SCIENCE CHINA
Life Sciences

THEMATIC ISSUE: Stem cells and regenerative medicine in China
• RESEARCH PAPER •

February 2014 Vol.57 No.2: 241–247
doi: 10.1007/s11427-013-4602-1

Distribution and characteristics of telocytes as nurse cells in the architectural organization of engineered heart tissues

ZHOU Jin[1†], WANG Yan[1†], ZHU Ping[2†], SUN HongYu[1], MOU YongChao[1], DUAN CuiMi[1], YAO AnNing[1], LV ShuangHong[1,3*] & WANG ChangYong[1*]

[1]*Department of Advanced Interdisciplinary Studies, Institute of Basic Medical Sciences and Tissue Engineering Research Center, Academy of Military Medical Sciences, Beijing 100850, China;*
[2]*The First Department of Cardiology, South Building, The General Hospital of People's Liberation Army, Beijing 100039, China;*
[3]*Laboratory of Oncology, Affiliated Hospital of Academy of Military Medical Sciences, Beijing 100071, China*

Received July 27, 2013; accepted October 12, 2013; published online January 14, 2014

Interstitial Cajal-like cells are a distinct type of interstitial cell with a wide distribution in mammalian organs and tissues, and have been given the name "telocytes". Recent studies have demonstrated the potential roles of telocytes in heart development, renewal, and repair. However, further research on the functions of telocytes is limited by the complicated *in vivo* environment. This study was designed to construct engineered heart tissue (EHT) as a three-dimensional model *in vitro* to better understand the role of telocytes in the architectural organization of the myocardium. EHTs were constructed by seeding neonatal cardiomyocytes in collagen/Matrigel scaffolds followed by culture under persistent static stretch. Telocytes in EHTs were identified by histology, toluidine blue staining, immunofluorescence, and transmission electron microscopy. The results from histology and toluidine blue staining demonstrated widespread putative telocytes with compact toluidine blue-stained nuclei, which were located around cardiomyocytes. Prolongations from the cell bodies showed a characteristic dichotomous branching pattern and formed networks in EHTs. Immunofluorescence revealed positive staining of telocytes for CD34 and vimentin with typical moniliform prolongations. A series of electron microscopy images further showed that typical telocytes embraced the cardiomyocytes with their long prolongations and exhibited a marked appearance of nursing cardiomyocytes during the construction of EHTs. This finding highlights the great importance of telocytes in the architectural organization of EHTs. It also suggests that EHT is an appropriate physical and pathological model system *in vitro* to study the roles of telocytes during heart development and regeneration.

telocytes, collagen/matrigel, scaffolds, engineered heart tissues, reconstruction

Citation: Zhou J, Wang Y, Zhu P, Sun HY, Mou YC, Duan CM, Yao AN, Lv SH, Wang CY. Distribution and characteristics of telocytes as nurse cells in the architectural organization of engineered heart tissues. Sci China Life Sci, 2014, 57: 241−247, doi: 10.1007/s11427-013-4602-1

The interstitial cells of Cajal were discovered in gut muscle and considered as pacemakers for gut motility [1,2]. In the past decade, interstitial Cajal-like cells (ICLCs) have been demonstrated to be present outside of the gastrointestinal tract and are found in the myometrium, placenta, pancreas, and mammary gland [3−8]. Demonstration of ICLCs in the heart was first reported by Popescu and Hinescu (2005), which were termed as telocytes in 2010 [9,10]. Telocytes are a novel type of interstitial cell that is widely distributed in the mammalian heart including the myocardium [11,12], epicardium [13,14], and endocardium [15]. The typical characteristic of telocytes is their extremely long and moniliform cellular processes called telopodes that can form an

†Contributed equally to this work
*Corresponding author (email: wcy2000@yahoo.com; lvsh2005@163.com)

interstitial network connecting different cells and release vesicles for heterocellular signaling. Considering the distinct characteristics and distribution of telocytes in the mammalian heart, it has been proposed that telocytes might play a pivotal role in cardiac function, and there is an increasing initiative to determine the roles of telocytes in the heart.

