

## Culture of rat endometrial telocytes

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### Abstract

Previous studies have shown that telocytes are found in a variety of tissues. Here, we report the presence of telocytes in the human endometrium. In addition, telocytes were isolated from the rat endometrium and cultured. Immunohistochemistry was performed *in vitro* and *in vivo*. Cultured cells showed that telocytes expressed CD34, and similar results were found in the uterine tissue. In both species, telocytes also stained positive for vimentin and connexin 43. Telopodes were observed connecting cell colonies and connecting distant cells. Our findings suggest that telocytes may have a role in cell-to-cell communication over short and long distances within the endometrium.

**Keywords:** endometrium • filopodia • stromal cells • telocytes

### Introduction

Telocytes have been described as interstitial cells with long prolongations, called telopodes, that extend from the cell body [1]. These prolongations can reach exceptional lengths, up to hundreds of micrometres [1], and they form attachments to a variety of cells. Although telocytes display electrical activity [2] and have been observed to form attachments to myocytes [1, 3], they are dissimilar from pacemaker interstitial cells of Cajal (ICCs) [4, 5] and can be distinguished from ICCs by their location, morphology, ultrastructural features and the presence of telopodes [1]. Telocytes have been studied in the gut [1], pancreas [6], lung [7, 8], mammary gland [9], myometrium [3, 10] and placenta [11]. The function of telocytes is not well understood; however, evidence points towards a role in facilitating long-distance cell-to-cell communication. Emerging theories include the role of telocytes in immune surveillance [12] and regulation of the stem cell niche [8, 13]. Telocytes form attachments to smooth muscle cells [1], eosinophils, plasma cells, basophils and macrophages [8, 14]. Ends of telopodes can be loaded with vesicles [1], which suggest that telocytes are equipped to communicate *via* their extensions. We suggest that telocytes are also present in tissues that are low in cell density, with significant space between neighbouring cells. In such a tissue matrix environment, telopodes could facilitate

cell-to-cell contact over long distances. We tested this hypothesis by looking for telocytes in the endometrium, a niche which, unlike the myometrium, becomes loose during decidualization. Functional studies are necessary to better understand the role of telocytes in tissue homeostasis and pathology, and thus we sought to develop a technique to isolate and culture telocytes from the endometrium.

### Materials and methods

#### Human tissue collection

The study was approved by the Ethics Committee of Harbin Medical University. Adult, non-pregnant women aged 22–49 years were recruited ( $n = 5$ ), and samples were taken from healthy areas of uterine tissue. Samples were fixed in 4% paraformaldehyde, dehydrated, blocked in paraffin, cut into 5  $\mu\text{m}$  sections and collected on glass slides.

#### Rat tissue collection and cell culture

Animal procedures were approved by the University Health Network Animal Care Committee. Adult, virgin, female Wistar rats ( $n = 8$ ) were killed and uteri were dissected. A small portion of the tissue (1 cm length of the horn) was processed for immunohistochemistry, and the remaining tissue was used for cell culture. For cell culture, the parametrial connective tissue surrounding the serosa was removed. The

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uterine horns were excised and cut longitudinally, rinsed with PBS and enzymatically digested. Digestion started with 0.1% trypsin in DMEM for 55 min. at 4°C and then 60 min. at 37°C. Tissue was briefly vortexed, and the enzymatic reaction was stopped with 10% FBS. Tissue was rinsed in PBS and incubated in 0.1% collagenase in DMEM for 60 min. at 37°C. The tissue was subsequently placed on a plastic dish with the luminal side facing up, and the endometrial layer of the uterus was scraped off using a razor. The tissue was incubated in 0.1% collagenase for an additional 30 min., dispersed by pipetting and passed through a 100 µm sieve and then a 40 µm sieve. The cells were washed with 10% FBS to stop the enzymatic reaction and then washed in PBS. Cells were plated on T75 flasks and cultured in DMEM with 10% FBS and 1% penicillin/streptomycin for 2 hrs. The media containing slowly adhering cells was re-plated into a new flask and maintained in culture. The tissue collected for immunohistochemistry was fixed in 4% paraformaldehyde for 16 hrs at 4°C, washed in PBS, equilibrated to a 2:1 mixture of 20% sucrose in PBS to OCT matrix (Fisher Scientific, Ottawa, ON, Canada), prepared as frozen blocks and cryosectioned at 5 µm.

