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Ligamentum arteriosum and its telocytes: An ultrastructure description

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Abstract

Results from my study have shown in 2022 the existence of telocytes (TCs) in mice ligamentum arteriosum (LA). Telocytes (TCs) are unique interstitial or stromal cells of mesodermal origin, defined by long cellular extensions called telopodes (Tps) which form a network, connecting them to surrounding cells. These Tps have dilated portions named podoms (usually containing mitochondria, endoplasmic reticulum and caveolae) and very thin segments (below resolving power of light microscopy), called podomers. Generally, transmission electron microscope revealed the existence of Tps with various conformations: (a) long, flattened irregular veils (ribbon-like segments) with knobs, corresponding to podoms, and (b) tubular structures (podomers) with uneven caliber because of irregular dilations (knobs)—the podoms. Also shown were numerous extracellular vesicles and exosomes released by the TCs which sometimes made direct contact with telopodes. Telopodes were observed communicating with each other through adherens junctions. Telopodes sandwiched between myocytes or in close proximity (0.01 μm) from nerve terminals were also observed. These data might be useful for understanding the role(s) of TCs in intercellular signaling and communication, neuromodulation as well as comprehension of pathologies like structural remodeling within the LA.

KEYWORDS

extracellular vesicles, intercellular signaling, ligamentum arteriosum, telocytes, telopodes

1 | INTRODUCTION

Over a century ago, peculiar cells localized between gut musculature and their nerve ending, referred to as intestinal neurons were discovered (Cajal 1911). They were renamed as intestinal cells of Cajal (ICC) after transmission electron microscope (TEM) studies reported these cells were not neurons but interstitial cells with the sole function of pace-making for gut motility. Further on, new cells similar to ICC but with consistently long prolongations were identified within and outside the gastrointestinal musculature and named telocytes (Popescu & Faussone-Pellegrini, 2010). Generally, telocytes are ultrastructurally characterized by a small cell body and very long processes that are called telopodes with a moniliform appearance consisting of alternating regions of thin

segments (podomers) and thick bead-like portions (podoms, Faussone-Pellegrini & Popescu, 2011; Popescu & Faussone-Pellegrini, 2010). These cells can range from several tens to hundreds of micrometers in length and form junctions with a number of cells (Popescu & Faussone-Pellegrini, 2010; Popescu & Nicolescu, 2013; Xiao et al., 2013). Numerous roles such as the provision of mechanical support to connective tissue, enhancement of cell-to-cell communication between smooth muscle cells, microvessels, immune cells, and nerve bundles, through either direct cell-to-cell contacts or paracrine signaling pathway, have been attributed to them (Faussone-Pellegrini & Popescu, 2011; Kondo & Kaestner, 2019; Pieri et al., 2008). There have been recent reports and growing evidence which have exposed a distinctive association between telocytes and stem cell niches in, but not limited to, organs such as the heart, lung, and skin (Rosa et al., 2021). Numerous studies have revealed the presence of telocytes described ultra-structurally in many organs (Chen et al., 2013; Yang et al., 2014; Zheng et al., 2011) and this led to the assumption that telocytes may exist in all organs. However, the existence of telocytes within the ligamentum arteriosum (LA) has not yet been reported.

2 | MATERIALS AND METHODS

Wild-type C57BL/6J mice (Janvier Labs, Le Genest-Saint-Isle, France, Cat#5751862, $n = 8$) were housed under specific-pathogen-free conditions (10 h dark, 14 h light) with free access to food and water. The study was carried out in accordance with the recommendations of the European Communities Council Directive of November 24, 1986 (86/609/EEC). The protocol was approved by the local authorities, that is, Regierungspräsidium Giessen, Germany (reference no. 571_M). All samples were taken after mice were euthanized by inhalation of an overdose of 5% isoflurane (Abbott, Wiesbaden, Germany) and exsanguination through abdominal blood vessels.

LA of wild-type mouse ($n = 8$) was fixed overnight in a fixative mixture consisting of 1.5% glutaraldehyde and 1.5% paraformaldehyde in 0.1 M phosphate buffer. After fixation, tissues were washed in 0.15 M HEPES buffer, osmicated in aqueous 1% osmium tetroxide, and washed in distilled water. Specimens were contrasted in aqueous 1% uranyl acetate overnight, washed in distilled water, and dehydrated with increasing concentration of ethanol. Then, dehydrated in 100% ethanol, then in 100% ethanol + propylene oxide, then in propylene oxide + epon, and embedded in epon overnight. The next day, samples were put in desiccators and put in fresh epon and left in 37°C. Resulting sample blocks were trimmed. Furthermore, semi-thin sections of were cut and stained with ready-to-use methylene blue solution in 1% tri-potassium phosphate. This was followed by cutting ultra-thin sections, which were viewed using TEM.