Previous studies have already demonstrated the potential roles of telocytes in heart development, renewal, and repair [16–19]. Notably, telocytes participate in formation of the stem cell niche in the subepicardial region of the heart to guide cardiac stem cell development as well as closely embrace and nurse the growing cardiomyocytes as protector cells throughout the entire heart [16]. In addition, *in vitro* studies have shown that telocytes can intervene in the aggregation of cardiomyocyte clusters, which provides cues to guide the assembly of cardiomyocytes [18]. Indeed, the functions of telocytes have been mainly recognized based on their typical ultrastructural features, but their functions in the myocardium remain unclear. Because the *in vivo* environment is complicated and affected by various factors, the use of *in vitro* models would be a potential approach to reveal the functions of telocytes in the heart. Cardiac tissue engineering approaches mainly rely on the use of synthetic or biological matrix materials and seed cells to reconstitute contractile cardiac muscle-like tissue *in vitro*, which has been used as an *in vitro* model for drug screening and developmental biology research [20–22]. Over the past decade, several groups have contributed to the field of cardiac tissue engineering. One of the most exciting achievements in myocardial tissue construction *in vitro* was by Zimmermann et al., in which collagen/Matrigel were used as scaffold materials [23,24]. Based on these findings, we have established a system to construct engineered heart tissues (EHTs) *in vitro* [25,26]. The myocardial tissue constructed in our system not only has structural similarity to the natural tissue, but its function and mechanical strength are also comparable to those of normal myocardial tissue. To investigate the involvement of telocytes in the function and regeneration of the heart, EHTs can be used as a promising model to study the distribution and characteristics of telocytes and to gain more biological insights into the roles of telocytes in the heart.

We have previously isolated and cultured telocytes from neonatal rat cardiac tissue in two- and three-dimensional environments *in vitro* [27]. Our previous study confirmed that typical telocytes existed in primary culture *in vitro* and some c-kit, vimentin, or CD34-positive cells in EHTs exhibited the morphology of telocytes. Three-dimensional EHTs will provide a foundation to elucidate the functional relationships of telocytes and cardiomyocytes. This study aims to confirm the existence of telocytes in EHTs and further investigate the appearance, distribution, and characteristics of telocytes in EHTs, and especially the potential roles of telocytes in the architectural organization of EHTs.

1 Materials and methods

1.1 EHT construction

EHTs were constructed by combining freshly isolated cardiac cells from one-day-old Wistar rats with a collagen/Matrigel mixture as described previously [25,27]. In brief, 0.5 mL 2× H-DMEM (Invitrogen, Carlsbad, CA, USA) containing 20% fetal bovine serum (Invitrogen) was mixed with 0.5 mL liquid collagen type I and Matrigel (4∶1, (v/v); Becton Dickinson Biosciences, San Jose, CA, USA). Then, the mixture was neutralized immediately by titrating with 0.1 mol L^{-1} NaOH. A total of 1×10^7 freshly isolated cardiac cells were combined with the mixture and pipetted into casting molds for incubation. One milliliter of H-DMEM containing 10% fetal bovine serum was added to the dish after 60 min of incubation, and then the culture medium was changed daily.

1.2 Histology and toluidine blue staining

After 14 d of culture, the EHTs were fixed in 4% formaldehyde and embedded in paraffin. Sections of 4 μm thickness were prepared for hematoxylin and eosin staining by standard procedures. For toluidine blue staining, the sections were incubated in a working solution of toluidine blue for 2 min followed by brief rinsing under running tap water (Pacific Pathology Associates, Salem, USA). Then, the sections were dehydrated and mounted with synthetic medium for microscopic analyses.

1.3 Immunofluorescence staining and confocal microscopy

To investigate the appearance of telocytes in EHTs, immunofluorescence staining was carried out. The primary antibodies used were anti-vimentin (1∶800; Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-c-kit (1∶200; Santa Cruz Biotechnology), and anti-CD34 (1∶500; R&D Systems, Minneapolis, CA, USA). The sections were incubated with primary antibodies overnight at 4°C and then FITC-labeled goat anti-mouse IgG and Cy3-labeled goat anti-rabbit IgG (1∶500; Invitrogen) secondary antibodies. The sections were then counterstained with Hoechst 33258 and observed under a Zeiss confocal microscope with BioRad confocal software. Primary antibodies were omitted for negative controls.

1.4 Transmission electron microscopy (TEM)

EHT sections were fixed with 2.5% glutaraldehyde in 0.1 mol L^{-1} sodium cacodylate buffer (pH 7.4) for 6 h, post-fixed in 1% phosphate-buffered OsO_4 (pH 7.4) for 2 h, and then embedded in epoxy resin. Semi-thin sections were obtained with a LKB NOVA ultra-microtome, stained with toluidine blue in 0.1 mol L^{-1} borate buffer, and then ob-

served under a light microscope. Ultrathin sections of areas of interest were prepared and examined under a transmission electron microscope (Technai10; Philips, Eindhoven, The Netherlands).

