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Tissue engineering and regenerative medicine – where do we stand?

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Abstract

Tissue Engineering (TE) in the context of Regenerative Medicine (RM) has been hailed for many years as one of the most important topics in medicine in the twenty-first century. While the first clinically relevant TE efforts were mainly concerned with the generation of bioengineered skin substitutes, subsequently TE applications have been continuously extended to a wide variety of tissues and organs. The advent of either embryonic or mesenchymal adult stem-cell technology has fostered many of the efforts to combine this promising tool with TE approaches and has merged the field into the term Regenerative Medicine. As a typical example in translational medicine, the discovery of a new type of cells called Telocytes that have been described in many organs and have been detected by electron microscopy opens another gate to RM. Besides cell-therapy strategies, the application of gene therapy combined with TE has been investigated to generate tissues and organs. The vascularization of constructs plays a crucial role besides the matrix and cell substitutes. Therefore, novel *in vivo* models of vascularization have evolved allowing axial vascularization with subsequent transplantation of constructs. This article is intended to give an overview over some of the most recent developments and possible applications in RM through the perspective of TE achievements and cellular research. The synthesis of TE with innovative methods of molecular biology and stem-cell technology appears to be very promising.

Keywords: tissue engineering • regenerative medicine • cell transplantation • gene transfer • mesenchymal stem cells • AV loop • vascularization • angiogenesis • telocytes • embryonal stem cells

Introduction

Questions of the quality of life (QOL) of any individual human being have to be inevitably considered when tackling the problems of ageing, because many people are seriously concerned about their future and are afraid of depending on medical machinery and nursing. With all the tremendous achievements of modern medicine, we have become able to extend human life span considerably. Nevertheless,

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Fig. 1 'The Fountain of Youth' is the title of a painting of Lucas Cranach the elder of 1546. The picture represents a bath in which from one side aged women rise in the water which they leave on the other side rejuvenated. Source : Wikipedia[http://de.wikipedia.org/w/index.php?title=Datei:Lucas_Cranach_d._%C3%84._007.jpg&filetimestamp=20050519080127

this achievement often comes along with a dramatic loss of the QOL. The hope to find Fountains of Youth is possibly one of the oldest dreams of mankind and stands for the desire to live a long and fulfilled life without loss of quality (Fig. 1).

When the idea of constructing living tissue equivalents and/or organs in the laboratory by means of cell-culture techniques and the use of biomaterials first came up and was then coined 'Tissue Engineering' this multi-disciplinary approach to regenerate lost tissue or organ functions was conceived as the light at the end of the tunnel to overcome organ shortage in transplantation medicine and to overcome the hitherto unsolved donor site morbidity problems associated with tissue transfer. The mere idea of creating tissue in the laboratory has led to many collaborations between clinicians and specialists from various basic areas in biomedicine and engineering and applied sciences. Hence, researchers in the field of tissue engineering and RM are now applying the principles of cell culture and transplantation, material science and bioengineering to construct biological substitutes that will restore and maintain normal function in diseased and injured tissues [1–3]. Scientific specialties that seem not to be involved in the first place are nevertheless also intrigued by this emerging field, as can be seen by international conferences of Computational Biology and Bioinformatics that strive to identify the rapidly growing body of knowledge with statistical techniques, genetic networking, comparative genomics, computational biochemistry and biophysics, computational biomodelling, macromolecular structure prediction, mathematical biology and medical informatics, to name just a few examples.

However, despite tremendous efforts and progress in standardizing cell culture techniques and developing customized biomaterials to substitute lost organ functions, the translation of laboratory achievements into clinical scenarios has not been equally successful so far. This is partly due to the three dimensional attitude of organs and tissue that necessitate a microvascular network to allow for sufficient blood flow and oxygenation of cells even in the middle of any given construct to keep them viable. Extrinsic vascularization depends on the ingrowth of a vascular network from the outside. This is a natural limitation to cell survival within three dimensional scaffolds during the initial phase following implantation of tissue-engineered substitutes into recipient organisms.

Several ways have been followed to overcome these problems. One way was to combine cell-seeded tissue substitutes by including endothelial cells to achieve earlier vascular ingrowth [4]. However, this idea may be helpful in the long-term, but does not circumvent the critical problem of the initial lack of vascular supply. This holds also true for the addition of various growth factors that may enhance any kind of vascularization, but still depends on the ingrowth of capillaries from the recipient into the middle of any given construct, which is a process of at least several days. Other groups therefore have introduced more surgical approaches by creating a vascular network first and then transplant completely vascularized constructs using arterial and venous loop models. Such models are by far more complex and depend on an enormous microsurgical expertise to guarantee successful vascular connections on an supramicrosurgical level [5]. Utilizing this technique in small animal models and in a clinically relevant scale in large animal models [6], first clinical results with long-term success over more than 4 years are now on the verge.