## Immunohistochemistry

Cultured cells were fixed for 5 min. using 100% methanol pre-chilled with dry ice for immunohistochemistry against vimentin and connexin 43. Fixation was performed with 4% paraformaldehyde for 30 min. at room temperature for the study of CD34. Following fixation, cells were rinsed with water and incubated with TBS 0.5% Triton X-100 for 3 min. Buffer was removed, and cells were blocked using DAKO Serum-Free Blocking Solution (DAKO, Mississauga, ON, Canada) for

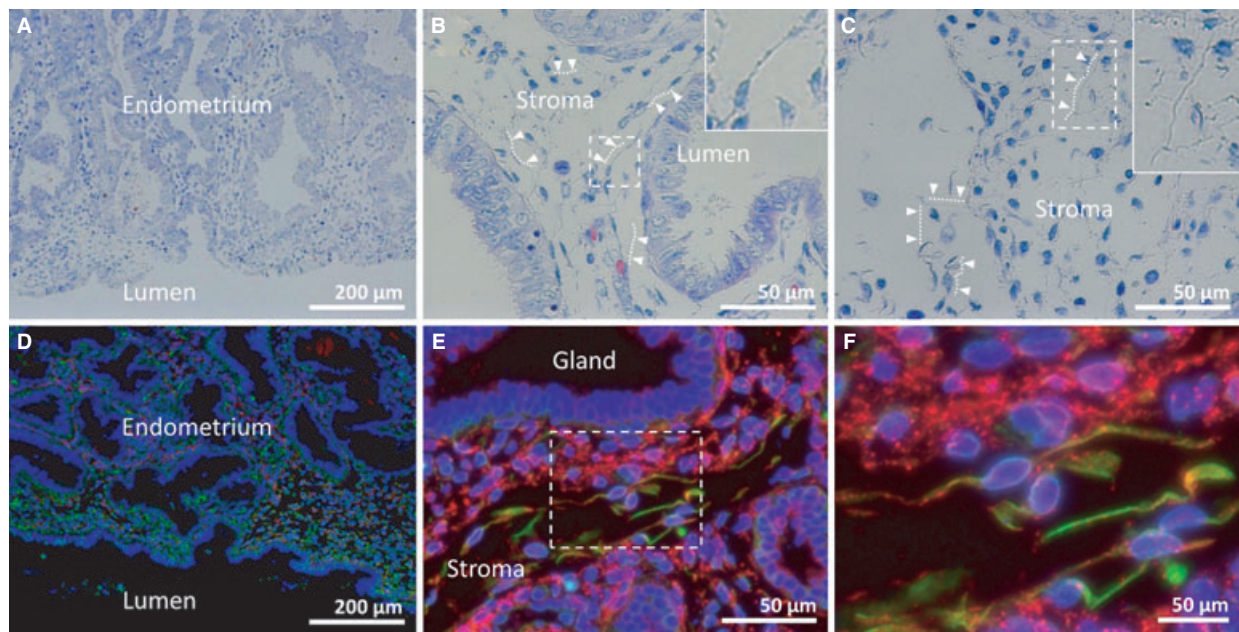
30 min. at room temperature. Immunostaining was performed with mouse anti-vimentin (Sigma-Aldrich, Mississauga, ON, Canada), rabbit anti-connexin 43 (Sigma-Aldrich) and goat anti-CD34 (R&D, Minneapolis, MN, USA). Primary antibodies were diluted in DAKO Antibody Diluent (DAKO) at a dilution of 1:40 for vimentin, 1:400 for connexin 43 and 1:20 for CD34 and incubated for 2 hrs at room temperature. Cells were washed and incubated with secondary Alexa 488 anti-mouse, Alexa 568 anti-rabbit and Alexa 488 anti-goat antibodies (Invitrogen, Mississauga, ON, Canada) at 1:500 for 30 min. at room temperature. Cells were washed and counterstained with DAPI. Cultures were also stained with Giemsa; cells were incubated overnight in Giemsa, rinsed with water and differentiated in 0.5% acetic acid. Staining was photographed using a Nikon Ti Eclipse microscope (Nikon, Mississauga, ON, Canada).

