Chapter 11
Telocytes in Exocrine Glands Stroma

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Abstract Stroma is viewed as the supportive framework of a predominant epithelial organ, comprising mostly of connective tissue, blood vessels and nerves. Since the discovery of telocytes one decade ago (Popescu and Faussone-Pellegrini J Cell Mol Med 2010;14(4):729–40), their presence was proven in several exocrine gland stromata, including major and minor salivary glands, mammary glands as well as exocrine pancreas.

Telocytes have been found in a close connection with acinar and ductal structures but also with their stromal neighbours – nerves, blood vessels or other connective elements, either cells or collagen fibres.

The approaches used to reveal the telocytes' location were immunohistochemistry and electron microscopy.

11.1 Salivary Glands

Saliva is produced by major and minor salivary glands. The first are responsible for about 90 % of the total salivary secretion. In humans, major/main salivary glands are the parotid, submandibular and sublingual pairs. The differences between their saliva are due to the differences in their cellular repertoire, namely, exclusively serous acini in parotids and mixed in the other two, with a mucous predominance in the sublinguals. Intriguingly, the telocytes' location did not show any significant difference between the major salivary glands. Their existence was suggested by immunohistochemistry and proven by electron microscopy, the most reliable technique for the direct identification of a cell or group of cells in a given tissue.

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In parotid glands, vimentin-positive cells were making stromal networks surrounding the acini, while others resided in septa (Fig. 11.1). Solitary CD117^{+} with long, moniliform processes were identified within the intraparotid large septa, around ducts and blood vessels. Although a defining trait of myoepithelial cells, smooth muscle α-actin also showed positive results on interacinar cells, outside their basal laminae, that sent long prolongations between and around parotid acini [5].

In adult submandibular glands (Fig. 11.2), CD117 exhibited positivity around ducts (panel A) and nerves (B and D panels) or in scarce interstitial location (panel C). Only excretory ducts presented surrounding CD117^{+} cells, while no positivity was encountered around striated or intercalary ducts in foetal samples (Fig. 11.3). In major salivary glands, CD117 marked preferentially the actual body of telocytes, while telopodes (the long, peculiar, moniliform prolongations of telocytes) were positive for vimentin and smooth muscle α-actin. Both structures showed selective positivity for CD34.

Interlobular telocytes were identified under electron microscopy in rat and human parotid samples (Fig. 11.4), with their long moniliform telopodes (Figs. 11.4 and 11.5). Interacinar telocytes confirmed the location suggested by

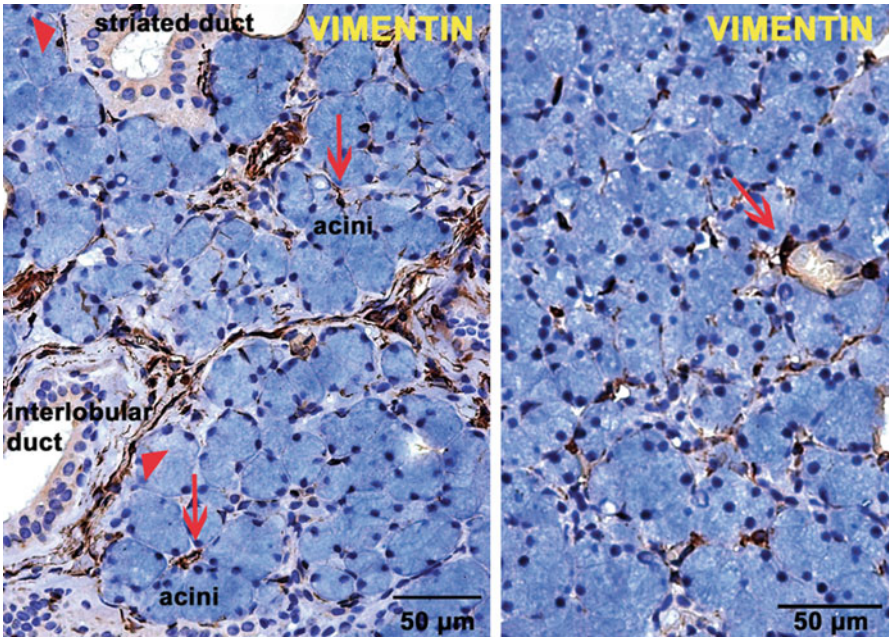

Fig. 11.1 Human parotid gland: vimentin immune staining. Spindle-shaped vimentin-positive cells with long prolongations are identified in septa, where they contribute to stromal networks. Periductal positive cells (*arrowhead*) are mostly uni-/bipolar, while peri-/interacinar ones (*arrows*) often have a multipolar appearance sending prolongations in the periphery of the neighbouring acini (Reproduced with permission from Nicolescu [5])