While all 'classical' TE approaches depend on the use of autologous adult or progenitor cells with more or less capacity of cell renewal and limited cell division cycles, to avoid the problem of immunosuppression, another brilliant perspective was added when techniques of harvesting and modulating adult or embryonic stem cells were standardized, and then logically became a target in TE. The utilization of the stem-cell capacity seems to be a logical step within the TE concept and has spread much optimism among the TE community. Especially for the substitution of worn out tissue, it is more than promising that organ regeneration with stem cells, formerly unknown in adult mammals, seems to be as logical as the basic TE concept was thought to be when the idea first came up in the early 1990s.

According to Atala, the stem-cell field is also advancing rapidly, opening new avenues for this type of therapy. For example, therapeutic cloning and cellular reprogramming may one day provide a potentially limitless source of cells for TE applications. While stem cells are still in the research phase, some therapies arising from TE endeavours have already entered the clinical setting successfully, indicating the promise RM holds for the future [7]. The focus in TE and RM for human applications is currently directed towards adult stem cells, mesenchymal stem cells (MSC) and induced pluripotent stem cells [8]. The latter ones are somatic cells (such as skin-derived fibroblasts etc.), reprogrammed into an embryonic cell-like state. This is achieved with somatic cell nuclear transfer or through ectopic expression of pluripotency-specific transcription factors with subsequent culturing under embryonic stem-cell conditions [9]. Although numerous laboratories around the world are now engaged in the development of new tools such as stem cells and biologically active scaffolds, it is not yet clear if this technique really is at the threshold of maturity as a clinical method for restoration of organ function in humans, as some authors propagate [7].

Nevertheless, in general, it has become clear that the stem-cell promise has entered the field of TE and the term Regenerative Medicine is now conceived as the superordinate concept [10–12]. It has

been said that scientists now are taking fresh looks at well-known clinical problems of replacement of a large variety of organs, such as the bone [13], skin [14], the spinal cord [15], peripheral nerves [16], articular cartilage [17], the conjunctiva [18], heart valves [19] and urological organs [20], while still other investigators are working out the mechanistic pathways of regeneration and the theoretical implications of growing back organs in an adult [21].

Intrinsic and extrinsic vascularization of TE constructs – the AV-loop model

The basic potential of regeneration of three dimensional tissue structures depends on the presence of a suitable biomaterial that can promote cell growth and proliferation. Only when such biomaterials can effectively interact with the surrounding tissue and incite the host to populate the graft with new tissue, the survival of any transplanted cell within a construct is made possible and can ultimately lead to the regeneration of lost or malfunctioning tissue [22]. The induction of angiogenesis by means of microsurgical creation of an arterio-venous loop has been shown to be one very effective way to achieve full blood supply to TE constructs from the very first moment after transplantation [23–29] (Fig. 2).

The clinical scenario encompasses a number of difficult problems to solve as radiation therapy has become one of the main treatment options within the concept of multimodal tumour therapy. Following irradiation, the recipient site for any kind of tissue transfer lacks a normal vascularization potential. Neovascularization cannot easily appear from irradiated wound beds, and makes the common approach of transplanting TE constructs that solely rely on extrinsic vascularization from the periphery of the construct to appear compulsively ineffective [30]. As oxygen and nutrition supply of the cells is limited to a maximum range of 200 μm into a given matrix, the nutritional supply by diffusion alone necessarily brings difficulties with itself. To overcome the oftentimes limited survival of cells in the centre of a large construct – because of the initially lacking vascularization – the *in vivo* creation of arterio-venous loops evolved over the last years. It aims to generate constructs with a predictable and dedicated neovascular network that allows for sufficient vascular supply directly after the vascular connection to the recipient bed has been created [31] (Fig. 3). This necessitates microsurgical skills and makes this approach highly dependent on expert microsurgeons. This concept is clinically used in microsurgical centres to customize tissues that are thought to be transplanted into a special problem wound or defect zone as so called pre-fabricated or pre-laminated free flaps [32–44] that rely on the intrinsic mode of vascularization and are not depending on extrinsic vascularization. Although this type of flap is usually not the first line of defect coverage, the requirements for more complex clinical tissue replacement with various surfaces or customized soft- and hard-tissue flaps is increasing. However, this modification clinically depends on at least two different interventions when 3D complex tissue substitutes are to be implanted following their prevascularization [45, 46]. The critical influence of the local recipient environment is minimized with this technique.

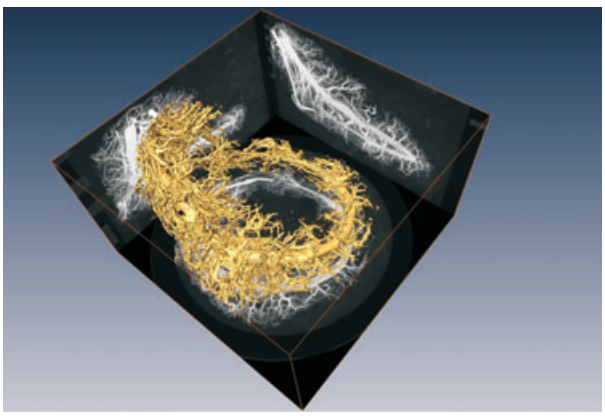

Fig. 2 Micro Ct of TricOS® matrix in an AV-loop within an isolation chamber at 2 weeks after implantation showing sprouting of new microvessels out of the arteriovenous loop.

