Uterine Telocytes: A Review of Current Knowledge 1

Authors: Roatesi, Iurie, Radu, Beatrice Mihaela, Cretoiu, Dragos, and Cretoiu, Sanda Maria

Source: Biology of Reproduction, 93(1)

Published By: Society for the Study of Reproduction

URL: https://doi.org/10.1095/biolreprod.114.125906

BIOLOGY OF REPRODUCTION (2015) **93**(1):10, 1–13
Published online before print 18 February 2015.
DOI 10.1095/biolreprod.114.125906

Minireview

Uterine Telocytes: A Review of Current Knowledge[1]

Iurie Roatesi,[3,4] Beatrice Mihaela Radu,[5,6] Dragos Cretoiu,[3,4] and Sanda Maria Cretoiu[2,3,4]

[3]*Victor Babeş National Institute of Pathology, Bucharest, Romania*
[4]*Division of Cell Biology and Histology, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania*
[5]*Department of Neurological and Movement Sciences, University of Verona, Verona, Italy*
[6]*Department of Anatomy, Animal Physiology and Biophysics, Faculty of Biology, University of Bucharest, Bucharest, Romania*

ABSTRACT

Telocytes (TCs), a novel cell type, are briefly defined as interstitial cells with telopodes (Tps). However, a specific immunocytochemical marker has not yet been found; therefore, electron microscopy is currently the only accurate method for identifying TCs. TCs are considered to have a mesenchymal origin. Recently proteomic analysis, microarray-based gene expression analysis, and the micro-RNA signature clearly showed that TCs are different from fibroblasts, mesenchymal stem cells, and endothelial cells. The dynamics of Tps were also revealed, and some electrophysiological properties of TCs were described (such as membrane capacitance, input resistance, membrane resting potential, and absence of action potentials correlated with different ionic currents characteristics), which can be used to distinguish uterine TCs from smooth muscle cells (SMCs). Here, we briefly present the most recent findings on the characteristics of TCs and their functions in human pregnant and nonpregnant uteri.

endometrium, myometrium, nonpregnant uterus, pregnant uterus, TCs, telopodes

INTRODUCTION, TERMINOLOGY, AND HISTORY OF DISCOVERY

The physiology of uterine contractility poses interesting challenges for obstetrics and gynecology specialists in their attempt to solve a major clinical problem, such as premature birth. Although the myometrium (ultra)structure does not appear to be very complex at a glance, the human uterus is capable of shifting its contractile intensity between weak contractions (as during the intervals between menstrual cycles and during a great period of time during pregnancy) to very powerful contractions during parturition. These variations in contractile status are due to the excitability of myometrial smooth muscle cells (SMCs), and they are particularly linked to cell membrane ion channel activity. Numerous studies have shown that the excitability levels of SMCs of the myometrium depend on an interaction between hormonal [1, 2], biochemical, and neurovegetative factors [3, 4].

Over the last decade, a new concept has evolved that refers to the role of telocytes (TCs) as possible cellular elements that participate in uterine physiology. The uterine cell population was reassessed, and increasing evidence supports the existence of a new cell type, the TC, in the interstitial (stromal) space. This novel cell type was described by Popescu et al. [5], from Bucharest, Romania, who coined the name TCs (using the Greek prefix ''telos,'' meaning goal, end, or fulfillment), suggesting that TCs accomplish their tasks through their extremely long prolongations, called telopodes (Tps) [5]. Tps measure up to hundreds of micrometers and consist of alternating thin segments, known as podomers (frequently below the resolving power of light microscopy), and dilated segments called podoms (which accommodate mitochondria, rough endoplasmic reticulum, and caveolae) [5–7]. TCs are frequently described in the myometrium and endometrium under confusing terms, which share the consensus of a membership of the stromal space. Various names include c-kit immunopositive interstitial cells (Cajal-type) [8], vimentin-positive c-kit-negative interstitial cells [9], interstitial Cajal-like cells (ICLC) [10–14], and TCs [15, 16].

Ciontea et al. [8] first described TCs in the uterus in 2005; initially, these cells were considered similar to interstitial cells of Cajal (ICCs). However, further studies revealed that there are no similarities between TCs and ICCs; therefore, there is no justification for the ICC-like or ICLC appellation. TCs were located close to SMCs of the myometrium and were also described in the endometrium; they were observed in the stromal space between the endometrial glands in mammals and reptiles [16, 17].

A large body of evidence on the description of TCs and their importance in many mammalian organs was gathered over time [18, 19].

[1]Supported by Sectorial Operational Programme Human Resources Development (SOP HRD), financed by European Social Fund and Romanian government contract POSDRU/89/1.5/S/141531 to I.R., Italian ministerial fellowship D.M. 198/2003 to B.M.R., and Romanian National Authority for Scientific Research CNCS-UEFISCDI project numbers 82/2012 and 194/2014.
[2]Correspondence: S.M. Cretoiu, Division of Cell Biology and Histology, Carol Davila University of Medicine and Pharmacy, Bucharest, 050474, Romania. E-mail: sanda@cretoiu.ro

Received: 26 October 2014.
First decision: 1 December 2014.
Accepted: 10 February 2015.

eISSN: 1529-7268 http://www.biolreprod.org
ISSN: 0006-3363

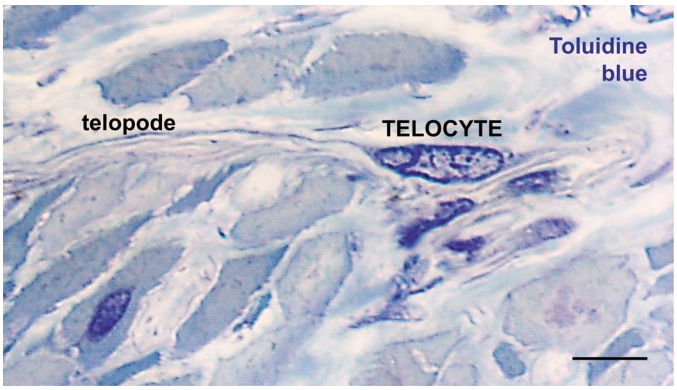

FIG. 1. Human pregnant myometrium (39 wk gestation). Semithin sections (0.5- to 1-μm thick) of the uterine muscular layer were embedded in Epon resin and stained with toluidine blue. There is a very long process of a TC squeezing obliquely between cut SMCs. Bar = 5 μm. Reproduced with permission from [119].

IN SITU IDENTIFICATION OF TCS

In situ, the presence of TCs is revealed through transmission electron microscopy (TEM), immunohistochemistry, and immunofluorescence. TEM is considered the most accurate method for identifying TCs [20]. Semithin sections of Epon-embedded tissue fragments stained with toluidine blue are generally useful for determining their general distribution in myometrium (Fig. 1). TC identification is facilitated by ultrastructural features, allowing for their differentiation from other interstitial space resident cells, such as (myo)fibroblasts, mesenchymal stem cells, and immune reactive cells [12, 21, 22]. Observation of a small cell body with a few (2–5) long and thin cytoplasmic prolongations (Tps) is sufficient to define the TC (Table 1). Frequently, TCs form close relationships with capillaries, nerve endings, and immune cells (Figs. 2 and 3). These details can only be observed after two-dimensional (2D) reconstruction of successive microscopic TEM fields (frequently between 8 and 12 photomicrographs) [15], reflecting the precise reality of the cellular network in the tissue (Fig. 4). Moreover, advanced techniques, such electron tomography (ET) and focused ion beam scanning electron microscopy (FIB-SEM), were applied to obtain detailed 3D images of TCs located in the pleura, lung, and heart [23–26].

In human myometrium, a detailed characterization of Tps was observed with morphometric analysis. Although the podomers are thicker in nonpregnant myometrium than in pregnant myometrium (~82 vs 75 nm), the podoms were thicker in pregnant myometrium (~316 vs 269 nm) [7]. Because uterine remodeling in pregnancy is associated with morphological and functional changes of multiple cells, for example, SMCs and TCs, differences observed in thickness and ramification of Tps may be important in determining their significance in pregnancy and/or other pathological conditions.

It is well known that in vivo cells communicate to exchange information under different physicochemical conditions [36]. Many cell types can generate extracellular small-membrane-bounded vesicles by a process referred to as ectocytosis [37]. Ectocytosis describes the direct vesicle formation from the cell membrane, and it is different from exocytosis, which indicates the release of preformed vesicles [38, 39]. In published reports, these vesicles are identified by various names, including exosomes, ectosomes, microparticles, microvesicles, and nanovesicles [39–42]. TCs release at least three types of extracellular vesicles, exosomes (45 ± 8 nm), ectosomes (128 ± 28 nm), and multivesicular cargos (MVCs; 1 ± 0.4 μm) [43], from their Tps and, occasionally, from the cell body. The precise site or area from which exosomes/ectosomes are released may determine their target destination and function. For the human uterus, there are no significant differences between exosomes/ectosomes released from TCs in nonpregnant myometrium and those from pregnant myometrium [7]. Mean diameters of these vesicles in pregnant myometrium were approximately the same as those in nonpregnant myometrium, which were 75 μm for exosomes and 185 μm for ectosomes (Table 2). Our current understanding of ectocytosis is limited, and more research is needed to support this phenomena. Edelstein and Smythies [44] proposed a possible mechanism by which the exosomal system operates. They suggested that during the process of tissue differentiation

TABLE 1. Ultrastructural characteristics of TCs.

