Morphology of luminal and glandular epithelial cells from pig endometrium grown on plastic or extracellular matrices

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ABSTRACT: Luminal (LE) and glandular epithelial (GE) cells from d-13 pregnant pigs were cultured on plastic, matrix secreted from endometrial stroma, and EHS matrix (Matrigel) in culture medium (RPMI-1640) supplemented with 20 and 10% fetal bovine serum, respectively. After culture for 7 and 14 d, GE and LE cells were prepared for transmission and GE cells for scanning electron microscopy. The two types of endometrial epithelial cells displayed different morphological characteristics when grown on different culture substrates. On plastic, the GE and LE cells formed flattened monolayers. However, stroma-secreted matrix directed the polarization of endometrial epithelia. The GE and LE cells reacted differently to thick Matrigel coatings; LE cells formed a colony after 7 d of culture and then proliferated further to form a colony with a cavity, but GE cells organized to form a colony with a shallow depression in the center at 7 d and developed duct-like structures after 14 d in vitro. Luminal epithelial cells grown on either diluted or thin-coated Matrigel and grown on stroma-secreted matrix formed a monolayer but no three-dimensional structures.

Key Words: Endometrium, Cell Culture, Pigs


Introduction

The endometrium in pigs and other mammals is composed of luminal (LE) and glandular epithelial (GE) cells, stromal components, and an intimately associated extracellular matrix. Fertilized porcine embryos enter the uterus about d 4 of gestation (onset of estrus is d 0; Dziuk, 1977). They first attach to the uterus on d 13 of gestation (Keys and King, 1992) and are nourished by uterine secretions in both pre- and postattachment stages. Interaction between the embryo and the maternal endometrium is a prerequisite to successful implantation and embryo development (Thatcher et al., 1985; Xie et al., 1990).

Investigations of the role of endometrial cells in modulating the local physiology of pregnancy have benefited from cell culture (Davis and Blair, 1993). However, epithelial cells grown on plastic culture dishes exhibit changes in morphology and may have altered expression of certain genes (Gospodarowicz and Tauber, 1980; Lee et al., 1984; Cooke et al., 1986; Thomas et al., 1992). Previous studies indicated that extracellular matrix (ECM) components, such as matrix from Englebreth-Holm-Swarm (EHS) tumors grown in C57B1/6 mice, can restore the polarity and secretion of various epithelial cells in vitro, including the uterine epithelium from rodents (Jacobs et al., 1990). Epithelia from thyroid (Toda and Sugihara, 1990), mammary gland (Hammamoto et al., 1988), kidney (Hall et al., 1982), and cornea have expressed differentiated characteristics and formed organ-like structures when grown on ECM, and human uterine epithelial cells generate gland-like structures when grown on EHS-secreted matrix (Rinehart et al., 1988).

Thus, our objective was to determine whether the two types of endometrial epithelial cells, LE and GE, from d-13 pregnant pigs would show different morphologies when grown on different culture substrates.

Materials and Methods

Animals and Collection of Endometrium. Six d-13 pregnant crossbred sows were used in the experiment. Sows were artificially inseminated on d 0 (first day of estrus) and d 1. Endometrium was collected surgically from anesthetized sows (Zhang et al., 1991; Guo et al., 1998). Pregnancy was confirmed by flushing elongated embryos from the uterus with PBS (pH 7.4). The uterus then was opened longitudinally along the antimesometrial surface, and endometrial strips were cut from the...
lateral and mesometrial surfaces with scissors (Zhang et al., 1991).

Cell Isolation and Culture Procedures. Three culture wells of GE and LE were prepared from endometrium from each donor. Endometrial cells were isolated and prepared for culture using modifications of previously reported procedures (Zhang et al., 1991). Endometrial tissue was washed with incomplete Hank’s buffered salt solution (IHBSS, Ca++ and Mg++ free, pH 7.4) containing penicillin (62 μg/mL), streptomycin (100 μg/mL), gentamicin (.8%, vol/vol), and Fungizone (.2%, vol/vol) (Sigma Chemical Co., St. Louis, MO). The tissue was digested with dispase/pancreatin (.4%) at room temperature for 2 h with occasional shaking. The digested tissue was rinsed with IHBSS (30 mL), and the pellet was resuspended in IHBSS with additional shaking and centrifugation as needed to free individual cells. Luminal epithelial cells were collected by centrifugation (200 × g), washed twice with IHBSS, resuspended, and cultured in 60-mm (culture area 21 cm²; Gibco, Grand Island, NY) dishes in RPMI-1640 (Gibco) supplemented with 20% fetal bovine serum (FBS; Gibco) and the same antibiotics and antimycotics used in IHBSS.