Frozen section immunohistochemistry for CD34 was performed as described above. For immunostaining of the paraffin sections, slides were dewaxed, rehydrated and antigen retrieval was performed with 10 mM citrate at 97°C for 30 min. Staining procedures were performed as above for the cultured cells.

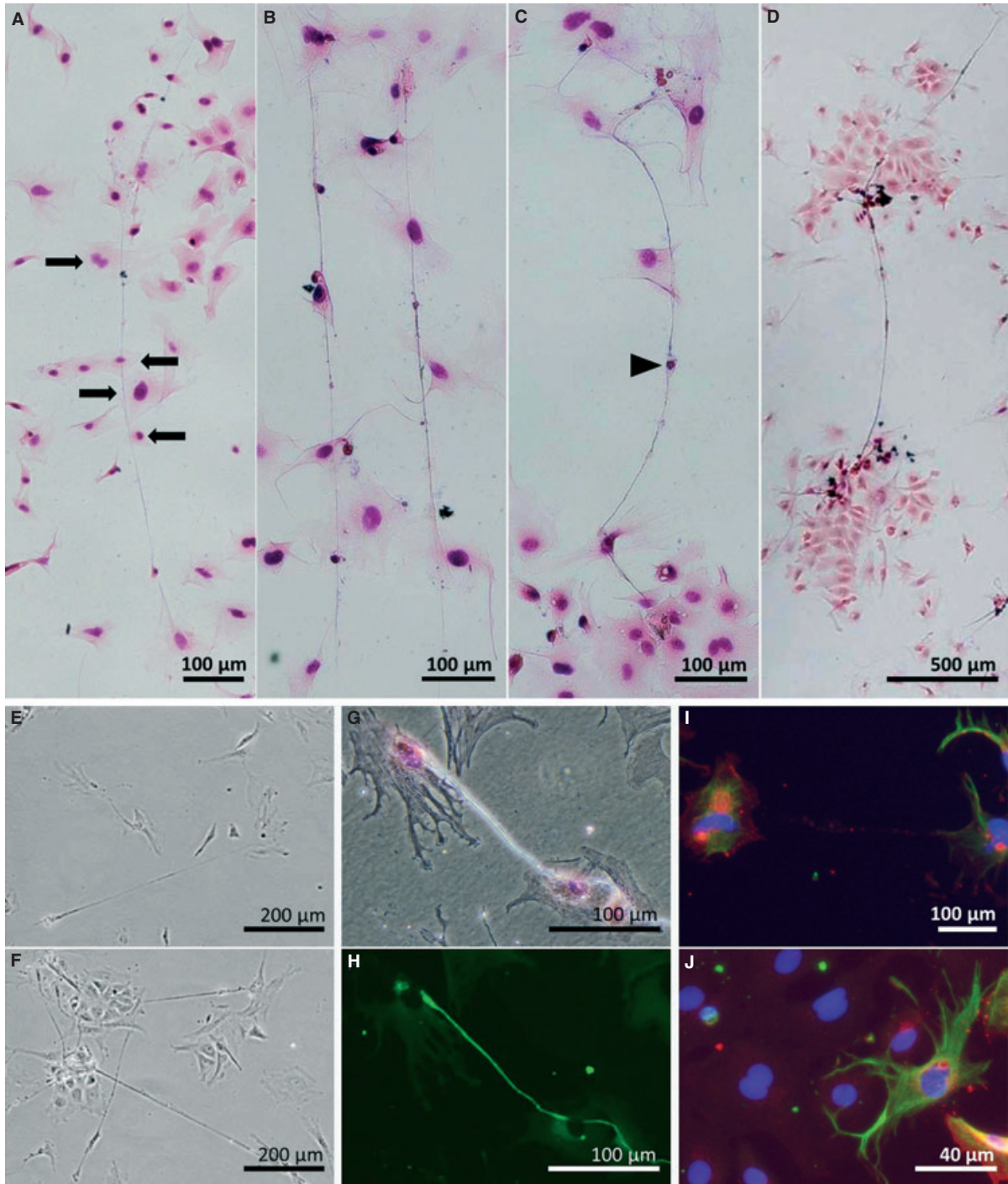
## Results

Telocytes were observed in the human endometrium (Fig. 1). Long cells with double extensions were found in the endometrial stroma (Fig. 1A–C) and stained positive for both vimentin and connexin 43 (Fig. 1D–F).

Endometrial telocytes were isolated and cultured from the rat uterus. Telopode extensions were often observed connected to stro-



**Fig. 1 (A–C)** Human endometrial tissue samples were stained with Giemsa. Dotted lines and arrowheads outline cells with long extensions. Boxed regions are enlarged. **(D–F)** Tissue samples were immunostained for vimentin (green), connexin 43 (red) and DAPI (blue). Boxed region in **E** is enlarged and shown in **F**.



**Fig. 2 (A–D)** Rat endometrial cell cultures were stained with Giemsa at 48 hrs. **(A)** Prolongations of telocytes extended over nearby stromal cells (arrows) and connected to distant ones. **(B)** On rare occasions, these extensions ran parallel to each other. **(C)** Arrowhead indicates podom along the extension. **(D)** Telocytes formed connections between stromal