FINE STRUCTURE OF FIBRILLAR COMPLEXES ASSOCIATED WITH THE BASEMENT MEMBRANE IN HUMAN ORAL MUCOSA

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INTRODUCTION
Palade and Farquhar (1) have recently reported the presence of a unique fibril in the skin of Amphibia and, in less well developed form, in various sites in the rat. Previous to that report, Brody (2) and Younes (3) observed similar fibrils in human skin and cervix, respectively, but did not emphasize their unusual character. Recent observations in our laboratory indicate that a similar fibril is present in human oral mucosa.

MATERIALS AND METHODS
Biopsy specimens of clinically healthy human oral mucosa (buccal and alveolar), obtained from middle-aged females, were fixed in 4% glutaraldehyde (4) in s-collidine buffer (5) for 2-3 hr at 0°C, washed in buffer overnight, postfixed in 1% OsO₄ for 3 hr, and embedded in Araldite (6). All sections were doubly stained with uranyl acetate (7) and lead citrate (8) and examined in the Philips EM-200.

RESULTS
A scalloped network of densely staining fibrils was observed immediately adjacent to the basement membrane (basal lamina or lamina densa) at its connective tissue side (Figs. 1–3). The network was composed of individual fibrils measuring 200–400 Å in diameter (Figs. 2–5) which, in turn, appeared to consist of a large number of closely packed fine filaments (Figs. 4 and 5). Although the length of the fibrils could not be determined due to their branching and curving course and their numerous interconnections, some were observed for nearly 250 µm. The fibrils demonstrated a distinctive banding pattern with no definite repeating unit (Fig. 4). Before reaching the basement membrane, the fibrils usually branched and broke up into smaller groups of individual fine filaments (20 Å in diameter) which fanned out into a spray (Figs. 4 and 5). Most of the filaments of this spray appeared to enter the basement membrane, while some seemed to traverse the dense portion of the basement membrane and the adjacent less dense region (lamina lucida) to reach the cell membrane of the basal epithelial cell. This relationship appeared to be most evident at the regions of the hemidesmosomes (Figs. 2, 4, and 5). In some sites, usually at or near a hemidesmosome, as the limit of resolution (imposed by our specimens) was approached, it was the impression that continuity existed between these extracellular filaments and intracellular filaments (Fig. 5). Unequivocal confirmation of this relationship is lacking.

Other fibrils have been noted to course perpendicular to the anchoring fibrils through the spaces formed by the anastomosis of adjacent anchoring fibrils (Figs. 2 and 5). It was difficult to determine with assurance the nature of these fibrils when viewed in cross-section. However, the morphology and periodicity of similar fibrils observed in longitudinal section indicated that they were collagen fibrils intertwined with the anchoring fibrils.
FIGURES 1 through 5 are perpendicular sections through the basement membrane region of human oral mucosa.

**General Abbreviations:**

- Epithelial cell: E
- Hemidesmosome: H
- Basement membrane: B
- Lamina propria: L
- Anchoring fibril: F
- Collagen fibril: C

**Figure 1** Two epithelial cells (E) comprise the major portion of this figure. Hemidesmosomes (H) are numerous along the basal surface of these cells. A continuous basement membrane (B) closely follows the contours of the epithelial cells at their junction with the lamina propria (L). Small, densely staining fibrils (F) approach the basement membrane from its connective tissue side and appear to attach to the basement membrane predominantly at the sites of the hemidesmosomes. × 20,000.

**Figure 2** Higher magnification of basement membrane region shown in Fig. 1. Anchoring fibrils (F) appear to branch and break up into fine filaments and then enter the basement membrane (B). Collagen fibrils (C) in cross-section are present within the tunnels formed by the network of anchoring fibrils. × 60,000.

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FIGURE 3 The scalloped network formed by interconnecting anchoring fibrils (F) on the lamina propria (L) side of the basement membrane (B) appears to house collagen fibrils (C) within its "tunnels." X 70,000.

In Fig. 6, a composite diagram of the relationships of the elements described above is represented in three-dimensional form.