Since intrinsic vascularization was found to be highly effective in transplanting viable cells within a 3D tissue-engineered construct, our group has combined this method with application of fibrin-gel immobilized angiogenetic growth factors [24, 25] because of its controlled release using fibrin gel as a drug-release system [47–56]. We also combined the arteriovenous loop model with the standard approach of extrinsic vascularization to enhance the ingrowth of nourishing vessels and the arborization of the microvasculature. In summary, Arkudas *et al.* were able to show that the combination of extrinsic and intrinsic pathways significantly accelerates axial vascularization of bioartificial tissues [57]. When the arteriovenous loop model that combines extrinsic and intrinsic vascularization modes to enhance vascularization of bioartificial matrices was modified in an experimental setting, an arteriovenous loop was created in the medial thighs of 24 rats and this loop was placed in a newly developed titanium chamber. At various explantation time-points between 2 and 8 weeks, constructs were perfused by differently coloured dyes to determine the amount of tissue vascularized by either the intrinsic or the extrinsic vascular pathway. Although an equal number of blood vessels were found originating from the centre and the periphery, 83% of all vessels were found to have a connection to the intrinsic arteriovenous loop system as soon as 2 weeks after implantation [57]. In this study, a continuous increase of the relative proportion of vessels connected to the arteriovenous loop was found over the observation period. At 8 weeks, communications between the newly formed vessels and the arteriovenous loop were visible in 97% of all vessels [57]. By this study, it was shown for the first time that an enhancement of angiogenesis in an axially vascularized tissue construct by an additional extrinsic vascular pathway is feasible. By 2 weeks, both pathways showed connections. This finding indicates that transplantation of the entire construct using the AV-loop as a pedicle can be performed at an earlier time-point than using either technique alone.

Gene transfer techniques

Bleiziffer *et al.* gave an overview on the therapeutic potential of gene transfer strategies in combination with TE and RM [58]. This group

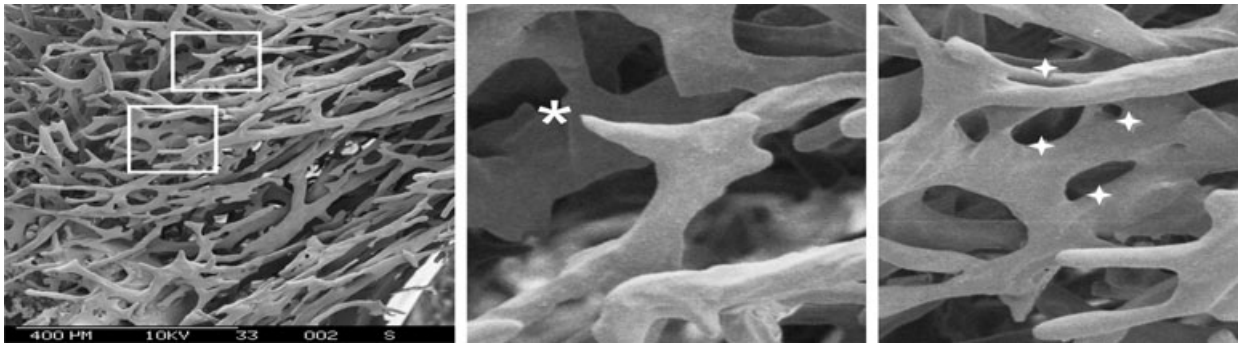

Fig. 3 Corrosion Cast of an axial neovascular assembly, *in vivo* vascular construct, 2 weeks after vascular induction. Left: Overview of a nascent capillary network. Middle: sprouting angiogenic event. A new capillary sprout emerging from the parent vessel by endothelial pericytic proliferation Right: intussusceptive angiogenic events. The parent vessel is divided into two distinct new vessels for the purpose of vascular growth or remodelling.

also studied the application of endothelial progenitor cells to be utilized for TE and RM purposes [4, 59]. In summary, modulating the genetic code of cells utilized in TE and RM holds promise in various ways and has been under intense investigation for years. Viral vectors in particular have the advantage of superior transduction efficiency, but their use is limited by safety concerns [60–71]. Large-scale transduction of target cells is most efficiently achieved using adenovirus at high titres, which involves the risk of vector toxicity [72]. Long-term gene expression can be achieved using retrovirus, but insertional viral remnants remain a major concern. Polymer-based gene delivery systems offer promising advantages over traditional gene delivery systems by prolonging gene expression, avoiding distribution to distant tissues and systemic circulation, reduced toxicity and decreased immune response [73].

Optimization and control of gene expression remains a challenge that needs to be addressed, given the deleterious effects of inadequately high levels of transgene expression [74]. Several technical concepts have been developed to address these issues. Yao *et al.* developed a Tetracycline-repressor-based highly sensitive tetracycline-dependent transcription switch (T-Rex™ System, Invitrogen). The T-Rex™ system was integrated into a replication-deficient HSV-1 vector. It could be demonstrated that *in vitro* infection of different cell types using the tet-conditional virus resulted in tetracycline dependent 300–1000-fold regulation of expression [74].