Telocyte	Features	References
Cell body	A small cell body, 9−15 μm; Small amount of cytoplasm surrounding the nucleus; Plasmalemma with caveolae (2−3%); Cytoplasmic organelles are scarce: mitochondria (~3%) and endoplasmic reticulum (1-2%) of cell volume	[5, 15, 19, 24, 25, 27]
Nucleus	One nucleus, oval or rod shaped; Contains moderately dense chromatin (40−45% euchromatin, 55−60% heterochromatin)	[8, 28]
Telopodes	Number 1−5, average of 2−3; Length 10−1000 μm; Organized in a labyrinthine or convoluted system that forms a three-dimensional network; Dichotomous branching pattern; Consists of long and thin segments called podomers (~75−80 nm) interspersed with 250- to 300-nm cistern-like dilations called podoms; Podomers alternating with podoms provide a bead-on-a-string appearance; Podoms accommodate mitochondria, endoplasmic reticulum, and caveolae; Podomers are anchored by homocellular and heterocellular junctions, consisting of: Classical cell–cell junctions, such as gap junctions Close contacts and nanocontacts Atypical homocellular junctions with tiny puncta adhaerentia minima or processus adhaerentes Heterocellular junctions with blood capillaries, nerve bundles, muscle fibers, stem cells, resident and non-resident connective cells, and extracellular matrix elements (e.g., collagen and elastin fibers) Release exosomes (60- to 100-nm vesicles) and ectosomes (250- to 350-nm vesicles)	[5, 8, 29] [15, 27, 28, 30] [7, 31] [18, 23, 32] [7, 16, 27, 33-35]

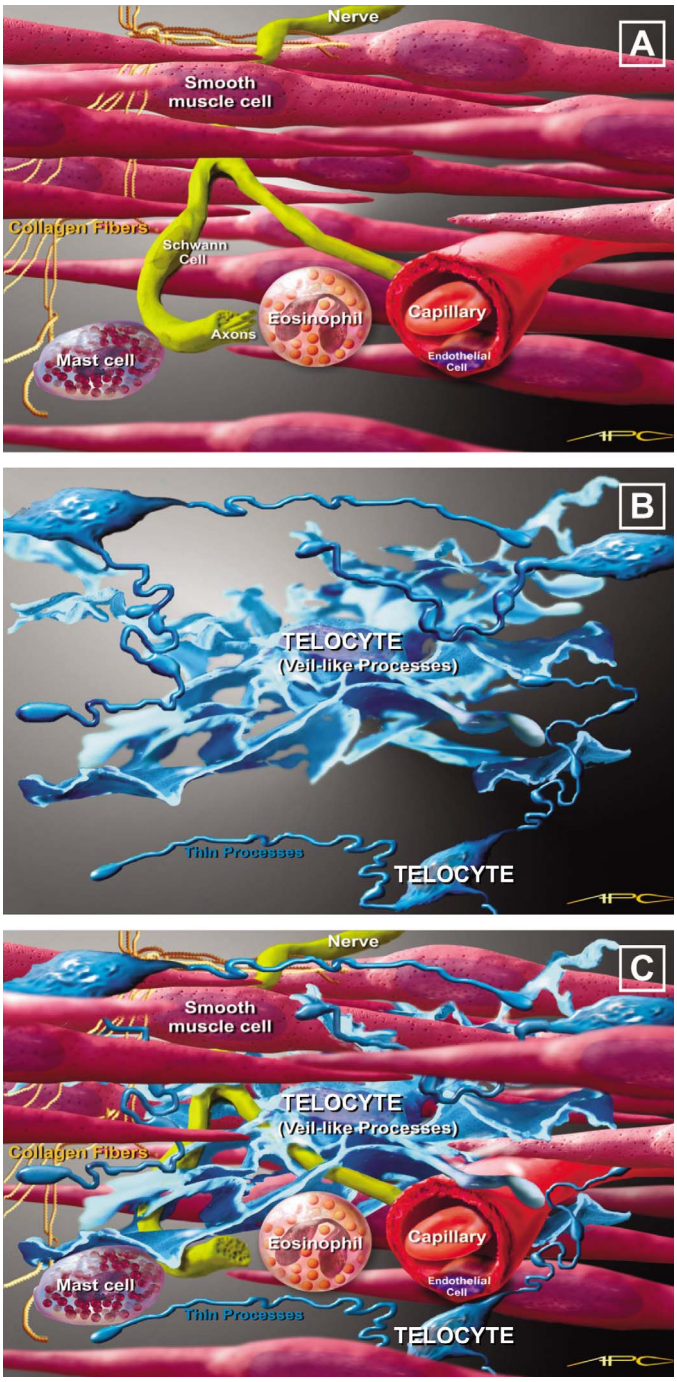

FIG. 2. **A**) Myometrial background with smooth muscle fibers, nerve fibers, and some connective tissue cells. **B**) Two possible ways to imagine the TCs in 3D, including cells with thin, broad, perforated, and veil-like processes as well as cells with long, thin processes. **C**) Close proximity of TC processes and SMCs, unmyelinated nerves, capillaries, collagen fibers, and immunoreactive cells. Scale bar = 7 μm (erytrocyte diameter). Reproduced with permission from [12].

and organ development, TCs randomly infiltrate the tissue, forming multiple synapses on a variety of specific target cells (STC); the two commence to exchange exosomes. The exosomes from the STC establish a molecular profile/mechanism in an immediately adjacent section of the TC (e.g., via exosome uptake sites or puncta adhaerentia), which is specific for the processing of signals from that type of STC. Subsequently, when the STC is damaged, it emits molecular signals, detailing the components of such event (e.g., hemoglobin in the case of a damaged blood vessel). Furthermore, injured cells rapidly change the specific RNA content of their emitted exosomes [44]. With respect to the main function of ectosomes, they may act as delivery vesicles that carry and transport information from TCs to target cells [45]. By releasing microvesicles, TCs play a role in intercellular communication; therefore, we suggest that they are responsible for maintaining the general tissue homeostasis of the main cell populations [46, 47], and of progenitor cells [48, 49].

Although ultrastructural characteristics are currently the method of choice for precisely identifying TCs, several immunohistochemical markers have been found that have variable expression in the TCs of the human genital system. Immunophenotypes of TCs include primarily CD34, CD117/c-Kit, PDGF-Rα and -β, and vimentin, as well as CD44, desmin, and cadherin-11, according to the TC location [8, 50–53]. Other markers were identified as (co-)expressed on TCs, including connexin 43, CD44, and nestin [31, 54]. It should be noted that TCs are immunohistochemically negative for procollagen 1, CD31/PECAM-1 (endothelial cells), α-SMA (myofibroblasts, pericytes, and vascular SMCs), CD11c (dendritic cells and macrophages), CD90/Thy-1 (fibroblasts) and, sometimes, c-kit/CD117 (mast cells) [55, 56]. TCs were originally considered CD117/c-kit positive based on their resemblance to ICCs. However, studies have reported controversial results from the presence [8] or absence [9] of c-kit expression at the uterine TC level, which is explained by a ''switching'' phenotype behavior in similar cell populations [53] or by differences in the technical procedures [57]. Some other studies have noted that TCs could sometimes be CD34-positive and c-kit-negative; also, c-kit positivity could depend on the type of antibody used [28, 58]. Hitherto, double-positive immunostaining with CD34/c-kit (mainly for cell body) or CD34/vimentin (mainly for Tps) were considered useful markers for TCs. Conversely, c-kit positivity alone or CD34 is insufficient to diagnose a TC [32]. In addition, TCs have been identified in human endometrium as cells positive for CD34, vimentin, and connexin 43 [16]. Therefore, using immunohistochemistry alone, we cannot differentiate between interstitial c-kit-positive cells that could be stem cells, mast cells [59, 60], or TCs; therefore, immunocytochemistry is recommended for evaluating cell cultures.

IN VITRO IDENTIFICATION OF TCS

The identity of TCs in myometrial primary culture and subsequent passages can easily be distinguished from SMCs by phase-contrast microscopy after 4 days in culture, which is before the cells reach confluence (Fig. 5) [8, 10]. TCs display a particular morphology, defined by very long, moniliform Tps that are not convoluted; they often create intercellular contacts between SMCs or other TCs. Frequently, dichotomous Tps branch out, forming an interlacing network of long lines. In vitro, as we have previously shown, TCs were initially identified using methylene blue vital staining (Fig. 6) [8]. Because the in situ ultrastructure of TCs demonstrated that they are rich in mitochondria, located especially in the podoms, the next step was to verify whether Tps in culture display such organelles. Hence, other vital techniques have been tried, such as Janus green B staining and MitoTracker Green FM, demonstrating that mitochondria are located in the cellular body and, particularly, in the podoms [7].

In vitro, uterine TCs express the same markers as in situ, at levels that are detectable by immunofluorescence [20]. To