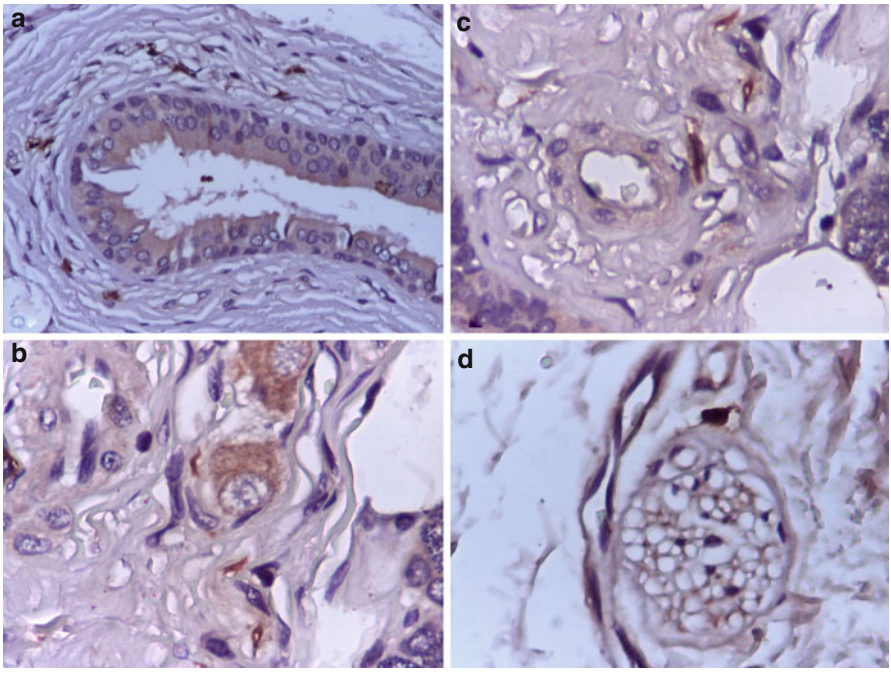

Fig. 11.2 Submandibular gland (human, adult): CD117 immunohistochemistry. (**a**) Positive cells around ducts. Ob. 20×. (**b**) Positive cellular processes around vegetative neural cells. Ob. 40×. (**c**) Positive interstitial cells. Ob. 40×. (**d**) Positive perineural cells. Ob. 40× (Reproduced with permission from Nicolescu [3])

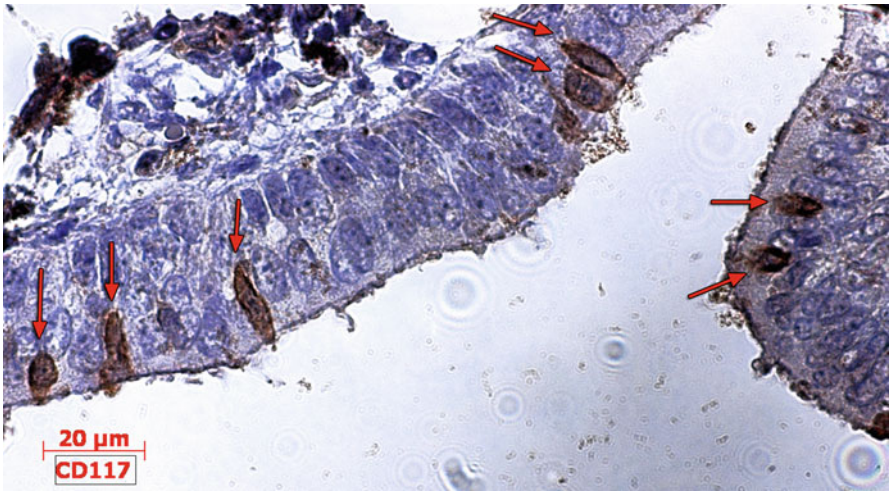

Fig. 11.3 Submandibular gland (human, foetal, 20 weeks): CD117 immunohistochemistry. *Arrows* indicate positive apical duct cells (Reproduced with permission from Nicolescu [3])