**DISCUSSION**

The fibrils reported here appear to be quite similar to, although substantially smaller than, the "anchoring fibrils" described by Palade and Farquhar (1). Their conclusion that anchoring fibrils represent a new type of specialized fibril, quite distinct morphologically from collagen or reticulin fibrils, is substantiated and extended by the present report. Other examples of the anchoring fibrils may be the "reticular filaments" of skin, which may be continuous with the basement membrane (2), and fibrils described in the human cervix (3).

An interesting relationship appears to exist among these fibrils, the basement membrane, and the basal epithelial cells. The fine filaments which arise from the anchoring fibril seem to diverge and run in various directions in the basement membrane. In so doing, they may constitute a major portion of the fine filamentous network of the basement membrane in this region. In addition, some of these fine filaments give the appearance of passing through the basement membrane (lamina densa) to cross the light space (lamina lucida) and attach to the cell membrane of the basal epithelial cells. Stern (9) has reported the presence of similar filaments (60 A in diameter) in the human gingiva and rat cheek. Younes et al. (3) have also observed fine filaments (40 A in diameter) running between the basement membrane and hemidesmosomes in the human cervix.

In the present study, these anchoring filaments, as well as the anchoring fibrils, appear to be most prominent at the sites of hemidesmosomes. There is also a suggestion that some of these fine filaments may pass through the cell membrane and into the epithelial cell. Fawcett has similarly indicated that the cytoplasmic filaments in epidermal cells of larval *Amblystoma* may converge upon the hemidesmosome and extend through the cell membrane to end in the dense layer of the basal lamina (10). This intriguing aspect necessitates further investigation of the anchoring fibril and its various ramifications in both normal and pathological conditions.

That portion of the human oral mucosa which lines the oral aspect of the lips, the cheek, the vestibule, and the alveolar bone (exclusive of the gingiva) consists of nonkeratinized stratified squamous epithelium and an underlying lamina propria, which are firmly bound together under normal circumstances. The results of the present study indicate that anchoring fibrils and filaments may play an important role in maintaining this firm attachment between epithelium and connective tissue in human oral mucosa. This attachment seems to involve the interrelationship of adjacent anchoring fibrils, collagenous fibrils, basement membrane, and even the epithelial cells themselves.
FIGURE 4 Longitudinal section of an anchoring fibril. At its deepest point in the lamina propria (L), the anchoring fibril (F) is seen to form a loop in which a collagen fibril (C) is cradled. Note the branching of the anchoring fibril before it enters the basement membrane (B). Some of the fine filaments can be seen running into the basement membrane and others seem to pass through to reach the basal epithelial cell (E). X 130,000.

SUMMARY

A specialized fibril of the lamina propria of human oral mucosa has been demonstrated. This fibril was observed beneath the basement membrane. A network of these fibrils appears to attach to the basement membrane and the basal epithelial cells and to interdigitate with collagen fibrils of the lamina propria. These fibrils may be important in maintaining the firm attachment of the epithelium to the connective tissue in this portion of the human oral mucosa.

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FIGURE 5 The fine filaments (30 A in diameter) of the anchoring fibril (F) fan out into a spray before entering the basement membrane (B). Some of these “anchoring filaments” seem to traverse the light space or lamina lucida (A) adjacent to the basement membrane to reach the epithelial cell membrane (E) in the area between the arrowheads. Filaments of the same size can be seen approaching the plasma membrane from the inside of the cell. The plasma membrane is located obliquely with respect to the plane of the section, thus creating a somewhat less dense appearance over a broader area than would be so if the membrane were oriented perpendicularly. In this area on the original micrograph, suggestions of filamentous continuities are present that have not been visualized in sites in which the membrane has high density due to its perpendicular orientation. A collagen fibril (C) can be seen running perpendicular to the anchoring fibril. X 100,000.

FIGURE 6 Three-dimensional representation of relationships among anchoring fibril, collagen, basement membrane, and epithelial cell. Evidence for the existence of penetrating filaments, indicated by a question mark (?), is not yet conclusive.
REFERENCES


