THE EXTRACELLULAR MATRIX OF HUMAN AMNIOTIC EPITHELIUM: ULTRASTRUCTURE, COMPOSITION AND DEPOSITION

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SUMMARY

Ultrastructural comparisons have been made between human amnion extracellular matrix in tissue and cell culture. Immunochemical analysis of matrix deposited by monolayers of cultured amnion epithelial cells has also been undertaken. The basal cell surfaces are highly invaginated with an associated basal lamina that is more electron dense at the distal tips of basal cell processes where hemidesmosomes are frequent. Immediately below the lamina densa is a zone rich in collagen bundles. In the underlying stroma two types of fibril predominate, one striated of 50 nm diameter and one of 18 nm diameter. The observations suggest that at gestational term the epithelial cells are still active in the production of matrix. Secretion appears to occur into invaginations in the basal cell surface where a loosely organized mixture of stromal-type and basal laminal-type aggregates is formed. In culture on plastic, cells also deposit a mixture of basal laminal (type IV collagen + laminin) and stromal (collagens type I + III) components as well as fibronectin. However, segregation into a true basal lamina with underlying stroma does not occur in vitro, suggesting the need for an organized subcellular template to complete matrix morphogenesis. The in vitro and in vivo evidence suggest that the epithelium contributes to the subjacent dense collagenous zone as well as to the basal lamina.

INTRODUCTION

Epithelial cells grow on a basement membrane, and in most anatomical locations stromal connective tissue, which is compositionally and morphologically distinct, provides the underlying support (Aplin & Hughes, 1982; Grant, Heathcote & Orkin, 1981). In human amnion, the compact layer of stroma beneath the epithelium is relatively thick (5–20 μm) and is likely to make an important contribution to the tensile strength of the tissue (Bourne, 1960). Its acellular nature also points to a possible origin in the epithelial cells. The assembly and maintenance of amniotic extracellular matrix is of clinical interest since rupture of the foetal membranes is a frequent factor leading to premature labour, and has been linked to ascorbate deficiency (Wideman, Baird & Bolding, 1964).

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In the present study we sought to determine by ultrastructural means the extent to which extracellular matrix laid down by amnion epithelial cells in culture resembles that seen in different subepithelial levels in tissue; whether electron microscopic or immunocytochemical evidence could be obtained in support of the idea that the epithelial cells secrete components of both basal lamina and stroma at their basal surfaces in vivo; and if so, how these components are sorted and separately assembled. It was also important to determine whether matrix secretion might still be occurring at or near gestational term.

MATERIALS AND METHODS

Culture
Normal placentas were obtained at Caesarian section in cases where surgery was undertaken because of cephalo–pelvic disproportion or previous history of section. Pure suspensions of amniotic epithelial (PAE) cells were isolated with trypsin–EDTA (Flow Laboratories, Paisley, U.K.), according to the method previously described by Aplin, Campbell & Foden (1984). Only those cultures that reached confluence within 5–7 days were used, after preliminary experiments demonstrated that cultures of low plating efficiency did not generate an abundant matrix visible in the phase-contrast microscope. The culture medium was a 1:1 (v/v) mixture of Ham's F12 and Dulbecco's Modified Eagle's Medium containing 9% (v/v) foetal calf serum. In some cultures the commercial synthetic medium supplement Ultroser G (LKB, Croydon, U.K.) was used in place of serum. A vial of dried supplement was reconstituted into 12 ml distilled water, then 125/4 was added to 100 ml culture medium. The medium was supplemented (Aplin et al. 1984) with 50 µg/ml L-ascorbic acid (Hopkin & Williams, Chadwell Heath, U.K.) on the tenth day of culture and subsequently every 2 or 3 days when fresh medium was added. All results reported here were obtained from primary confluent cultures, which were maintained for a maximum of 24 days.