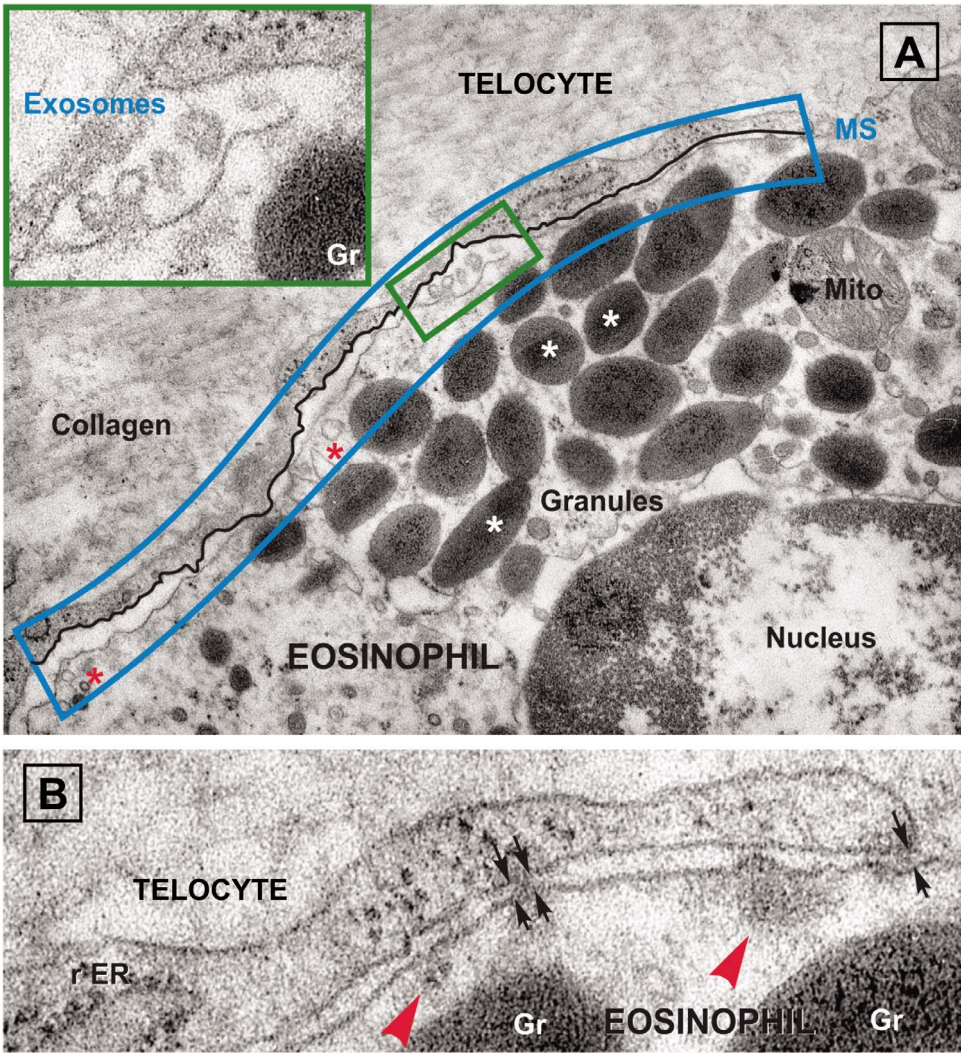

FIG. 3. Electron micrograph of a stromal synapse between a TC process and an eosinophil in rat myometrium. Original magnification ×19 000 (**A**). (Inset) Four membrane-bound vesicles with an average diameter of 54 ± 7 nm and homogenous content are shown in the synaptic cleft. The cleft is dilated and delimited by close contact points of both cells. Extracellular vesicles (exosomes) are similar in size and structure to subplasmalemmal vesicles of the eosinophil (black asterisks). Gr = granules with electron-dense crystalloid (white asterisks); Mito = mitochondrion. **B**) At higher magnification, electron-dense structures ("feet") are visible between the two cell membranes (arrows), as are focal densities located underneath the synaptic membrane (arrowheads). Gr = eosinophil-specific granules; rER = rough endoplasmic reticulum. Reproduced with permission from [21].

provide evidence that TCs are present, it is necessary to perform double-immunolabeling for CD34 and α-smooth muscle actin (α-SMA). CD34 expression is associated with the distinct morphology of TCs (very long Tps) and is considered to be in favor of a positive diagnosis [18]. In contrast, cells that are positive for α-SMA, with widened cellular bodies and without cytoplasmic extensions, are considered SMCs. The advantages of cell culture compared to conventional histological slides include the potential to examine individual cells. This advantage was exploited when myometrial TCs were shown to express estrogen and progesterone receptors [10, 61] (Fig. 7). Additionally, uterine TCs were found to be double-positive for CD34 and PDGFRα as well as to express T-type Ca^{2+}channels ($Ca_v3.1$ [α1G], Cav3.2 [α1H] members), from nonpregnant and pregnant myometrium on the cell body and in Tps [62].

During culture growth, the dynamic behavior of TCs was observed by time lapse videomicroscopy [6]. Tps growth and ramification may contribute to the guidance of surrounding cells or influence regional homeostasis by releasing signaling molecules, as we previously suggested [7]. Moreover, the mechanisms for Tps growth, adherence and extension seem to depend on various matrix proteins, and TC behavior is completely different from that of fibroblasts [63].

INTEGRATING TCS IN UTERINE PHYSIOLOGY

It is well known that uterine tissue has a complex physiology that is controlled and influenced by hormone-dependent changes during nonpregnant, pregnant, and postpartum states. TCs are present in both the endometrium and the myometrium of rat uterus, where, because of their different reproductive states, the uteri undergo numerical, morphological, and phenotypic trait changes. Therefore, related research has attempted to identify TC changes during these phases and may explain their functions. A comparison during different reproductive states reveals that immature uteri contain a small number of TCs in both the endometrium and myometrium, and the count is significantly increased in adult nonpregnant uteri. Pregnant uteri showed a further increase in endometrial TCs with a significant decrease in myometrial TCs, which is

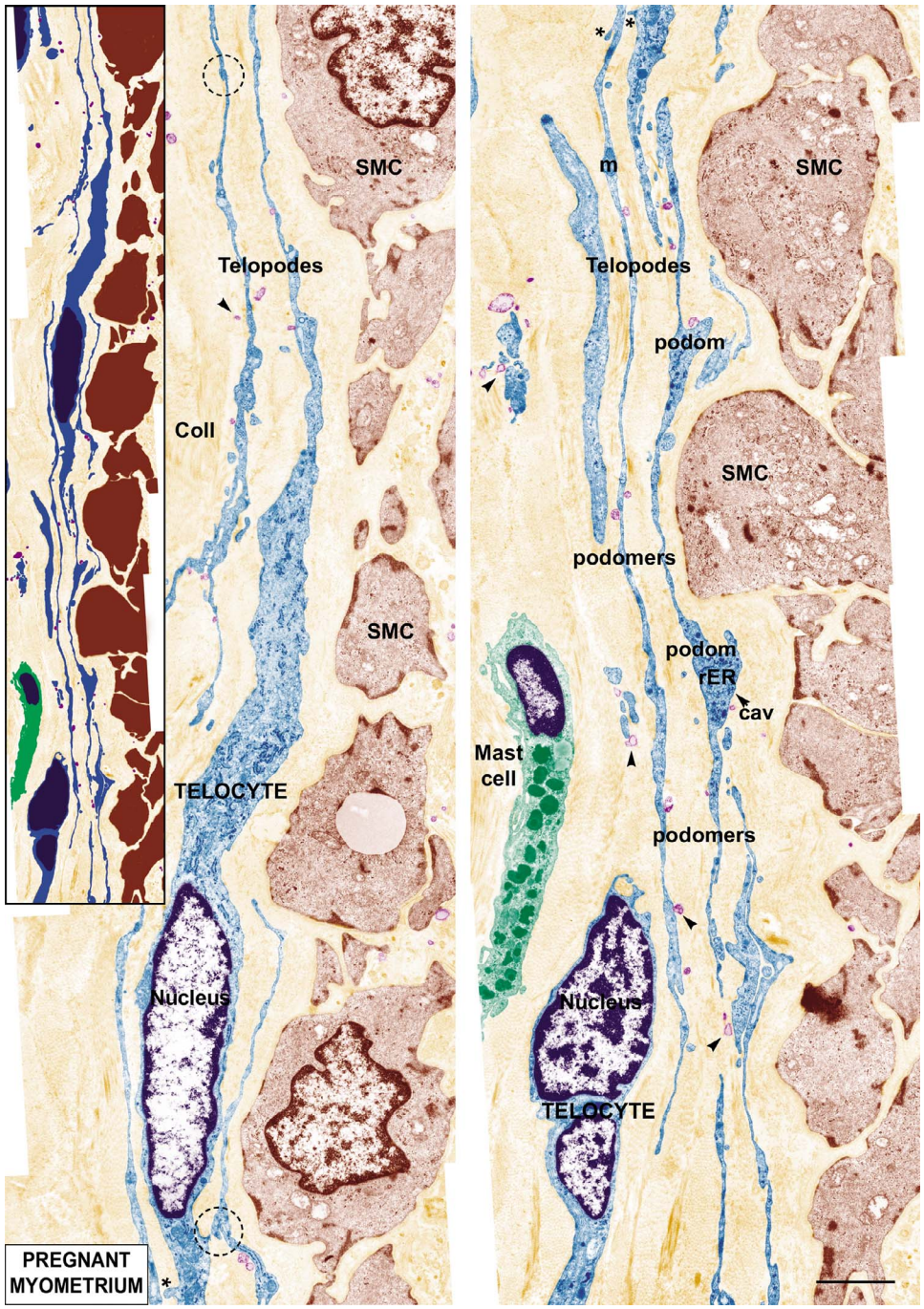

FIG. 4. Representative ultrathin section of human pregnant myometrium. Two-dimensional sequenced concatenation from 11 serial electron micrographs showing the 3D network of TCs (blue) interconnected by homocellular junctions (dotted circles). SMCs are shown in cross-section and were digitally colored brown. In their vicinity, numerous Tps (blue) establish a network and release extracellular organelles (exososomes and shedding vesicles [arrowheads]) digitally colored purple. One mast cell (green) is in the vicinity of this network. Some vesicles were captured at the moment of being shed from Tps (*). Cav = caveolae; coll = collagen; m = mitochondria; rER = rough endoplasmic reticulum; N = nucleus. Bar = 2 μm. Reproduced with permission from [15].

possibly to prevent preterm delivery. The highest count of myometrial TCs was recorded in the postpartum uteri and could reflect a role in postpartum involution [64]. Hence, Hatta et al. [16] hypothesized that TCs are frequently present in tissues that have a low cell density and significant space between neighboring cells. This hypothesis could be supported by the present finding that the number of endometrial TCs in the pregnant group was significantly higher than in other groups because the endometrium, unlike the myometrium, becomes loose with less cellularity during pregnancy [65]. This condition necessitates the presence of more TCs to facilitate cell-to-cell contact over long distances. Moreover, TCs express connexin 43, a gap junction protein, which most likely has a vital role in decidua maturation, as its decrease is associated with recurrent pregnancy loss [66]. The opposite condition is present in the endometrium of the immature uterus where the stroma is compact, densely cellular, and contained few glands. This state requires fewer TCs for glandular support and stromal

TABLE 2. Comparison between the extracellular membranous vesicles found in the human uterus, exosomes, and SMVs.

	Released vesicle diameters (nm)		Exosome diameters (nm)		Shedding vesicle diameters (nm)	
Parameter	Nonpregnant	Pregnant	Nonpregnant	Pregnant	Nonpregnant	Pregnant
Mean	160.64	171.60	75.97	78.32	185.38	182.71
Standard error	6.89	4.56	1.99	1.61	6.96	4.37
Median	151.03	170.30	76.07	78.63	170.58	176.59
Standard deviation	73.92	62.53	10.16	7.22	65.67	56.63
Range	347.77	296.62	33.74	26.44	308.45	267.27
Minimum	57.85	65.20	57.85	65.20	97.16	94.55
Maximum	405.61	361.82	91.59	91.64	405.61	361.82
Count	115	188	26	20	89	168
Confidence level (95.0%)	13.66	9.00	4.10	3.38	13.83	8.63

Reproduced with permission from Cretoiu et al. [7].

cell communication, which might explain why the lowest count of endometrial TCs was detected in the immature group. The functions of TCs in the endometrium seem to be glandular support and stromal cell communication by forming a scaffold around them; they may also play an active role in endometrial maintenance, whereas they possibly initiate control and coordinate myometrial contractility in the myometrium.