Another promising direction is the use of stem cells and progenitor cells as a vehicle for gene delivery. Currently, the ethical issues associated with the use of embryonic stem cells make adult stem cells and progenitor cells a particularly attractive choice. Adult tissues require these cell types for continuous self-renewal [23, 75–77]. Multipotent stem cells are found in most adult tissues and can generate a certain spectrum of differentiated cell lineages depending on their location. Progenitor cells, on the other hand, are unipotent, capable of generating one specific cell type. In the case of shortage of an autologous cell source, allogenic or xenogeneic sources become an option. Even elimination by the host immune system is the key obstacle to xenotranspantation that must be solved to guarantee success. Taken together, gene delivery in combination with TE applications may greatly enhance therapeutic options to (re-)generate tissue severed or lost by disease or trauma. Polymeric release and substrate-mediated gene delivery from natural or synthetic scaffolds can be carried out through both viral and non-viral vector systems. The efficacy of gene delivery systems in TE could be further enhanced by employing gene expression regulation, co-transplantation of stem cells or progenitor cells and use of xenogenic tissue or cell sources.

Combining mesenchymal stem cells with the AV-loop model of intrinsic vascularization

As the clinical application of MSC to regenerate defect or lost tissue would face serious regulatory problems in terms of producing and transplanting such cells outside the operating room before they can be retransplanted to the donor and recipient, we investigated ways to circumvent this barrier. In a large animal model we were able to show that directly auto-transplanted MSC induce bone formation in a ceramic bone substitute in an ectopic sheep model [78].

As bone defect regeneration is believed to be one of the more challenging issues in TE, we have chosen this critical model to study the effect of various vascularization processes in TE [23, 26, 79–81] (Fig. 4). Recently, there has been an increasing focus on the use of MSC in this context [82]. In most animal transplantation models, MSC are isolated and expanded before auto cell transplantation, which might be critical for clinical application in the future. Hence, this study compares the potential of directly auto-transplanted *versus* *in vitro*-expanded MSC with or without bone morphogenetic protein-2 (BMP-2) to induce bone formation in a large volume ceramic bone substitute in the sheep model. In these experiments, MSC were isolated from bone marrow aspirates and either directly auto-transplanted or expanded *in vitro* and characterized using fluorescence-activated cell sorting (FACS) and RT-PCR analysis before subcutaneous implantation in combination with BMP-2 and β-tricalcium phosphate/hydroxyapatite (β-TCP/HA) granules. Constructs were explanted after 1 to 12 weeks followed by histological and RT-PCR evaluation. Sheep MSC were CD29(+), CD44(+) and CD166(+)

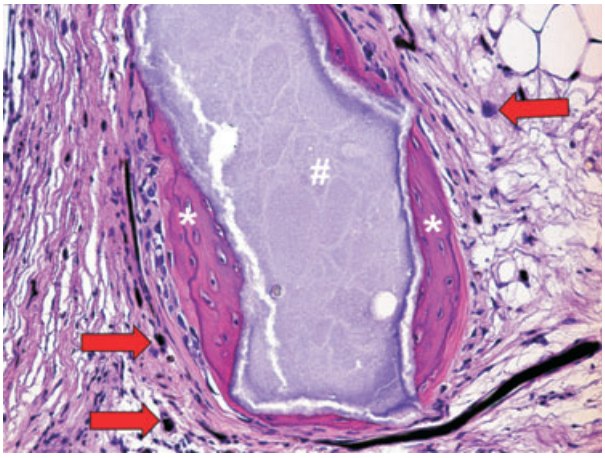

Fig. 4 HE staining of newly formed bone (*) attached to a HA/TCP matrix (TricOS®, #) 8 weeks after implantation of an arteriovenous loop in the medial thigh of a rat and application of primary osteoblasts and 2.5 + g BMP2. Vascularization of matrices is visible by India Ink-filled vessels (arrows).

after selection by Ficoll gradient centrifugation, while directly auto-transplanted MSC-populations expressed CD29 and CD166 at lower levels [78]. Both, directly auto-transplanted and expanded MSC, were found to be constantly proliferating, and showed a decreasing apoptosis over time *in vivo*. Directly auto-transplanted MSC led to *de novo* bone formation in a heterotopic sheep model using a β-TCP/HA matrix comparable to the application of 60 μg/ml BMP-2 only or implantation of expanded MSC. Bone matrix proteins were up-regulated in constructs following direct auto-transplantation and in expanded MSC as well as in BMP-2 constructs. Up-regulation was detected using immunohistology methods and RT-PCR. Dense vascularization was demonstrated by CD31 immunohistology staining in all three groups. As, in this model, ectopic bone could be generated by using directly auto-transplanted or expanded MSC with β-TCP/HA granules alone, it can be concluded that BMP-2 stimulation might become dispensable in the future. This would provide an attractive and a clinically feasible approach to bone TE [78].