2 Results

2.1 Distribution of telocytes in EHTs

Scattered cells with typical small round cell bodies were widespread in collagen/Matrigel scaffolds (Figure 1A). The nuclei of these cells were compact and stained with toluidine blue. In addition, these cells preferred to surround cardiomyocytes (Figure 1B). Toluidine blue staining further showed many scattered stellate cells distributed in the EHTs, which formed networks (Figure 2A). These cells presented a small round cell body and several moniliform prolongations (Figure 2B). The putative telocytes contained smaller and more toluidine blue-stained nuclei, while the telopodes showed a characteristic dichotomous branching pattern with endings on other cells nearby.

2.2 Confocal microscopy of telocytes in EHTs

Consistent with our previous findings, $CD34^+$, c-kit^+, or $vimentin^+$ cells were observed in EHTs. The CD34- and vimentin-positive cells showed typical features of long moniliform processes emanating from the cell body (suggestive of telocytes) (Figure 3). c-kit-positive cells showed a plaque-positive appearance around the cell membrane and along their cell processes (Figure 4A). Double immunofluorescence staining for both cTnT and c-kit showed that c-kit-positive cells were located around cardiomyocytes (Figure 4B). Interestingly, some cTnT- and c-kit-positive areas were colocalized around the cell membrane of cardiomyocytes (Figure 4C), which might have been immature cardiomyocytes or telopode fragments around cardiomyocytes.

2.3 TEM of telocytes in EHTs

Identification of telocytes in EHTs was further confirmed by electron microscopy. Myocardial telocytes in EHTs showed typical features: a small oval-shaped cell body surrounded by a ring of cytoplasm and extremely long but thin prolongations (Figure 5). Remarkably, we found that multi-vesicular bodies were emanating from the telopodes in EHTs (Figure 5A). The vesicles were near the connecting protuberances and also appeared in the extracellular matrix. This observation suggested the delivery of vesicles for cell

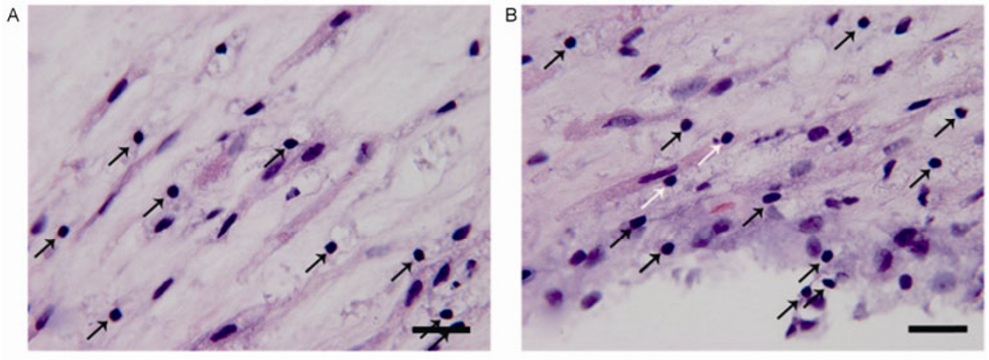

Figure 1 Hematoxylin and eosin staining of EHTs. A, Putative telocytes formed interstitial networks in EHTs. B, Some putative telocytes were located around cardiomyocytes. Black arrows indicate putative telocytes. White arrows indicate telocytes surrounding cardiomyocytes. Scale bar, 50 μm.

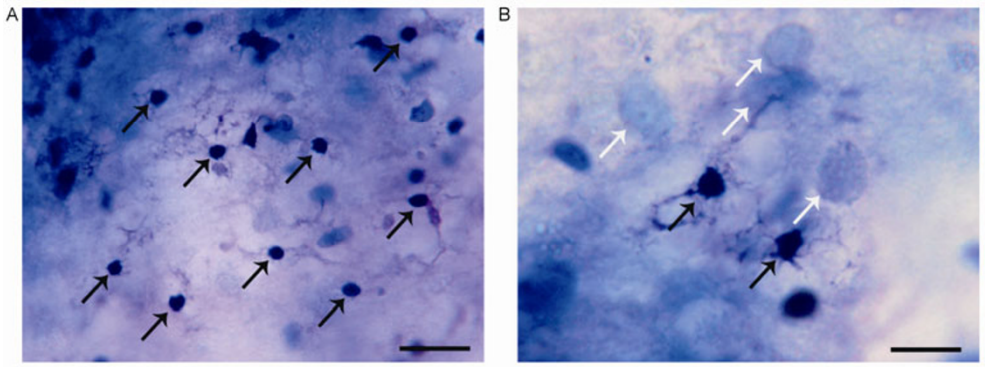

Figure 2 Toluidine blue staining of EHTs. A, Putative telocytes with a blue-stained appearance formed interstitial networks in EHTs. Scale bar, 50 μm. B, Putative telocytes showed small round nuclei and several prolongations emanating from the cell body with a moniliform aspect. Scale bar, 20 μm.

