Chapter

Telocytes: New Connecting Devices in the Stromal Space of Organs

Dragos Cretoiu, Maria Giuliana Vannucchi, Yihua Bei, Mirko Manetti, Maria Simonetta Faussone-Pellegrini, Lidia Ibba-Manneschi, Junjie Xiao and Sanda Maria Cretoiu

Abstract

Telocytes (TCs) represent a new type of interstitial cells, and were discovered by Prof. Popescu and his collaborators from Bucharest in 2005, and described as Interstitial Cajal-Like Cells (ICLCs). In 2010, Prof. Popescu and Prof. Faussone-Pellegrini from Florence, based on their expertise in morphology, agreed that in fact ICLCs were a brand-new entity and they renamed them telocytes. TCs are characterized by specific veil- or ribbon-like extensions called telopodes. Telopodes aid TCs in forming homo- or hetero-cellular contacts; thus, assembling three-dimensional networks that organizes the stromal and the parenchymal components of the organs. TCs can transfer information to neighbor cells ensuring a short-distance communication, and remotely by the release a wide variety of extracellular vesicles: exosomes, ectosomes, and multivesicular bodies. Here, we reviewed the evolution of the interest regarding TCs in different organs, in normal and pathological conditions. The main focus was on the role of TCs in gastrointestinal tract, urinary bladder, reproductive tract, and heart. This chapter sums up information about the possibilities that TCs are capable to behave as sensors/mediators in nervous activity, to represent mesenchymal stem cell precursors in adulthood, and to control and determine the differentiation/maturation of other cell types either during development or in postnatal life.

Keywords: telocytes, telopodes, podoms, podomers, 3D-network

1. Introduction: the telocytes

Discovered 13 years ago, telocytes (TCs) still represent a subject of debate regarding their role. TCs were first described in 2005, in the interstitial space surrounding exocrine acini in the pancreas, and considered as cells closely resembling the interstitial cells of Cajal (ICCs) by Romanian scientists from Carol Davila University of Medicine and Pharmacy in Bucharest, Romania, which entitled their first publication *Interstitial Cells of Cajal in Pancreas* [1]. Subsequent publications of this team described these cells under the name of interstitial Cajal-like cells and characterized them with the aid of electron microscopy and immunoctyo(histo) chemistry [2–6]. In 2010, professor Popescu, the Bucharest team leader, along with

professor Faussone-Pellegrini, who is considered to be the international leading expert in ICCs (the gut pacemaker cells), have agreed that TCs and ICCs can be regarded as completely different cell populations based on the ultrastructural peculiarities, and went on to give them the name of "telocytes" [7]. In the following years, TCs have been described in numerous organs, and appear to be omnipresent in stromal space of humans and laboratory mammals where they form networks [8–10] by making contacts with each other (homo-cellular contacts). This is possible due to their unique and exceptionally long (several tens to hundreds of μm) cell prolongations called telopodes [7]. As professor Popescu liked to say "the shortest possible definition for telocytes is cells with telopodes" [11]. Telopodes are characterized by a succession of thin, filamentous regions (podomers) and dilated areas (podoms) with bead-like appearance [12]. TCs were revealed to have a third dimension that was only recently observed by FIB-SEM tomography, telopodes being represented by a veil- or ribbon-like extensions that compartmentalize the interstitial space [13]. Apart from the ability to form networks, TCs also can provide local communication with different cells and also to deliver extracellular vesicles to establish distant communication [14–18]. A detailed representation of the TCs contacts is illustrated in **Figure 1**.

To find out the function(s) of these cells, many methods of investigation have been employed, ranging from classical microscopy—optical and electronic—to advanced genomics and proteomics techniques. Thus, differentiation of TCs from mesenchymal stem cell adipocytes, fibroblasts, and endothelial cells was achieved [19–22].

The discovery and description of TCs have given rise to many controversies. From the publication of the first article to the present, the interest generated by TCs can be traced in the graph summarizing the number of articles published annually in PubMed (**Figure 2**). Though not yet described in speciality treatises as a distinct cellular type, this prospect does not seem to be far, the evidence being the increase in the number of articles published in the most prestigious journals such as Nature [23–25]. The discussion of these controversies is dealt with in detail by two recent reviews, in which there are enough arguments for and against the existence of TCs as a new cell type [26, 27]. We believe that in the shortest time new biomarkers will

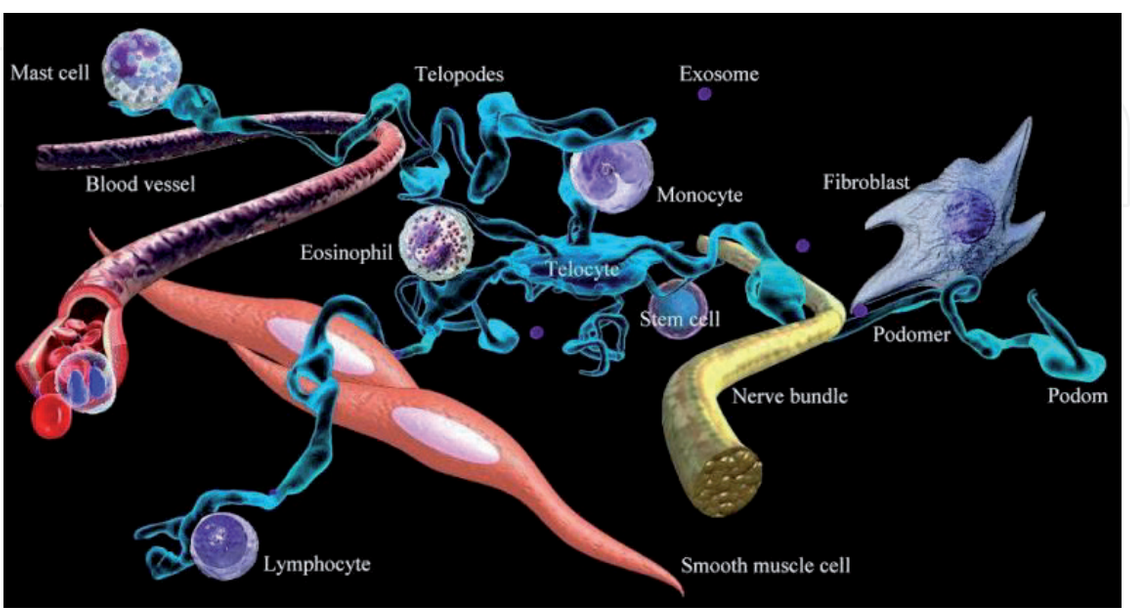

Figure 1.
Artistic representation of a 3D view of the contacts of a telocyte. TCs are regarded as interconnection devices due to their homo- and hetero-cellular junctions, as well as to their proximity to structures like blood vessels, nerve fibers, and muscle fibers. Image courtesy of Iurie Roatesi. Reproduced with permission from Ref. [75].

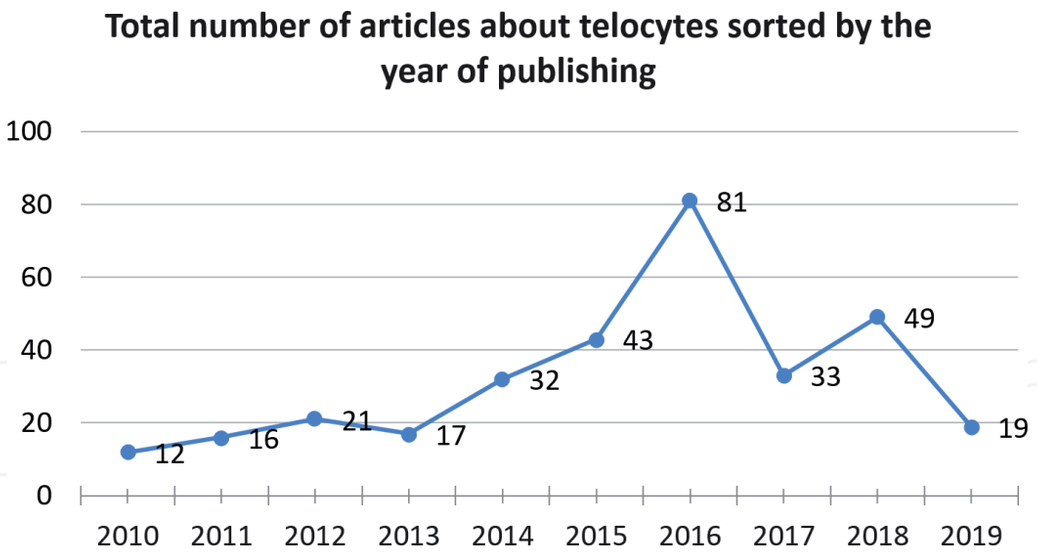

Figure 2.
Trends of publications searched in PubMed with "Telocytes" as a key word between 2010 and 2019.