TE and RM in the context of cancer research

Naturally, many of the advances in cell culture and the studies of cell–cell as well as cell–biomaterial interactions in TE and RM have also gained attraction from other sides in medical research. A better understanding of cell adherence phenomena and the ability of cell-scaffold constructs to mimic biological processes, especially with regard to the vascularization cascades, could also help to better understand mechanisms of tumour angiogenesis by analysing the basic mechanisms of morphogenesis, differentiation and cancer development and progression [83]. Models to study the behaviour of tumour cells under culture conditions and in the context of biomaterials as a carrier of malignant cells in various experimental conditions could also be used to develop anti-cancer therapies. Hence, technology platforms originally developed for TE applications produce valuable models that mimic three-dimensional (3D) tissue organization and function to enhance the understanding of cell/tissue function under normal and pathological situations [84]. Investigating angiogenetic processes and factors in tumourigenesis can be seen as a key to establish ways of targeting angiogenesis in tumours. As an offspring from angiogenesis research, meanwhile several anti-angiogenic agents have been accepted for clinical application as attractive targeted therapeutics for the treatment of cancer. When the areas of tumour angiogenesis, combination therapies and drug delivery systems are combined, this knowledge is closely related to the understanding of the basic principles that are applied in TE models. Studies with 3D model systems have repeatedly identified complex interacting roles of matrix stiffness and composition, integrins, growth factor receptors and signalling in growth and cancer [84]. These insights suggest that plasticity, regulation and suppression of these processes can provide strategies and therapeutic targets for future cancer therapies. Hutmacher *et al.* have stated that the historical perspective of the fields of TE and controlled release of therapeutics, including inhibitors of angiogenesis in tumours, is becoming clearly evident as a major future advance in merging these fields. New delivery systems are expected to greatly enhance the ability to deliver drugs locally and in therapeutic concentrations to relevant sites in living organisms. Investigating the phenomena of angiogenesis and anti-angiogenesis in 3D *in vivo* models such as the Arterio-Venous (AV) loop mode in a separated and isolated chamber within a living organism adds another and reproducibly significant horizon to this perspective and opens new modalities for translational research in this field [84, 85].

Newly discovered cells of potential benefit for RM

Another exciting discovery are telocytes, a new type on interstitial cells, that might well influence RM approaches, especially in the context of cardiac regeneration. The short history of telocytes detection was published in 2010, as a case of serendipity [86]. The existence of these cells was reported within interstitium of many cavitary and non-cavitary organs [87–103], as well as in heart (epicardium [104], myocardium [87, 88] and endocardium [105]) (Fig. 5).

Telocytes have unique features, as it was demonstrated in cell cultures and by electron microscopy. The main characteristic that clearly distinguishes telocytes from all other interstitial cell types is the presence cell body prolongations (usually 1-5 visible cell prolongations), which were termed telopodes. Thus, the shortest definition of telocyte is: cell with telopodes. The main features of telopodes are: (1) their length: usually tens to hundreds of μm, (2) the monofiliform aspect: an alternation of dilated segments (termed podoms) and thin segments (termed podomers), (3) podomers are very thin (in some regions thinner than 0.2 μm, being under the resolving power of light microscopy), (4) podoms accommodate mitochondria, elements of endoplasmic reticulum and caveolae, which are involved in calcium

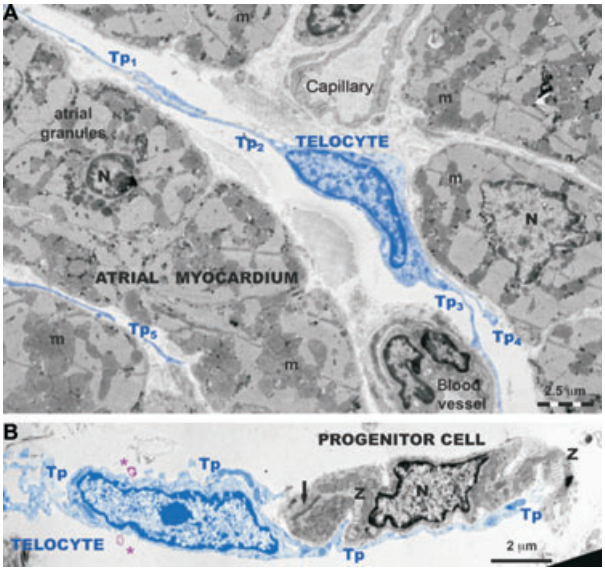

Fig. 5 Transmission electron microscopy. (**A**) Human atrial myocardium. A telocyte (digitally coloured in blue) is located among atrial cardiomyocytes (the atrial granules are obvious). Telopodes are situated in between cardiomyocytes (Tp1, Tp2, Tp4, Tp5), and another telopode (Tp3) with close spatial relation with the blood vessels. The apposition of Tp1 and Tp2 suggests that telocytes are realizing a network, by homo-cellular junctions. (**B**) 1-yr old mouse subepicardial stem-cell niche. Close relationship in between a telocyte and its telopode with a cardiomyocyte progenitor (the cell containing leptofibrills – 'zebra-like' striations – Z). The arrow indicates desmosome – the origin for a future intercalated disc. The telocyte have shedding vesicles (asterisks) – digitally coloured in violet. N – nucleus, m – mitochondria Kindly provided by Prof. L.M. Popescu, National Institute of Pathology, Bucharest, Romania.

movements. Telopodes have a dichotomous branching pattern, making a three-dimensional network due to homo- and heterocellular junctions. Telocytes release shed vesicles and/or exosomes, thus sending macromolecular signals to neighbouring cells and thereby modifying their transcriptional activity, eventually.

