to modified dermal-melanocyte junctions, in which the intercellular cytoplasmic filaments do not come together at an attachment plaque, the laminar components tend to be thinner, and the anchoring fibrils beneath the lamina densa are fewer. A trilaminar membrane also was interposed between the connective and epithelial tissues on the outside of the follicle, but nothing that resembled a hemidesmosome or any other type of anchoring structure was seen. The difference in structure of the junctional complex between skin and hair follicles probably reflects the relatively permanent state of the epidermis, compared to the dynamic processes involved during the anagen phase of the hair follicle. Key words: dermal papilla/ultrastructure. J Invest Dermatol 104: 90-94, 1995

The structure and ultrastructure of the dermopidermal junction (DEJ) are well documented [1-4]; its composition and formation [5,6] and the roles of hemidesmosome attachment points and the various anchoring mechanisms in skin are clearly understood [7,8]. In contrast, most descriptions of the ultrastructure of hair follicles have concentrated on the appearance of the connective tissue and epithelial cells, their topographic organization with respect to each other, and the changes in appearance due to development and differentiation [9-12]. The junctions between the connective and epithelial tissues in the human hair follicle have been neglected, except by Puccinelli et al [13], probably because the important role of the connective tissues in the regulation of hair growth was not appreciated fully at the time.

The terms basement membrane and basal lamina are both used to describe the thin layer intervening between epithelial and connective tissues. Confusion exists because many biologists have used "basal lamina" to describe the structure that is now called the lamina densa [14]. The terms recommended by the International Anatomical Nomenclature Committee [15] have been adopted here, i.e., basement membrane for light microscopy and basal lamina at the electron-microscope level [16].

In transverse sections, the DEJ is seen as a basement membrane that follows an irregular, undulating line. Briggaman and Wheeler [1] described at the ultrastructural level the details of the junction, hemidesmosomes, and modified attachment points associated with melanocytes. The ultrastructure of the junctional zone between the dermal papilla and epithelial components (DPEJ) in mouse and rat hair follicles during early and late catagen also has been examined [17-19]. An additional component, the hyaluronic acid, was described on the outside of the hair follicle [17]. This is an outer layer of the follicle, adjacent to the basal lamina, which is seen as two layers of orthogonally arranged collagen fibers, with the inner layer parallel to the long axis of the hair. Puccinelli et al [13] did not describe this hyaluronic acid in human follicles.

Because the dermal papilla is now known to play a major role in influencing the form and dynamics of the hair follicle [20,21], which probably involves regulatory substances crossing the basement membrane [22], we investigated the ultrastructure of connective tissue-epithelial junctions (CTEJ) on the outside of the hair bulb and around the dermal papilla of normal anagen hair follicles. Samples of post-auricular skin were processed as controls.

MATERIALS AND METHODS

Collection of Skin Samples. Samples of normal human post-auricular skin were obtained from routine surgical excisions of benign lesions and plastic surgery. Samples were immersed immediately in a solution of 3% glutaraldehyde in 6.1 M Sörensen phosphate buffer at pH 7.2, cut into approximately 2-mm cubes, and transported to the laboratory in the...
glutaraldehyde in a sterile container. Scalp biopsy specimens were taken from the occipitoparietal region of normal scalp from six volunteers with informed consent and Ethical Committee approval at the Dermatology Department, Leeds General Infirmary. Punch biopsy specimens of 4 mm in diameter were taken under 1% Lignocaine local anaesthesia and immersed immediately in 3% glutaraldehyde in 0.1 M Sorensen phosphate buffer to check autolytic changes during transport to the laboratory.

**Processing of Skin Samples and Individual Hair Folllicles** Skin tissue was dissected further to yield 1-mm cubes, which were immersed in the subcutaneous DEJ. After trimming, the samples were processed for transmission electron microscopy within 4 h of excision. Scalp specimens were transferred to 35-mm petri dishes in glutaraldehyde solution, where anagen hair follicles were microdissected from each specimen and transferred to a second dish. The follicles were cleaned of surrounding tissue and lipid, but the connective tissue sheath was left intact. Primary fixation of the dissected follicles was continued in similar 3% glutaraldehyde for a total time of 3–4 h, including transport time. After the first fixation, subsequent processing was done in a Lync Microscopy Tissue Processor (Leica UK Ltd.). All tissues were fixed routinely in glutaraldehyde and osmium tetroxide and embedded in Araldite CY 212 epoxy resin for electron microscopy.