Electron microscopy
The reflected membranes were dissected free of the placenta and washed briefly in phosphate-buffered saline (PBS) containing magnesium and calcium. On occasions the membrane was left for a further 10–15 min in PBS, in order to facilitate separation of amnion and chorion. Pieces of amnion or chorioamnion were then cut from areas remote from tears or cuts, fixed in 2-5% gluteraldehyde in Sorenson's phosphate buffer (pH 7.2) for 24 h, postfixed in 1% osmium tetroxide in the same buffer for 1 h, and routinely dehydrated and embedded in Epon. Sections were stained with uranyl acetate and lead citrate and viewed on an AE1801A transmission electron microscope at 80 kV accelerating voltage. Tissue prepared for scanning electron microscopy was fixed and dehydrated in a similar fashion, and critical point dried from liquid CO2 using 'Arklone' (ICI, U.K.) as transitional solvent. The tissue was bonded to aluminium stubs, sputter-coated with 5 nm of platinum and observed with an ISI SS40 scanning microscope at an accelerating voltage of 20 kV. In order to examine the stromal layers subjacent to the epithelium, critical-point-dried amnions were mounted on stubs, finely dissected with a microscalpel made from a hypodermic needle (Campbell & Bard, 1985), and then recoated. Cultures were prepared in a manner similar to that described above and as previously described by Campbell, Allen & Aplin (1984). For transmission electron microscopy cultures were double embedded and cut perpendicular or parallel to the plane of the growth surface.

Preparation of extracellular matrix in vitro
Substratum-attached extracellular matrix was prepared by adding 2% sodium deoxycholate (DOC, Koch-Light, Colnbrook, Berks, U.K.) with 0.02% EDTA (pH 8.2) to confluent cultures after the period of ascorbic acid supplementation. This treatment solubilized the cells within a few
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seconds, as assessed by light-microscopic examination. The resultant suspension was immediately washed away with copious amounts of distilled water and then the matrix was fixed in 2% formaldehyde in PBS for a minimum of 20 min. Circular portions of the culture dish were removed with a heated cork borer and stored in PBS at 4°C. At no time was the extracellular matrix air dried.

Cryosection of amnion

Pieces of amnion were obtained as above and quenched directly into liquid nitrogen. Sections were cut at 5 μm in a plane perpendicular or tangential to the epithelium, dried onto microscope slides in a stream of warm air and stored at −20°C for up to 1 month. Sections were then thawed and fixed in 2% formaldehyde in PBS for 15 min before use.

Indirect immunofluorescence

Cryosections of tissue and samples of extracellular matrix from tissue cultures were examined for the presence of cellular and extracellular matrix components using standard techniques. The first antibodies were applied to the sample for 45 min at ambient temperature. Slides were washed rapidly in PBS, then for 5–8 min in running tap water, whereas cultured material was washed by repeated dipping in three sequential baths of PBS and then distilled water. Fluorescein isothiocyanate (FITC)- or tetramethyl rhodamine-conjugated antibody was then used as a second layer and the washing procedure repeated. Control samples with second layer alone were processed in each case with both types of antibody. Samples were mounted in the non-fade mountant of Johnson & Nogueira Araujo (1981), which contained 1 mg ml⁻¹ 1-phenylenediamine, 9.9% PBS (pH 7.4) and 90% glycerol, and photographed within 24 h on Leitz Dialux microscope using Ilford HP5 film uprated to 16000 ASA with Paterson Acuspeed developer.

Antibodies

Rabbit anti-fibronectin and anti-laminin antibodies (Bethesda Laboratories, Paisley, U.K.) were used at a concentration of 1/50 on tissue sections and 1/10 on ECM. Mutual cross-reactivity was <10% by ELISA. As expected (Morgan & Garrod, 1984) neither antibody reacted with detergent-generated subcellular matrix produced by the cell line FL, which resembles HeLa. Neither antibody reacted with fibronectin-free human plasma by double diffusion, nor with trypsin-treated subcellular matrix generated by amnion epithelial cells (Aplin et al. 1984). Anti-fibronectin antibody reacted with human skin fibroblast monolayers and their detergent-treated matrix in a characteristic fibrillar pericellular pattern that was quite distinct from that seen in Fig. 12.

Guinea pig anti-collagen types III and IV were generously provided by Dr Shirley Ayad (Evans et al. 1983). They were raised to purified human pepsinized placental collagens. Anti-type III was absorbed against types I, IV and V and then eluted from immobilized type III. Anti-type IV was absorbed against types I, III and V before eluting from immobilized type IV. Rabbit anti-type I collagen was kindly provided by Dr John Anderson (Bartholomew & Anderson, 1983). It was affinity absorbed and then affinity purified using a similar protocol.

Antibodies were raised against keratins of human stratum corneum according to the procedure of Sun, Shih & Green (1979). Reactivity of this antiserum was tested against electroblotted keratins and it was found to react principally with components of 62000, 58000 and 48000 molecular weight.