cell colonies. **(E, F)** Phase-contrast micrographs of 24 hr cell cultures. **(G, H)** Giemsa staining was observed under phase-contrast and fluorescence. **(I)** Epi-fluorescence micrographs of 48 hr cell cultures show immunostaining for vimentin (green), connexin 43 (red) and DAPI (blue). Prolongations between endometrial stromal cells were localized with a connexin 43 antibody. **(J)** An endometrial stromal cell near a group of endometrial epithelial cells exhibits tentacle-like extensions.

mal cells (Fig. 2A–D). These prolongations spanned up to hundreds of micrometres in length. The terminal connections were often made not to the nearest stromal cell, but to distant cells, sometimes extending over other intervening stromal cells (Fig. 2A). The telopode structures were sometimes observed in parallel (Fig. 2B). These structures occasionally exhibited podoms along their length (Fig. 2C) and connected colonies of stromal cells (Fig. 2D). These extensions grew out from stromal cells and formed both long (Fig. 2E and F) and short prolongations (Fig. 2G and H). Shorter extensions could be visualized with fluorescence following Giemsa staining (Fig. 2H). Immunohistochemistry showed that the telocytes stained positive for vimentin and connexin 43 (Fig. 2I). Stromal cells associated with epithelial cell colonies occasionally formed tentacle-like extensions, appearing

