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Human myometrium – the ultrastructural 3D network of telocytes

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Abstract

Telocytes (TCs), a novel type of interstitial cells, were recently described in the interstitial space of tissues (www.telocytes.com). Telocytes TCs have several very long, moniliform extensions, namely telopodes (Tps). However, the functional role(s) of TCs is not yet understood. Successive photomicrographs of ultrathin sections were concatenated to capture the entire length of Tps which usually measure tens to hundreds of micrometres. Besides the podoms (dilations) and podomers (thin segments), ultrastructural features of Tps include the dichotomous branching and establishing homo- and heterocellular contacts. Telopodes make a labyrinthine system by 3D convolution and overlapping, their number being roughly estimated at approximately 20 per 1000 μm^2. Moreover, the presence of extracellular vesicles (shedding vesicles/exosomes) along the Tps suggests an active intercellular signalling (micro- and macromolecules), with possible significance in regulating uterine contractility.

Keywords: telocytes • telopodes • podoms • podomers • human uterus • extracellular vesicles

Introduction

Telocytes, were recently described as interstitial cells with specific cellular extensions called telopodes [1–3]. A telopode consists of a succession of thin segments called podomers and dilated regions named podoms. Usually podoms accommodate mitochondria, endoplasmic reticulum, and caveolae assembled as 'Ca^{2+}uptake/release units'. Telocytes were reported initially to display electrical activity [4–7] and described in close vicinity to myocytes, nerve endings and blood capillaries. Telocytes presumably correspond to former ICLCs [8], but their roles are now considered to be different [9].

Telocytes were identified in human myometrium by transmission electron microscopy and immunohistochemistry [10]. Recently, TCs were described in the endometrial stroma of the stratum functionalis, underlying the shape of the adjacent epithelial architecture [11]. Telocytes are also present in a wide variety of organs in humans and mammals [12–20].

In this study, we report ultrastructural evidence of 3D network created by homocellular contacts of the TCs in the human uterus. Extracellular vesicles (shedding microvesicles/exosomes) are frequently found in the close proximity of telopodes, a topography suggestive of the possible involvement of the TCs in the process of intercellular signal transmission.

Materials and methods

Human myometrial tissue samples

Tissue samples from human non-pregnant and pregnant myometrium were obtained and processed for ultrastructural investigation as previously described [2, 4].

Five biopsies of human myometrium were obtained from different hysterectomy specimens (benign indications) of premenopausal women. Other five specimens were obtained from uteri of pregnant