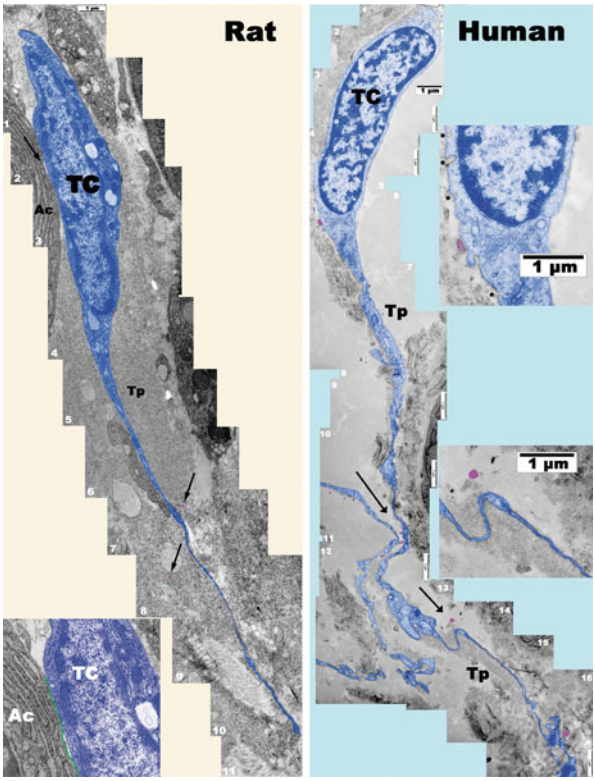

Fig. 11.4 *Left* panel – rat parotid gland: two-dimensional sequenced concatenation from 11 serial electron micrographs. *Right* panel – human parotid gland: two-dimensional sequenced concatenation from 16 serial electron micrographs. Stromal telocytes (*TC*, digitally colourized in *blue*) in rat (*left* panel) and human (*right* panel) parotid interstitium send off long telopodes (*Tp*). *Arrows* and *insets* indicate shedding microvesicles (digitally colourized in *purple*). *Left inset* shows (*bright green*) the stromal synaptic line between a telocyte and an acinar cell. *Right insets* prove why several sequenced electron micrographs are needed to observe a whole TC/Tp and why they were overlooked so far. *Asterisks* indicate TC caveolae. *Ac* acinar cell, *TC* telocyte, *Tp* telopode (Reproduced with permission from Nicolescu [5])

immunohistochemistry (Fig. 11.5). The long trajectory of telopodes includes very convoluted areas we consider to be elongation reserves, possibly needed for morphological reconfiguration of the cellular processes involved in intercellular signalling (Fig. 11.6).

Telocytes' close connection with neurovascular bundles (Fig. 11.7) presumably may support a role in regulation of vascular tonicity, by direct influence or via local neural pathways. Nevertheless, they might also influence the salivary flow and composition, by local interactions, e.g. with myoepithelial cells (Fig. 11.8). Heterocellular relations between telocytes and mast and plasma cells (Fig. 11.9) further emphasize the complexity of their interactions in salivary gland stroma.