**Selection and Examination of Ultrathin Sections** Three control samples of post-auricular skin from different donors were sectioned on a Reichert Ultracut D ultramicrotome. Silver to pale gold ultrathin sections, i.e., about 80–100 nm in thickness, were examined in two blocks from one source of skin and in one from each of the others. Sections were stained with uranyl acetate and lead citrate [23] and then examined in either JEOL 100S or 100CX transmission electron microscope. Because the DEJ is a continuous and ultrastructurally stable environment, only two or three sites were selected in each of the sections examined; these were considered representative of the junction as a whole.

Hair follicles were much more difficult to section than skin samples because of the importance of orienting the follicle correctly in section to the direction of hair growth and through the center of the follicle. Excess resin was trimmed carefully from the block to leave the follicle exposed on the top of a broad-based pyramid of resin. Semi-thin surface sections of thickness 350 nm taken at frequent intervals were stained with toluidine blue [24] and examined by light microscopy. We adjusted the orientation of the follicle repeatedly until it was aligned, ideally with its long axis parallel to the Z axis of the microtome and perpendicular to the X and Y planes. This ideal was not always achieved because follicles are rarely regular in shape; if any follicle was twisted or kinked in any way, we exposed as much of the bulb and lower part of the follicle as possible for sectioning. Longitudinal ultra-thin sections of the lower follicle, up to about three times papilla height, were stained and examined as described above. Only sections cut through the median planes of the follicle were examined.

Eleven follicles from the six biopsy specimens were selected for study; useful ultra-thin sections were cut successfully from eight. The CTEJ was examined in detail at six or seven sites in each follicle, including the apex, approximate mid-point, and base of the dermal papilla, and three corresponding sites on the outside of the follicle; if possible, an additional site was examined at about two times papilla height. These sites are marked on Fig 2.

**RESULTS**

**The Skin DEJ** The human skin DEJ resembled that described in other studies [1–3]. Hemidesmosomes were seen lining the plasma membranes of the basal keratinocytes (Fig 1), with tonofilaments coursing back from the hemidesmosome attachment plaques into the cytoplasm of the basal cells. The lamina lucida and lamina densa were clearly seen as pale and darkly stained bands, respectively, that faithfully followed the line of the basal cell membranes. The extracellular space of the dermis was mostly filled with transversely cut collagen bundles interleaved with the processes of connective tissue cells.

Fine structural detail of the junctional zone and connective tissue is shown in Fig 1. The lamina lucida was evident though not always very well defined, but in the lucent space, sub-basal dense plaques were seen associated with the attachment plaques. Tonofilament connection to the attachment plaques was very marked. A slight increase in staining density of the lamina densa was evident at the points where the membrane passed beneath a sub-basal dense plaque and an attachment plaque. Anchoring fibrils were drawn out from their connection with the lamina densa at one end and inserted into the extracellular matrix of the dermis at the other; some appeared to be in the form of a loop (Fig 1). In the dermis, collagen bundles and cytoplasmic processes were interspersed with strips of longitudinally cut collagen, which displayed characteristic periodic banding. Anchoring filaments, although not readily obvious in Fig 1, could be discerned at higher magnification, but neither dermal microfibril bundles nor anchoring plaques associated with anchoring fibrils were seen in any of the sections examined.

**The CTEJ in the Normal Hair Follicle** At the light-microscope level, the basement membrane of the hair follicle appeared as an amorphous, pale-staining or fine fibrillar band (Fig 2). It formed a continuous layer enclosing the dermal papilla, where it formed an unbroken barrier, and continued around the outer parts of the follicle between the connective tissue sheath and the outer root sheath. Ultrastructural examination revealed a high level of organization similar to that of the DEJ.

**Outside of the Hair Follicle** Electron microscopy of the CTEJ confirmed the smoothness of the membrane between the outer root sheath and the connective tissue sheath. At medium magnification, the CTEJ appeared as a three-layered structure (position 1; Fig 3a), which was less undulatory than the similar structure seen in skin. The plasma membrane of cells of the outer root sheath was very well defined and was invaginated in several places by infoldings into the cell cytoplasm. The lamina lucida was a narrow but easily seen clear space between the plasma membrane and the lamina densa, with some particulate content comparable in appearance and quantity to that seen in skin. The lamina densa was a smooth band of uniform thickness which at high magnifications had a fine granular appearance. Subjacent to the lamina densa were the orthogonal layers of collagen fibers, which make up the hyaline membrane.