The remaining tissue was cut into small strips (about 1 mm² in diameter) and digested further with combined trypsin, .04%; collagenase, .06%; and DNase-I, .01% (all from Sigma) at 37°C for 50 min with vigorous shaking every 10 min. Stromal cells and glandular fragments in the suspension from the digestion were separated with a 38-μm sterile sieve (Newark Wire Cloth Co., Newark, NJ). Stromal cells in the filtrate were pelleted by centrifugation (500 × g) and then washed twice with IHBSS. Glandular fragments were retained on the sieve and collected by back-washing, pelleted (200 × g), and washed twice with additional shaking to disperse GE into individual cells and some small clumps. Fragments of tubular structures were not present after this process. Stromal and glandular cells were cultured in RPMI-1640 enriched with 10% FBS in 60-mm dishes.

Stroma Matrix. Stromal cells from endometrium of d-13 pregnant pigs were cultured for 7 d. Attached cells were rinsed with IHBSS twice and treated with .01% NH₄OH to lyse the cells and expose the ammonium-insoluble matrix deposited on the dishes. The plates were rinsed again, and epithelial cells were plated on the matrix within 3 h.

EHS Matrix. Basement membrane matrix extracted from EHS tumors (Matrigel; Collaborative Research, Bedford, MA) was used according to manufacturer’s instructions. In thin- and thick-coated dishes, 50 and 150 μL of Matrigel/cm² of culture area was poured, respectively. For diluted coatings, the Matrigel was diluted with RPMI-1640 (1:3) and 50 μL/cm² culture area was poured.

Electron Microscopy

Transmission Electronic Microscopy. Confluent cultures were rinsed with IHBSS and fixed with 2.5% glutaraldehyde in culture dishes at room temperature for 2 h. Fixed cells were washed three times in PBS (pH 7.4), stained by immersion in 2 mL of OsO₄ (1%) in PBS for 30 min, and washed with distilled water three times. Urinal acetate (UA) (1% aqueous solution) staining also was applied by adding UA solution directly into culture dishes for 30 min. Cells were dehydrated serially with ethanol and immediately infiltrated with Epon 812 (100%; Ted Pella, Redding, CA), under vacuum, for 3 to 5 h. The Epon was decanted, and cells were cured in fresh Epon 812 at 60°C for 48 h. Cured samples were sectioned (90 to 120 μm) and viewed under a Philips EM201 (Philips Electronoptics, Mawah, NJ). Some samples of endometrium were not subjected to enzymatic procedures and were prepared for transmission electron microscopy (TEM) to visualize in vivo tissue architecture.

Scanning Electronic Microscopy. Confluent cells were fixed, stained, and dehydrated as for TEM. After critical-point drying, cells were coated with gold/palladium and examined with a scanning electron microscope (SEM).

Results

Stromal cells of the endometrium have no intercellular contacts in vivo. Rather, they are surrounded by ECM (Figure 1). They behaved similarly in vitro (Figure 2), and the ECM consisted not only of collagen but also of secretory vesicles. Observations with TEM showed that LE and GE cells cultured on plastic culture dishes appeared flattened (Figure 3). Although the cells were stretched over a thin basal lamina, actual contact was limited to scattered small regions of the cell membranes. The edges of adjacent cells overlapped slightly, but intercellular junctions were not observed. Culture on matrices other than plastic promoted development of cell interactions, polarity (as indicated by
Uterine epithelium grown on various substrates

Figure 2. Stromal cells grown in vitro and surrounded by collagen and secretory vesicles (arrows) (bar = 1 μm).

apical microvilli and tight junctions), and three-dimensional structures, as summarized in Table 1. When LE cells were cultured on stroma-secreted matrix and diluted Matrigel, polarity developed (Figure 4). The cells remained in a monolayer but had apical microvilli and tight junctions between cell membranes (Figure 4a). Relatively advanced cell contacts were present in LE cells cultured on diluted Matrigel (Figure 4b). The GE cells cultured on diluted Matrigel developed polarity and cell contacts but not more complex structures. When GE cells were grown on stroma-matrix, they formed combinations of clumps, and multilayered structures (Figure 5) developed characteristics of polarity and colonies with central cavities. Higher magnification (Figure 6) demonstrated that cells in the clumps had numerous apical microvilli with associated glycocalyx, basally located nuclei, and tight junctions between their apical regions. A prominent feature was the basal secretion of collagen and some electron-dense vesicles in the lumens of the clumps (Figure 7).