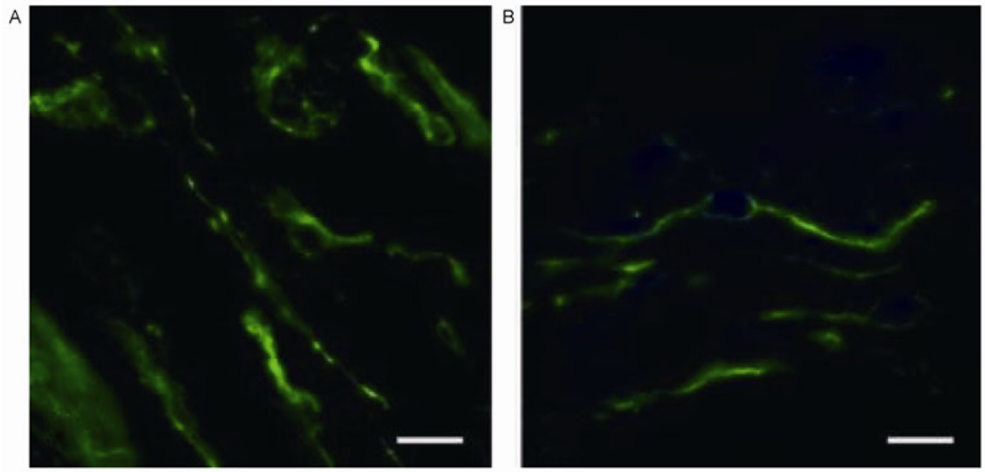

Figure 3 Confocal microscopy of telocytes in EHTs. A, CD34-positive telocytes with moniliform processes in EHTs. B, Vimentin-positive telocytes with long processes. Scale bar, 20 μm.

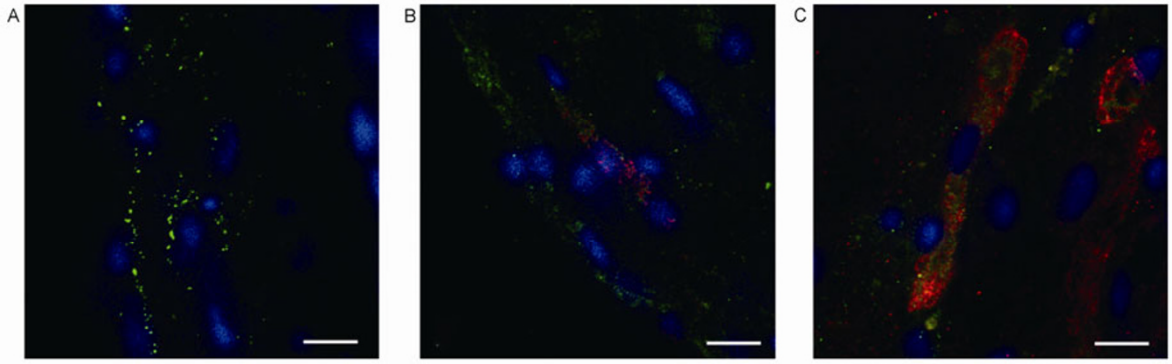

Figure 4 Double immunofluorescence staining and confocal microscopy of telocytes in EHTs. A, c-kit-positive telocytes with a plaque-positive appearance in EHTs. B, Double immunofluorescence staining for both cTnT (red) and c-kit (green) showed that c-kit-positive cells were located around cardiomyocytes. C, Some cTnT (red)- and c-kit (green)-positive areas were colocalized around the cell membrane of cardiomyocytes. Scale bar, 20 μm.