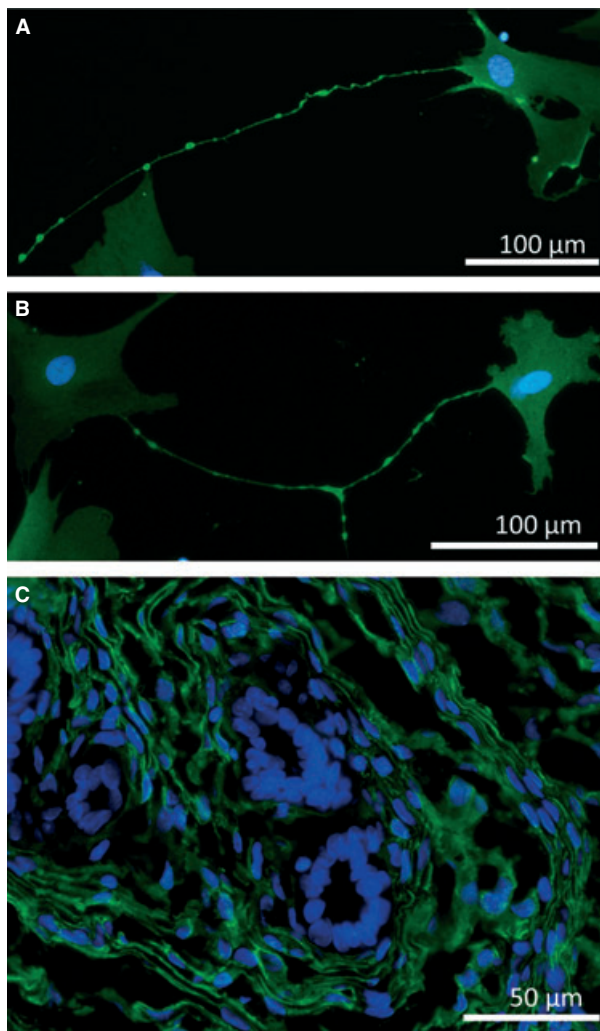
‘octopus like’ (Fig. 2J). Cultured rat endometrial telocytes expressed CD34 (Fig. 3A and B). Telopodes frequently formed connections to neighbouring cells (Fig. 3B). Thin, elongated telocytes were observed around the glands of the rat endometrium and were positive for CD34 (Fig. 3C).

## Discussion

In the human endometrium, we observed telocytes in the endometrial stroma of the stratum functionalis. In the loose stromal space between glandular epithelium or near the luminal epithelium, telocytes adopted an orientation that contoured the shape of the adjacent epithelial architecture. Similar cells have been reported in the human basal endometrium after menstruation. With the surface epithelium absent from shedding, thin stromal projections around the collar of endometrial gland stumps have been observed [15]. Given their location, they may support the structure of the gland and nearby stroma as a functional unit by forming a scaffold around them. Alternatively, their position in the loose connective tissue may suggest that they participate in loosening the matrix or secreting factors during decidual formation.

The rat endometrial stromal cells with telopode projections we observed *in vitro* were similar to cultured telocytes described by others [2, 3, 10, 16]. Projections were singular, double or forked. However, stromal cells adjacent to epithelial cells occasionally appeared ‘octopus-like’, forming tentacle-like protrusions similar to those described in fallopian tube telocytes [2]. Co-culture studies have shown that uterine stromal cells are able to enhance epithelial cell growth [17], and therefore tentacle-like prolongations may play a role in epithelial cell maintenance. We additionally observed telopode projections connecting individual endometrial stromal cells and also colonies of stromal cells. It has been reported that telocytes may regulate stem cell niches [13]. It could be postulated that telocytes regulate the expansion of endometrial stromal cells or progenitors. Through their connection to stromal cells, they may play an active role in endometrial maintenance by communicating across large distances. The telocytes we observed *in vivo* and *in vitro* stained positive for connexin 43, a protein involved in gap junctions. Connexin 43 is involved in decidual maturation, and decreased expression is associated with recurrent pregnancy loss [18]. The presence of connexin 43 does not definitively prove the existence of gap junctions, and due to its short half-life, further work is necessary to understand the possible role of gap junctions in endometrial telocytes.

Our observation that telocytes express CD34 is consistent with previous reports [19]. Additional work is necessary to determine the function of endometrial telocytes, and additional phenotyping with other telocyte-related markers, such as c-Kit/CD177, would strengthen these observations. Our results are consistent with the notion that telocytes occur in various tissues and that subtypes of organ-specific telocytes may exist [20]. Although contact-dependent signals could be relayed through a network of neighbouring cells in compact tissue, propagation of similar signals may rely on



**Fig. 3** Rat endometrial telocytes were labelled for CD34 (green) and counterstained with DAPI (blue). CD34+ telocytes were observed in culture (A, B) and in the stromal space around glands (C).

telopodes in tissues with a matrix environment that is low in cell density, such as the decidua.

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## Conflict of interest

The authors confirm that there are no conflicts of interest.

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