Colonies of LE cells were observed under the light microscope after 14 d of culture on either thin- or thick-coated Matrigel. Under TEM, the LE cells on thin Matrigel showed multiple layers and indications of migration into the matrix on the edge of the colonies (Figure 8). When grown on thick-coated Matrigel, LE cells actually reached the plastic dish and formed a colony surrounding a cavity (Figure 9); however, tubular structures were not observed.

When GE cells were cultured on thin or thick Matrigel, small colonies formed and at first showed little or very slow expansion. Observation by SEM showed structures resembling gland openings located in the center of the colonies, as shown in Figure 12. These structures were shallow depressions after 7 d and deep holes after 14 d of culture, resulting in a tubular duct-like structures over a period of 7 to 14 d (Figure 10). The TEM demonstrated that lumens formed by GE cells were surrounded by columnar, polarized cells with numerous ciliated cells. Undifferentiated cells and secretory cells were identified. In secretory cells, secretory granules were accumulated in supranuclear regions, and microvilli were present at the apical plasma membranes (Figure 11). Interdigitating lateral membranes and apical tight junctions were distributed among all the cells (Figure 11). The SEM revealed the formation of tubular-like structures (Figure 12). The cell surface displayed microvilli of different heights and buried cell boundaries under the microvilli (Figure 13).

Discussion

Cell culture has provided a tool for studying many aspects of cell growth and function under controlled conditions. However, the utility of culture systems has been limited because of discrepancies in cell behavior in vitro and in vivo and the possibility that cells may lose their identity and capacity for normal function in vitro. Some epithelial cells in culture have exhibited marked changes in shape, protein-synthetic patterns, hormone responsiveness, and function compared with intact epithelial cells in vivo (Gospodarowicz and Tauber, 1980; Lee et al., 1984; Cooke et al., 1986). During our initial studies, LE cells collected after trypsin
digestion attached poorly to a plastic culture surface, even in the presence of serum. Cells that did attach retained a round morphology, and small aggregates were found rather than flattened monolayers. The cells had only a limited interaction with the plastic, in agreement with observations by Thomas et al. (1992) on cultured neonatal choroid plexus epithelia. Cell numbers decreased over the culture period, apparently because of cell detachment and removal during media changes rather than significant cell death. However, when dispase and pancreatin digestion were used to collect LE, the cells performed much better in vitro. They formed small colonies and a flattened single layer but grew much more slowly than GE cells.

Pig endometrial gland cells are capable of reconstructing duct-like tubules and structures similar to developing secretory glands when they are plated on dishes coated with thick or thin layers of Matrigel. Clumps of glandular cells with central lumens also were formed when glandular cells were cultured on matrix secreted by stromal cells. Differentiated monolayers of cells were observed with culture on plastic or diluted Matrigel surfaces.

Matrigel is a mixture of many components, including laminin, Type IV collagen, heparin sulfate proteoglycan, nidogen, and entactin (Kleinman et al., 1986). Also, Vigny et al. (1988) reported that EHS sarcomas contain large amounts of fibroblast growth factor. Kleinman et al. (1986) indicated that EHS basement membrane complexes have normal structure and function.

Benali et al. (1992, 1993) observed tubule formation by cultured epithelial respiratory cells and suggested that the process of tubule formation is dependent on both basal and apical sides of epithelial cells being covered by Matrigel. Duct-like structures may be due to the physical properties of the matrix (i.e., a loose and flexible fibrillar structure that permits changes in cell shape and density; Gierer, 1977). Alternatively, the interaction of cell surface integrin receptors with the Matrigel components (e.g., collagen) may play a role. Another hypothesis, proposed by Toda and Sugihara (1990), is that epithelial cells lose their polarity if embedded in a three-dimensional gel, and an intracytoplasmic cavity develops. An increase in the size of the cavity and the reappearance of polarities of cells surrounding the cavity result in the tubule structure.

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<sup>a</sup>Matrigel (Collaborative Research, Bedford, MA).
Uterine epithelium grown on various substrates 135

Figure 4. Luminal epithelial cells grown on stroma-secreted matrix (a) and diluted Matrigel (b). Numerous microvilli (arrow heads) are located on the apical surface and tight junctions (arrows) are visible (bar = 2.5 µm).