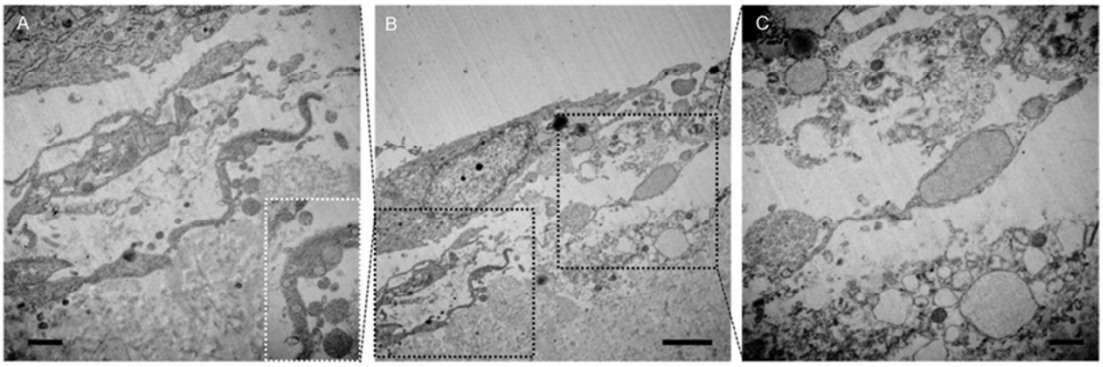

Figure 5 Electron microscopy of telocytes in EHTs. A, Enlarged view of the black inset in B. White inset shows multi-vesicular bodies emanating from telopodes. Scale bar, 500 nm. B, Typical telocytes in EHTs: a small oval-shaped cell body surrounded by a ring of cytoplasm and extremely long but thin prolongations. Scale bar, 2 μm. C, Enlarged view of the black inset in B. Scale bar, 500 nm.

signaling in EHTs. After 14 d of culture, the telocytes in EHTs surrounded the cardiomyocytes with several telopodes (Figure 6).

We also evaluated the morphofunctional interactions between cardiomyocytes and telocytes in EHTs. TEM images showed that telocytes formed an extended network embracing the cardiomyocytes. These cardiomyocytes showed features of necrosis, such as few myofibrillae and numerous mitochondria. A telocyte was closely located to a cardiomyocyte and nursing it with its long prolongations

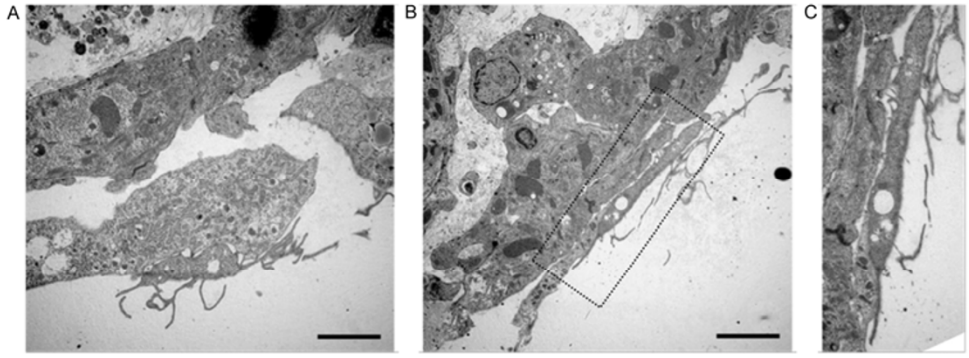

Figure 6 Electron microscopy of telocytes in EHTs. A and B, Telocytes in EHTs were distributed around cardiomyocytes with several telopodes. C, Enlarged view of the black inset in B. Scale bar, 2 μm.

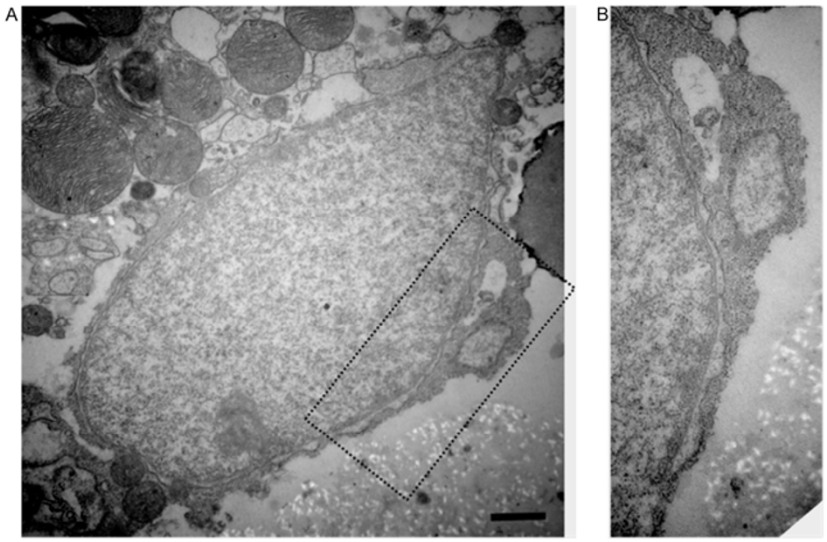

Figure 7 Electron microscopy of telocytes in EHTs. A, Telocytes formed an extended network embracing cardiomyocytes and nursing them with their long prolongations. B, Enlarged view of the black inset in A. Scale bar, 500 nm.