Rosenbaum et al. [67] demonstrated the presence of small-conductance calcium-activated potassium (SK3) channels in CD34-positive TCs in human nonpregnant myometrium and their absence in pregnant myometrium. SK3 channels are known to participate in myometrial relaxation in vitro [68]; therefore, we might hypothesize that their presence or absence in myometrial TCs regulates myometrial contractility in the nonpregnant versus the pregnant state.

In vitro clamp recordings of TCs in primary cultures have identified several electrophysiological characteristics that distinguish these cells from SMCs (Table 3), such as membrane capacitance, input resistance, membrane resting potential, or the presence/absence of different outward and inward currents. It worth noting that SMCs membrane surface areas vary, ranging from 33.5 to 181.9 μm^2, with a correction factor relative to the presence of caveolae [69] and important implications in the calculus of the membrane capacitance per unit area. As previously described, the presence of caveolae on the plasmalemma of TCs [6, 7] should be expected to have the same variability of the electrical membrane capacitance [8, 10]. Moreover, the membrane capacitance of SMCs from the pregnant human myometrium is 2.3-fold larger than that for SMCs from nonpregnant myometrium due to differences in the cell surface [70]. Although voltage-gated potassium channels have not yet been characterized in TCs from human myometrium, there are recent data from indicating their presence in TCs from human epicardium [71]. Further electrophysiological characterization is necessary to understand the role of TCs in uterine contraction mechanisms. The major bias of in vitro TC electrophysiological recordings, particularly in human myometrium preparations, is due to the reduced percentage of these cells (~6%) in the uterus compared to that on SMCs [9], which makes extensive characterization difficult.

Innovative concepts have recently been proposed for the role of TCs in bioelectrical signaling and introducing the concept of ''primitive nervous system'' constituted by TCs, exosomes, gap junctions, and the cytoskeleton [44, 79]. Despite the absence of regular slow waves of depolarization in TCs [7, 9], recent indirect observations indicate the presence of T-type/L-type calcium currents [75] that might improve our understanding uterine bioelectrical signaling mechanisms.

FUNCTIONAL OUTLOOK

The functions of TCs under both physiologic and pathologic conditions can be considered a versatile phenomenon, as suggested by phenotype adjustment (variable and inconsistent expression of some markers in the same organ or in different organs) that depends on the surrounding physical and chemical conditions. This includes signal reception and transmission of information via extracellular vesicles or by direct cellular and contact junctions [7, 25, 80].

A new approach was considered for describing the cellular genotype as well as for collecting proteomic data, providing a clear vision about the expression of structural proteins as well as enzymatic profile and surface antigens [81–84]. The role of TCs has been investigated by evaluating the bioelectrical signals and modulating physicochemical environment involved in morphogenesis [44, 49], remodeling [85], renewal [34], aging [86], cancer [87], and other pathological conditions [88–90].

In both physiological states (nonpregnant or pregnant), the human uterus is known to develop myogenic contractions. However, numerous attempts to study the myometrial pacemaker cells that are similar to those in the gut have failed [14, 91]. Instead, a new cell type was described compared with what we know today as TCs, a heterogeneous population of cells that are found among SMCs [7, 15]. TCs may contribute to broad physiological functions and, for the moment, only supposed roles can be inferred.

Intercellular Signaling

The first and most important function hypothesized for TCs is participation in intercellular signaling. All studies have shown that, on one hand, TCs are responsible for long-distance communication intermediated by very long (~100-μm-long) Tps, which establish physical contacts with nerve endings, blood vessels, and different types of progenitor cells [24, 25, 92]. On the other hand, TCs can influence the surrounding environment by either paracrine or juxtacrine secretion, which is mediated by extracellular vesicle release [7, 15]. These vesicles can transport different macromolecular signals to the surrounding cells and can induce epigenetic changes or can be involved in the regulation of inflammation or immunity [43, 93, 94]. Zavadil et al. [95] suggested that micro-RNAs could be responsible for uterine leiomyoma formation; therefore, because TCs were shown to express significant amounts of miR-21, -22, -29, and -199a and to express both estrogen and progesterone receptors, their role in this process should not be overlooked. Recently, Diaz-Flores et al. [96] demonstrated that TCs also have endocytic properties, which could explain the

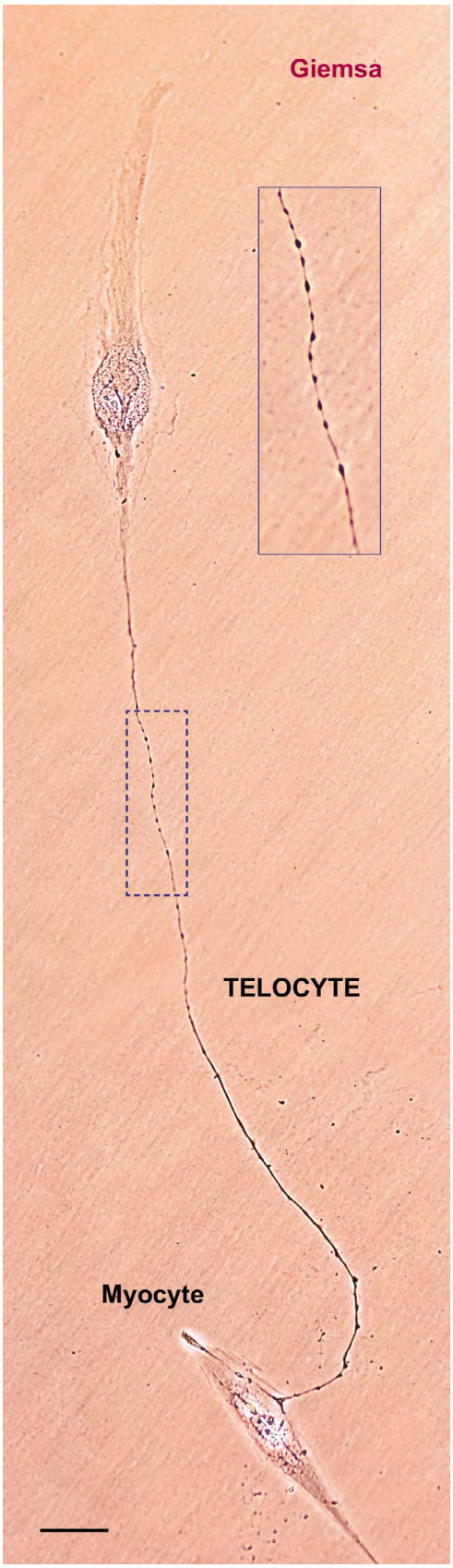

FIG. 5. Human nonpregnant myometrium in cell culture; Day 3 is the first passage. Giemsa staining. One TC established contact with an SMC by a Tp that is approximately 65 mm long. Photographic composition of four sequential phase-contrast images. Bar = 5 μm. (Rectangles) A higher magnification clearly shows the moniliform aspect; at least 40 specific dilations (podoms) interconnected by thin segments (podomeres) are visible in a beadlike fashion. Reproduced with permission from [8].

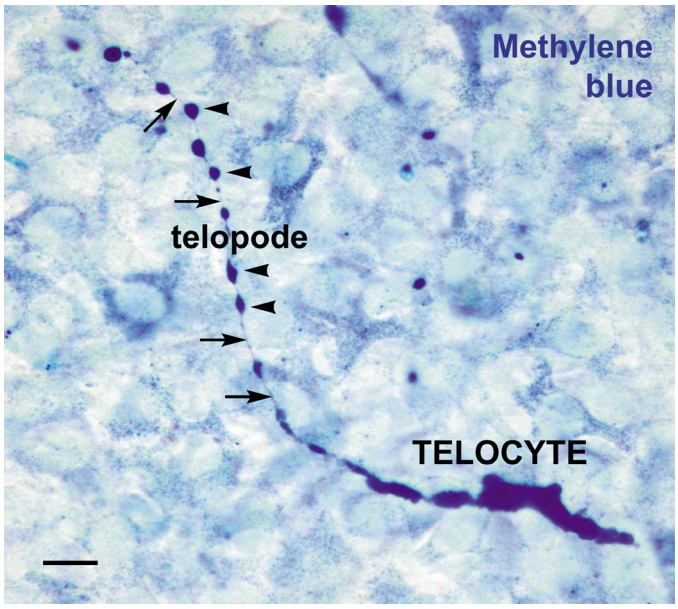

FIG. 6. Human pregnant myometrium. Primary confluent cultures (Day 8) showing a TC with at least 7 "beads" per process. Bar = 5 μm. Reproduced with permission from [8].

bidirectional information exchange between TCs and other proximity cells.

The existence of the 3D network of TCs is very important at the uterine level, as well as in other organs, for maintaining their functionality. Recent studies report that TC network damage is responsible for an impaired function by dysregulating intercellular signaling, leading to fibrosis of the intestinal wall, skin, and oviduct wall [56, 86, 89, 97].

Progenitor Cells and Uterine Renewal

Although remarkable progress has been made in reproductive medicine, there are still many unanswered questions, such as how the human uterus undergoes a 500- to 1000-fold increase in volume and a 24-fold increase in weight (from 50 to 1100 g) during pregnancy evolution [98]. The uterine stroma, which contains TCs, provides a supportive matrix for SMCs as well for blood vessels, nerves, and lymphatics. TCs might act as tissue organizers at least in some organs [5, 25, 99]. Although changes in the microvasculature of the human uterus during pregnancy are less well known, neoangiogenesis undoubtedly accompanies myometrial hypertrophy. Currently, angiogenesis is induced mainly by growth factors, creating tube formation in the vascular endothelial cells and promoting endothelial cell proliferation [100]. Studies have demonstrated that TCs located in the interstitial space significantly increase in number around the neocapillaries in mice with acute myocardial infarction [101]. Zheng et al. [102] demonstrated that the level of new vessel formation is correlated with the level of VEGF and EGF in TC supernatant [101]. TCs may participate in uterine neoangiogenesis and promote regeneration and reparation of the injured tissues.