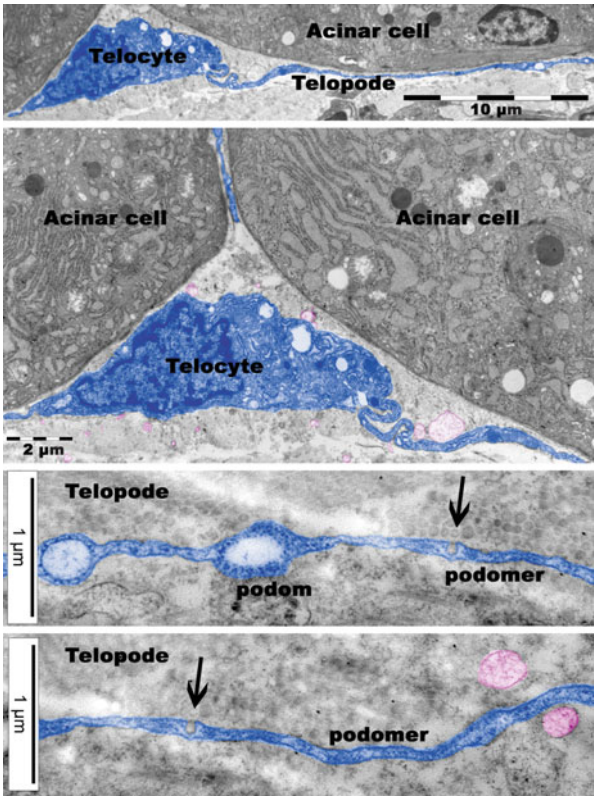

Fig. 11.5 Rat parotid gland: electron micrographs. A typical telocyte cell body with an interacinar localization is shown in the upper panels, digitally colourized in *blue*. Shed microvesicles were digitally colourized in *purple*. Note the moniliform aspect of telopodes (digitally colourized in *blue*). *Arrows* indicate caveolae. Note the alternation of thin segments (*podomeres*) and thicker portions (*podoms*) (Reproduced with permission from Nicolescu [5])

More recent studies showed, on minor salivary glands, the impairment of telocytes in autoimmune pathologies such as Sjogren's syndrome [1].

11.2 Mammary Glands

Telocytes have been previously reported as "interstitial Cajal-like cells" (ICLCs) in several organs [7], including human resting mammary gland stroma [2].

Toluidine blue-stained semithin sections showed long cellular processes [8], which were to be later named telopodes. Their ultrastructural details were thoroughly reviewed under transmission electron microscopy. Telopodes present an alternation of thin, long segments – podomeres – and dilations accommodating mitochondria and calcium-releasing units, podoms [10].

Telocytes and various cell types (plasma cells, lymphocytes, fibroblasts, mast cells and macrophages) presented multi-contact stromal synapses of variable length (up to several micrometres), comprising of close plasmalemmal apposition segments, with the cell membranes only 10–20 nm apart, as detailed in [2].

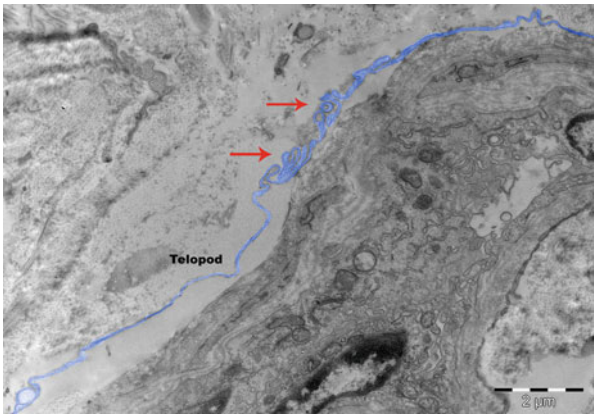

Fig. 11.6 Human submandibular gland: transmission electron microscopy. A telopode (digitally colourized in *blue*) shows seriated elongation reserve areas (*red arrows*) along its thin segments (podomeres) (Reproduced with permission from Nicolescu [3])

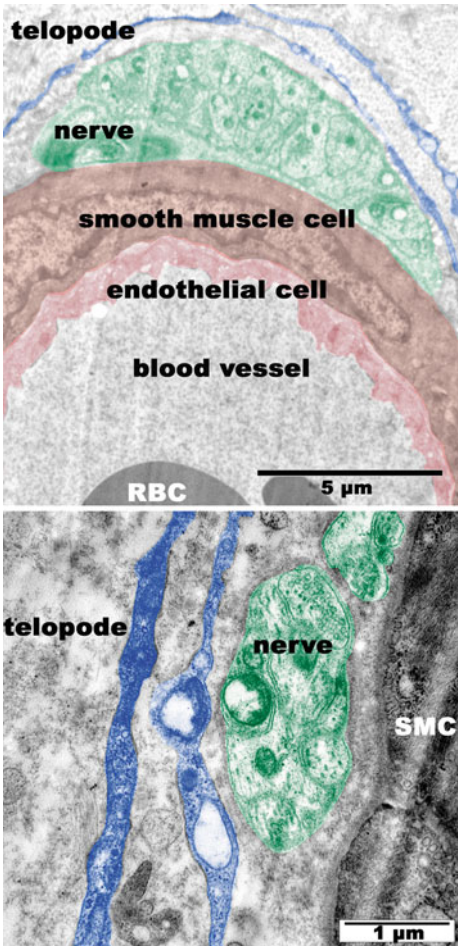

Fig. 11.7 Rat parotid gland: electron micrographs. Two telopodes (digitally colourized in *blue*) are bordering nerves (digitally colourized in *green*) and an arteriolar wall – digitally colourized in *brown* (muscular layer) and *red* (endothelium). *Lower panel* shows a cholinergic nerve between an arteriole and a telopode. *RBC* red blood cell, *SMC* smooth muscle cell (Reproduced with permission from Nicolescu [5])