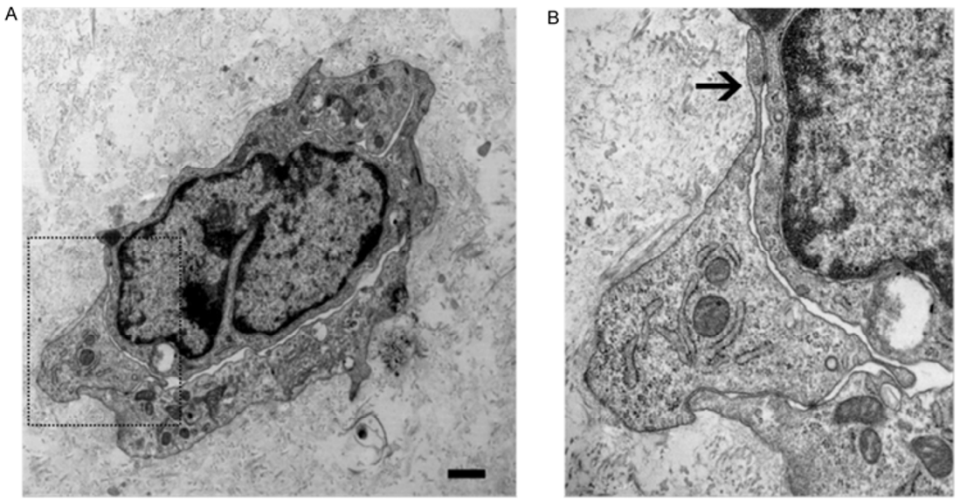

Figure 8 Electron microscopy of telocytes in EHTs. A, Putative telocytes distributed around a developing cardiomyocytes, which established direct membrane-to-membrane contacts with the cardiomyocytes and contacted each other to form junctions. B, Enlarged view of the black inset in A. Scale bar, 500 nm.

(Figure 7). Another TEM image showed multiple putative telocytes distributed around a developing cardiomyocyte and establishment of direct membrane-to-membrane contacts with cardiomyocytes. In addition, the putative telocytes contacted each other to form junctions. Both the cardiomyocyte and telocyte membranes had protuberances near the connecting sites, and a shedding vesicle was found at the connecting site.

3 Discussion

In this study, we presented a series of visual evidences to show the characteristics and distribution of telocytes in the architectural organization of EHTs. Our results demonstrated that telocytes formed interstitial networks with their long processes in EHTs. They also preferred to distribute around cardiomyocytes and transmitted information to cardiomyocytes through membrane contacts and vesicle release. Furthermore, some TEM images showed that telocytes embraced the cardiomyocytes (necrotic or developing) with their long prolongations and showed a marked appearance of nursing the cardiomyocytes. This finding highlights the potential importance of telocytes in the architectural organization of the myocardium. In addition, it suggests that EHT is an appropriate model to study the functions of telocytes in the myocardium.

In terms of the morphological characteristics of telocytes in EHTs, although the ultrastructural features were not completely consistent with their appearance *in vivo*, the main characteristics were quite consistent, such as the long moniliform prolongations and the shedding vesicles released from the telopodes [11–15]. The main reason for differences in the morphological characteristics of telocytes in EHTs and the heart *in vivo* might be the scaffold materials used to construct the EHTs. Because collagen I is the main component of the extracellular matrix in normal heart, we used collagen I as the fundamental scaffold, and combined it with Matrigel to add several growth factors that are necessary for cardiomyocyte development. Even though we used natural materials and factors to construct EHTs and mimic the mechanical environment *in vivo* by exerting static stretch, the microenvironment was still disparate between EHTs and normal heart *in vivo*. Thus, it is understandable that the morphology of telocytes in EHTs was not as typical as their appearance *in vivo*. The mechanisms of the extracellular matrix influencing the morphology of telocytes should be determined in the future.