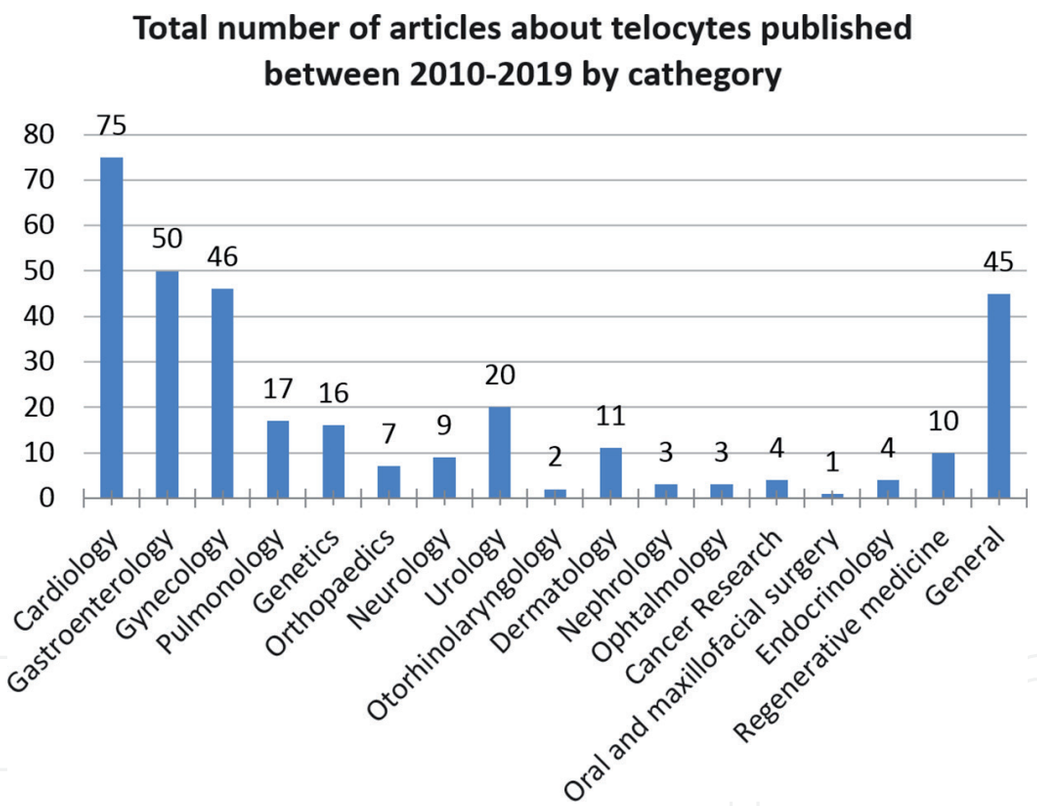

Figure 3.
Histogram of publications on telocytes categorized by categories of interest.

be found to prove the existence of this particular type of cell, and here is just one step to the description of their possible functions.

Our attention in this chapter is focused on synthesizing the available information relating to the main categories of interest that emerge from the chart shown in **Figure 3**, namely gastrointestinal tract, urinary bladder, reproductive tract, and heart.

1.1 The telocyte network

The ability of these cells to form 3-D stromal networks can be considered a discriminant element for their recognition under the light microscope [8],

especially because no specific immunomarkers are available [28]. Indeed, the cell surface glycoprotein CD34, a marker shared with vascular endothelial cells, is currently considered one of the most suitable for the immunohistochemical identification of the TCs, which are also referred to as CD34+ stromal cells/TCs by some authors [29, 30]. Through extensive homo-cellular networks, TCs are believed to build the stromal scaffold whose continuity and adaptability guarantees the maintenance of the integrity of tissues/organs every time they are subjected to mechanical forces, such as distension and stretching. Moreover, TCs are universally considered key organizers of the connective tissue and eventually, they may contribute to the production and shaping of the extracellular matrix (ECM) in cooperation with fibroblasts. This has been observed in TCs located in the female genital tract where these cells express both estrogen and progesterone receptors [4, 31] whose activation is followed by significant changes of the TCs that acquire fibroblast-like features and become capable to produce the ECM [8]. Likely, the homo-cellular TCs contacts are also involved in the intercellular exchange of molecular or ionic signaling. Alongside the aforementioned roles, probably shared by all the TCs, many other roles have been attributed to these cells [30]. Therefore, each of the TCs subtypes is likely to play its organ-/tissue-specific role [8].

Although the TCs homo-cellular contacts are commonly observed, a variety of cell-to-cell contacts between TCs and other cell types (referred to as hetero-cellular contacts) are also observed [8, 32–35]. They consist of minute junctions (point contacts, nanocontacts, and planar contacts) whose mean inter-membrane distance is 10–30 nm, but more often by variably extended simple apposition of the contiguous plasma membranes that might act either as mechanical cell-to-cell attachments or as sites of intercellular communication [18]. Among these contacts, there are the so-called "stromal synapses" [36], a term used to describe those contacts occurring between TCs and several types of connective tissue cells such as mast cells, macrophages, myofibroblasts, and fibroblasts [8, 18, 35, 37]. The networks built by these hetero-cellular contacts are named "mixed networks." Collectively, the existence of mixed networks in addition to the homo-cellular TCs networks, the morphological and immunohistochemical differences reported for the TCs among organs and tissues, the existence of TCs subtypes, the interactions that TCs make with the ECM and, finally, the TCs vicinity to nerve endings and vascular cells, have substantiated the hypothesis that these cells may be part of integrated systems playing tissue-/organ-specific roles [8, 30, 32, 33, 35, 38].

1.2 Specific roles of the telocyte network: hollow organs

A common role proposed for the 3-D TCs scaffold in the hollow organs is to follow organ distension and relaxation avoiding anomalous deformation and controlling blood vessels closure or rheology. However, because of the anatomical complexity of such districts and the great variety of cell populations herein interacting with the TCs, many other roles are conceivable, suggesting that these cells, as connecting devices in the stromal space, might take center stage in the integration of all the information coming from the vascular, nervous, and immune systems, as well as from tissue-resident stem cells.

The present overview of the literature focused on the spatial organization, morphological, and histochemical peculiarities of the TCs, according to their location in different organs. This may help to point out the presumptive roles of the homo- and hetero-cellular TCs networks. With this aim, the TCs networks located in some representative hollow organs that have been more intensively studied, such as the

gastrointestinal and reproductive tracts, the urinary bladder, and in the cardiac parenchyma, in both healthy and disease conditions, have been taken into consideration.

2. Gastrointestinal tract

The gastrointestinal tract consists of different hollow organs which have some similar and some different shapes and functions. Every organ modifies its lumen caliber and thickness several times throughout the day, following food transit. Food intake might happen several times per day, with different types and quantities, and the food transit varies according to the different regions, from the stomach to the colon. The cells of the lining epithelium do not change their shape, while microvilli height importantly changes. Under the mucosa, made by the epithelium, the lamina propria, and the muscular mucosae, there is the submucosa that has different morphological organization and function. Finally, the muscle coat is responsible for gastrointestinal contractility. Two motile activities, coordinated by the enteric nervous system and the ICCs, are present: peristalsis, a constant ab-oral movement that does not importantly modify the lumen caliber, and the relaxation/contraction related to food arrival/mixing for digestion and absorption/transit which promotes sustained lumen caliber changes. Region-specific mechanical and functional interrelationships between all the components of this complex apparatus are at the basis of the correct and coordinated behavior of the apparatus.