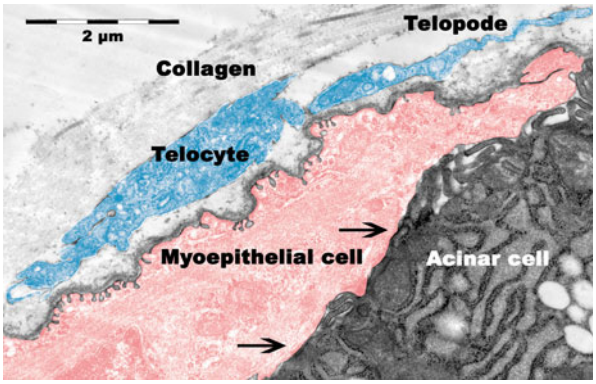

Fig. 11.8 Human parotid gland: electron micrograph. A telocyte (digitally colourized in *blue*) is present between collagen fibres and a myoepithelial cell (digitally colourized in *red*). *Arrows* indicate junction points between the myoepithelial cell and a parotid acinar cell (Reproduced with permission from Nicolescu [5])

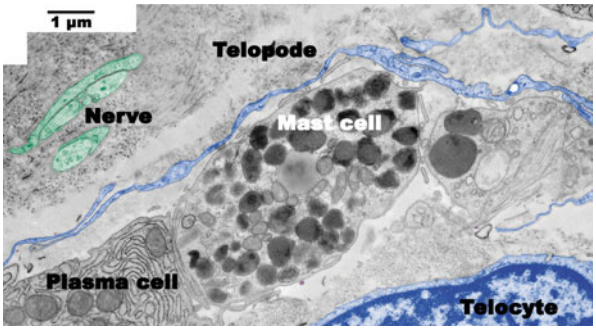

Fig. 11.9 Human parotid gland: electron micrograph. A mast cell surrounded by plasma cells establishes a multi-contact stromal synapse with a telopode (digitally colourized in *blue*). Shed microvesicles were digitally colourized in *purple*. A nearby nerve is digitally colourized in *green* (Reproduced with permission from Nicolescu [5])

Networks of CD34+ stromal cells surrounding microvessels and excretory units were reported in the human mammary gland stroma. Furthermore, $CD34^{+}/CD10^{\pm}/CD117^{-}$/vimentin^{-} cells of inter-/intralobular mammary gland stroma showed signs of mesenchymal potency [6].

11.3 Exocrine Pancreas

As the case of other organs [7], too, pancreatic telocytes were initially described as ICLCs [9]. In human pancreas, telocytes and their telopodes form an extensive lattice network, especially around serous acini (Fig. 11.10). This network is based on

homocellular contacts (between the same type of cells – here, between the long podomeres of telocytes), as they may be seen in Fig. 11.11. All the same, heterocellular connections are also present between telocytes and mast cells (Figs. 11.11b and 11.12a), macrophages (Fig. 11.12b) and stellate cells (Fig. 11.13a).

Branching pattern of telopodes might enhance the cellular telocytes signalling in the interstitial microenvironment. However, less periductal telocytes were encountered in exocrine pancreas compared to major salivary glands, where telocytes are more frequent around ducts than between acinar units [5]. As a true interconnecting hub, telocytes establish relations with all functional elements of the pancreas – acinar