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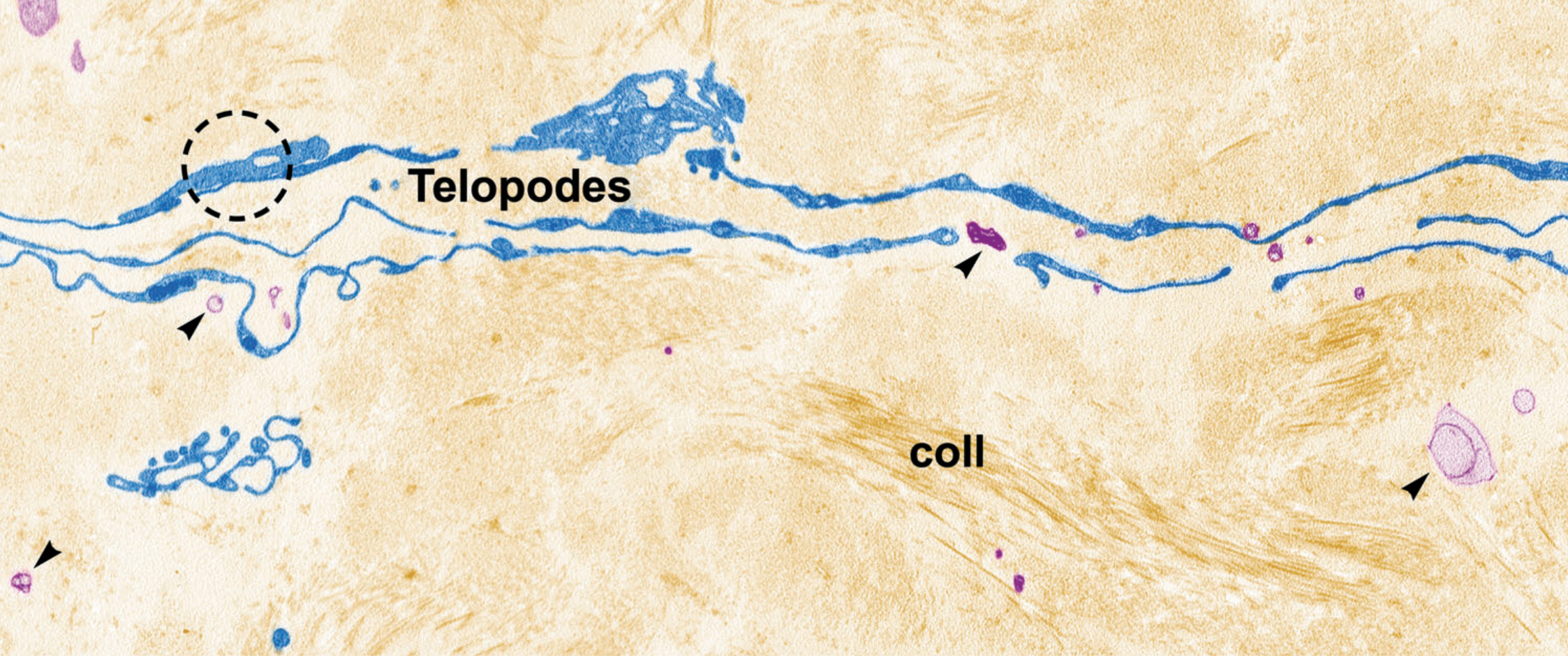
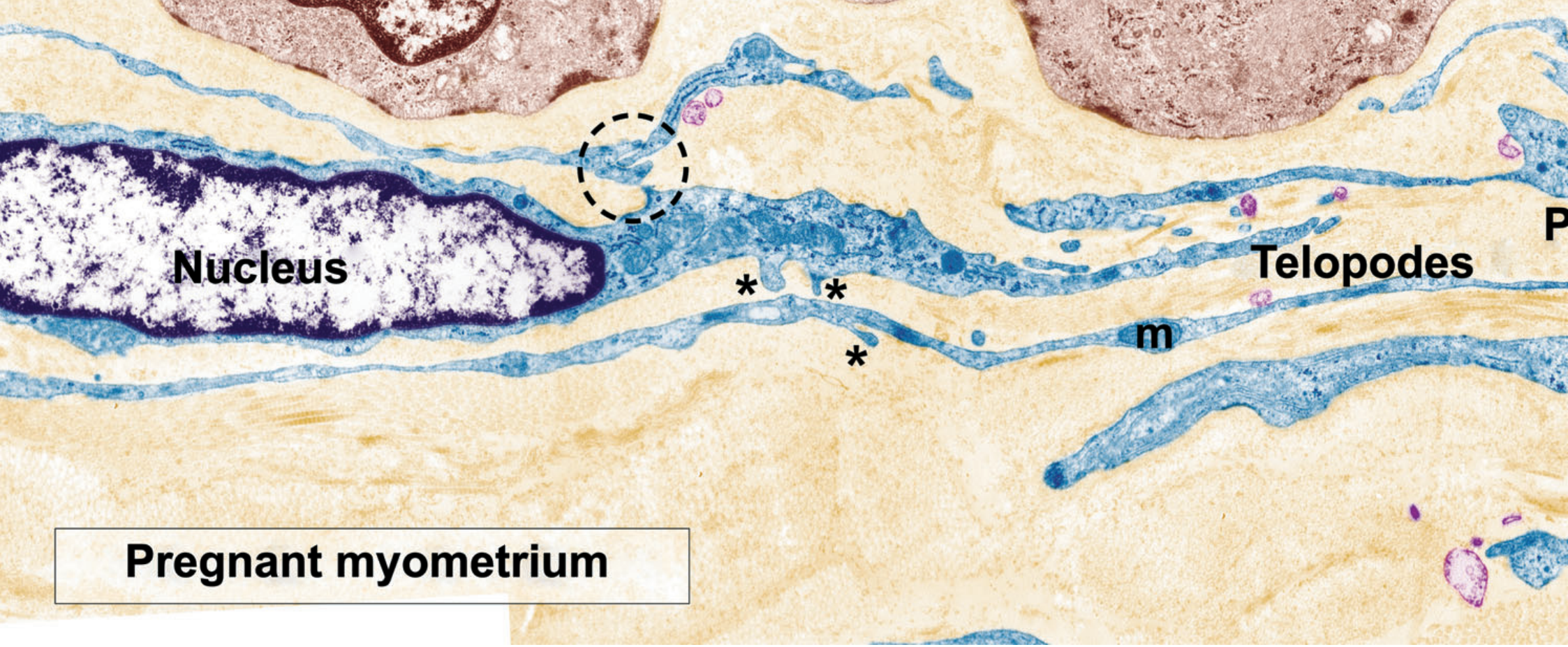