2.1 Gastrointestinal telocyte network in healthy condition

In the human and mouse gastrointestinal tract, the TCs form widespread networks in the mucosa, submucosa, muscle layers, at the myenteric plexus level, at the submucosal border of the muscular mucosae, in the circular muscle layer, and around nerve strands, blood vessels, funds of gastric glands, and intestinal crypts [32, 39]. Immunohistochemically, all the TCs residing in the different layers of the gastrointestinal tract wall can be identified as CD34+/PDGFRα+ interstitial cells [32]. In the lamina propria and submucosa, the 3D homo-cellular TCs network has a structural role, forming the scaffolding that can direct the collagen fibers/bundles and define the spaces where the several elements of the connective tissue accommodate. Although it cannot be excluded that these TCs could eventually be recruited for the ECM synthesis, the abovementioned structural function is likely to be the main one. However, the role attributed to the TCs lining the basal-lateral surface of the glandular crypts [32, 39], where epithelial stem cells are located is particularly intriguing, since these TCs have been proposed to influence the proliferation and differentiation of stem cells due to their ability to produce and secrete a variety of molecules [40], the close relationships they recurrently establish with the "stem cell niches" [34, 40], and the expression on their surface of the functional receptor PDGFRα whose activation is critical in mammalian organogenesis [39]. In this context, it is worth mentioning that a very recent study demonstrated that the subepithelial plexus formed by PDGFRα+ TCs acts as a crucial source of Wnt proteins, which are essential to support intestinal crypt stem cell proliferation and epithelial renewal [23]. In the muscle coat, by both immunohistochemistry (PDGFRα and CD34 immunolabeling) and electron microscopy, the TCs processes were observed to constitute 3D networks intermingling with those of the ICCs and to establish cell-to-cell contacts with them [32, 34]. Interestingly, within the gut muscle layers the TCs and ICCs networks can be clearly distinguished based on their different immunophenotypes, as the TCs are CD34+/PDGFRα+ and negative for c-kit, and vice versa, the ICC are c-kit+ and negative for either CD34 or PDGFRα [32]

(**Figure 4A–I**). This mixed TCs/ICCs meshwork and areas of simple apposition occurring between the TCs and the smooth muscle cells have suggested that the intramuscular TCs might support the spreading of the slow waves generated by the ICCs, which are electrically coupled to the smooth muscle cells, thus contributing to the regulation of gastrointestinal motility [32, 41]. In favor of this hypothesis, it has been recently reported that the "smooth muscle cells are electrically coupled to both ICCs and PDGFRα+ cells (i.e., the TCs) forming an integrated unit called the SIP syncytium" [42]. Another possible role attributed to the TCs located in the gut muscle coat is that they might eventually differentiate into ICCs. This hypothesis is mainly based on the existence of the ICCs/TCs mixed network, where the two interstitial cell types are often intercalated (**Figure 4I**) [32, 34, 37]. In support of this hypothesis, despite apoptotic ICCs have been described in the colon of human healthy subjects of different ages, no decrease in the number of ICCs was observed in relation to aging and no ICCs was ever seen undergoing mitosis [43], while mitotic TCs rich in rough endoplasmic reticulum can be detected in the interstitial spaces usually occupied by the ICCs (personal unpublished observation). Taken together, these data suggest that TCs might represent a pool of ICCs precursors being responsible for the physiological replacement of aged ICCs. Furthermore, it has been demonstrated that in culture stromal cells expressing CD34 (a typical marker of the TCs) proliferate and progressively lose their CD34-positivity to acquire the c-kit-positivity (a typical marker of the ICCs) [44]. Reasonably, in adulthood the TCs, wherever they are located, might be considered as a pool of mesenchymal stromal cells and, in the gut, to be important for ICCs renewal [37].

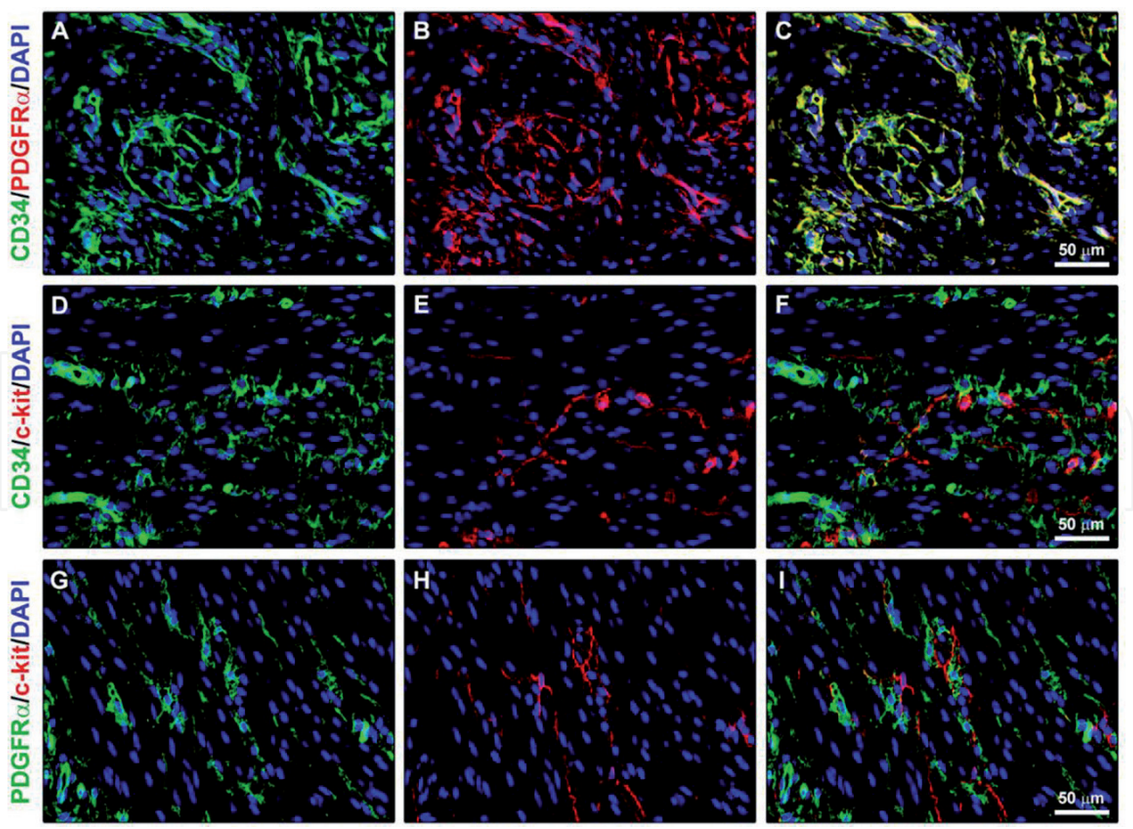

Figure 4.
TCs and ICCs in the human gastrointestinal tract. TCs and ICCs form intermingled networks in the muscularis propria of the human intestine. Representative images of human colon sections double immune-stained for: (A–C) CD34 (green) and PDGFRα (red), (D–F) CD34 (green) and c-kit (red), and (G–I) PDGFRα (green) and c-kit (red) are shown. Nuclei are counterstained with DAPI (blue). Merge images are shown in the right panels. All the TCs are CD34+/PDGFRα+ (A–C), while the ICCs are c-kit+ and negative for either CD34 (D–F) or PDGFRα (G–I). Scale bar: 50 μm.