By transmission electron microscopy (TEM) were identified cardiac stem-cell niches in subepicardium [106, 107], pulmonary subepithelial niches in the bronchiolar tree [97], as well as non-satellite (resident) progenitor cell niches among skeletal muscle fibres [95]. In all aforementioned structures, telocytes and telopodes were identified in close contact with progenitor cells. Moreover, electron microscope tomography revealed complex nanoscopic junctions between telocytes, or between telocytes and resident progenitor cells [93, 94, 106]. Apparently, telopodes provide tracks for the 'evolution' (sliding) of precursor cells towards their mature condition, and also their integration into organ microscopic architecture. Telocytes, *via* their paracrine secretion (including microRNAs), produce an adequate microenvironment for precursor cells.

Cardiac regeneration might have the potential to reverse the consequences of myocardial infarction. In this context, stem-cell therapy was promoted as a potential solution.

Experimental myocardial infarction models have been implemented for studying the ultrastructural recovery after the acute obliteration of coronary artery. Telocytes are (in)directly involved in neo-angiogenesis after (experimental) myocardial infarction [108]. By TEM, immunocytochemistry and analysis of expression of several proangiogenic microRNAs were provided evidence for telocytes involvement in neo-angiogenesis after myocardial infarction. Ultrastructurally, there are close spatial relationships between telocytes and neoangiogenic elements. Telocytes have multiple direct nanocontacts with endothelial cells, where the extracellular space seems obliterated. On the other hand, telocytes are involved in neoangiognesis, presumably *via* paracrine secretion, as shown by immunocytochemistry for VEGF or NOS2. In addition, by RTqPCR was demonstrated the positive expression of telocytes for several angiogenic microRNAs, such as let-7e, 10a, 21, 27b, 100, 126-3p, 130a, 143, 155 and 503.

These findings suggest an important participation of telocytes in neo-angiogenesis during the late stage of myocardial infarction. This adds to our understanding of cellular and molecular events and opens another perspective of potential keys to solve the present day problems in TEand RM.

Summary

In summary, this article systematically seeks to comment some of the most recent advances in the field of TE and RM. The evolving area of stem cells will have to be addressed in a separate overview. By highlighting selected topics of specific advances with various technical approaches, the variety of interconnections and possibilities is clearly visible. The dynamic of these developments underlines that different fields of biotechnology must be seen as a driving force in the development of new disciplines that might change conventional medicine and health systems considerably in the near future. Following the initial hype with TE, it is now the broader term of RM that is concerned with the development and application of innovative medical therapies that aim to support the regeneration of damaged organs or to fully or partially restore damaged parts of the human organism. This means that in addition to the more out-of-the-human-body *ex vivo* approach of the original TE concept, RM seeks to activate the natural healing resources of the body to achieve a full restoration of health, which might ultimately and optimally be achieved with a one-time treatment.

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Conflicts of Interest Statement

The authors confirm that there are no conflicts of interest.