Several studies have reported the presence of TCs in the stem cell niche microenvironment (e.g., in the heart [27, 99,

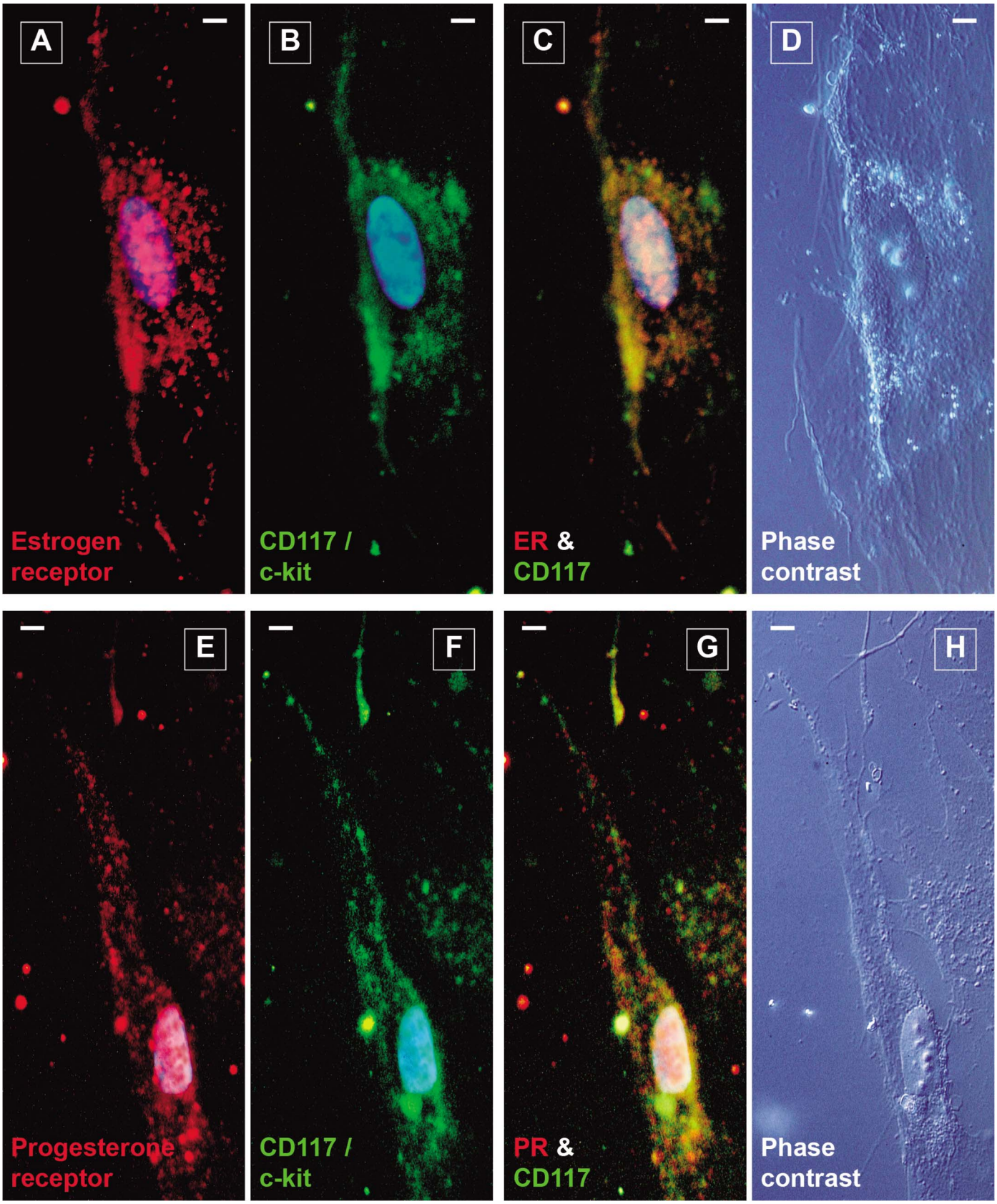

FIG. 7. **A–H**). Human myometrial cell culture at the fourth passage. Immunofluorescent labeling marks estrogen (**A**) and progesterone (**E**) receptors (red), which are present inside both the nucleus and the cytoplasm. c-kit/CD117 only (green) was found in the cytoplasm (**B**, **F**) and double labeling for both markers (**C**, **G**), where coexpression appears as yellow areas. Hoechst 33342 (blue) dye was used for nuclear counterstaining. Phase contrast microscopy focused on the same cells, typical TCs with long and moniliform prolongations (**D**, **H**). Bar = 2 μm. Reproduced with permission from [10].

TABLE 3. Electrophysiological characteristics of TCs versus those of SMCs in uterine myometrium.

Electrophysiological characterization	TCs/species (mean ± SD)	SMCs/species (mean ± SD)
Membrane capacitance	84.8 ± 18.1 pF/human, rat [9]; 35.7 ± 12.1 pF/human [8]	118 ± 26 pF/human, rat [9]; Differences pregnant vs nonpregnant/human, rat [70]; 1.57 pF/cm$^{2/human}$ [69]
Input resistance	3.04 ± 0.5 GΩ/human, rat [9]; 5.2 ± 1.0 GΩ/human [8];	1.94 ± 1.1 GΩ/human, rat [9]; 6.06 kΩ-cm^2/human [69]
Membrane resting potential	–58 ± 7/human, rat [9]	–65 ± 13 mV/human, rat [9]; –15 ÷ –85 mV (mean –49.4 mV)/human [69]
Small-conductance calcium-activated potassium (SK3) currents	Only nonpregnant/human [67]	Very low expression/human [67]; Present/rat [72]
Large conductance calcium-activated potassium (BK_{Ca}) currents	Not characterized	Differences pregnant vs nonpregnant/human, rat [70]
Delayed rectifier voltage-gated potassium currents	Not characterized	Present/human [73]
A-like voltage-gated potassium currents	Not characterized	Present/human, rat [73]
Outward rectifying potassium currents	Present/human, rat [9]	Present/human [69]
Na^+ currents	Absent/human, rat [9]	Present/rat [74]
T-type/L-type calcium currents	Present/human [62]; Present (indirect evidence by mibefradil effect)/human [75] Absent/human, rat [9]	Present/human, rat [74, 76-78]
Hyperpolarization-activated current	Present/human [7]	Present/rat [74]
Passive electronic and action potentials	Absent/human, rat [9]	Present/human, rat [9]
Regular slow waves of depolarization	Absent/human [7, 9]	Present/rat [74]

103], lungs [24], liver [20, 104], skin [55], skeletal muscle [48, 105, 106], meninges and choroid plexus [35], and eye [107]). However, the detailed regulatory mechanisms by which TCs and stem/progenitor cells interact are unknown. We speculate that there is close cross-talk between these cells, considering a possible protective role exerted by TCs relative to that of stem cells against extrinsic oxidative stress [108]. Furthermore, TCs can inconsistently express stem cell markers, such as c-kit, Sca-1, and Oct-4 [80, 109], and they have been suggested to be involved in the differentiation process of mesenchymal stem cells [55]. In any case, TCs are constantly present in the stem cell niche, indicating they make an important contribution to the tissue microenvironment, playing a crucial role in the cell signaling and regulation of normal and malignant cell function, which was suggested by Horch et al. [110].

Mechanochemical Sensors

The hypothesis that TCs might act as mechanoreceptors has been proposed in rat mesentery [111]. The TCs are located in the human uterus at the border of SMCs and between them, thus justifying the presumption that TCs could be capable of detecting and translating stretch information to the nuclear factors and activate the genes responsible for protein synthesis [112]. Indeed, it was recently shown that TCs from nonpregnant and pregnant myometrium have different sensitivities to low-level laser stimulation [75] and that mibefradil, a T-type calcium channel antagonist, modulates this effect. Therefore, TCs act as a stretch sensor and might play an important role in the uterine contraction mechanism in a direct relationship with the pregnancy status. The proteomic analysis of TCs compared with fibroblasts and endothelial cells shows that myosin-14 is up-regulated in TCs, suggesting the role in mechanical sensing and mechanochemical conversion [83, 108].

TCs could also be ''hormone sensors'' in human myometrium and the fallopian tube because they express estrogen and progesterone receptors in vitro. There is evidence that some uterine stromal cells play a role in endometrial growth and differentiation in a hormone-dependent manner [10, 113, 114]. Rehman et al. [115] showed by microarray analysis that the two estrogen receptor isoforms, α and β, had differential expression levels in the nonpregnant and the pregnant myometrium. The switch from ERα to ERβ expression in the myometrium occurs with the progression of pregnancy and may help delay labor until term [115].

Morphogenetic Bioelectrical Signaling

TCs may play an essential role in morphogenetic bioelectrical signaling in nearly all organs in the body [44, 79]. The TC system seems to have the necessary equipment to form a major part of the bioelectric ''information pathway,'' which is postulated to exist between cells in most tissues by Levin and Stevenson [116] and Levin [117]. The system may operate as follows: because the TC network is based on homo- and heterocellular junctions, and some of them are gap junctions; we can suppose that it could modulate the electrical activity in an organ and the membrane potential of these cells [44]. Indeed, TCs possess a vast communication network, including nerve endings, blood vessels, progenitor cells, immune cells, SMCs [24, 25, 30, 92], and bioelectrical signaling pathways, which have yet to be unraveled.

Pacemakers

The presence of specialized pacemaking cells is consistent with the spontaneous activity at least for gastrointestinal tract [118]. The paradox in regard to the uterus is that it must have periods of intense contractility alternating with periods of total absence of contractions [119]. Questions about whether uterine TCs perform this function remain a subject of debate. In our opinion, based on the scientific data regarding ICC pacemaking, this function is only mandatory in organs that require permanent peristaltic wave action to remove their content (e.g., gut [120], vasculature [121], and urinary tract [33]), and there is no such need for the uterus [75]. In fact, the lack of regular slow waves of depolarization in TCs already has been shown [7, 9], reinforcing previous idea.