llustrates the diagram of the interstitial network built-up by TCs and Tps with uneven calibres: podoms and podomers. Exosomes and shedding vesicles (arrowheads) are digitally coloured in purple. coll: collagen; m: mitochondria; mvb: multivesicular bodies; N: nucleus. Scale bar = 5 µ



work and release extracellular organelles (exosomes and shedding vesicles) (arrowheads) digitally coloured in purple. One mast cell (green) is in the vicinity of this network. Some vesicles are captured at the moment of being shed from Tps (marked with *). Cav: caveolae; coll: collag

women (between 38 and 40 weeks of gestation), at the time of caesarean section. All patients received information about the study and signed an informed consent file. All experiments have been carried out in accordance with the EU guidelines and approved by the Bioethics Committee of 'Carol Davila' University of Medicine Bucharest.

Electron microscopy (TEM)

Tissue samples were immersed in 4% buffered glutaraldehyde during transportation from the hospital to the laboratory. Each biopsy was cut into 1 mm^3 small fragments and fixed for 4 hrs in 4% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4 at 4°C. The fragments were post fixed for 1 hr in buffered 1% OsO_4, dehydrated in an ethanol series and then processed for Epon 812 embedding. One-micrometre-thick sections stained with 1% toluidine blue were examined for a precise orientation of the subsequent thin sections. The ultrathin sections were cut using a MT-7000 ultramicrotome (Research Manufacturing Company Inc., Tucson, AZ, USA), mounted on 50-mesh grids, and double stained with uranyl acetate and lead citrate. The grids were examined on a CM 12 Philips electron microscope (Eindhoven, The Netherlands), at an acceleration voltage of 60 kV. Digital electron photomicrographs (negatives) were taken with Olympus Morada CCD camera (16 bit, 11 Mpx) (Olympus Soft Imaging Solutions, Münster, Germany) on the electron microscope. Images were processed using Adobe Photoshop© (Adobe Systems, San Jose, CA, USA) to outline cell contours.

Two-dimensional reconstructions

Successive images of microscopic fields were captured at high magnification, then aligned with each other and merged to form a collage. Alignment and merging were performed using Adobe Photoshop software (Adobe Systems).

Digital colouring of electron micrographs

Transmission electron microscopy images were digitally coloured to increase the visual contrast between several structures: TCs, telopodes and microvesicles. The purpose of such a technique is to make them more visible for the untrained eye. Contours of all structures have been manually traced in Adobe Photoshop software (Adobe Systems) using a Wacom digital tablet (Wacom Europe GmbH, Krefeld, Germany).