2.2 Telocyte network in gastrointestinal diseases

Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are complex disorders in which chronic relapsing and inflammation progressively evolve into extensive fibrosis of the intestinal wall [41, 45]. Both CD and UC are characterized by abdominal pain and diarrhea, mainly as a result of the progressive fibrotic process that leads to a stiff intestine unable to properly carry out peristalsis and resorptive functions [45–47]. The evidence that intestinal dysmotility with a reduction in the number of ICCs is a feature of IBD and that the ICCs and TCs networks are intermingled in the gut neuromuscular compartment has prompted an investigation of the TCs distribution either in the terminal ileum of CD patients or in the colon of UC patients [32, 41, 48–51]. Interestingly, in both conditions, the gut wall fibrosis was strictly paralleled by a reduction in TCs [50, 51]. In fact, in the CD intestinal wall, which is histopathologically characterized by discontinuous signs of inflammation and fibrosis (referred to as "skip lesions"), TCs were normally distributed in all layers of the healthy-looking areas from the mucosa to the subserosa, while they were markedly reduced in the fibrotic areas displaying severe architectural derangement [50]. In particular, the network of TCs was discontinuous or even completely lost among smooth muscle bundles and around myenteric plexus ganglia [50]. As far as UC is concerned, TCs were investigated in tissue specimens from both patients in early and those in advanced phases of colonic wall fibrotic remodeling [51]. In the early phase, fibrosis is confined to the muscular mucosae and submucosa, while in the advanced one it extends affecting wide areas of muscle layers and the myenteric plexus. Of note, TCs were significantly reduced in the muscular mucosae and submucosa of both early and advanced fibrotic UC cases [51]. On the contrary, the intramuscular and myenteric plexus TCs networks were severely compromised in the advanced but not in the early fibrotic UC cases [51]. Through double immunofluorescence, it was possible to further reveal that in both forms of IBD the losses of TCs and ICCs occurred in parallel in the muscle layers and around the myenteric ganglia [50, 51]. Based on these findings, it has been proposed that the simultaneous reductions in the TCs and ICCs might significantly account for intestinal dysmotility in IBD. Several assumptions have also been made concerning the possible causes and pathophysiologic implications of this TCs impairment [41, 50, 51]. As reported in the failing human heart [41, 52], the progressive alteration in the ECM composition and entrapment of TCs in such fibrotic ECM may provoke profound cell sufferance and eventually lead to cell death. Both the ECM accumulation/rearrangement and the parallel reduction of TCs may profoundly impair their hetero-cellular networks with immune cells, fibroblasts, smooth muscle cells, ICCs, blood vessels, and nerve endings, thus hampering the TCs intercellular signaling functions [41]. Whether the loss of the TCs might even precede the onset of fibrosis rather than being merely a consequence of tissue fibrotic remodeling is difficult to demonstrate. In line with the proposed role of TCs as a guide for the correct tissue shaping during organ morphogenesis, it cannot be excluded that the loss of TCs might contribute to the altered 3D ECM organization in the fibrotic intestinal wall [46]. For instance, it has been proposed that the disappearance of TCs might favor an uncontrolled activation of ECM synthesizing fibroblasts and their transition to profibrotic α-smooth muscle actin (α-SMA)+ myofibroblasts [46]. Noteworthy, hetero-cellular contacts between TCs and fibroblasts/myofibroblasts have been described in different organs, suggesting that the TCs could contribute to tissue homeostasis by controlling the synthetic activity of such partners through inhibitory signals [46]. In support of this hypothesis, in

UC colonic specimens the loss of TCs was paralleled by an increase in the number of α-SMA+ myofibroblasts [51]. This last observation suggests that during pathologic processes a subset of TC could undergo phenotypic changing, possibly contributing to the increase in the profibrotic myofibroblast population [41]. However, double immunolabeling for CD34 (as TC marker) and α-SMA did not reveal the presence of CD34+/α-SMA+ transitioning stromal cells in the colonic wall of UC patients, which makes unlikely the aforementioned hypothesis [51]. Also, electron microscopy investigations of different pathological tissues (e.g., failing human heart, fibrotic skin) have clearly shown that fibrosis is accompanied by TC degenerative processes rather than by activation/transformation into myofibroblasts [52, 53]. As the findings in the CD and UC intestinal tissues, a loss of TCs have also been reported in the neuromuscular compartment of the fibrotic gastric wall of patients with systemic sclerosis, where it likely contributes to gastric dysmotility clinically manifesting as delayed gastric emptying or gastroparesis [54].

Besides IBD, recent evidence suggests that the TCs might be a cell source of certain kinds of gastrointestinal stromal tumors (GIST) [55]. While ICCs hyperplasia has been identified as a crucial pathogenic feature of KIT-mutant GIST, it has been proposed that the TCs could represent the physiological counterpart of PDGFRa-mutant GIST and inflammatory fibroid polyps [55]. Indeed, a pathogenic relationship between TCs hyperplasia and both inflammatory fibroid polyps and PDGFRa-mutant GIST has been suggested. Moreover, the term "telocytoma" was proposed for defining inflammatory fibroid polyps, since it conveys both the pathogenic (neoplastic) and histotypic ("telocytary") nature of this tumor [55].

3. Human urinary bladder

The urinary bladder is a complex organ which modifies its volume and shapes several times during the day following filling and micturition. Filling happens gradually during the time while micturition happens in a unique and sustained emission of the urine. The cells of the lining epithelium (urothelium) dramatically changes their shape and height; under the urothelium, there are two regions: the upper lamina propria (ULP) and the deep lamina propria (DLP), with different morphological organization and function; the detrusor is the muscle coat responsible for organ relaxation and contraction. Region-specific mechanical and functional interrelationships between all the urinary bladder components are at the basis of correct and coordinated behavior.

3.1 Bladder telocyte network in healthy condition

In the bladder, unlike in other organs, a more complex picture comes out [33, 35]. As is the case in the gut, the bladder TCs form complex networks and make contacts either between themselves or with other cell types; however, depending on their location, they show different immunohistochemical properties and ultrastructural peculiarities. The main differences are detected between the TCs located in the sub-urothelial connective tissue (upper lamina propria, ULP) and those located in the submucosa (also referred to as deep lamina propria) and detrusor. The TCs in the ULP is PDGFRα+ and CD34- while those in the submucosa and detrusor are CD34+ and PDGFRα- (**Figure 5A** and **B**). Moreover, while the TCs immediately below the urothelium express only the PDGFRα and form a homo-cellular network, the other sub-urothelial TCs are also α-SMA+ (**Figure 5A**)

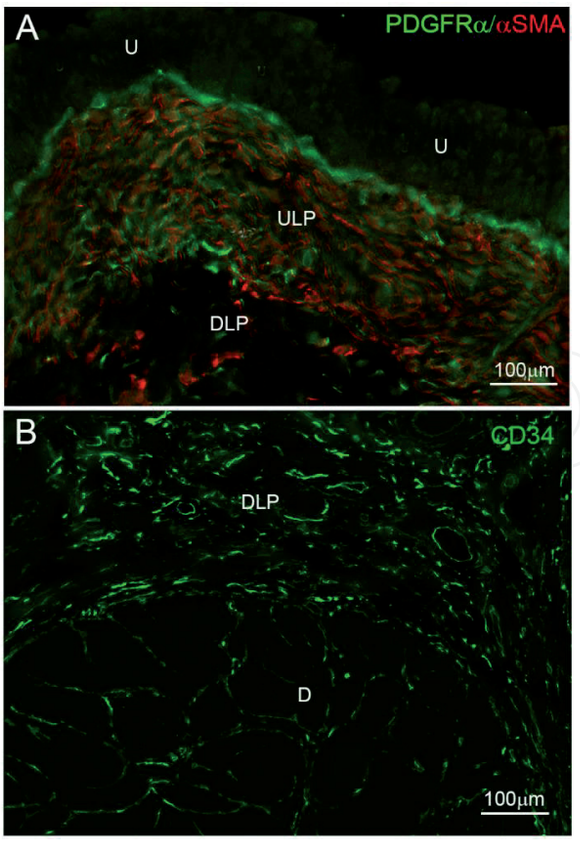

Figure 5.
TCs networks in the human urinary bladder. (A) PDGFRα (green) and α-SMA (red) immunolabeling. A monolayer of PDGFRα+ TC lines the urothelium (U). In the remaining upper portion of the lamina propria (ULP) a mixed network made by PDGFRα+/α-SMA+ TC (hybrid TC) and α-SMA+ myofibroblasts is present. (B) CD34+ TC form a homo-cellular network in the deep lamina propria (DLP) and detrusor (D). Scale bar: A, B = 100 μm.