The second layer consisted of either FITC-conjugated sheep anti-rabbit immunoglobulin (Wellcome, London, U.K.; 1/50 dilution) or fluorescein- or tetramethyl rhodamine-conjugated anti-guinea pig (Miles, Slough, U.K.; 1/50).

Phallacidin staining

NBD-labelled phallacidin (Molecular Probes, Inc., Junction City, Oregon) was used to stain F-actin at a concentration of 165 μg ml⁻¹ according to the method of Barak, Yocum, Nuthnagel & Webb (1980) except that the non-fade mountant (see above) was used.
RESULTS

Structure of amniotic epithelium and basal lamina

The epithelium of the amnion consists of a single layer of cells resting on a basal lamina, supported by a dense acellular collagenous matrix. The cells are cuboidal, with apical microvilli (Figs 1, 2). The lateral cell borders are convoluted with frequent desmosomes and no obvious tight junctions. The basal epithelial cell surfaces (Fig. 2) are highly convoluted with frequent hemidesmosomes at the distal termini of cell processes. Wavy filament bundles – probably cytokeratin – are seen in the adjacent cytoplasm (Fig. 3). The basal lamina appears to follow the contours of the basal cell surfaces for most of their length.

At higher magnification (Figs 3, 4) the basal lamina is seen to consist of a lamina rara and lamina densa. Beneath the distal tips of basal cell processes, at hemidesmosomes, the lamina rara is narrow (30 nm) with frequent small parallel fibrils (4–12 nm diameter) running from the lamina densa to the cell surface. Here the lamina densa is compact and condensed. Within basal cell surface invaginations, the organization of the lamina densa becomes looser and fine fibrils (4–12 nm) can be distinguished similar to those seen crossing the lamina lucida. These are occasionally seen to be organized in a meshwork (Fig. 4) with associated amorphous deposits. In thee invaginations, fibrillar material can still be seen in association with the cell surface at discrete points. However, the matrix becomes more loosely organized and generally at points >2 μm from the hemidesmosomes (proximal to the cell body) it disappears entirely. This also occurs between the borders of adjacent cells.

Ultrastructure of the compact layer

In Fig. 1, tissue is shown after fracturing to expose inner layers of connective tissue. Because the dried tissue contains no discrete fracture plane (Figs 2, 3, 4), it is likely that the basal lamina remains adherent to the basal cell surface and is therefore not exposed except in small patches. As a result, acellular layers of collagenous fibrils are visible. Their packing density decreases in deeper locations. The uppermost layer is a particularly tightly packed meshwork of fibrils.

By transmission electron microscopy (TEM) (Figs 3, 4) a dense fibrillar transitional zone is also visible between the lamina densa and stroma. Here bundles of parallel fibrils can be seen in cross-section or running in the plane of section. The most abundant of these (F1, Figs 3, 4) are indistinguishable from 18 nm diameter fibrils that occur throughout the deeper layers and often bundle at or below the

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Fig. 1. SEM of the surface and fractured edge of the amnion, showing the surface epithelium (e) and underlying collagenous matrix (c). Where the cells have been fractured away from the surface of the matrix, some remnants of basal lamina may remain (arrows), although the majority of the basal lamina is interpreted to have remained attached to the cells. ×1750.

Fig. 2. TEM of vertical section through the amniotic epithelium (e), basal lamina (bl), and underlying collagenous matrix. The cell on the right is sectioned near its lateral border, which is complex and interdigitated with that of an adjacent cell. ×16000.
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transitional zone. In addition, occasional 25 nm fibrils are present (f2, Fig. 4), and appear to be located exclusively in the transitional zone.

In deeper layers, a class of thicker (50 nm) fibrils is visible by TEM (f, Figs 3, 4), in which striations are clearly observed. Numerous examples are seen in Figs 3 and 4 of 50 nm diameter fibrils in cross-section or in the plane of section. Variation is observed amongst different amniotic membranes in the distribution of 50 nm fibrils, accumulations of which can sometimes also be found immediately subjacent to the basal lamina. In addition, an overall faintly staining background (Fig. 4) suggests the presence of an amorphous ‘filler’ material, possibly proteoglycan. No elastin is evident in the micrographs.