References

1. **Langer R, Vacanti JP.** Tissue engineering. *Science.* 1993; 260: 920–6.
2. **Vacanti CA.** History of tissue engineering and a glimpse into its future. *Tissue Eng.* 2006; 12: 1137–42.
3. **Atala A.** Bioengineered tissues for urogenital repair in children. *Pediatr Res.* 2008; 63: 569–75.
4. **Bleiziffer O, Hammon M, Naschberger E, *et al.*** Endothelial progenitor cells are integrated in newly formed capillaries and alter adjacent fibrovascular tissue after subcutaneous implantation in a fibrin matrix. *J Cell Mol Med.* 2011; 15: 2452–61.
5. **Polykandriotis E, Drakotos D, Arkudas A, *et al.*** Factors influencing successful outcome in the arteriovenous loop model: a retrospective study of 612 loop operations. *J Reconstr Microsurg.* 2011; 27: 11–8.
6. **Beier JP, Hess A, Loew J, *et al.*** De novo generation of an axially vascularized processed bovine cancellous-bone substitute in the sheep arteriovenous-loop model. *Eur Surg Res.* 2011; 46: 148–55.
7. **Atala A.** Human embryonic stem cells: early hints on safety and efficacy. *Lancet.* 2012; 379: 689–90.
8. **Polykandriotis E, Popescu LM, Horch RE.** Regenerative medicine: then and now–an update of recent history into future possibilities. *J Cell Mol Med.* 2010; 14: 2350–8.
9. **Xu RH, Peck RM, Li DS, *et al.*** Basic FGF and suppression of BMP signaling sustain undifferentiated proliferation of human ES cells. *Nat Methods.* 2005; 2: 185–90.
10. **Arkudas A, Beier JP, Pryymachuk G, *et al.*** Automatic quantitative micro-computed tomography evaluation of angiogenesis in an axially vascularized tissue-engineered bone construct. *Tissue Eng Part C Methods.* 2010; 16: 1503–14.
11. **Roemeling-van Rhijn M, Weimar W, Hoogduijn MJ.** Mesenchymal stem cells: application for solid-organ transplantation. *Curr Opin Organ Transplant.* 2012; 17: 55–62.
12. **Cossetti C, Alfaro-Cervello C, Donega M, *et al.*** New perspectives of tissue remodelling with neural stem and progenitor cell-based therapies. *Cell Tissue Res.* 2012; in press: doi: 10.1007/s00441-012-1341-8
13. **Mistry AS, Pham QP, Schouten C, *et al.*** *In vivo* bone biocompatibility and degradation of porous fumarate-based polymer/alumoxane nanocomposites for bone tissue engineering. *J Biomed Mater Res A.* 2010; 92: 451–62.
14. **Jin G, Prabhakaran MP, Nadappuram BP, *et al.*** Electrospun Poly(l-Lactic Acid)-co-Poly(varepsilon-Caprolactone) Nanofibres Containing Silver Nanoparticles for Skin-Tissue Engineering. *J Biomater Sci Polym Ed.* 2012; in press: doi: jbs3388 [pii] 10.1163/156856211X617399
15. **Liu T, Houle JD, Xu J, *et al.*** Nanofibrous Collagen Nerve Conduits for Spinal Cord Repair. *Tissue Eng Part A.* 2012; in press: doi: 10.1089/ten.TEA.2011.0430
16. **Suri S, Han LH, Zhang W, *et al.*** Solid free-form fabrication of designer scaffolds of hyaluronic acid for nerve tissue engineering. *Biomed Microdevices.* 2011; 13: 983–93.
17. **Trimborn M, Endres M, Bommer C, *et al.*** Karyotyping of human chondrocytes in scaffold-assisted cartilage tissue engineering. *Acta Biomater.* 2011; 8: 1519–29.
18. **Silva GA, Silva NF, Fortunato TM.** Stem cell and tissue engineering therapies for ocular regeneration. *Curr Stem Cell Res Ther.* 2011; 6: 255–72.
19. **Wong ML, Leach JK, Athanasiou KA, Griffiths LG.** The role of protein solubilization in antigen removal from xenogeneic tissue for heart valve tissue engineering. *Biomaterials.* 2011; 32: 8129–38.
20. **Kollhoff DM, Cheng EY, Sharma AK.** Urologic applications of engineered tissue. *Regen Med.* 2011; 6: 757–65.
21. **Atala A.** Regenerative medicine strategies. *J Pediatr Surg.* 2012; 47: 17–28.
22. **Cassell OC, Hofer SO, Morrison WA, Knight KR.** Vascularisation of tissue-engineered grafts: the regulation of angiogenesis in reconstructive surgery and in disease states. *Br J Plast Surg.* 2002; 55: 603–10.
23. **Arkudas A, Beier JP, Heidner K, *et al.*** Axial prevascularization of porous matrices using an arteriovenous loop promotes survival and differentiation of transplanted autologous osteoblasts. *Tissue Eng.* 2007; 13: 1549–60.
24. **Arkudas A, Tjiawi J, Bleiziffer O, *et al.*** Fibrin gel-immobilized VEGF and bFGF efficiently stimulate angiogenesis in the AV loop model. *Mol Med.* 2007; 13: 480–7.
25. **Arkudas A, Pryymachuk G, Hoereth T, *et al.*** Dose-finding study of fibrin gel-immobilized vascular endothelial growth factor 165 and basic fibroblast growth factor in the arteriovenous loop rat model. *Tissue Eng Part A.* 2009; 15: 2501–11.
26. **Kneser U, Polykandriotis E, Ohnolz J, *et al.*** Engineering of vascularized transplantable bone tissues: induction of axial vascularization in an osteoconductive matrix using an arteriovenous loop. *Tissue Eng.* 2006; 12: 1721–31.
27. **Erol OO, Spira M.** New capillary bed formation with a surgically constructed arteriovenous fistula. *Surg Forum.* 1979; 30: 530–1.
28. **Mian R, Morrison WA, Hurley JV, *et al.*** Formation of new tissue from an arteriovenous loop in the absence of added extracellular matrix. *Tissue Eng.* 2000; 6: 595–603.
29. **Lokmic Z, Stillaert F, Morrison WA, *et al.*** An arteriovenous loop in a protected space generates a permanent, highly vascular, tissue-engineered construct. *Faseb J.* 2007; 21: 511–22.
30. **Polykandriotis E, Horch RE, Arkudas A, *et al.*** Intrinsic *versus* extrinsic vascularization in tissue engineering. *Adv Exp Med Biol.* 2006; 585: 311–26.
31. **Polykandriotis E, Tjiawi J, Euler S, *et al.*** The venous graft as an effector of early angiogenesis in a fibrin matrix. *Microvasc Res.* 2008; 75: 25–33.
32. **Schipper J, Boedeker CC, Horch RE, *et al.*** The free vastus lateralis flap for reconstruction in ablative oncologic head and neck surgery. *Eur J Surg Oncol.* 2006; 32: 103–7.
33. **Horch RE, Schipper J, Laszig R.** Retroauricular skin flap and primary Z-plasty for donor site closure in partial ear reconstruction. *J Laryngol Otol.* 2003; 117: 487–9.
34. **Schipper J, Ridder GJ, Maier W, *et al.*** Laryngotracheal reconstruction using prefabricated and preconditioned composite radial forearm free flaps. A report of two cases. *Auris Nasus Larynx.* 2007; 34: 253–8.
35. **Garfein ES, Orgill DP, Pribaz JJ.** Clinical applications of tissue engineered constructs. *Clin Plast Surg.* 2003; 30: 485–98.
36. **Pribaz JJ, Fine N, Orgill DP.** Flap prefabrication in the head and neck: a 10-year experience. *Plast Reconstr Surg.* 1999; 103: 808–20.
37. **Pribaz JJ, Fine NA.** Prelamination: defining the prefabricated flap–a case report and review. *Microsurgery.* 1994; 15: 618–23.
38. **Horch RE.** [The development of plastic surgery: retrospective view of 80 years of "Der Chirurg" (The Surgeon)]. *Chirurg.* 2009; 80: 1132–9.
39. **Shen TY.** Microvascular transplantation of prefabricated free thigh flap. *Plast Reconstr Surg.* 1982; 69: 568.
40. **Hyakusoku H, Okubo M, Umeda T, Fumiiri M.** A prefabricated hair-bearing island flap