Telocytes in EHTs appeared to be involved in cell signaling and heterocellular communication. Cell communication plays an important role in the architectural organization and functional establishment of the myocardium [28,29]. Because the heart is a complex organ composed of many cell types, including cardiomyocytes and non-cardiomyocytes such as cardiac fibroblasts, smooth muscle cells, and endothelial cells, heterocellular communication between cardiomyocytes and non-cardiomyocytes is essential for cardiac development [18,19], heart physiology [30], and pathology [31]. It has been shown that myocardial cell maturation and function depend on the presence of endocardial endothelium cells [32], while cardiac fibroblasts mainly provide structural support during ventricular wall thickening from embryogenesis to adulthood [33]. In this study, we demonstrated that telocytes, serving as an important interstitial cell type, formed networks in EHTs with their long prolongations and communicated with cardiomyocytes through the formation of junctions and vesicle shedding. Because telocytes appear to be the main interstitial cell type in normal heart, the results from the three-dimensional model *in vitro* further confirmed the roles of telocytes in cardiac development, regeneration, renewal, as well as heart physiology and pathology.

Regarding the role of telocytes in heart regeneration, we showed that telocytes participated in the repair and regeneration of cardiomyocytes in EHTs. At present, the functions of telocytes in the heart are still obscure, but some studies have proposed some relevant and potential roles. Previous reports have demonstrated the distinct roles of telocytes during heart development. Telocytes can nurse and guide cardiac precursor cells to form the correct three-dimensional tissue architecture [16]. In addition, telocytes participate in formation of the stem cell niche in the subepicardial region to embrace the developing cardiomyocytes [17–19]. Very important insights strongly support the concept that telocytes are involved in cardiac stem cell regulation and heart regeneration. In this regard, the TEM images showed that telocytes with their telopodes may guide and nurse the cardiomyocytes (necrotic or developing) in EHTs. This observation further confirms the potential distinct roles of telocytes in myocardium repair and regeneration. Elucidation of the distribution and characteristics of telocytes in EHTs provides insights to better understand the functions of telocytes in the heart.

In conclusion, we provide insights to better understand the characteristics of telocytes using EHT as an *in vitro* model. Our results support the previous hypothesis that telocytes may participate in pacemaking and arrhythmogenesis or nursing cardiomyocytes for regeneration. In addition, they suggest the importance of telocytes in the architectural organization and function of EHTs. Further research on telocytes in EHTs may reveal more information to improve the quality of EHTs.

We thank Prof. L. M. Popescu from the Department of Cellular and Molecular Medicine, Carol Davila University of Medicine and Pharmacy in Romania for excellent technical assistance and guidance, and all of the members from his laboratories for helpful comments regarding the manuscript. This work was supported by the National High Technology Research and Development Program of China (2012AA020506), Key Program of National Natural Science Foundation of China (31030032), Na-

tional Natural Science Funds for Distinguished Young Scholar (31025013), and the National Natural Science Foundation of China (31100697).