and under the transmission electron microscope show a larger body and cell processes possessing attachment plaques with the connective tissue like the fibronexuses typical of myofibroblasts. Further, these TCs establish extended regions of simple apposition with the myofibroblasts, thus forming a 3D mixed network (**Figure 5A**). This mixed network together with the homo-cellular networks that the TCs make in the remaining portions of the bladder constitutes the scaffolding to guarantee the organ integrity during distention and relaxation [33, 35, 37, 56]. However, other specific roles have been assumed for the bladder TCs, particularly for those located in the ULP. The TCs lining the urothelium is quite peculiar, because of their location and immunolabeling [33, 38]. As already discussed, the PDGFRα+ TCs located immediately beneath the intestinal crypts appear to be cells engaged in controlling the proliferation and differentiation of the stem cells resident in the crypts [23]. Likewise, sub-urothelial TCs could play similar functions. Immediately below those TCs, there is the TCs/myofibroblast network and both cell types form gap junctions and express the Cx43 protein, are close to nerve varicosities, express the vanilloid, the ATP, the purine and the muscarinic receptors and contain the cGMP, the target molecule of NO [35]. These features support the hypothesis that these cells have a role as intermediaries in propagating chemical or electrical stimuli locally generated, as well as a role as the target of the paracrine activity of the urothelium and the nervous stimuli [35, 38, 57]. The importance of these roles is plenty understood, since the ULP and the urothelium constitute a sensory system capable of perceiving mechanical and chemical stimuli and whose integrated responses control the efferent pathways on the detrusor and the micturition.

3.2 Telocytes in bladder diseases

The micturition reflex is the result of a complex integration among involuntary and voluntary nervous mechanisms. Several pathological conditions of the low urinary tract compromise this function causing detrusor dysfunctionality. The most frequent is the idiopathic detrusor overactivity, the neurogenic detrusor overactivity (NDO), the bladder pain syndrome/interstitial cystitis, and the partial bladder outlet obstruction. All these diseases are characterized functionally by excessive sensitivity of the detrusor/bladder to filling [58], and histologically by an intense inflammation especially in the lamina propria [35, 38, 59, 60]. Since it is well known that TCs and myofibroblasts produce cytokines and other molecules able to recruit immune cells and express receptors for the cytokines released by the immune cells, both cell types likely intervene in the inflammation intensity, quality, and duration. Furthermore, in the presence of detrusor hyperactivity, both ULP-TC and myofibroblasts showed an increase of the Cx43 protein labeling that was interpreted as an augmentation of the gap junctions and signs of cellular activation (clear nuclei and larger bodies) [35, 38, 59, 61]. Additionally, the TCs expressing both PDGFRα and α-SMA were significantly increased in comparison with controls suggesting a shift toward a myofibroblast phenotype [35, 38]. All these cell changes were considered signs of adaptability because, despite the presence of inflammation, the 3D cell network was preserved [38, 59]. However, this integrity not necessarily means adequate functionality of the sensory system made by the urothelium and the ULP; in fact, the higher thickness of the ULP, due to the intense cell infiltrate and edema, forcing the net meshes to enlarge, could cause an increase in the distances among the cells, between them and the nerve endings, and between all of them and the urothelium, likely affecting the sensitivity to volume changes and the capability of responding to the molecules released by the nerve terminals and by the urothelium. Finally, because the ULP thickening was uneven alongside the organ [35, 38, 60], foci of hypersensitivity could alternate to others less responding, further prejudicing the correct integration of the afferent stimuli [57]. Finally, it was reported that the TCs forming the monolayer underlying the urothelium did not show any significant changes in hyperactive bladders. These data were explained as follow: the location of these TCs could spare them from the damages caused by the cell infiltrate. Further, if these TCs are engaged in cell proliferation and differentiation of the overlying epithelium, the absence of epithelial cell death signs in NDO might account for their sparing.

4. Reproductive system

The female reproductive system includes, besides external sex organs, the internal sex organs: the ovaries, fallopian tubes, and uterus. Immature at birth, these organs continue to develop and reach maturity at puberty when they can produce gametes, and to carry a fetus to full term. Fallopian tubes integrity is capital for fertilization which usually occurs in the external third of the tubes. The traveling zygote will form the blastocyst that will be implanted in the uterine endometrium. To obtain and maintain a pregnancy, the integrity and functionality of these organs need to be at a maximum.

4.1 Telocytes in uterus and fallopian tubes in healthy condition

Currently, TCs are found in uterine tubes and uterus, including endometrium, myometrium, and cervix, and also in the vagina [2, 3, 62–65]. Among the first

locations in which TCs were described are the organs of the female genital apparatus: the uterus and the fallopian tubes [2, 3, 5, 10, 66]. Since the beginning, their characterization was based on conventional microscopy methods and techniques such as methylene blue staining and silver impregnation, in situ and in vitro [2, 3], followed by the description of the "gold standard" for their identification with the aid of electron microscopy [5]. In parallel, various immunohistochemical markers have been used to enhance TCs better characterization, which has varied over time from vimentin, α-SMA, progesterone receptor, desmin, estrogen receptor, and S100 protein, to stabilize what we consider nowadays to best describe the phenotype of these cells—CD34 and PDGFRα [2, 3, 67]. A book chapter refers to the immunohistochemistry of TCs in female genital organs [28]. However, it should be pointed out that TCs in the uterus and fallopian tubes express receptors for estrogen and progesterone [4, 31]. Nowadays, the most suitable methods for TCs identification are electron microscopy and double staining for CD34 and PDGFRα or PDGFRβ or vimentin [68, 69].

In the human uterus, TCs establish homo- and hetero-cellular junctions, demonstrated by electron microscopy images of the telopodes (**Figure 6**) [70]. Homocellular junctions are typically established between two telopodes, but might be observed between a telopode and a TCs' body [71]. The most frequently observed interactions are simple appositions of the plasma membranes; however, puncta adhaerentia minima, processus adhaerens, recessus adhaerens, and manubria adhaerentia can be captured in electron microscopy images [10, 18]. Sometimes, even gap junctions were captured [18]. Hetero-cellular junctions are usually seen between telopodes and fibroblasts, myofibroblasts, pericytes, stem cells, macrophages, mast cells, lymphocytes, plasma cells, Schwann cells, endothelial cell, neurons, cardiomyocytes, and smooth muscle cells, as described in the literature [12, 56, 72–74]. The contacts made between the two membranes are of the type of point contacts, nanocontacts, planar contacts, or simple apposition of plasma membranes [18].

TCs can release exosomes (from multivesicular bodies), ectosomes (shredded directly from plasma membrane), and multivesicular cargos (multiple tightly packed endomembrane-derived vesicles) [75]. The three types of extracellular vesicles emitted by TCs are evidence of the involvement of these cells in intercellular distance communication. Shed vesicle number and diameter are not correlated with the reproductive state, while the quantity of TCs in the endometrium and the myometrium varies with it [17]. Moreover, it was demonstrated that the morphology of telopodes is correlated with the presence or absence of gestation [70].