Assembly of basal laminal and stromal matrix

Close examination of the basal aspect of the cells reveals that small discontinuities and areas of loosely associated matrix are quite frequent within the basal lamina, occurring more than twice under each cell in each plane of section. At high magnification it is clear that at or near these discontinuities (d, Fig. 3) fibrils characteristic of the stromal layers, especially the abundant 18 nm class (f1, Fig. 3) occur directly adjacent to the basal plasma membrane of epithelial cells, above the basal lamina. Areas of stromal fibrillar material also occur within basal surface invaginations (Fig. 3) and in places the finer fibrillar and amorphous material characteristic of lamina densa is associated with it (Fig. 3). Numerous ribosomes and extensive rough endoplasmic reticulum and Golgi stacks are observed in the cytoplasm of the epithelial cells (not shown). Coated pits are also frequently associated with plasma membrane within basal surface invaginations (Figs 3, 4) and at lateral cell borders, but are much less frequent at the distal ends of basal cell processes near hemidesmosomes. Thus at gestational term the morphology of the epithelial cells is consistent with the synthesis and secretion of a mixture of stromal and basal laminal macromolecules into basal invaginations.

Extracellular matrix deposition by amnion epithelial cells in culture

Cells in culture in the presence of foetal calf serum, or the commercial synthetic substitute Ultroser G, grow to confluence as a pure epithelial monolayer and display a more flattened morphology than in tissue, with under- and overlapping lateral

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Figs 3, 4. Vertical sections through the epithelial cell/basal lamina/matrix region.

Fig. 3. Both lamina rara (lr) and lamina densa (ld) follow the cell processes for approximately two thirds of the total length, and in areas of hemidesmosomes (hd) there is increased material traversing the lamina rara. Intermediate filaments (if) run into the cell extensions. Coated pits (c) are also seen on the basal cell surface. Cross striations are visible on some deeper fibrils (f) whereas other fibrils show no striations (f1). Discontinuities in the basal lamina (d) are occasionally apparent. In some basal surface invaginations, fibrillar stromal material is visible (unlabelled arrow) next to the plasma membrane.

Fig. 4. A tangential section through adjacent hemidesmosomes (hd). Most of the fibrils immediately beneath the lamina densa are of the f1 type, but occasionally thicker fibrils are seen (f2). Fig. 3, ×37,000; Fig. 4, ×42,000.
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processes and fewer apical microvilli. Desmosomes are prominent at lateral surfaces, and some interdigitation is maintained resembling that in the tissue. Extracellular matrix is deposited and increases markedly after feeding the cells with ascorbate (50 µg ml⁻¹). As in tissue, coated pits can be seen at the plasma membrane near areas of secreted matrix (Fig. 5). In this type of culture, where cells are seeded directly onto plastic, no organized basal lamina is visible, and correspondingly, no hemidesmosomes are formed (Figs 5, 6). The matrix can also be examined by scanning electron microscopy (SEM) (Figs 7, 8) in areas where cells have retracted spontaneously (without degeneration). It consists of a fine network of substratum-associated fibrils gathered up at intervals into thicker bundles, which may reach 5 µm in diameter. Bundles can also be seen in the light microscope at high magnification, where it is clear that when fully hydrated, they run up out of the plane of the substratum.

In sections cut parallel to the growth surface, the extracellular matrix deposited in serum-supplemented culture contains strips and foci of amorphous material distributed amongst thin fibrils (5—15 nm) (Fig. 5). Further from the cell underside, bundles of thicker (30 nm) fibrils are formed (Fig. 6), while the amorphous deposits are associated into larger aggregates. Vertical sections show similar materials deposited in dense bundles under the basal membrane. Changes in culture conditions can give rise to alterations in the organization of matrix accumulated by the cultures. Thus in the presence of Ultroser G, bundles of matrix are no longer visible in phase contrast at sites of cell retraction. This is due to the fact that the matrix is now present adjacent to the substratum as a thin sheet consisting of smaller fibrils. No bundling into larger structures occurs (Fig. 9). The small fibrils are oriented approximately anisotropically within the sheet. Substantial quantities of this type of matrix also appear in association with the upper surfaces of the cells running between apical microvilli (Fig. 10).

