MONOLAYER CULTURE OF HUMAN ENDOMETRIUM: METHODS OF CULTURE AND IDENTIFICATION OF CELL TYPES

V. A. VARMA, SUSAN A. MELIN, THOMAS A. ADAMEC, B. HUGH DORMAN,
JILL M. SIEGFRIED, LESLIE A. WALTON, CHARLES N. CARNEY,
CAROL R. NORTON, AND DAVID G. KAUFMAN

Department of Pathology, School of Medicine, University of North Carolina at Chapel Hill, Chapel
and D. G. K.) and Department of Obstetrics and Gynecology, University of North Carolina and
North Carolina Memorial Hospital, Chapel Hill, North Carolina 27514 (L. A. W.)

(Received January 4, 1982; accepted July 7, 1982)

SUMMARY

Monolayer cultures can be established from human endometrial tissue after enzymatic
dispersal into isolated glands or single cells. Three cell types that have distinct morphology
by light and electron microscopy are observed in the resulting primary cultures. One cell
type, an elongated spindle cell, is similar in appearance to fibroblasts derived from other
tissues. A second cell type forms colonies of tightly cohesive cells, ranging in shape from
oval to polygonal. These cells have typical organelles and junctional complexes charac-
teristic of epithelial cells from the endometrium. The third cell type assumes a pavement-
lke appearance composed of polygonal cells when viewed by phase contrast microscopy,
but lacks distinctive ultrastructural features of epithelial cells. These cells in culture resemble
the endometrial stromal cell, the predominant cell type of the human endometrium in
vivo. The epithelial cell does not survive subculturing but the other two cell types can be
passaged through several generations and can be stored in liquid nitrogen and subse-
quently returned to culture.

Key words: human endometrium; stromal cell.

INTRODUCTION

The human endometrium is a complex tissue consisting of glands lined by columnar epithelium
and supported by a network of mesenchymal cells unique to the endometrium. The biology of the
human endometrium is complicated by the fact that it undergoes morphological and biochemical
alterations during the menstrual cycle and pregnancy (1,2). These alterations are not confined
only to the epithelium. The endometrial stromal cells, unlike mesenchymal cells in other tissues,
are also highly sensitive to estrogen and progesterone during the menstrual cycle. The Stromal
cells react to hormonal stimulation by first proliferating and later forming secretory substances
(1) in a manner similar to the epithelium, and in
the event of pregnancy evolve into decidual cells.

Any attempt to culture and identify cells from human endometrium must take into consideration
both the complexity of this tissue and the characteristics of the various cell types. Several inves-
tigators have described culture techniques for human endometrium with varying degrees of suc-
cess with long term propagation (3-11). There is
some confusion in the literature concerning the identity and lifetime in culture of the various
endometrial elements. There is general agreement
that elongated bipolar cells typical of fibroblasts
found in other tissues can be subcultured from
endometrium for periods of many months. In
most cases, however, epithelial cells did not sur-
vive longer than a few weeks and could not be
subcultured (7-10). Recently, Trent et al. (10)
indicated that epithelial cells could be isolated
and subcultured from monolayer outgrowths of
explanted normal human endometrium. Liu and

*Current address: Bowman Gray School of Medicine,
  Wake Forest University, Winston-Salem, North
  Carolina 27103.

+Current address: Alaskan Native Medical Center,
  Box 7-741, Anchorage, AK 99510.
Tseng (12) have also described endometrial epithelial cell lines that can be subcultured.

Confusion concerning the identity and growth characteristics of human endometrial elements in culture may exist for a number of reasons. First, the terms “stromal cells” and “fibroblasts” are sometimes used imprecisely and interchangeably in describing the supportive elements of tissues in general. This terminology creates confusion when applied to the endometrium because the endometrial stromal cell is not simply a fibroblast. Second, tissues undergo changes in culture which complicate the identification of individual cell types. Third, because endometrial stromal cells share some characteristics of the epithelial cells, it may be difficult to distinguish these two cell types in culture.

We have attempted to identify human endometrial cell types in monolayer culture based on these considerations. In this report we describe culture methods that permit the emergence of three cell types in primary culture, two of which can be subcultured through multiple passages. The three cell types have been tentatively identified by comparison of their morphologic features in culture with the well-documented features of endometrial cells in vivo (1,2).

METHODS

Tissue obtainment. Endometrial tissue was obtained from hysterectomy specimens. Donors were patients offering informed consent who had no clinical history of hormonal administration or infectious, hyperplastic or neoplastic endometrial disease. Tissue was collected in a sterile environment within the surgical suite immediately after removal of the uterus. Each specimen was opened coronally from cervix to fundal dome and inspected for gross evidence of intrinsic disease (hyperplasia, polyps, tumor, infection). If no gross abnormality was evident, the endometrium from a segment of the uterus was removed by scraping the surface with a large scalpel blade held perpendicularly to the surface. The tissue was then placed in 10 ml of chilled complete medium (described below) containing high concentrations of penicillin (1,000 U/ml), streptomycin (1,000 µg/ml), and gentamicin (500 µg/ml) and transported to the tissue culture laboratory.

Tissue dispersion. In the tissue culture laboratory the tissue was minced into 2 mm cubes and washed several times with calcium and magnesium-free Hanks’ balanced salt solution (CMF-HBSS) containing 25 mM HEPES buffer, pH 7.4. To obtain isolated glands the tissue was dissociated using a modification of a procedure previously described by Satyaswaroop et al. (13). The tissue fragments were incubated at 37°C in 0.25% collagenase (Sigma, St. Louis, MO) in CMF-HBSS (5 ml/0.5 cm² tissue). At 15 min intervals the tissue was pipetted up and down several times to facilitate dissociation; the progress of dissociation was monitored by phase contrast microscopy. Within 1 to 2 h this procedure yielded glands relatively free of nonepithelial cells along with a suspension of single cells of mixed origin. Any undigested fragments were collected by sedimentation and the glands plus supernatant cell suspension were removed with a pipet. An enriched gland component was obtained by repetitive washings with CMF-HBSS followed by gentle centrifugation at 500 rpm for 15 sec. The glands were then plated at a density of 20 glands/cm². After the glands were isolated the supernatant fluid containing single cells was centrifuged at 1200 rpm for 10 min. The pelleted cells were resuspended in 10 ml of culture medium, counted in a Coulter model ZBI particle counter, and plated at 4.5 × 10⁴ cells/cm².

Two other methods were employed in initiating cell cultures. Using the techniques described above, a triple enzyme solution consisting of 0.1% trypsin, 0.1% hyaluronidase, and 0.05% collagenase in CMF-HBSS was used instead of the collagenase solution. After approximately 1 h the tissue was reduced to free cells and a few connective tissue strands. The cell suspension, diluted with 5 ml of fetal bovine serum (FBS), was filtered through a 100 mesh nylon screen; the suspension was centrifuged and the pelleted cells were resuspended and plated as described above. Cell cultures consisting primarily of epithelial cells were also obtained from tissue explants. Mincend endometrial tissue was placed directly onto culture dishes and partially covered with culture media. Within 5 to 7 d outgrowths began to emerge from the explants. Confluent cultures were obtained after approximately 3 wk.

Subculture and storage. Primary cell cultures, initiated using one of the three described methods, were grown to confluency. To passage cultures a solution consisting of 0.1% trypsin plus 0.02% EDTA in CMF-HBSS was used. For growth kinetic studies cultures were seeded at 9.5 × 10⁴ cells/cm². Generally after two or three subcultures an aliquot of each culture in complete medium plus 10% dimethyl sulfoxide at a concentra-