All these morphological, immunohistochemical, and electrophysiological observations have led to several hypotheses on the TC functions in the uterus and fallopian tubes. The existence of homo-cellular junctions leads to a presumptive function in controlling the shape of the tissues which are subjected to of dynamic changes, such as the pregnant uterus that hypertrophies and expands as the fetus grows [17]. In support of this assumption stands the hypothesis that TCs contribute to smooth muscle growth in areas with high mechanical forces due to TCs mechanical sensitivity [76]. The mechano-sensing function should also be considered, due to the presence of catenins that make up the junctions [18, 71]. Moreover, TCs express T-type calcium (CaV3.1 and CaV3.2) channels and small-conductance calcium-activated potassium channels (SK3) and calcium-dependent hyperpolarization-activated chloride inward channels, the levels of expression being dependent on the physiological state, pregnant or non-pregnant [70, 77, 78]. This point to a TCs' involvement in calcium signaling mechanisms with neighboring cells [79]. Extracellular matrix remodeling is also emphasized by some studies and can be considered as applicable to the uterus [80, 81]. The existence of (ERα)

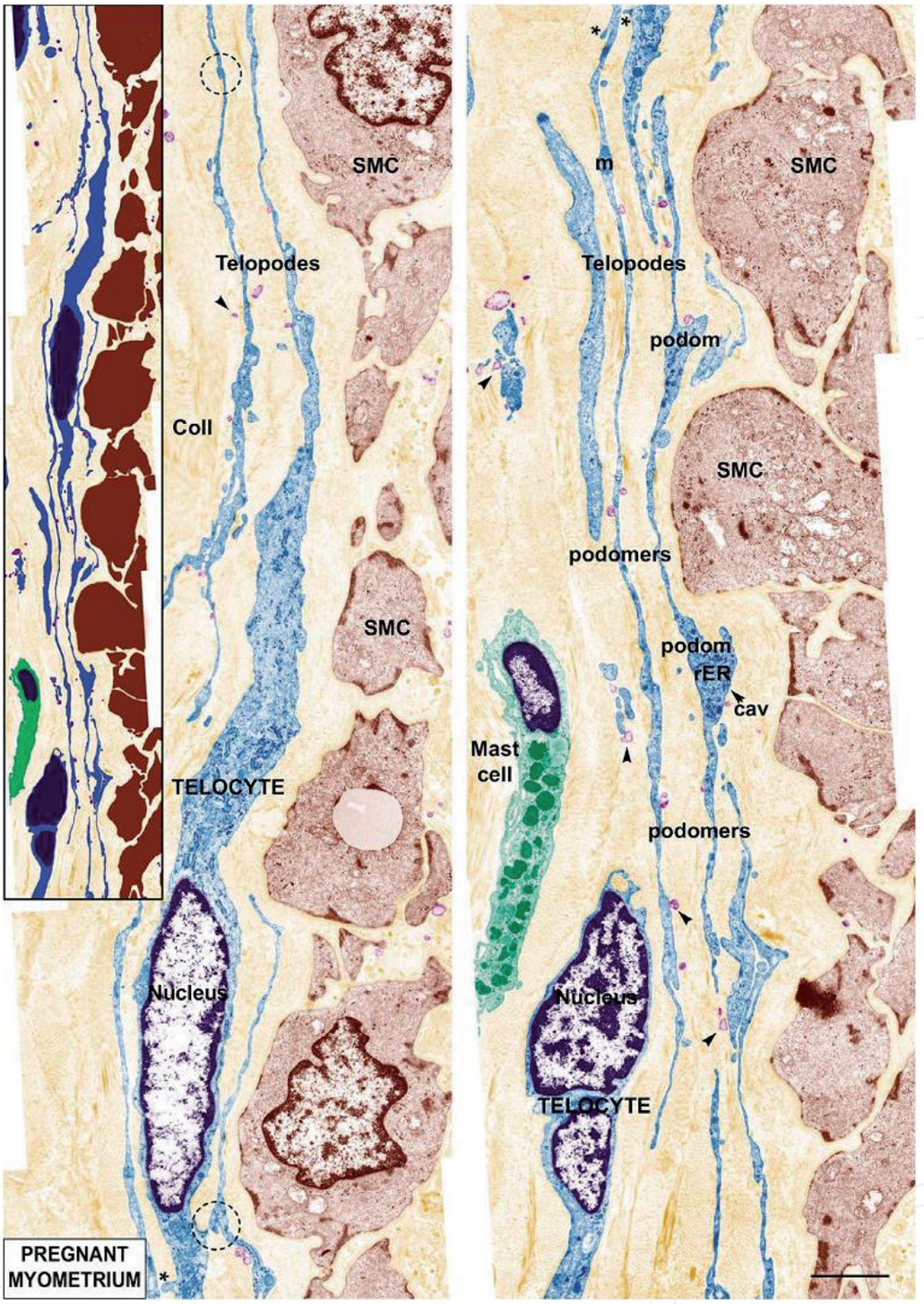

Figure 6.
Representative ultrathin section of human pregnant myometrium. Two-dimensional sequenced concatenation from 11 serial electron micrographs showing the 3D network of TCs (blue) interconnected by homo-cellular junctions (dotted circles). SMCs are shown in cross-section and were digitally colored brown. In their vicinity, numerous Tps (blue) establish a network and release extracellular organelles (exosomes 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; N¼nucleus. Bar¼2 lm. Reproduced with permission from Ref. [10].

and progesterone receptor A (PR-A) on the surface of uterine TCs suggests their involvement as sensors for steroid hormones levels. Although little is known about the existence of stem cells in the uterus, they certainly exist, and the secretome of the TCs could influence the cellular microenvironment, controlling their proliferation, and differentiation [15]. Also, TCs secretome factors could participate in decidua formation [17]. Some studies pointed to the angiogenic properties of TCs

due to vascular endothelial growth factor (VEGF) expression [15, 82, 83], while others indicated the anti-oxidative properties of TCs because the TCs specific morphology can be changed by modifying the redox balance of their environment or by aging due to their richness in SOD2 (mitochondrial superoxide dismutase) [21, 84].

Recently, TCs were found to activate and "educate" peritoneal macrophages (pMACs) with the aid of telopodes by direct physical contact through heterocellular junctions or using a TCs-conditioned media through paracrine mechanisms [85]. This is suggestive for a role in immunosurveillance [85].

In the fallopian tubes, TCs have been described throughout the thickness of the wall, their density decreasing from the mucosa to the muscular, from ~18 to ~7.5% [3]. TCs are also found in the fimbriae of Fallopian tubes [17, 28]. A panel of antibodies was used to identify tubal TCs. The telopodes possess all the features described above, creating a 3D network and establishing contacts with different structures, such as blood vessels, nerves, and muscle fibers [86, 87]. Both, homo- and hetero-cellular junctions are described, and also, a new hypothesis was emitted that telopodes that contact the immune cells (plasma cells and lymphocytes) might stimulate antibody production [88, 89]. As suggested by Cretoiu et al., the tubal peristalsis might suffer influences from TCs which also express PR-A and ERα receptors [31]. The tubal movements seem to be amplified by estrogen and decelerated by progesterone [90]. Recently, additional markers were tested for TCs identification, such as Podoplanin (D2–40) and Dog-1 but proved to be inappropriate [91].

4.2 Telocytes in uterine and tubal diseases

The pathogenesis of uterine leiomyomas, the most frequent benign tumors in women might be determined, among other factors, by the loss of TCs [26]. Varga et al. proposed three hypotheses regarding TCs' involvement: (i) loss of TCs as steroid sensors leads to an increased density of estrogen receptors at smooth muscle cells level followed by a cell cycle disruption; (ii) considered as progenitor cells, TCs absence can favor the rise of new leiomyoma cells; and (iii) in the absence of antioxidant protection conferred by TCs, leiomyoma cells grow numerically due to local hypoxia that blocks their apoptosis [26].

A recent study shows, for the first time, that there is an interplay between telocytes and autonomic innervation in leiomyomata [92]. TCs decreased in numbers in the leiomyomatous myometrium, suggesting a role for these cells in the control of the microenvironment [92].

The integrity of the 3D network of TCs appears to be fundamental in exercising the function of the fallopian tubes that are regarded as a major organ in the reproduction. Several studies showed that by affecting the 3D organization and number of TCs perturbations occur in the local homeostasis, leading to angiogenesis and interstitial fibrosis [88, 89]. Neo-angiogenesis plays a major role in endometriosis and adenomyosis pathogenesis, and even in tubal ectopic pregnancy [93–95]. TCs were shown to be involved in all these processes [96, 97]. In pelvic endometriosis and tubal ectopic pregnancy, the decrease in the number of TCs is probably due to the overproduction of iNOS, COX-2, LPO, and estradiol [49, 69, 88].