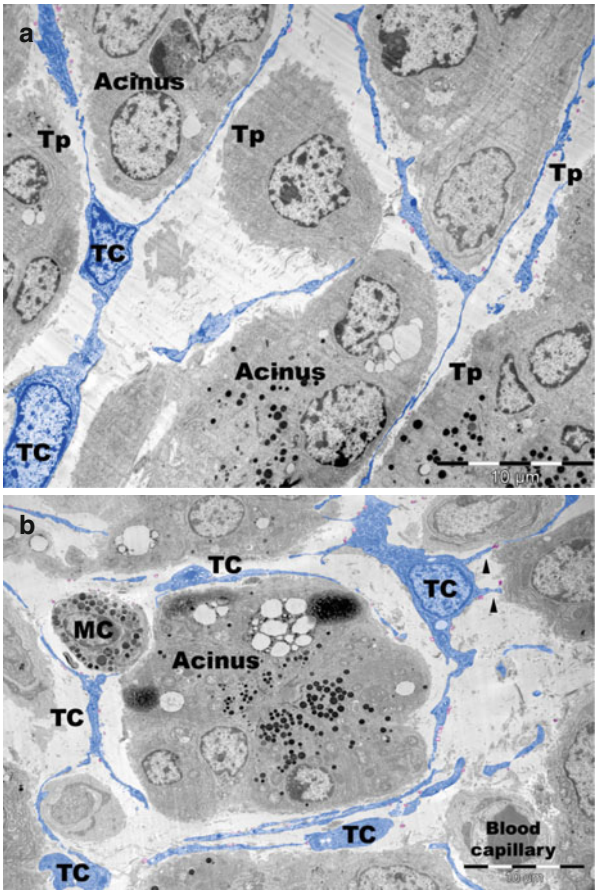

Fig. 11.10 Overview of telocytes' network in the pancreatic interstitium. Human exocrine pancreas: transmission electron microscopy. Telocytes (TC) were digitally colourized in *blue*, shed microvesicles in *purple*. (**a**) General topography of acini and the interstitial TC. Note the small TC cell body, with a nucleus and several very long and thin telopodes (Tp). (**b**) One human pancreatic acinus is circumvented by long Tp belonging to different TC. Arrowheads indicate two Tp in close contact with neighbour pancreatic acinar cells. Blood capillary and a mast cell (MC) are also present (Reproduced with permission from Nicolescu and Popescu [4])

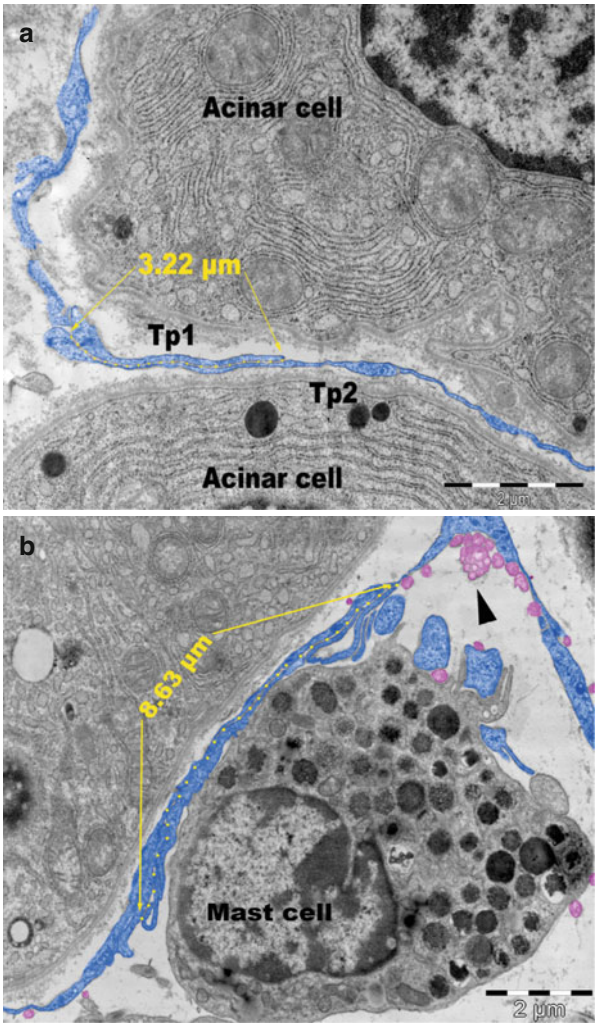

Fig. 11.11 Interpodomeric connections. Human exocrine pancreas: transmission electron microscopy. Telocytes digitally colourized in *blue*, shed microvesicles in *purple*. (**a**) Note the long (3.22 µm, *dotted yellow line*) linear plain contact between podomeres of two telopodes (*Tp1*, *Tp2*) in their intra-acinar trajectory. (**b**) Several Tps located between an acinar cell and a mast cell, bordering both of them. They establish a close intertelopodic convoluted plain contact (*dotted yellow line*) 8.63 µm long. A multivesicular body (*arrowhead*) is present at a Tp bifurcation (Reproduced with permission from Nicolescu and Popescu [4])