There was no evidence of anything resembling a hemidesmosome and no tonofilaments reaching back into the cytoplasm of cells of the outer root sheath. Located at irregular intervals along the membranes were small clouds of particulate matter (Fig 3a).
Near the base of the inner part of the lobe (position 4; Fig 3d), the orthogonal arrangement of the collagen layers was broken almost completely, though the fibers tended to lie parallel to the lamina densa. The orthogonal arrangement of fibers seen on the outside of the follicle was never observed within the dermal papilla.

The plasma membranes of the basal epithelial cells had a few thickenings possibly associated with attachment points to the basal lamina. The lamina densa appeared to follow the line of the epithelial plasma membrane, though there were several widenings of the lamina lucida. The lamina lucida was mostly filled with a substance similar in staining density and appearance to the cytoplasm of the epithelial cells. No invaginations similar to those seen on the outside of the follicle were seen in this area.

The interface between the epithelium and connective tissue, at mid-papilla height (position 5), was well defined by a slightly undulating line, which had few gaps or invaginations (Fig 3e). There were some slight thickenings on the plasma membrane, but these were not convincing as attachment plaques. The lamina lucida clearly had an evenly distributed, fine granular content (Fig 3e). The lamina densa was seen as a very thin, well-defined line, which paralleled the course of the epithelial cell plasma membranes. Collagen fibers were distributed intermediately along the length of the junction. The fibers amassed nearest to the lamina densa seemed to be preferentially end-cut.

At the top of the papilla (position 6; Fig 3f), the plasma membranes displayed several gaps and invfoldings similar to those on the outside of the hair follicle. Thickenings of the plasma membrane and associated increases in density of the lamina densa were seen at several points along the junction (Fig 3f). The lamina lucida contained an evenly distributed, fine-grained inclusion, and the lamina densa was continuous though of variable density. Collagen fibers were collected in irregularly sized clumps along the edges of the lamina densa. Anchoring fibril-like structures were attached at one end to the lamina densa of the junction and at the other end inserted into the extracellular matrix of dermal papilla (Fig 1).

Concentrations of fine fibrillar material, similar to dermal microfibril bundles [1], also were seen in the dermal papilla extracellular matrix.

Table 1 summarizes the observations described in the Results.

**DISCUSSION**

The ultrastructure of the CTEJ has been examined here in two main areas of the human scalp anagen follicle: on the outside between the connective tissue sheath and outer follicular tissues from the base of the follicle up to about twice the dermal papilla height, and on the inside between the dermal papilla and the epithelial tissues of the hair bulb. These junctions are the CTEJ and the DPEJ, respectively. Because our parallel control samples of skin clearly showed the normal structures reported for the human DEJ [1], our processing technique seems valid. The follicular junctions were similar to those seen in the skin; on the outside the junction appeared as a smooth line between the epithelial and connective tissue components, whereas where the junction divides the dermal papilla from the epithelial tissue, it was undulatory, though less so than in the skin.

The basement membrane was resolved into the three layers of peripheral cell membranes: lamina lucida, lamina densa, and the additional layers of the hyalin membrane reported in mouse follicles [17]. The mechanical connection between the hair follicle and the connective tissue components was much weaker than that between the corresponding components in skin. Nothing was seen that corresponded to the skin hemidesmosome with tonofilaments on either the external connective tissue or dermal papilla junctions. However, the appearance of particulate clouds of matter (Fig 3e) in the regions of the lamina densa and adjacent epithelium on the outside of the follicle were reminiscent of the thickening densities reported around and below attachment plaques in the dermal-melanocyte junction [1]. The increases in tissue density may be a similar kind of loose anchoring structure. Putative anchoring structures similar to those described in the skin dermal-melanocyte
junction also were seen around the top of the dermal papilla (Fig 3f). A similar absence of substantial anchoring components can be seen in a single micrograph of a human dermal papilla [13], though Puccinelli et al made no comment about this in their paper. Hemidesmosomes were reported as absent also from the rat hair matrix basement membrane [25]. It is interesting that bullous pemphigoid antigen, which is found in association with hemidesmosomes in the DEJ [26], was not present in the DPEJ of human anagen follicles [27] or in developing hair bulbs in mouse and rat skin [28,29].