Some other pathologies that might affect the 3-D network of TCs were described, such as Chlamydia infection responsible for the activation of macrophages or pelvic inflammatory disease [89, 98, 99]. In inflammation and ischemia, TCs were shown to be lost and to suffer major ultrastructural changes, a process followed by interstitial fibrotic remodeling [99]. Abd-Elhafeez et al. proposed a role for tubal TCs in the regulation of the epithelial function necessary for the final gamete maturation, fertilization, and early embryo development [100].

5. Heart

Cardiac TCs are among the most well described in the body [101]. Cardiac TCs are unique interstitial cells in the heart [101]. Intramyocardial TCs account for less than 1% of interstitial cells in the human heart [56]. Cardiac TCs have been identified in heart valves, left and right atrium and ventricle, epicardium, myocardium, endocardium, sub-endocardium, and myocardial sleeves [101, 102], in mice, rat, porcine, and human [56, 103]. The whole ultrastructural anatomy of human cardiac TCs has been reconstructed by focused ion beam scanning electron microscopy (FIB-SEM) [104]. The electrophysiology of human cardiac atrial and ventricular TCs has also been reported [105].

Cardiac TCs are completely different from other types of myocardial interstitial cells, especially from cardiac fibroblasts [106]. Cardiac TCs and cardiac fibroblasts are completely different in immunophenotypes, as cardiac TCs are positive for CD34/PDGFR-α, CD34/PDGFR-ß, or CD34/Vimentin while cardiac fibroblasts are only positive for PDGFR-ß and Vimentin [106]. CD34/PDGFR-α positive TCs account for one-third of the total cells among TCs enriched rat cardiac interstitial cell population [106, 107]. Some studies have also reported that cardiac TCs inconstantly express CD34/c-kit [56]. Besides, cardiac TCs are also distinct from pericytes, since cardiac TCs are CD34 positive and α-SMA weak positive while pericytes are CD34 negative and α-SMA positive [106]. Moreover, cardiac TCs are CD34/PDGFR-ß positive while pericytes are CD34 negative and PDGFR-ß positive [106]. Interestingly, cardiac TCs are positive for CD29 (a mesenchymal marker) but negative for CD45 (a hematopoietic marker), suggesting that cardiac TCs could be a source of cardiac mesenchymal cells [106]. Interestingly, the telomerase concentration in CD117 and CD34 positive cardiac TCs is significantly higher than that in cardiomyocytes and is 2.5-times and 1.5-times lower than that in bone mesenchymal stem cells and cardiac fibroblasts [108].

Cardiac TCs can form tight junctions with all other types of cells within the heart including cardiomyocytes, vascular smooth muscle cells, endothelial cells, and pericytes. The functions of cardiac TCs are not fully known but are proposed as follows: (1) intercellular signaling; (2) mechanoreceptors/transducers; and (3) cardiac homeostasis and repair [101, 109]. In the heart, telocytes participate in cardiac development and physiology, and diverse cardiovascular diseases (**Figure 7**).

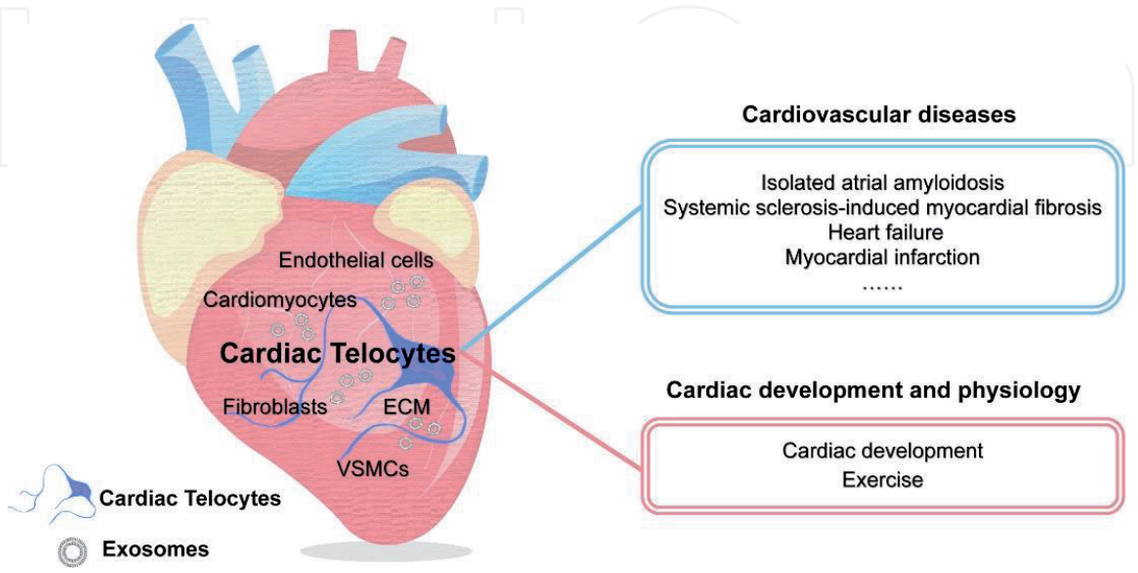

Figure 7.
Cardiac telocytes are in tandem with different types of cells in the heart, and participate in cardiac development and physiology, and diverse cardiovascular diseases. ECM, extracellular matrix; VSMCs, vascular smooth muscle cells.

5.1 Cardiac telocytes in heart development and physiological growth

Involvement of cardiac TCs and cardiomyocytes during development have been investigated using myocardium from embryonic (E14, E17), newborn (P0, P6), and adult (2 months) CD1 mice by using transmission electron microscopy and immunohistochemistry [110]. It was found that TCs were present from early embryonic to adult life in the mouse heart [110]. Besides, cardiac TCs demonstrated immature features in early embryonic hearts while cardiac TCs exhibit a more differentiated phenotype in newborn hearts [110]. Cardiac TCs played a fundamental role during cardiac development by forming a correct three-dimensional myocardial architecture and nursing cardiomyocyte precursors [110]. Intriguingly, cardiac TCs were found negative for c-kit and CD34 during the embryonic stage [110]; however, CD34 was expressed in a few TCs in the heart of a newborn mouse, and in most TCs in adult hearts [110]. This suggests a phenotype switch of cardiac TCs during development.

Exercise can induce cardiac physiological growth, which is characterized by increased cell size of cardiomyocytes and the formation of new cardiomyocytes [111, 112]. Three double-immunostainings, including CD34/PDGFR-α, CD34/PDGFR-ß-, and CD34/vimentin, have been used to determine the number of cardiac TCs in exercise-induced physiological cardiac growth [81]. The number of cardiac TCs was found to be significantly increased in the exercised heart [81]. The increased cardiac TCs in exercise might communicate with cardiomyocytes through direct contacts or telocyte-shed vesicles, balance angiogenesis, and maintain the normal 3D-organization of ECM [81]. This study suggested a potential role of cardiac TCs in exercise-induced cardiac growth [81].

5.2 Cardiac telocytes in cardiovascular diseases

Isolated atrial amyloidosis (IAA) is frequently found in long-standing atrial fibrillation patients [113]. By electron microscopy, telopodes are found surrounding the amyloid deposits, which limit their spreading into the interstitium [113]. This indicates that TCs might participate in amyloidogenesis by gathering masses of amyloid fibrils.

Systemic sclerosis represents a complex connective tissue disease featured with fibrosis of the skin and various internal organs [54]. TCs, as defined by CD34-positivity/CD31-negativity, were checked in the fibrotic areas of systemic sclerosis myocardium and were found to be almost undetectable [54]. However, in the control myocardium, numerous TCs were found located in the interstitium surrounding cardiomyocytes [54]. This indicates that loss of cardiac TCs contributes to myocardial fibrosis caused by systemic sclerosis.