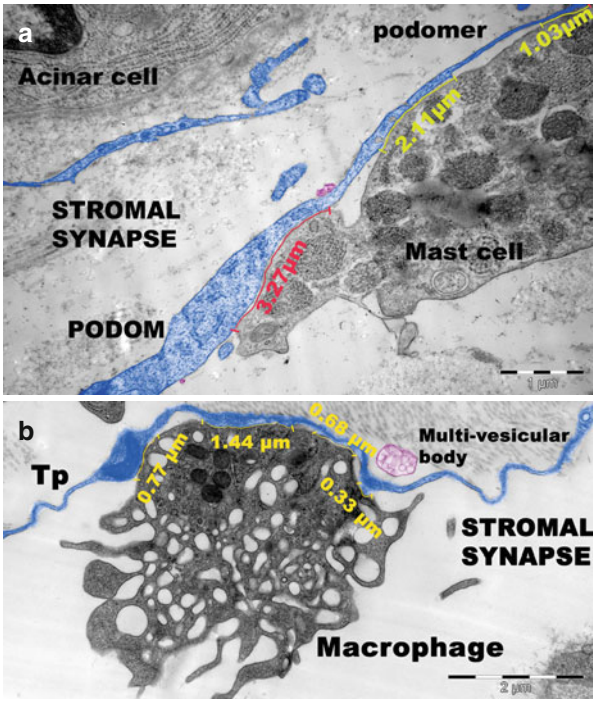

Fig. 11.12 Stromal synapses. Human exocrine pancreas: transmission electron microscopy. Telocytes digitally colourized in *blue*, shed microvesicles in *purple*. (**a**) Multi-contact (*bright yellow*) and "kiss-and-run" (*bright red*) stromal synapses between a telopode and a mast cell. Note that the contacts are established at both podomic and podomeric levels. (**b**) Multi-contact stromal synapse between a long telopode (*Tp*) and a macrophage. Note also a multivesicular body (Reproduced with permission from Nicolescu and Popescu [4])

cells, ducts, blood vessels and nerves (Figs. 11.13, 11.14, and 11.15). Telocytes release microvesicles to communicate with other cells, probably as alternative to stromal synapses for a telecrine/remote noncontact cellular cross talking. Thus, they act not as simply inert stromal elements but as active participants in regulating specific microenvironment [4].

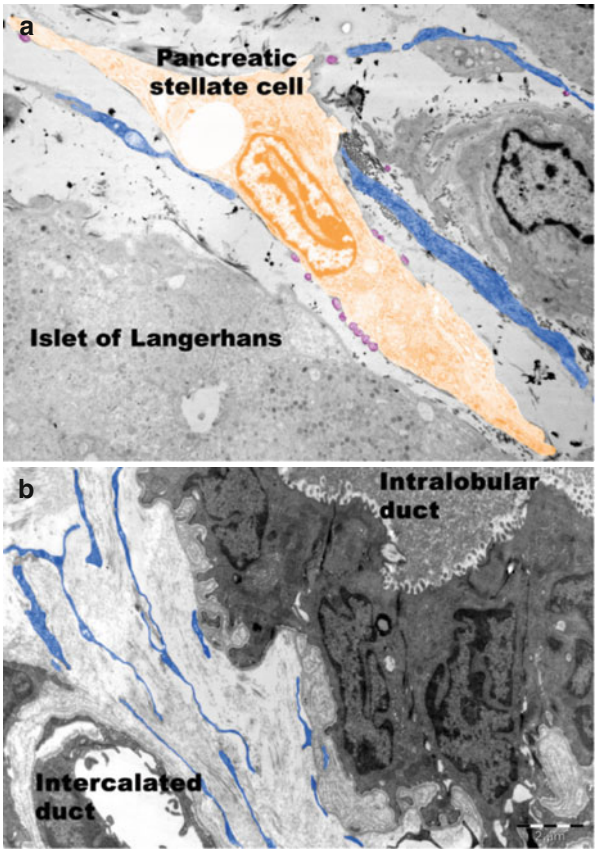

Fig. 11.13 Telocytes near stellate cells and pancreatic ducts: transmission electron microscopy. Telocytes and telopodes digitally colourized in *blue*, shed microvesicles in *purple*. (**a**) Human endocrine pancreas. Pancreatic stellate cell (digitally colourized in *brown*) in close relation to several telopodes. Note also abundant microvesicles. (**b**) Rat exocrine pancreas. Parallel telopodes bordering cells from the walls of pancreatic intercalated and intralobular ducts (Reproduced with permission from Nicolescu and Popescu [4])