Contractility Modulators

Drawing a parallel between gut TCs that are suggested to be cells that might directly or indirectly influence gut motility [58, 91, 122], we can assume that this is also valid for TCs described between uterine SMCs. Myogenic uterine contractility modulation under hormonal control [14] could involve the TCs either by transferring bioactive molecules or by direct stimulation of SMCs because TCs express steroid hormone receptors, at least in vitro [10, 119].

Guidance of Immune Cells

Judging from our previous reports that demonstrated stromal contacts between TCs and immune cells (eosinophils, macrophages, and plasma cells) in rat myometrium [30], we can hypothesize a role in immune surveillance. Leukocytes are known to be crucial for pregnancy maintenance and for the mechanism of uterine activation during labor [123, 124]. In our opinion, TCs, SMCs and leukocytes form a correlated orchestra that plays a role in the pregnancy maintenance or onset of labor.

FUTURE CONSIDERATIONS

The growing body of scientific evidence on TCs demonstrate that these still enigmatic cells cannot be considered negligible players in myometrial contractile coordination and uterine regeneration. Presently, there are many drawbacks to the TC research progress. These can include the absence of a selective marker and lack of valuable data for the phylogenesis of this heterogeneous cell population.

It will therefore be important to establish the following goals:

1. Whether TCs are involved in endometrial or myometrial renewal because fundamental studies have reported the presence of epithelial and stromal stem/progenitor cells [125] involved in endometrial growth and differentiation in a hormone-dependent manner. In vivo, estrogen is responsible for inducing regeneration, and progesterone is important for withdrawal-induced regression of human endometrial tissue [126, 127]. TCs could be a subpopulation of the stromal mesenchymal stem cells because they express some of the described markers of the subepithelial stromal niche cells (see Gargett et al. [128]).
2. The implication of TCs in immune surveillance and the presence of steroid hormone receptors could make them a promising target for therapeutic (non)hormonal interventions. Unraveling the existence of all members that are supposed to exist in a TC population and defining their functions will be essential for selective pharmacological targeting.
3. Future studies will need to address several important functional mechanisms of intercellular communication, electrical, and mechanical, or chemical, that may improve our understanding of the tissue-level signaling in the myometrium. These studies will most likely escalate when the specific immunohistochemical markers for TCs are identified, allowing us to obtain a magnetic bead purified and enriched culture. Another direction in these studies might be based on characterization of the laser-capture micro-dissected TC gene profile. After these goals are met, we will be able to run comparative animal experiments based on different pathological models (e.g., adenomyosis, uterine synechiae, and premature birth).

Future advances in understanding myometrial regeneration and its contractility must not ignore TCs as important members of the uterine nonmuscle cell populations.

REFERENCES

1. Soloff MS, Jeng YJ, Izban MG, Sinha M, Luxon BA, Stamnes SJ, England SK. Effects of progesterone treatment on expression of genes involved in uterine quiescence. Reprod Sci 2011; 18:781–797.
2. Garfield RE, Shi L, Shi SQ. Use of progesterone and progestin analogs for inhibition of preterm birth and other uterine contractility disorders. Facts Views Vis Obgyn 2012; 4:237–244.
3. Lammers WJ. The electrical activities of the uterus during pregnancy. Reprod Sci 2013; 20:182–189.
4. Greenwood IA, Tribe RM. Kv7 and Kv11 channels in myometrial regulation. Exp Physiol 2014; 99:503–509.
5. Popescu LM, Faussone-Pellegrini MS. Telocytes—a case of serendipity: the winding way from interstitial cells of Cajal (ICC), via interstitial Cajal-like cells (ICLC) to telocytes. J Cell Mol Med 2010; 14:729–740.
6. Suciu L, Nicolescu MI, Popescu LM. Cardiac telocytes: serial dynamic images in cell culture. J Cell Mol Med 2010; 14:2687–2692.
7. Cretoiu SM, Cretoiu D, Marin A, Radu BM, Popescu LM. Telocytes: ultrastructural, immunohistochemical and electrophysiological characteristics in human myometrium. Reproduction 2013; 145:357–370.
8. Ciontea SM, Radu E, Regalia T, Ceafalan L, Cretoiu D, Gherghiceanu M, Braga RI, Malincenco M, Zagrean L, Hinescu ME, Popescu LM. C-kit immunopositive interstitial cells (Cajal-type) in human myometrium. J Cell Mol Med 2005; 9:407–420.
9. Duquette RA, Shmygol A, Vaillant C, Mobasheri A, Pope M, Burdyga T, Wray S. Vimentin-positive, c-kit-negative interstitial cells in human and rat uterus: a role in pacemaking? Biol Reprod 2005; 72:276–283.
10. Cretoiu D, Ciontea SM, Popescu LM, Ceafalan L, Ardeleanu C. Interstitial Cajal-like cells (ICLC) as steroid hormone sensors in human myometrium: immunocytochemical approach. J Cell Mol Med 2006; 10: 789–795.
11. Popescu LM, Vidulescu C, Curici A, Caravia L, Simionescu AA, Ciontea SM, Simion S. Imatinib inhibits spontaneous rhythmic contractions of human uterus and intestine. Eur J Pharmacol 2006; 546:177–181.
12. Popescu LM, Ciontea SM, Cretoiu D. Interstitial Cajal-like cells in human uterus and fallopian tube. Ann N Y Acad Sci 2007; 1101: 139–165.
13. Cretoiu SM, Cretoiu D, Suciu L, Popescu LM. Interstitial Cajal-like cells of human Fallopian tube express estrogen and progesterone receptors. J Mol Histol 2009; 40:387–394.
14. Cretoiu SM, Simionescu AA, Caravia L, Curici A, Cretoiu D, Popescu LM. Complex effects of imatinib on spontaneous and oxytocin-induced contractions in human non-pregnant myometrium. Acta Physiol Hung 2011; 98:329–338.
15. Cretoiu SM, Cretoiu D, Popescu LM. Human myometrium—the ultrastructural 3D network of telocytes. J Cell Mol Med 2012; 16: 2844–2849.
16. Hatta K, Huang ML, Weisel RD, Li RK. Culture of rat endometrial telocytes. J Cell Mol Med 2012; 16:1392–1396.
17. Ullah S, Yang P, Zhang L, Zhang Q, Liu Y, Chen W, Waqas Y, Le Y, Chen B, Chen Q. Identification and characterization of telocytes in the uterus of the oviduct in the Chinese soft-shelled turtle, *Pelodiscus sinensis*: TEM evidence. J Cell Mol Med 2014; 18:2385–2392.
18. Faussone-Pellegrini MS, Popescu LM. Telocytes. Biomol Concepts 2011; 2481–2489.
19. Cretoiu SM, Popescu LM. Telocytes revisited. Biomol Concepts 2014; 5: 353–369.
20. Xiao J, Wang F, Liu Z, Yang C. Telocytes in liver: electron microscopic and immunofluorescent evidence. J Cell Mol Med 2013; 17:1537–1542.
21. Popescu LM, Gherghiceanu M, Cretoiu D, Radu E. The connective connection: interstitial cells of Cajal (ICC) and ICC-like cells establish synapses with immunoreactive cells. Electron microscope study in situ. J Cell Mol Med 2005; 9:714–730.
22. Zheng Y, Bai C, Wang X. Telocyte morphologies and potential roles in diseases. J Cell Physiol 2012; 227:2311–2317.
23. Hinescu ME, Gherghiceanu M, Suciu L, Popescu LM. Telocytes in pleura: two- and three-dimensional imaging by transmission electron microscopy. Cell Tissue Res 2011; 343:389–397.