The imbalance between cardiac TCs apoptotic death and cardiac TCs proliferation is responsible for the depletion of cardiac TCs in cardiac diseases leading to heart failure [52]. In human heart failure patients' myocardium, the number of cardiac TCs and telopodes decreases over twofold. Additionally, the apoptotic cardiac TCs increases threefold in the diseased heart while the percentage of proliferating cardiac TCs remains unchanged, suggesting that the decreased cardiac TCs population in heart failure is mainly due to increased apoptosis [52]. Interestingly, the number of cardiac TCs and telopodes has been found to depend on the composition of the extracellular matrix which is correlated negatively with mature fibrillar collagens and positively with degraded collagens [52].

The changes of cardiac TCs have been determined in an acute myocardial infarction rat model induced by isoproterenol (ISO) [114]. It was found that CD117/CD34 positive cardiac TCs were undetectable with immunohistochemical

staining 1 day after ISO treatment. Interestingly, treatment with grape seed extract (GSE) could significantly increase cardiac TCs numbers and enhance angiogenesis in myocardia but not in other tissues; in fact, it was found suppressing angiogenesis in tumor tissues instead [114]. Thus, GSE was regarded to promote angiogenesis by modulating cardiac TCs, which subsequently stimulated endothelial cells [114].

Also, in a rat myocardial infarction model induced by coronary occlusion, cardiac TCs were reported undetectable in the infarction zone from 4 days to 4 weeks [115]. Simultaneous transplantation of cardiac TCs could significantly decrease infarct size and improve heart function 2 weeks after myocardial infarction [115]. Moreover, the protective effects of intramyocardial transplantation of cardiac TCs were also observed 14 weeks after myocardial infarction as evidenced by improved heart function, decreased infarct size, increased angiogenesis, and decreased myocardial fibrosis [116].

Currently, no single specific immunophenotype for cardiac TCs has been identified [101]. For in-depth studies of cardiac TCs, it is highly needed to identify a specific immunostaining marker for them. Most isolated cardiac TCs are either not pure enough (as cardiac fibroblasts grow much faster than cardiac TCs) or only containing subtypes of cardiac TCs. It would be beneficial to investigate the therapeutic effects of cardiac TCs or cardiac TCs-derived exosomes. Moreover, the immunoregulatory effects of cardiac TCs should be thoroughly investigated. Finally, other organs or tissue-specific TCs are worthy to be studied.

6. Conclusion and future overlook for telocytes' study

In conclusion, this review of the literature data indicates that TCs, depending on their location, may display different immunohistochemical properties, ultrastructural peculiarities and form complex networks making contacts either between themselves or with other cell types. Further, our current knowledge of TCs allows the following conclusions on the role(s) that these cells may play, some of which might be common to the different organs and some be organ-/region-specific.

1. In the stromal space of all the organs taken into account, TCs appear as connecting cells. Reasonably, TCs, due to their homo- and hetero-cellular contacts, can be considered as *connecting devices* playing either common or region-specific roles. These contacts might be merely mechanical or sites of cross-talking between TCs and other cell types establishing intercellular molecular exchanges. Spatial relationships also suggest an involvement of the TCs network in the coordination of tissue homeostasis in response to local functional demands.

The involvement in tissue homeostasis might be explained by the heterogeneity of TCs depending on their location.

2. TCs might be engaged in *controlling the proliferation and differentiation of the stem cells* and, either in the adulthood or during organ differentiation, wherever they are located, these cells might be considered as a pool of mesenchymal stromal cells

As an example, in the gastrointestinal tract and the urinary bladder, the subepithelial plexus formed by TCs likely supports the stem cell proliferation and epithelial renewal and, the TCs located in the muscle coat, might differentiate into ICCs

and, undergoing to phenotypic changes, become a cell source of gastrointestinal stromal tumors such as GIST. A shift toward a myofibroblast phenotype has been proposed for the TCs located in the urinary bladder lamina propria.

In the female genital tract, although there are no reported interactions with stem cells, a role for TCs in this direction cannot be overlooked because neo-angiogenesis undoubtedly accompanies myometrial hypertrophy.

Moreover, in the heart, a special inter-relation exists between TCs and cardiac stem cells based on the exchange of information via extracellular vesicles which shuttle miRNA or by direct connections through typical and atypical junctions. The secretome of TCs might enhance the proliferation and differentiation of cardiac stem cells. Suggestions were also made regarding the possibility of their role in the re-activation of dormant myocardial precursors during the repair of the adult heart, while in embryo TCs act as inductors/regulators of differentiation during morphogenesis.

3. The TCs scaffold located in all the hollow organs follows organ distension and relaxation likely to avoid anomalous organ deformation and to control blood vessels closure or rheology. This is *a mechanical role* whose importance has been demonstrated in some gastrointestinal pathologies, where TCs loss provokes severe architectural derangement and contributes to the altered 3D ECM organization in the fibrotic intestinal wall. In the uterus, TCs can function as a sensor for the mechanical stress exerted on the uterine wall, allowing uniform uterine growth during pregnancy, by mechanosensitive coordination due to the existence of different ionic channels which can be modulated by pharmacological interventions.

Moreover, TCs can also be regarded as chemical sensors as it was hypothesized for the human uterus and fallopian tube, where TCs might play an important role in the uterine contraction mechanism due to the presence of estrogen and progesterone receptors at their level.

4. Because of the anatomical complexity of the hollow organs and the great variety of cell populations interacting with the TCs, many different and organ-specific TCs roles are conceivable suggesting that these cells might take center stage in *the integration of the overall interstitial information* from the vascular, nervous, and immune systems, as well as from tissue-resident stem cells

In the gastrointestinal tract, a particular role is played by the intramuscular hetero-cellular TCs network in *supporting the spreading of the slow waves generated by the ICCs*, which are electrically coupled to the smooth muscle cells, thus contributing to the regulation of gastrointestinal motility. In agreement with this hypothesis is the evidence that the simultaneous reductions in the TCs and ICCs account for the intestinal dysmotility characterizing the IBD. In the urinary bladder, the sub-urothelial TCs likely play a role as intermediaries in propagating chemical or electrical stimuli locally generated being the target of the paracrine activity of the urothelium and the nervous system. The importance of these roles is plenty understood since the ULP and the urothelium constitute a sensory system capable of perceiving mechanical and chemical stimuli and whose integrated responses control the efferent pathways on the detrusor and the micturition, responses that are lost in urinary pathologies such as the NDO.

In female genital tract, TCs seems to lack in regular slow waves indicating that they are not involved in triggering or supporting the peristalsis of these organs, but more detailed studies are necessary.

Currently, for a more undoubted TCs identification and careful characterization, no single specific immunophenotype marker is adequate. Transmission electron microscope is still considered the instrument necessary for the identification of TCs with certainty. Recently, FIB-SEM tomography was used to confirm that TCs come in contact with each other thus forming networks that constitute the scaffold organizing the stromal and the parenchymal components of the organs. For in-depth studies of TCs roles, it is highly needed to find out functional markers or receptors for the specific TC subtypes and TCs functional molecules.

Conflict of interest

The authors declare no conflict of interest.

Author details

Dragos Cretoiu[1,2†], Maria Giuliana Vannucchi[3†], Yihua Bei[4†], Mirko Manetti[3], Maria Simonetta Faussone-Pellegrini[3], Lidia Ibba-Manneschi[3], Junjie Xiao[4] and Sanda Maria Cretoiu[1*]

1 Department of Cell and Molecular Biology and Histology, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

2 Alessandrescu-Rusescu National Institute of Mother and Child Health, Fetal Medicine Excellence Research Center, Bucharest, Romania

3 Department of Experimental and Clinical Medicine, Section of Anatomy and Histology, University of Florence, Florence, Italy

4 Cardiac Regeneration and Ageing Lab, Institute of Cardiovascular Sciences, School of Life Science, Shanghai University, Shanghai, China

*Address all correspondence to: sanda@cretoiu.ro

† These authors are contributed equally.

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