We (Y. L. Zhang, unpublished data) have observed that uterine gland cells cocultured with stromal cells in vitro form cavities by the degeneration of the cytoplasm in the central cells.

The process of tubule formation on Matrigel in our study is similar to angiogenesis or tumor invasion, in that gland genesis occurs through the remodeling of the ECM. Benali et al. (1993) detected the secretion of collagenase, a member of the matrix metalloproteinase family, when they cultured surface respiratory cells on a three-dimensional collagen lattice, and the cells formed tubules. Cultured canine kidney cells showed similar properties (Montesano et al., 1991). Perhaps similar enzymatic processes occur when pig uterine gland cells are grown on Matrigel.

The coiled, branching uterine glands are exposed in vivo to a matrix and stromal-cell microenvironment. Both epithelia and stroma secrete components of the ECM. In our study, duct-like structures were reconstructed in vitro on Matrigel but not on diluted Matrigel or matrix from stroma. Matrigel alone carries at least part of the information for organogenesis. Matrix from stroma also has the signals to restore the polarity of gland cells. However, it seems that the thickness of the lattice on which the cells grow plays an important role in organogenesis, because tubules were not observed on the thin coating of Matrigel. The establishment of polarity when LE cells were cultured on matrix or diluted Matrigel demonstrated that ECM plays an important role in restoring the normal morphology of these epithelial cells.

The LE cells responded distinctively to Matrigel. Seemingly, an intercellular lumen was structured by migration of LE cells down and inside of the Matrigel after 14 d of culture. This could correspond to the in vitro method of cavity formation described by Toda and Sugihara (1990). Migration of cells among connective tissue and ECM has been observed in tumor invasion, invasive implantation in certain animals such as rodents and humans, angiogenesis, and disease states such as rheumatoid arthritis and has been related to effects of matrix proteinases, especially the metalloproteinases (Werb, 1989; Woessner, 1991). Matrix proteinases have been found in the endometria of mice (Brenner et al., 1989), rats (Woessner and Taplin, 1988; Roswit et al., 1993), humans (Rodgers, 1993; Osteen et al., 1994), and sheep (Salamonsen et al., 1991). Production of metalloproteinases may have helped LE cells of porcine endometrium to migrate through the Matrigel.

In summary, culturing porcine uterine epithelial cells on plastic results in abnormal cell morphology. The ECM plays an important role in the maintenance of cell morphology in vitro. Matrix secreted by endometrial stroma contains signals for the polarization of endometrial epithelial. Further, although GE and LE cells have

Figure 5. Glandular epithelial cells grown on stroma-secreted matrix. (a) Clumps and multiple layers of cells. (b) A clump with a central cavity filled with collagen fibers (CF) and secretory vesicles. Note basal location of nuclei (bar = 2.5 µm).
Figure 6a and b. High magnifications of glandular epithelial cells grown on stroma-secreted matrix. Numerous vesicles (Ve) are visible. Cell membrane contacts are observed between cells (arrows) (bar = 1 μm).

Figure 7a and b. High magnification of glandular epithelial cells grown on matrix. Note the basal secretion (arrow heads) and cell contacts (arrows) (bar = 1 μm).

Figure 8. Transverse section of luminal epithelial cells grown on a thin coating of Matrigel and organized as a colony (bar = 2.5 μm).

Figure 9. Cross-section of luminal epithelial cells grown on a thick coating of Matrigel showing the lumen of the cell colony (bar = 2.5 μm).

Figure 10. A duct-like structure formed by glandular epithelial cells after 14 d of culture on a thick coating of Matrigel (bar = 1 μm).
Uterine epithelium grown on various substrates

Figure 11. Higher magnification of two areas of Figure 10. The GE cells are organized around a lumen (L) with tight junctions (arrows). Microvilli line the lumen (bar = 1 μm).

the same embryonic origin, they behave differently in vitro and in a manner that seems consistent with in vivo architecture, that is, GE cells form duct-like structures on thick Matrigel, but LE cells do not.

Implications

Fertility is dependent on the function of uterine glandular and luminal epithelial cells to sustain embryonic development. The perimplantation period is a time of embryonal loss. In vitro models of uterine functions are useful for studying conceptus-uterine interactions. The responses of uterine cells to the matrices described in this report provide insights into the regulation of morphological development in vivo and enhance understanding of uterine functions that support pregnancy.

Literature Cited