Results

Telocytes were revealed in non-pregnant and pregnant myometrium by TEM as interstitial cells with long processes (Figs 1 and 2). These cells fulfil ultrastructural criteria for TCs: have long (up to 74 μm) and thin (50–200 nm) cellular processes called telopodes (Tps). Telopodes are branching in a dichotomous pattern having a moniliform appearance because of uneven width: podomers (thin segments) alternating with podoms (dilated segments). The entire length of a Tp cannot be surprised in the same section plane because of its tortuous trajectory. Table 1 illustrates the lengths values of the Tps visible in Figs 1 and 2. Telopodes number was estimated to be approximately 20 per 1000 μm^2 of interstitial space. In non-pregnant myometrium Tps were thinner and longer compared to those in pregnant myometrium. Telopodes are connected to each other by homocellular junctions and appear to form a 3D network in the interstitial space at the border of smooth muscle cell bundles. The very high resolution of the images was obtained after the concatenation of eight (Fig. 1) and 11 (Fig. 2) successive photomicrographs (1 μm equals 1.1 cm for Fig. 1 and 1.8 cm for Fig. 2 respectively) of ultrathin sections. The wide fields (115 μm for Fig. 1 and 70 μm for Fig. 2) allowed us to observe ultrastructural details such as the presence of calcium uptake/release units (caveolae, mitochondria and endoplasmic reticulum) in the podoms. Along Tps and sometimes emerging from it, we can observe numerous exosomes (60–100 nm vesicles) and shedding vesicles (diameters: 250–350 nm up to 1 μm). We can even describe Tps terminal endings structures, which we believe to be multivesicular bodies responsible for exosomes release (Fig. 1).

Table 1 Telopodes lengths in non-pregnant and pregnant myometrium

Telopode length (μm)	
Non-pregnant myometrium	**Pregnant myometrium**
9.3	4.4
9.3	7.1
10.8	8.4
11.9	12.1
12.7	12.5
13.8	15.7
17.0	19.1
30.2	22.9
39.9	25.0
73.2	42.5

Discussion

In both physiological states (non-pregnant or pregnant), human uterus is known to develop myogenic contractions [21]. However, numerous attempts to evidence myometrial pacemaker cells [22] similar to those in the gut [23] failed. Instead, we observed a new interstitial cell type – the TCs, a heterogeneous population of cells found in many organs in mammals. Uterine TCs can be investigated and identified using TEM, a reliable diagnostic tool, because usually podomers are below the resolving power of light microscope [1]. The

uterine interstitial cells that were observed in this study fulfilled all the necessary criteria for TCs: very long telopodes with alternating regions of podoms and podomers, which form a 3D network, by homocellular contacts, at the border of smooth muscle fascicles. In the close proximity of telopodes and/or even emerging from them, numerous extracellular organelles (exosomes and shedding vesicles) were observed and these are in correlation with similar aspects found in heart [24], lungs [25], mammary gland [10], pancreas [20] and parotid gland [14]. It is well known that cells use microvesicles released in the extracellular space as mediators of cell-to-cell communication, guaranteeing short- and long-range exchange of information [26–29]. The release of exosomes and clusters of shedding microvesicles suggested that uterine TCs are equipped to communicate *via* their Tps and could be involved in intercellular signalling and regulation, facilitating cell-to-cell contact over long distances. TCs may also contribute to a wide variety of (for the moment, only supposed) functions (for details, see [10, 30]).

To conclude, TCs are a rather unique cell type of the interstitial space of human myometrium. Our future advances in their study will have to establish whether: (*i*) TCs are involved in endometrial or myometrial renewal because fundamental studies witness the presence of such stromal stem/progenitor cells [31–33]; (*ii*) TCs may became a promising target for therapeutic (non) hormonal interventions as we have proved the existence of shedding microvesicles/exosomes.

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Conflict of interest

The authors confirm that there are no conflicts of interest.

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