1 Thuneberg L. Interstitial cells of Cajal: intestinal pacemaker cells? Adv Anat Embryol Cell Biol, 1982, 71: 1–130
2 Faussone-Pellegrini MS, Thuneberg L. Guide to the identification of interstitial cells of Cajal. Microsc Res Tech, 1999, 47: 248–266
3 Popescu LM, Ciontea SM, Cretoiu D. Interstitial Cajal-like cells in human uterus and fallopian tube. Ann NY Acad Sci, 2007, 1101: 139–165
4 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
5 Hinescu ME, Ardeleanu C, Gherghiceanu M, Popescu LM. Interstitial Cajal-like cells in human gallbladder. J Mol Histol, 2007, 38: 275–284
6 Lang RJ, Klemm MF. Interstitial cell of Cajallike cells in the upper urinary tract. J Cell Mol Med, 2005, 9: 543–556
7 McHale NG, Hollywood MA, Sergeant GP, Shafei M, Thornbury KT, Ward SM. Organization and function of ICC in the urinary tract. J Physiol, 2006, 576: 689–694
8 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
9 Hinescu ME, Popescu LM. Interstitial Cajallike cells (ICLC) in human atrial myocardium. J Cell Mol Med, 2005, 9: 972–975
10 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
11 Sucin L, Nicolescu MI, Popescu LM. Cardiac telocytes: serial dynamic images in cell culture. J Cell Mol Med, 2010, 14: 2687–2692
12 Hinescu ME, Gherghiceanu M, Mandache E, Ciontea SM, Popescu LM. Interstitial Cajal-like cells (ICLC) in atrial myocardium: ultrastructural and immunohistochemical characterization. J Cell Mol Med, 2006, 10: 243–257
13 Suciu L, Popescu LM, Regalia T, Ardelean A, Manole CG. Epicardium: interstitial Cajal-like cells (ICLC) highlighted by immunofluorescence. J Cell Mol Med, 2009, 13: 771–777
14 Gherghiceanu M, Popescu LM. Human epicardium: ultrastructural ancestry of mesothelium and mesenchymal cells. J Cell Mol Med, 2009, 13: 2949–2951
15 Gherghiceanu M, Manole CG, Popescu LM. Telocytes in endocardium: electron microscope evidence. J Cell Mol Med, 2010, 14: 2330
16 Gherghiceanu M, Popescu LM. Cardiomyocyte precursors and telocytes in epicardial stem cell niche: electron microscope images. J Cell Mol Med, 2010, 14: 871–877
17 Popescu LM, Gherghiceanu M, Manole CG, Faussone-Pellegrini MS. Cardiac renewing: interstitial Cajal-like cells nurse cardiomyocyte progenitors in epicardial stem cell niches. J Cell Mol Med, 2009, 13: 866–886
18 Bani D, Formigli L, Gherghiceanu M, Faussone-Pellegrini MS. Telocytes as supporting cells for myocardial tissue organization in developing and adult heart. J Cell Mol Med, 2010, 14: 2531–2538
19 Faussone-Pellegrini MS, Bani D. Relationships between telocytes and cardiomyocytes during pre- and post-natal life. J Cell Mol Med, 2010, 14: 1061–1063
20 Franchini JL, Propst JT, Comer GR, Yost MJ. Novel tissue engineered tubular heart tissue for *in vitro* pharmaceutical toxicity testing. Microsc Microanal, 2007, 13: 267–271
21 Bursac N, Loo Y, Leong K, Tung L. Novel anisotropic engineered cardiac tissues: studies of electrical propagation. Biochem Biophys Res Commun, 2007, 361: 847–853
22 Katare RG, Ando M, Kakinuma Y, Sato T. Engineered heart tissue: a novel tool to study the ischemic changes of the heart *in vitro*. PLoS ONE, 2010, 5: e9275
23 Zimmermann WH, Fink C, Kralisch D, Remmers U, Weil J, Eschenhagen T. Three-dimensional engineered heart tissue from neonatal rat cardiac myocytes. Biotechnol Bioeng, 2000, 68: 106–114
24 Zimmermann WH, Schneiderbanger K, Schubert P, Didié M, Münzel F, Heubach JF, Kostin S, Neuhuber WL, Eschenhagen T. Tissue engineering of a differentiated cardiac muscle construct. Circ Res, 2002, 90: 223–230
25 Zhao YS, Wang CY, Li DX, Zhang XZ, Qiao Y, Guo XM, Wang XL, Dun CM, Dong LZ, Song Y. Construction of a unidirectionally beating 3-dimensional cardiac muscle construct. J Heart Lung Transplant, 2005, 24: 1091–1097
26 Guo XM, Zhao YS, Chang HX, Wang CY, E LL, Zhang XA, Duan CM, Dong LZ, Jiang H, Li J, Song Y, Yang XJ. Creation of engineered cardiac tissue *in vitro* from mouse embryonic stem cells. Circulation, 2006, 113: 2229–2237
27 Zhou J, Zhang Y, Wen X, Cao J, Li D, Lin Q, Wang H, Liu Z, Duan C, Wu K, Wang C. Telocytes accompanying cardiomyocyte in primary culture: two- and three-dimensional culture environment. J Cell Mol Med, 2010, 14: 2641–2645
28 Loewenstein WR. Junctional intercellular communication: the cell-to-cell membrane channel. Physiol Rev, 1981, 61: 829–913
29 Musil LS, Goodenough DA. Gap junctional intercellular communication and the regulation of connexin expression and function. Curr Opin Cell Biol, 1990, 2: 875–880
30 Li J, Radice GL. A new perspective on intercalated disc organization: implications for heart disease. Dermatol Res Pract, 2010, 2010: 207835
31 Faussone-Pellegrini MS, Bani D. Cordial connections: molecular ensembles and structures of adhering junctions connecting interstitial cells of cardiac valves in situ and in cell culture. Cell Tissue Res, 2009, 337: 63–77
32 Li J, Radice GL. Impaired myocardial angiogenesis and ischemic cardiomyopathy in mice lacking the vascular endothelial growth factor isoforms VEGF164 and VEGF168. Nat Med, 1999, 5: 495–502
33 Ottaviano FG, Yee KO. Communication signals between cardiac fibroblasts and cardiac myocytes. J Cardiovasc Pharmacol, 2011, 57: 513–521