Epithelial matrix cells surrounding the dermal papilla appear to be joined only loosely to the connective-tissue components, because it is nearly impossible to obtain dermal papillae by plucking anagen hairs [30]. The lack of definite anchoring mechanisms in the hair-follicle junctions may allow flexibility of movement while the cells undergo the dynamic processes of the growth cycle. The upward progression of the matrix epithelial cells [31] and the proposed downward movement of cells of the outer root sheath during anagen [32] would be highly restricted if they were tightly bound at the epithelial-connective tissue junctions.

We observed invaginations of the plasma membranes of cells of the outer root sheath adjacent to the CTEJ (Fig 3b,c), which apparently were not described before for the human anagen hair

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**Figure 3. Details of the CTEJ on the outside of the follicle (a–c) and the DPEJ (d–f).** See text and Fig 2 for definitions of positions 1–6. a: The junction (position 1) between the outer root sheath (top) and the connective tissue sheath (below) shows a three-layered structure comprising the basal-cell plasma membrane (PM), lamina lucida, and lamina densa (LD). Below the junction are two orthogonal layers of longitudinal (Cl) and transverse (Ct) collagen fibers. Invaginations (In) are seen in this part of the junction. Small clouds of particulate matter are diffused in the epithelial cell cytoplasm and across the basal lamina into the connective tissue layers. b: CTEJ at mid-bulb (position 2) between the hair matrix (above) and the connective tissue sheath (below). There are pronounced invaginations (In) in the basal-cell plasma membranes. Longitudinal (Cl) and transverse (Ct) collagen fibers persist in orthogonal layers but are not as well defined as higher in the follicle. Infusions of particulate matter (PC) are present in the lamina densa and connective tissue regions, but these do not persist into the epithelial tissues. c: CTEJ in the lower part of the bulb (position 3) between the division zone above and the connective tissue sheath. The trilaminar arrangement of the junction (CTEJ) is still evident and there are invaginations (In) in the plasma membranes, but the ordered composition of the collagen layers (C) has broken down. d: DPEJ at the base of the papilla (position 4). The two-layer orthogonal arrangement of collagen fibers (C), as seen on the outside of the follicle, is no longer evident. The junction is generally well defined, though the basal-cell plasma membranes (PM) and lamina densa (LD) are slightly diffuse. e: DPEJ at mid-papilla height (position 5) has a very well-defined interface with few gaps or invaginations. Small thickenings (A) of the plasma membranes on the hair-matrix side are not convincing as attachment plaques. Collagen fibers (C) in the dermal papilla are much sparser than in lower regions of the follicle. f: Putative anchoring system in the DPEJ near the apex of the papilla (position 6). The presumptive cortex is seen above, with the dermal papilla below. This anchoring system, which has a few similarities to a hemidesmosome, comprises a dense plate or attachment plaque (D) and a complementary thickening of the subjacent lamina densa only. Anchoring fibrils (AF) protrude into the extracellular matrix (ECM) of the dermal papilla, and microfibril bundles (MB) are interconnected with collagen fibers. Bars, 0.4 μm (a,b,d,f); 0.8 μm (c,e).
The differences in attachment points between the connective and epithelial tissues are obvious morphologic characteristics that distinguish the junction in skin from that of the hair follicle. Further studies, such as those by Shimizu et al [33] using immunohistochemistry, could be useful to define the differences between skin and both types of hair-follicle junctions. It would also be interesting to investigate changes to both ultrastructure and immunomorphology during the hair cycle, as changes have been demonstrated in the amount and distribution of basement membrane components [34].

In summary, these investigations into the comparative ultrastructure of normal human anagen hair-follicle bulbs have produced some interesting findings. Although follicles showed some marked similarities in the CTEJ, there were also clear differences both on the outside of the follicle and around the dermal papilla as compared with the similar junction in skin. In particular, neither hemidesmosomes nor tonofilaments, as seen in the DEJ, were observed in any hair-follicle junctions. Anchoring fibrils appeared well organized and interconnected with extracellular matrix collagen in the dermal papilla, but no anchoring fibrils were detected at the junction on the outside of the follicle. The lack of complexity of the junctions in the hair follicle as compared with the similar junction between dermis and epidermis is consistent with the need for motility of follicular tissues, in contrast to the relatively permanent relation between basal epithelial cells and the basement membrane. It will be interesting to examine the ultrastructure of the important DPEJ in hair follicles from patients with hair diseases, such as alopecia areata, to see whether it is altered in any way.

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