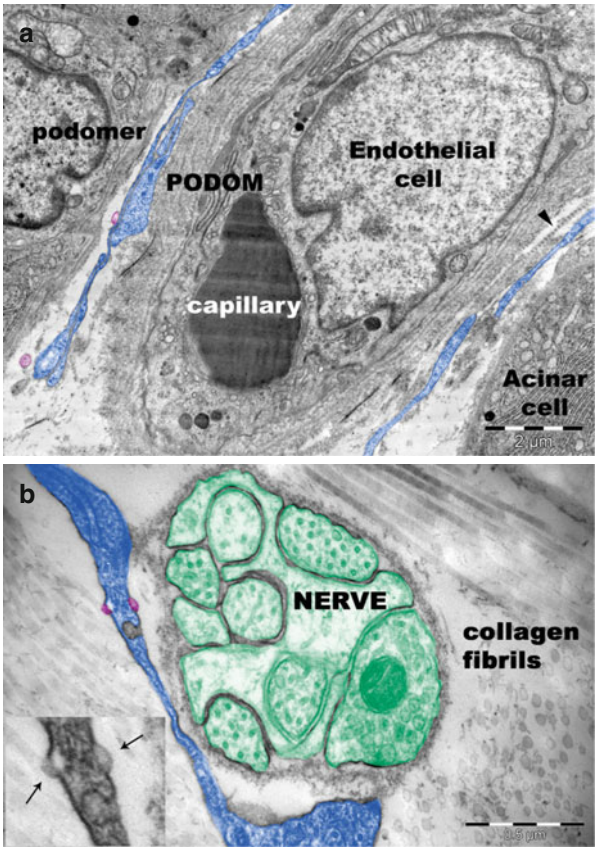

Fig. 11.14 Telocytes near blood vessels, nerves and collagen fibres: transmission electron microscopy. Telocytes and telopodes digitally colourized in *blue*, shed microvesicles in *purple*. (**a**) Human exocrine pancreas. Several telopodes are passing very close to endothelial cells. Note that telopodes establish contacts with other telopodes (podomere-podomere and podomere-podom). Note the similar diameter of podomere segments and collagen fibrils (*arrowhead*). (**b**) Rat exocrine pancreas. Telopode neighbouring a nerve (digitally colourized in *green*) in pancreatic interstitium. *Inset* shows nascent shed microvesicles (*arrows*). Also present transversal- and cross-cut collagen fibrils (Reproduced with permission from Nicolescu and Popescu [4])

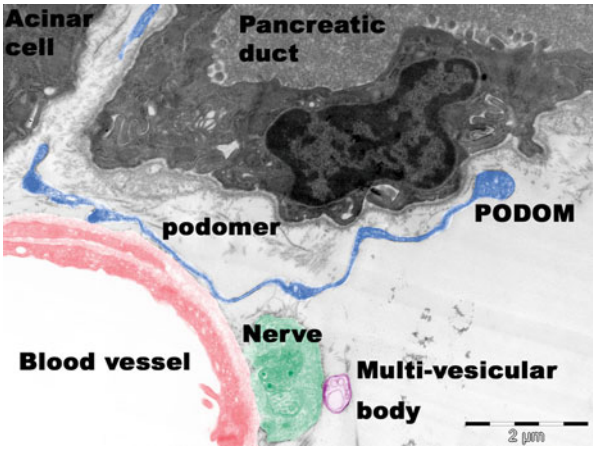

Fig. 11.15 Integrative role of telocytes. Rat exocrine pancreas: transmission electron microscopy. Telocytes digitally colourized in *blue*. Note the same telopode bordering blood vessel (digitally colourized in *red*), nerve (digitally colourized in *green*) and pancreatic acinar and ductal cells. Also note a multivesicular body (digitally colourized in *purple*) (Reproduced with permission from Nicolescu and Popescu [4])

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My current publication record consists of 2 book chapters and 20 peer-reviewed publications, with more than 600 citations, resulting in a Hirsch index of 10. I submitted over 60 meeting abstracts at national/international scientific meetings (most of them as principal author) and served as a reviewer for 10 international ISI scientific journals.