Immunofluorescence examination of matrix deposited in culture

Confluent cultures established in serum and fed for 10 days with ascorbate were treated with deoxycholate and EDTA to dissolve the cells and leave extracellular matrix associated with the substratum (Aplin et al. 1984). A comparison of matrix present between cells in spontaneously retracting cultures with that seen in detergent-treated cultures shows that the extraction procedure does not affect the organization of the matrix. This is shown by immunofluorescence with antibody to fibronectin in Figs 11 and 12, and can be confirmed by SEM (not shown). The

Figs 5, 6. TEM sections cut en face parallel to the growth surface of a primary amniotic epithelial cell culture in 10% foetal calf serum with 50 µg ml⁻¹ ascorbate. Where the plane of section is close to the cell underside (Fig. 5) cell–matrix contacts can be seen with microtubules (mi) in the adjacent cytoplasm. The extracellular matrix consists of thin disorganized fibrils with associated strips and foci of amorphous material. Further away from the cell underside (Fig. 6) the fibrils are thicker and gathered into bundles and the amorphous deposits (arrows) are also in larger patches. Fig. 5, ×19 000; Fig. 6, ×19 000.
residual matrix does not contain quantities of actin or keratin detectable by immunofluorescence and therefore appears to be free of cytoplasmic components. It was stained with antibodies to basal laminal or stromal matrix components (Figs 11–16). Staining for laminin (Fig. 13) and type IV collagen (Fig. 14) consists of punctate deposits superimposed on a fainter-staining reticular array. Collagen type I (Fig. 15) and type III (Fig. 16) show more extensive, continuous regular filamentous networks with bundling into thicker fibrils, which run out of the plane of the substratum. Fibronectin (Fig. 12) appears still more abundant, appearing to be present in most areas of matrix as a reticular array organized in a fashion similar to matrix observed by SEM (Fig. 7).

Cells grown in monolayer culture in Ultroser G proved resistant to solubilization using DOC–EDTA, cell residues and nuclei being clearly visible in contrast to the

Figs 9, 10. SEM of area of cultured PAE cells grown in Ultroser G, which appears to stimulate extracellular matrix production as a sheet rather than a reticular pattern (Fig. 9) and also over the upper surface of the cells, as a dense fibrous meshwork (Fig. 10). Fig. 9, ×4500; Fig. 10, ×15 000.

Figs 7, 8. SEM preparations of cultured primary amniotic epithelial cells in areas of spontaneous retraction, allowing visualization of the subepithelial matrix deposited on the plastic growth surface. At low power (Fig. 7) the matrix has a roughly reticular distribution, and at high power (Fig. 8) the bundling of the denser areas of fibrils is apparent. Culture conditions as in Figs 5, 6. Fig. 7, ×2500; Fig. 8, ×13 000.
cell-free matrix remaining from cultures in serum. This confirms the variation in phenotype with conditions of culture, and resistance to extraction probably arises from the fact that cells grown in Ultroser G accumulate matrix adjacent to their

Figs 11–16. Immunofluorescence of matrix components secreted by cultured amnion cells.

Fig. 11. A spontaneously retracting culture stained with anti-fibronectin antibody. Three cells are in view. The plane of focus is on the cytoplasm, which is seen to be rich in fibronectin. Reticular staining is also visible on surrounding substratum. Some of the substratum-associated fibronectin projects upward into the plane of focus.

Figs 12–16. Immunofluorescence of detergent-extracted matrix with the plane of focus on the substratum. Fig. 12 shows fibronectin with organization similar to substrate-associated material in Fig. 7; Fig. 13, laminin; Fig. 14, type IV collagen; Fig. 15, type I collagen; Fig. 16, type III collagen. Figs 11, 12, 14, ×500; Figs 13, 15, 16, ×640.
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apical surfaces in addition to the basolateral surfaces. This matrix was not analysed by immunofluorescence.

**Immunofluorescence of human amnion at gestational term**

Whilst actin is concentrated in apical (Fig. 17) and lateral (Fig. 18) areas of epithelial cell cytoplasm, keratin is visible throughout epithelial cell cytoplasms including the basal areas where hemidesmosomes occur (Fig. 19). Laminin (Figs 20, 21) occurs in the basal lamina and the cytoplasm. In transverse sections (Fig. 20) it appears as a continuous lamina near the basal cell surface. However, in glancing sections (Fig. 21) spots and streaks of laminin are resolved near the basal cell surface, consistent with the basal cell surface invaginations and basal laminal organization seen by TEM (Figs 2, 3). Type III collagen occurs throughout the stroma and weakly in epithelial cell cytoplasms (Fig. 24) while type I collagen (Fig. 22) is visible only as sparse punctate distributions. Thus type III may be the more abundant of the two. Surprisingly, an array of type I collagen is present within the epithelial cell cytoplasm. Fibronectin is abundant in all layers (Fig. 23) as well as being very weakly detectable within epithelial cell cytoplasms. Fig. 23 also shows the demarcation between the acellular compact layer and the underlying stromal layers populated by fibroblasts. Type IV collagen staining (not shown) occurred in the basal laminal area essentially as described by Alitalo *et al.* (1980).