3 | RESULTS

There was the presence of telocytes with cell bodies and nuclei surrounded by minimal cytoplasm. Extending from the cell bodies were elongated projections referred to as telopodes with a moniliform aspect, representing segments with dilations or "bead on a string" appearance due to the formation of podoms and podomers, which are described as the dilated, cistern-like regions and thin fibrillar segments respectively. There was also dichotomous branching of podomers which ultimately terminated into long convoluted podomer. Collagen fibers running in different orientations are seen in proximity to the telopodes (Figure 1). Telopodes were usually in direct or indirect contact with other telopodes or heterogeneous surrounding cell types such as muscle cells, nerve terminals, extracellular vesicles, and exosomes (Figure 2). Telopodes making direct adherens contact with each other are also observed (Figure 3).

4 | CONCLUSION

This study provides the first demonstration that telocytes exist in mouse LA and agreed with the diagnostic criteria for telocytes suggested by Popescu and Faussone-Pellegrini (2010). The present ultrastructural study revealed the existence of telocytes and their long thin telopodes with alternating regions of podomers and podoms in mouse LA. The present study observed telocytes or telopodes in close proximity to collagen fibers and may be postulated to be involved in the remodeling, regeneration, and repair of the interstitial tissue of the LA. These findings are in line with previous studies that reported the presence of telocytes or telopodes in close proximity to collagen fibers and its involvement in remodeling, regeneration, and repair (Ceafalan et al., 2012; Rusu et al., 2012). Additionally, results from the study revealed telocytes in close proximity to nerve terminal bundles and myocytes. Its role in smooth muscle contraction or modulation of neurotransmission within the LA may only be postulated based on data published by Cretoiu et al. (2015), proving the presence of T-type calcium channels in telocytes which was implicated in myometrium contractions regulation. Telocytes are believed to be involved in intercellular communication either with