24. Popescu LM, Gherghiceanu M, Suciu LC, Manole CG, Hinescu ME. Telocytes and putative stem cells in the lungs: electron microscopy, electron tomography and laser scanning microscopy. Cell Tissue Res 2011; 345:391–403.
25. Gherghiceanu M, Popescu LM. Heterocellular communication in the heart: electron tomography of telocyte-myocyte junctions. J Cell Mol Med 2011; 15:1005–1011.
26. Cretoiu D, Hummel E, Zimmermann H, Gherghiceanu M, Popescu LM. Human cardiac telocytes: 3D imaging by FIB-SEM tomography. J Cell Mol Med 2014; 18:2157–2164.
27. Gherghiceanu M, Popescu LM. Cardiac telocytes—their junctions and functional implications. Cell Tissue Res 2012; 348:265–279.
28. Cretoiu D, Cretoiu S, Simionescu A, Popescu L. Telocytes, a distinct type of cell among the stromal cells present in the lamina propria of jejunum. Histol Histopathol 2012; 27(8):1067–1078.
29. Popescu LM, Hinescu ME, Ionescu N, Ciontea SM, Cretoiu D, Ardelean C. Interstitial cells of Cajal in pancreas. J Cell Mol Med 2005; 9: 169–190.
30. Popescu LM, Ciontea SM, Cretoiu D, Hinescu ME, Radu E, Ionescu N, Ceausu M, Gherghiceanu M, Braga RI, Vasilescu F, Zagrean L, Ardeleanu C. Novel type of interstitial cell (Cajal-like) in human fallopian tube. J Cell Mol Med 2005; 9:479–523.
31. Suciu L, Popescu LM, Gherghiceanu M. Human placenta: de visu demonstration of interstitial Cajal-like cells. J Cell Mol Med 2007; 11: 590–597.
32. Zheng Y, Li H, Manole CG, Sun A, Ge J, Wang X. Telocytes in trachea and lungs. J Cell Mol Med 2011; 15:2262–2268.
33. Gevaert T, De Vos R, Van Der Aa F, Joniau S, van den Oord J, Roskams T, De Ridder D. Identification of telocytes in the upper lamina propria of the human urinary tract. J Cell Mol Med 2012; 16:2085–2093.
34. Zheng Y, Zhu T, Lin M, Wu D, Wang X. Telocytes in the urinary system. J Transl Med 2012; 10:188.
35. Popescu BO, Gherghiceanu M, Kostin S, Ceafalan L, Popescu LM. Telocytes in meninges and choroid plexus. Neurosci Lett 2012; 516: 265–269.
36. Frühbeis C, Fröhlich D, Kuo W, Krämer-Albers E. Extracellular vesicles as mediators of neuron-glia communication. Front Cell Neurosci 2013; 7: 182.
37. Turturici G, Tinnirello R, Sconzo G, Geraci F. Extracellular membrane vesicles as a mechanism of cell-to-cell communication: advantages and disadvantages. Am J Physiol Cell Physiol 2014; 306(7):621–633.
38. Sadallah S, Eken C, Schifferli J. Ectosomes as modulators of inflammation and immunity. Clin Exp Immunol 2010; 162:26–32.
39. Smythies J, Edelstein L, Ramachandran V. Molecular mechanisms for the inheritance of acquired characteristics-exosomes, microRNA shuttling, fear and stress: Lamarck resurrected? Front Genet 2014; 5:133.
40. Johnstone R. Exosomes biological significance: A concise review. Blood Cells Mol Dis 2006; 36:315–321.
41. Gupta A, Pulliam L. Exosomes as mediators of neuroinflammation. J Neuroinflammation 2014; 3:68.
42. Record M. Intercellular communication by exosomes in placenta: a possible role in cell fusion? Placenta 2014; 35(5):297–302.
43. Fertig ET, Gherghiceanu M, Popescu LM. Extracellular vesicles release by cardiac telocytes: electron microscopy and electron tomography. J Cell Mol Med 2014; 18:1938–1943.
44. Edelstein L, Smythies J. The role of telocytes in morphogenetic bioelectrical signaling: once more unto the breach. Front Mol Neurosci 2014; 7:41.
45. Smythies LE, Smythies JR. Exosomes in the gut. Front Immunol 2014; 5: 104.
46. Yu B, Zhang X, Li X. Exosomes derived from mesenchymal stem cells. Int J Mol Sci 2014; 15:4142–4157.
47. Tan CY, Lai RC, Wong W, Dan YY, Lim SK, Ho HK. Mesenchymal stem cell-derived exosomes promote hepatic regeneration in drug-induced liver injury models. Stem Cell Res Ther 2014; 5:76.
48. Bojin FM, Gavriliuc OI, Cristea MI, Tanasie G, Tatu CS, Panaitescu C, Paunescu V. Telocytes within human skeletal muscle stem cell niche. J Cell Mol Med 2011; 15:2269–2272.
49. Bani D, Nistri S. New insights into the morphogenic role of stromal cells and their relevance for regenerative medicine. Lessons from the heart. J Cell Mol Med 2014; 18(3):363–370.
50. Vannucchi MG, Traini C, Manetti M, Ibba-Manneschi L, Faussone-Pellegrini MS. Telocytes express PDGFRalpha in the human gastrointestinal tract. J Cell Mol Med 2013; 17:1099–1108.
51. Mou Y, Wang Y, Li J, Lu S, Duan C, Du Z, Yang G, Chen W, Zhao S, Zhou J, Wang C. Immunohistochemical characterization and functional identification of mammary gland telocytes in the self-assembly of reconstituted breast cancer tissue in vitro. J Cell Mol Med 2013; 17: 65–75.
52. Diaz-Flores L, Gutierrez R, Garcia MP, Saez FJ, Diaz-Flores L Jr., Valladares F, Madrid JF. CD34+ stromal cells/fibroblasts/fibrocytes/telocytes as a tissue reserve and a principal source of mesenchymal cells. Location, morphology, function and role in pathology. Histol Histopathol 2014; 29:831–870.
53. Vannucchi MG, Traini C, Guasti D, Giulio DP, Faussone-Pellegrini MS. Telocytes subtypes in human urinary bladder. J Cell Mol Med 2014; 18: 2000–2008.
54. Kostin S. Myocardial telocytes: a specific new cellular entity. J Cell Mol Med 2010; 14:1917–1921.
55. Ceafalan L, Gherghiceanu M, Popescu LM, Simionescu O. Telocytes in human skin–are they involved in skin regeneration? J Cell Mol Med 2012; 16:1405–1420.
56. Manetti M, Guiducci S, Ruffo M, Rosa I, Faussone-Pellegrini MS, Matucci-Cerinic M, Ibba-Manneschi L. Evidence for progressive reduction and loss of telocytes in the dermal cellular network of systemic sclerosis. J Cell Mol Med 2013; 17:482–496.
57. Popescu LM, Hinescu ME, Radu E, Ciontea SM, Cretoiu D, Leabu M, Ardeleanu C. CD117/c-kit positive interstitial (Cajal-like) cells in human pancreas. J Cell Mol Med 2005; 9:738–739.
58. Pieri L, Vannucchi MG, Faussone-Pellegrini MS. Histochemical and ultrastructural characteristics of an interstitial cell type different from ICC and resident in the muscle coat of human gut. J Cell Mol Med 2008; 12: 1944–1955.
59. Terada T. Gastrointestinal stromal tumor of the uterus: a case report with genetic analyses of c-kit and PDGFRA genes. Int J Gynecol Pathol 2009; 28(1):29–34.
60. Cinel L, Aban M, Basturk M, Ertunc D, Arpaci R, Dilek S, Camdeviren H. The association of mast cell density with myometrial invasion in endometrial carcinoma: a preliminary report. Pathol Res Pract 2009; 205(4):255–258.
61. Cretoiu SM, Cretoiu D, Simionescu AA, Popescu LM. Telocytes in human fallopian tube and uterus express estrogen and progesterone receptors In: Kahn S (ed.), Sex Steroids. Rijeka: Intech; 2012; 91–114.
62. Cretoiu S, Radu B, Banciu A, Banciu D, Cretoiu D, Ceafalan L, Popescu L. Isolated human uterine telocytes: immunocytochemistry and electrophysiology of T-type calcium channels. Histochem Cell Biol 2015; 143: 83–94.
63. Niculite CM, Regalia TM, Gherghiceanu M, Huica R, Surcel M, Ursaciuc C, Leabu M, Popescu LM. Dynamics of telopodes (telocyte prolongations) in cell culture depends on extracellular matrix protein. Mol Cell Biochem 2015; 398:157–164.
64. Salama N. Immunohistochemical characterization of telocytes in ratuterus in different reproductive states. The Egypt J of Histo 2013; 36:185–194.
65. Grinnell F, Head JR, Hoffpauir J. Fibronectin and cell shape in vivo: studies on the endometrium during pregnancy. J Cell Biol 1982; 94: 597–606.
66. Nair RR, Jain M, Singh K. Reduced expression of gap junction gene connexin 43 in recurrent early pregnancy loss patients. Placenta 2011; 32:619–621.
67. Rosenbaum ST, Svalo J, Nielsen K, Larsen T, Jorgensen JC, Bouchelouche P. Immunolocalization and expression of small-conductance calcium-activated potassium channels in human myometrium. J Cell Mol Med 2012; 16:3001–3008.
68. Rosenbaum ST, Larsen T, Joergensen JC, Bouchelouche PN. Relaxant effect of a novel calcium-activated potassium channel modulator on human myometrial spontaneous contractility in vitro. Acta Physiol (Oxf) 2012; 205:247–254.
69. Pressman EK, Tucker JA, Jr., Anderson NC, Jr., Young RC. Morphologic and electrophysiologic characterization of isolated pregnant human myometrial cells. Am J Obstet Gynecol 1988; 159:1273–1279.
70. Zhou XB, Wang GX, Huneke B, Wieland T, Korth M. Pregnancy switches adrenergic signal transduction in rat and human uterine myocytes as probed by BKCa channel activity. J Physiol 2000; 524 Pt 2:339–352.
71. Sheng J, Shim W, Lu J, Lim SY, Ong BH, Lim TS, Liew R, Chua YL, Wong P. Electrophysiology of human cardiac atrial and ventricular telocytes. J Cell Mol Med 2014; 18:355–362.
72. Noble K, Floyd R, Shmygol A, Shmygol A, Mobasheri A, Wray S. Distribution, expression and functional effects of small conductance Ca-activated potassium (SK) channels in rat myometrium. Cell Calcium 2010; 47:47–54.
73. Knock GA, Tribe RM, Hassoni AA, Aaronson PI. Modulation of