**DISCUSSION**

Although extracellular matrices of numerous epithelia have been investigated either in tissue or cell culture, rarely does such a favourable situation occur for comparing the two as in human amnion. Pure epithelial cell populations can be obtained in large quantities and the epithelial surface of the tissue is accessible for examination and experimental manipulation. The principal conclusions of this comparative study are that amnion epithelial cells produce a mixture of stromal and basal laminal components; that they contribute to the extracellular matrix of both the basal lamina and the underlying acellular compact layer (Bourne, 1960); that active production of extracellular matrix continues in amnion epithelium up to gestational term; and that this is reflected in the behaviour of primary cell cultures. Organ culture experiments (S. Campbell, K. Lord & J. D. Aplin, unpublished) have also shown active synthesis of fibronectin and type III collagen in term amnion. Precedents for this type of behaviour exist in other tissues where stromal extracellular matrix abuts on the basal lamina, including cornea (Meier & Hay, 1975), lens (Tassin, Jacquemin & Courtois, 1983), mammary gland (David & Bernfield, 1981) and lung (Sage, Farin, Striker & Fisher, 1983). Vascular endothelial cells also produce both stromal-type and basal laminal-type matrix (Sage, Crouch & Borstein, 1979). On the basis of analyses performed on components secreted into culture medium, Alitalo *et al.* (1980) have suggested that cultured amnion epithelial cells produce considerably more stromal (types I and III) than basal laminal collagen (type IV). Evidence cited here, as well as unpublished biochemical observations,
confirms that more stromal than basal laminal matrix material is deposited by these cells in culture onto the underlying substratum.

Figs 17–24. Fluorescent staining of amnion tissue.
Figs 17, 18. Actin in the epithelial cell layer visualized by NBD-phallacidin binding; Fig. 17 is a perpendiccular section showing actin concentrated near apical and lateral cell borders; Fig. 18 is a glancing section showing actin concentrated near lateral cell borders.

Fig. 19. Immunofluorescence of keratin, which is seen throughout epithelial cell cytoplasmas.

Figs 20–24. Immunofluorescence of matrix components; Figs 20, 21, laminin in perpendicular and glancing section. Near the bottom of Fig. 21 small streaks and spots of laminin are seen, possibly coinciding with basal cell processes (Figs 2, 3); Fig. 22, type I collagen; Fig. 23, fibronectin; x, epithelial cell apical surface; arrow, sub-basal laminal zone; Fig. 24, type III collagen. Figs 17–21, ×320; Figs 22–24, ×380.
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Fig. 25. Model of matrix formation beneath amnion epithelial cells. Fibrillar stromal and 'amorphous' basal laminal material are secreted into the inner or proximal ends of basal cell surface invaginations. These are then segregated into basal lamina, which associates with plasma membrane via fibrils that cross the lamina lucida, and stromal matrix, which associates with existing stroma through discontinuities in the lamina densa. The basal lamina becomes more compact at the distal ends of foot processes where hemidesmosomes provide stable attachment.

Evidence from both SEM (Fig. 1) and TEM (Figs 3, 4) suggests the existence of a discrete transitional zone between the lamina densa and the stroma. The transitional zone contains a greater concentration of bundles of the fibrillar components typical of the stroma. Evidence that the epithelial cells play a role in collection and organization of such bundles will be published elsewhere. The transitional zone may also be compositionally distinct from the stroma of the compact layer, perhaps containing minor collagens (Odermatt, Risteli, van Delden & Timpl, 1983; Bentz et al. 1983).

The acellular compact layer clearly plays a major role in providing the mechanical strength of the amnion, whose function is to maintain the integrity of the foetal sac up to parturition (Bourne, 1960). Premature rupture of the membranes is associated with a considerable incidence of preterm birth, and has been linked to ascorbate deficiency (Wideman et al. 1964). Thus concentrations of ascorbate and other solutes in amniotic fluid, which bathes the apical epithelial cell surfaces, may influence the contribution made by these cells to the underlying matrix. Transfer of cultured amnion cells from foetal calf serum to Ultroser G causes a marked alteration in the organization of the secreted matrix (Figs 7-10). Similar effects have been seen in cultured lens epithelial cells in response to soluble growth factors (Tassin et al.)
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The presence of amniotic fluid in amnion cell culture supernatants influences cell morphology in short-term cultures (Campbell et al. 1984), and so it will now be necessary to examine the effect of fluid on matrix organization in vitro.