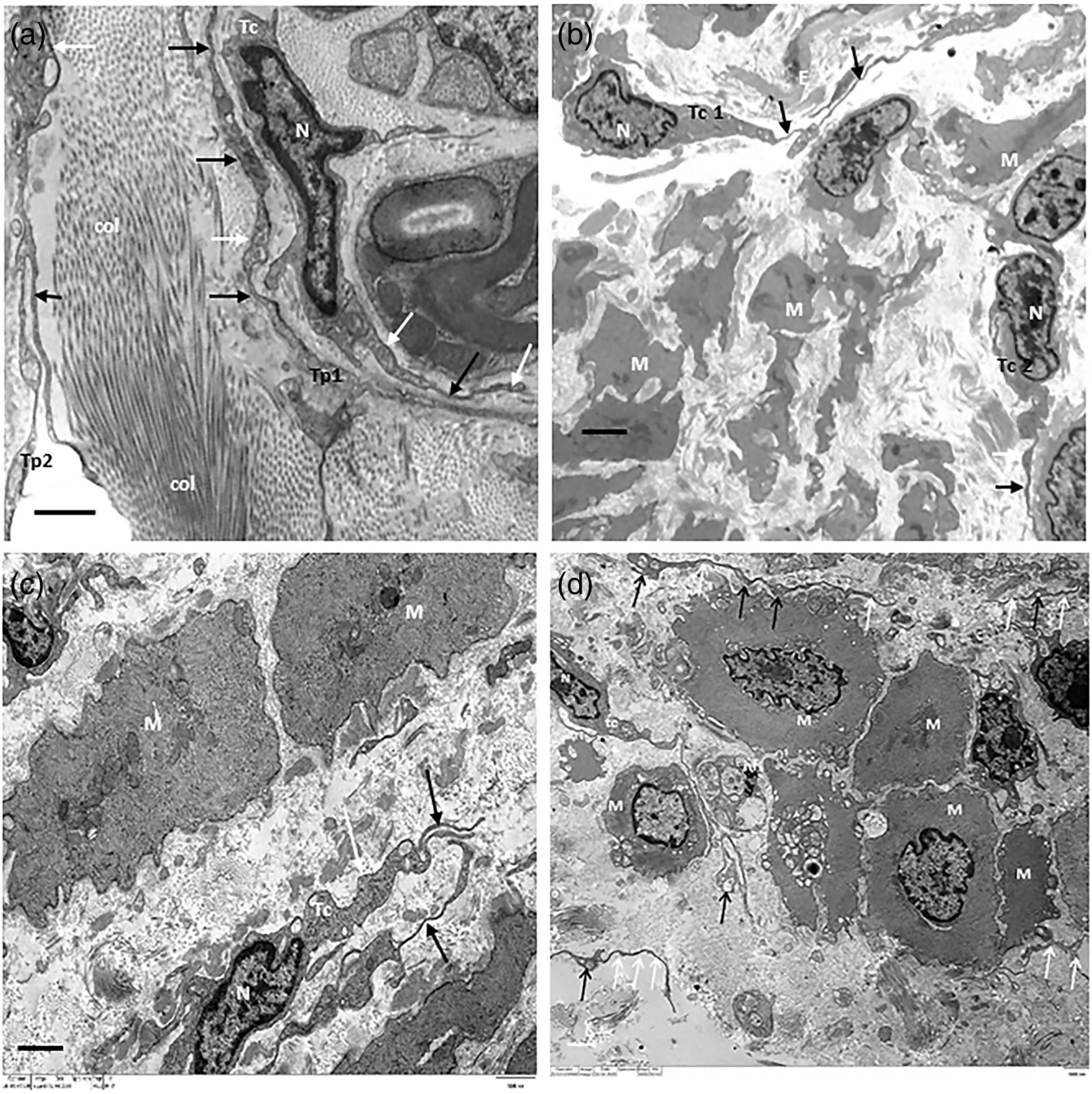

FIGURE 1 TEM of murine LA showing mono-nucleated (N) telocytes cell bodies. (a) Telocyte cell body (Tc) with and moniliform telopodes (white arrows indicative of podoms) as well as podomers (black arrows). An adjacent telopodes (Tp1) running parallel to this cell observed. Collagen fibrils (col) also seen in close proximity with second telopodes (Tp2) seen running lateral to it. (b) Two telocytes (Tc1 and Tc2) with the presence of elastin (E), moniliform telopodes (white arrows indicative of podoms), and podomers (black arrows) seen in close proximity to myocytes (M). (c) Telocyte cell body (Tc) with alternating regions of podom (white arrow) and podomers (black arrows) in proximity to myocytes (M). (d) Mono-nucleated (N) telocyte cell body (tc) and telopodes (white arrows) as well as podoms (black arrows). A region with podom and podomer appeared sandwiched between a nerve fiber bundle (nf) and a myocyte (M). Additionally, the presence of other telopodes (white arrows) and podoms (black arrows) seen in close proximity to myocytes (M). Scale bar = 1 μm

the aid of the cell to cell junctions, or through the release of extracellular microvesicles (Cretoiu, 2016). This way, they establish complex networks that sustain the physiological organ-wide co-ordination of heterocellular signaling. Numerous extracellular vesicles as well as exosomes were observed in close proximity to telopodes, and these shed vesicles are perhaps indicative of short- and long-distance inter-cellular communication regulation