potassium current characteristics in human myometrial smooth muscle by 17beta-estradiol and progesterone. Biol Reprod 2001; 64:1526–1534.
74. Tong WC, Choi CY, Kharche S, Holden AV, Zhang H, Taggart MJ. A computational model of the ionic currents, Ca2+ dynamics and action potentials underlying contraction of isolated uterine smooth muscle. PLoS One 2011; 6:e18685.
75. Campeanu RA, Radu BM, Cretoiu SM, Banciu DD, Banciu A, Cretoiu D, Popescu LM. Near-infrared low-level laser stimulation of telocytes from human myometrium. Lasers Med Sci 2014; 29:1867–1874.
76. Young RC, Smith LH, McLaren MD. T-type and L-type calcium currents in freshly dispersed human uterine smooth muscle cells. Am J Obstet Gynecol 1993; 169:785–792.
77. Fry CH, Sui G, Wu C. T-type Ca2+ channels in non-vascular smooth muscles. Cell Calcium 2006; 40:231–239.
78. Blanks AM, Zhao ZH, Shmygol A, Bru-Mercier G, Astle S, Thornton S. Characterization of the molecular and electrophysiological properties of the T-type calcium channel in human myometrium. J Physiol 2007; 581: 915–926.
79. Smythies J, Edelstein L. Telocytes, exosomes, gap junctions and the cytoskeleton: the makings of a primitive nervous system? Front Cell Neurosci 2014; 7:278.
80. Suciu L, Popescu LM, Gherghiceanu M, Regalia T, Nicolescu MI, Hinescu ME, Faussone-Pellegrini MS. Telocytes in human term placenta: morphology and phenotype. Cells Tissues Organs 2010; 192:325–339.
81. Cismasiu VB, Radu E, Popescu LM. miR-193 expression differentiates telocytes from other stromal cells. J Cell Mol Med 2011; 15:1071–1074.
82. Zheng Y, Zhang M, Qian M, Wang L, Cismasiu VB, Bai C, Popescu LM, Wang X. Genetic comparison of mouse lung telocytes with mesenchymal stem cells and fibroblasts. J Cell Mol Med 2013; 17: 567–577.
83. Zheng Y, Cretoiu D, Yan G, Cretoiu SM, Popescu LM, Wang X. Comparative proteomic analysis of human lung telocytes with fibroblasts. J Cell Mol Med 2014; 18:568–589.
84. Sun X, Zheng M, Zhang M, Qian M, Zheng Y, Li M, Cretoiu D, Chen C, Chen L, Popescu LM, Wang X. Differences in the expression of chromosome 1 genes between lung telocytes and other cells: mesenchymal stem cells, fibroblasts, alveolar type II cells, airway epithelial cells and lymphocytes. J Cell Mol Med 2014; 18:801–810.
85. Mandache E, Gherghiceanu M, Macarie C, Kostin S, Popescu LM. Telocytes in human isolated atrial amyloidosis: ultrastructural remodelling. J Cell Mol Med 2010; 14:2739–2747.
86. Manetti M, Rosa I, Messerini L, Guiducci S, Matucci-Cerinic M, Ibba-Manneschi L. A loss of telocytes accompanies fibrosis of multiple organs in systemic sclerosis. J Cell Mol Med 2014; 18:253–262.
87. Mirancea N, Morosanu AM, Mirancea GV, Juravle FD, Manoiu VS. Infrastructure of the telocytes from tumor stroma in the skin basal and squamous cell carcinomas. Rom J Morphol Embryol 2013; 54: 1025–1037.
88. Matyja A, Gil K, Pasternak A, Sztefko K, Gajda M, Tomaszewski KA, Matyja M, Walocha JA, Kulig J, Thor P. Telocytes: new insight into the pathogenesis of gallstone disease. J Cell Mol Med 2013; 17:734–742.
89. Milia AF, Ruffo M, Manetti M, Rosa I, Conte D, Fazi M, Messerini L, Ibba-Manneschi L. Telocytes in Crohn's disease. J Cell Mol Med 2013; 17:1525–1536.
90. Zheng Y, Bai C, Wang X. Potential significance of telocytes in the pathogenesis of lung diseases. Expert Rev Respir Med 2012; 6:45–49.
91. Faussone-Pellegrini MS. Interplay among enteric neurons, interstitial cells of Cajal, resident and not resident connective tissue cells. J Cell Mol Med 2009; 13:1191–1192.
92. Zheng YH, Li H, Ge JB, Gao HJ, Wang XD. [Location of telocytes in mouse bronchial and pulmonary tissues]. Zhonghua Bing Li Xue Za Zhi 2012; 41:172–175.
93. Colombo M, Raposo G, Thery C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. Annu Rev Cell Dev Biol 2014; 30:255–289.
94. Mulcahy LA, Pink RC, Carter DR. Routes and mechanisms of extracellular vesicle uptake. J Extracell Vesicles 2014; 3.
95. Zavadil J, Ye H, Liu Z, Wu J, Lee P, Hernando E, Soteropoulos P, Toruner GA, Wei JJ. Profiling and functional analyses of microRNAs and their target gene products in human uterine leiomyomas. PLoS One 2010; 5:e12362.
96. Diaz-Flores L, Gutierrez R, Garcia MP, Saez FJ, Aparicio F, Diaz-Flores L Jr, Madrid JF. Uptake and intracytoplasmic storage of pigmented particles by human $CD34^+$ stromal cells/telocytes: endocytic property of telocytes. J Cell Mol Med 2014; 18:2478–2487.
97. Yang XJ, Yang J, Liu Z, Yang G, Shen ZJ. Telocytes damage in endometriosis affected rat oviduct and potential impact on infertility. J Cell Mol Med 2015; 19:452–462.
98. Ramsey EM. Anatomy of the human uterus. In: Chard T, Grudzinskas JG (eds.), The Uterus. Cambridge: Cambridge University Press; 1994; 18–40.
99. Bani D, Formigli L, Gherghiceanu M, Faussone-Pellegrini M. Telocytes as supporting cells for myocardial tissue organization in developing and adult heart. J Cell Mol Med 2008; 14:2531–2538.
100. Holmes DI, Zachary I. The vascular endothelial growth factor (VEGF) family: angiogenic factors in health and disease. Genome Biol 2005; 6: 209.
101. Manole CG, Cismasiu V, Gherghiceanu M, Popescu LM. Experimental acute myocardial infarction: telocytes involvement in neo-angiogenesis. J Cell Mol Med 2011; 15:2284–2296.
102. Zheng Y, Chen X, Qian M, Zhang M, Zhang D, Bai C, Wang Q, Wang X. Human lung telocytes could promote the proliferation and angiogenesis of human pulmonary microvascular endothelial cells in vitro. Mol Cell Ther 2014; 2:1–7.
103. Gherghiceanu M, Popescu LM. Cardiomyocyte precursors and telocytes in epicardial stem cell niche: electron microscope images. J Cell Mol Med 2010; 14:871–877.
104. Wang F, Song Y, Bei Y, Zhao Y, Xiao J, Yang C. Telocytes in liver regeneration: possible roles. J Cell Mol Med 2014; 18:1720–1726.
105. Suciu LC, Popescu BO, Kostin S, Popescu LM. Platelet-derived growth factor receptor-beta-positive telocytes in skeletal muscle interstitium. J Cell Mol Med 2012; 16:701–707.
106. Ceafalan LC, Popescu BO, Hinescu ME. Cellular players in skeletal muscle regeneration. Biomed Res Int 2014; 2014:957014.
107. Luesma MJ, Gherghiceanu M, Popescu LM. Telocytes and stem cells in limbus and uvea of mouse eye. J Cell Mol Med 2013; 17:1016–1024.
108. Zheng Y, Cretoiu D, Yan G, Cretoiu SM, Popescu LM, Fang H, Wang X. Protein profiling of human lung telocytes and microvascular endothelial cells using iTRAQ quantitative proteomics. J Cell Mol Med 2014; 18: 1035–1059.
109. Galiger C, Kostin S, Golec A, Ahlbrecht K, Becker S, Gherghiceanu M, Popescu LM, Morty RE, Seeger W, Voswinckel R. Phenotypical and ultrastructural features of Oct4-positive cells in the adult mouse lung. J Cell Mol Med 2014; 18:1321–1333.
110. Horch RE, Boos AM, Quan Y, Bleiziffer O, Detsch R, Boccaccini AR, Alexiou C, Sun J, Beier JP, Arkudas A. Cancer research by means of tissue engineering–is there a rationale? J Cell Mol Med 2013; 17: 1197–1206.
111. Hinescu ME, Popescu LM, Gherghiceanu M, Faussone-Pellegrini MS. Interstitial Cajal-like cells in rat mesentery: an ultrastructural and immunohistochemical approach. J Cell Mol Med 2008; 12:260–270.
112. Shynlova O, Tsui P, Jaffer S, Lye SJ. Integration of endocrine and mechanical signals in the regulation of myometrial functions during pregnancy and labour. Eur J Obstet Gynecol Reprod Biol 2009; 144(Suppl 1):S2–10.
113. Bigsby R. Control of growth and differentiation of the endometrium: the role of tissue interactions. Ann N Y Acad Sci 2002; 995:110–117.
114. Cunha G, Cooke P, Kurita T. Role of stromal–epithelia interactions in hormonal responses. Arch Histol Cytol 2004; 67:417–434.
115. Rehman KS, Yin S, Mayhew BA, Word RA, Rainey WE. Human myometrial adaptation to pregnancy: cDNA microarray gene expression profiling of myometrium from non-pregnant and pregnant women. Mol Hum Reprod 2003; 9:681–700.
116. Levin M, Stevenson CG. Regulation of cell behavior and tissue patterning by bioelectrical signals: challenges and opportunities for biomedical engineering. Annu Rev Biomed Eng 2012; 14:295–323.
117. Levin M. Endogenous bioelectrical networks store non-genetic patterning information during development and regeneration. J Physiol 2014; 592: 2295–2305.
118. Zhu YF, Wang XY, Lowie BJ, Parsons S, White L, Kunze W, Pawelka A, Huizinga JD. Enteric sensory neurons communicate with interstitial cells of Cajal to affect pacemaker activity in the small intestine. Pflugers Arch 2014; 466:1467–1475.
119. Hutchings G, Williams O, Cretoiu D, Ciontea SM. Myometrial interstitial cells and the coordination of myometrial contractility. J Cell Mol Med 2009; 13:4268–4282.
120. Cantarero Carmona I, Luesma Bartolome MJ, Junquera Escribano C. Identification of telocytes in the lamina propria of rat duodenum: transmission electron microscopy. J Cell Mol Med 2011; 15:26–30.
121. Cantarero I, Luesma MJ, Junquera C. The primary cilium of telocytes in the vasculature: electron microscope imaging. J Cell Mol Med 2011; 15: 2594–2600.
122. Bassotti G, Villanacci V, Cretoiu D, Cretoiu SM, Becheanu G. Cellular

and molecular basis of chronic constipation: taking the functional/idiopathic label out. World J Gastroenterol 2013; 19:4099–4105.
123. Srikhajon K, Shynlova O, Preechapornprasert A, Chanrachakul B, Lye S. A new role for monocytes in modulating myometrial inflammation during human labor. Biol Reprod 2014; 91:10.
124. Lee YH, Shynlova O, Lye SJ. Stretch-induced human myometrial cytokines enhance immune cell recruitment via endothelial activation. Cell Mol Immunol 2015; 12:231–242.
125. Gargett CE, Chan RW, Schwab KE. Hormone and growth factor signaling in endometrial renewal: role of stem/progenitor cells. Mol Cell Endocrinol 2008; 288:22–29.
126. Maruyama T, Masuda H, Ono M, Kajitani T, Yoshimura Y. Human uterine stem/progenitor cells: their possible role in uterine physiology and pathology. Reproduction 2010; 140:11–22.
127. Masuda H, Matsuzaki Y, Hiratsu E, Ono M, Nagashima T, Kajitani T, Arase T, Oda H, Uchida H, Asada H, Ito M, Yoshimura Y, et al. Stem cell-like properties of the endometrial side population: implication in endometrial regeneration. PLoS One 2010; 5:e10387.
128. Gargett CE, Nguyen HP, Ye L. Endometrial regeneration and endometrial stem/progenitor cells. Rev Endocr Metab Disord 2012; 13: 235–251.