The organization of matrix deposited during culture in serum differs markedly from that seen beneath the epithelial cells in tissue. Instead of being collected into a continuous sheet of basal lamina, type IV collagen and laminin appear in immunofluorescence as discrete punctate deposits or as a faint fibrillar matrix (Figs 13, 14). It is reasonable to suppose that these may correspond to amorphous aggregates seen by TEM below cells in culture in association with fibrillar material more characteristic of stromal matrix (Figs 5, 6). The amorphous deposits may therefore resemble a disorganized form of the amniotic basal lamina. The fibrillar material also fails to polymerize in a fashion analogous to that in vivo; the fibril diameters (~30 nm within the bundles) do not coincide with any stromal fibril type, and more variation in fibril diameter is apparent. Considerable evidence exists in other systems to suggest that matrix organization below cells in culture depends on the nature of the growth substrate or ‘template’. Epithelial cells grown directly on plastic have generally been shown not to generate subcellular structures resembling basal lamina (Billig et al. 1982). In contrast, lens epithelial cells (Heathcote, Bruns & Orkin, 1984), corneal endothelial cells (MacCallum et al. 1982) and aortic endothelial cells (Schor, Schor & Allen, 1984) grown on type I collagen gels deposit a basal lamina-like matrix, as do microvascular endothelial cells cultured on extracellular matrix elaborated by endodermal cells (Kramer, Bensch, Davison & Karasek, 1984). Further publications will deal with the organization of matrix secreted by amnion cells onto collagenous substrata. The cell phenotype is clearly dependent on growth substrate as well as on the composition of culture medium.

The results presented raise questions about the mechanism of matrix organization by amnion epithelial cells. Evidence from TEM (Figs 3, 4) shows that the basal cell surface contains two types of region. At the distal ends of foot processes hemidesmosomes are frequent and the basal lamina is highly organized and compact with a narrow lamina lucida. These areas probably comprise stable adhesions and are associated with cytoplasmic filaments. Within basal surface invaginations, a more loosely woven basal lamina is evident, generally lacking hemidesmosomal specializations and with a wider lamina lucida (Figs 3, 4). Estimates based on these and other similar micrographs suggest that at least 20-fold more basal cell surface area is contained within the invaginations than at distal ‘anchorage points’.

It is reasonable to suggest (Fig. 25) that secretion of newly synthesized matrix materials occurs initially into the basal cell surface invaginations. A disorganized mixture of stromal-type and basal laminal-type matrix appears within invaginations (e.g. top right, Fig. 3). At this stage, therefore, fibrillar matrix can be seen between the basal lamina and the basal cell surface. A related form of basal surface organization has been observed in rhesus monkey amnion late in gestation (King, 1980). It is proposed that this mixture progressively segregates into basal laminal and stromal-type matrix. Mixtures of basal laminal components including laminin, type IV collagen and heparan sulphate proteoglycan have been shown to form aggregates
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in vitro (Kleinman, McGarvey, Hassell & Martin, 1983). This may be followed by association with existing basal lamina, a process not expected to occur in culture on plastic. Alternatively, the plasma membrane may play a role in organizing the basal lamina. Various distinct stages of organization from a loose to a more compact lamina densa can be seen in the various basal surface invaginations of Figs 3 and 4.

It may also be proposed that the extracellular matrix observed in culture (Figs 5, 6) is analogous to the mixture initially secreted in vivo; subsequently, no basal lamina is formed, although large-scale bundling of fibrillar elements takes place (Fig. 6). Thus one reason for the abnormal organization of matrix in vitro may be the absence of stable hemidesmosomal adhesions; in vivo, these appear to limit the size of the compartments into which new matrix is secreted. In tissue, newly secreted stromal-type components are postulated to associate with existing sub-basal laminal matrix through discontinuities in the lamina densa. TEM observations of human (Figs 3, 4) and rhesus monkey amnion (King, 1980) suggest that such discontinuities exist, and further evidence for their existence obtained using SEM will be presented elsewhere. The main features of this model are summarized in Fig. 25.

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REFERENCES


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