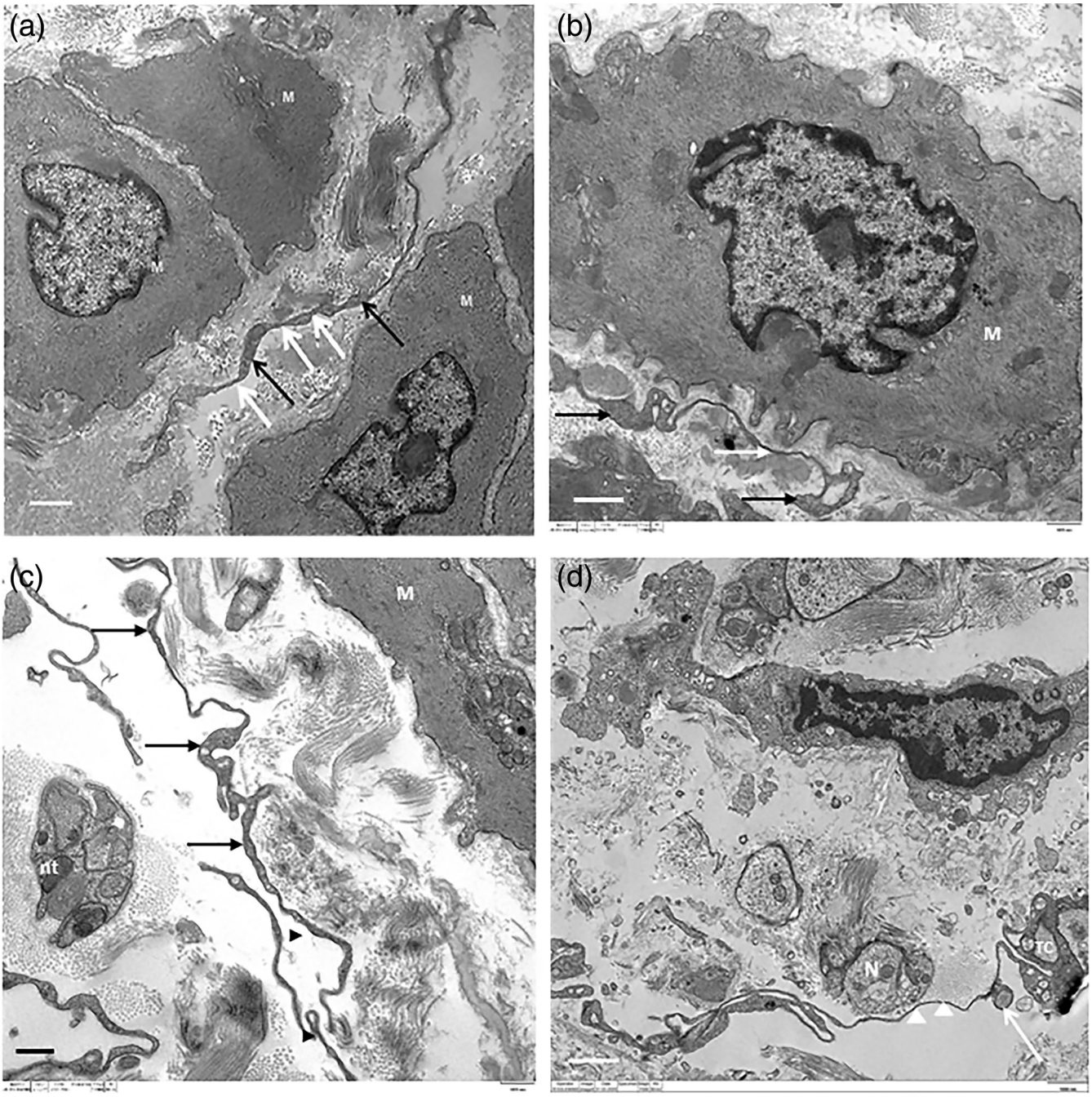

FIGURE 2 TEM of murine LA (a) showing a telopode moniliform appearance of alternating regions of podomers (white arrows) and podoms (black arrows) sandwiched between myocytes (M). (b) A telopode moniliform appearance of alternating regions of podomer (white arrow) and podoms (black arrows) less than 0.5 μm. From a myocyte (M). (c) Telopodes with moniliform appearances of alternating regions of podomer (black arrowheads) and podoms (black arrows) in proximity to a nerve terminal (nt) and a myocyte (M). (d) TEM showing mono-nucleated, oligosarcoplasmic telocyte cell body (TC), and telopodes (white arrow head) around a nerve bundle (N). Also observed is a podom (white arrow) and a dichotomous branching of podomers. Scale bar: (a) = 1 μm, (b–d) = 0.5 μm

within the LA through paracrine signaling. Additionally, functional units such as caveolae and mitochondria present within podoms may be involved in calcium uptake/release within the LA.

Are the telocytes within the LA functioning in the earlier postulated capacity or there may be a possibility that different locations of telocytes could be associated with different roles. Could the telocytes discovered within the LA be a target for tissue regenerative purposes, as those discovered in other organs are already being considered in this regard in present time? At present, the current data may offer new insights for

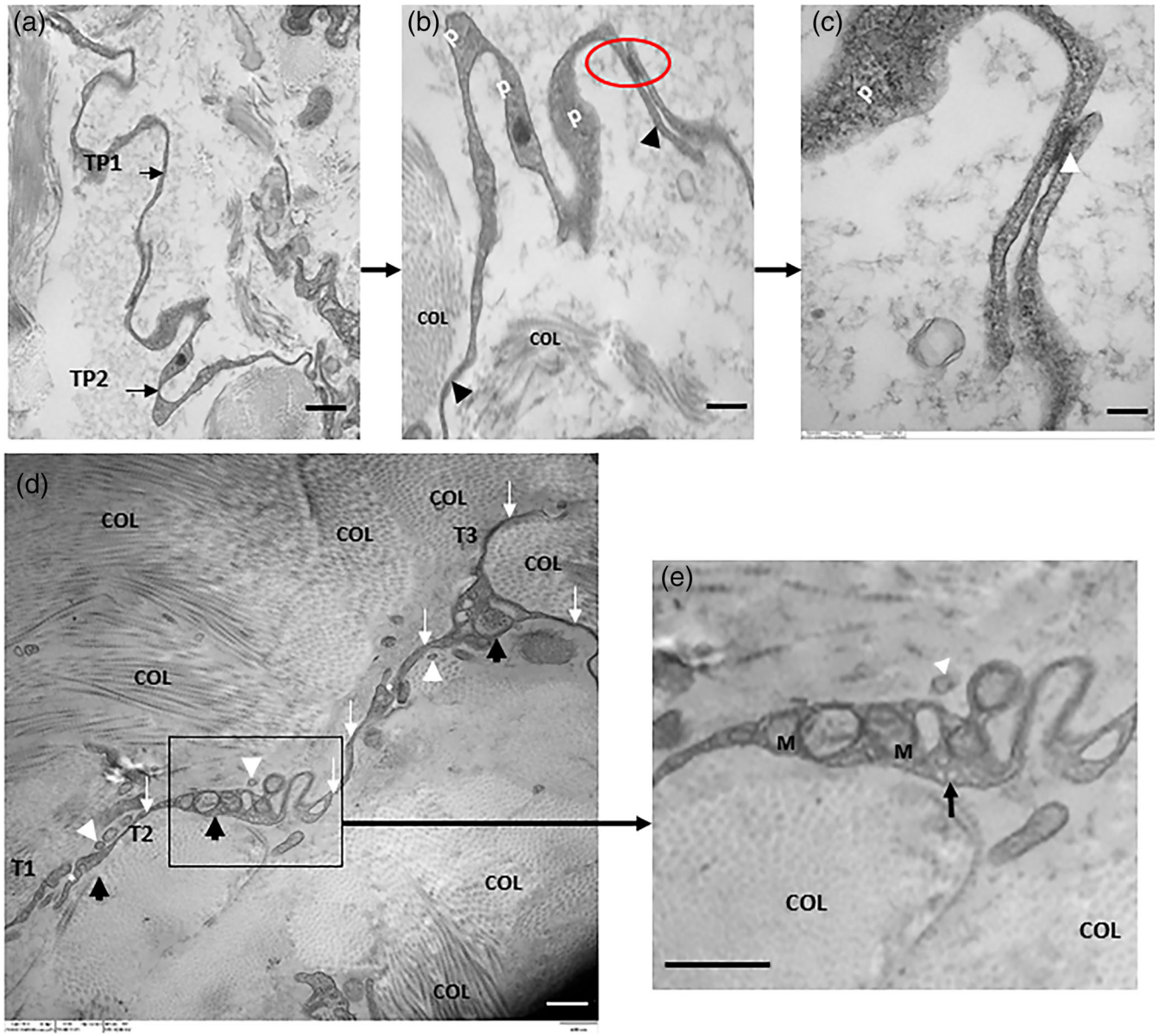

FIGURE 3 TEM of murine LA showing (a) two telopodes (TP1 AND TP2) making an adherens junction contact (red oval) with each other. Image (c) is the magnification of the red oval region in image (b) adherens contact shown (white arrowhead). Collagen (COL) also seen in proximity to telopodes. (d)TEM of murine LA showing multiple telopodes (T1–T3) with indirect cellular connections and alternating regions of podoms (black arrowheads) and podomers (white arrows) surrounded by collagen (COL) running in different orientations. Extracellular vesicles (white arrowheads) also seen in proximity to telopodes. (e) A magnified image of podom showing the presence of mitochondria (M), caveolae (black arrow), collagen (COL), and extracellular vesicle (white arrowhead). Scale bar: (a–c) = 1 μm, (d, e) = 0.5 μm

understanding the function of the LA. Further research into the role of the telocytes within the LA using immunohistochemical staining and 3D imaging is highly recommended.

AUTHOR CONTRIBUTIONS

Benedicta Quaye Mensah: Conceptualization (lead); data curation (lead); formal analysis (lead); funding acquisition (lead); investigation (lead); methodology (lead); project administration (lead); resources (supporting); software (lead); supervision (equal); validation (lead); visualization (lead); writing – original draft (lead); writing – review and editing (lead).

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CONFLICT OF INTEREST

The author declares no potential conflict of interest.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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