for lip reconstruction. *Br J Plast Surg.* 1987; 40: 37–9.

41. **Hirase Y, Valauri FA, Buncke HJ.** Neovascularized bone, muscle, and myo-osseous free flaps: an experimental model. *J Reconstr Microsurg.* 1988; 4: 209–15.
42. **Hirase Y, Valauri FA, Buncke HJ, Newlin LY.** Customized prefabricated neovascularized free flaps. *Microsurgery.* 1987; 8: 218–24.
43. **Morrison WA, Dvir E, Doi K, *et al.*** Prefabrication of thin transferable axial-pattern skin flaps: an experimental study in rabbits. *Br J Plast Surg.* 1990; 43: 645–54.
44. **Khouri RK, Upton J, Shaw WW.** Prefabrication of composite free flaps through staged microvascular transfer: an experimental and clinical study. *Plast Reconstr Surg.* 1991; 87: 108–15.
45. **Polykandriotis E, Arkudas A, Euler S, *et al.*** [Prevascularisation strategies in tissue engineering]. *Handchir Mikrochir Plast Chir.* 2006; 38: 217–23.
46. **Polykandriotis E, Arkudas A, Horch RE, *et al.*** Autonomously vascularized cellular constructs in tissue engineering: opening a new perspective for biomedical science. *J Cell Mol Med.* 2007; 11: 6–20.
47. **Hudlett P, Neuville A, Miternique A, *et al.*** Angiogenesis and arteriogenesis are increased in fibrin gel chambers implanted in prehypertensive spontaneously hypertensive rats. *J Hypertens.* 2005; 23: 1559–64.
48. **Jackson MR, MacPhee MJ, Drohan WN, Alving BM.** Fibrin sealant: current and potential clinical applications. *Blood Coagul Fibrinolysis.* 1996; 7: 737–46.
49. **Rifkin DB, Moscatelli D.** Recent developments in the cell biology of basic fibroblast growth factor. *J Cell Biol.* 1989; 109: 1–6.
50. **Quirinia A, Viidik A.** The effect of recombinant basic fibroblast growth factor (bFGF) in fibrin adhesive vehicle on the healing of ischaemic and normal incisional skin wounds. *Scand J Plast Reconstr Surg Hand Surg.* 1998; 32: 9–18.
51. **Sahni A, Baker CA, Sporn LA, Francis CW.** Fibrinogen and fibrin protect fibroblast growth factor-2 from proteolytic degradation. *Thromb Haemost.* 2000; 83: 736–41.
52. **Sahni A, Francis CW.** Vascular endothelial growth factor binds to fibrinogen and fibrin and stimulates endothelial cell proliferation. *Blood.* 2000; 96: 3772–8.
53. **Sahni A, Odrljin T, Francis CW.** Binding of basic fibroblast growth factor to fibrinogen and fibrin. *J Biol Chem.* 1998; 273: 7554–9.
54. **van Hinsbergh VW, Collen A, Koolwijk P.** Role of fibrin matrix in angiogenesis. *Ann N Y Acad Sci.* 2001; 936: 426–37.
55. **Wong C, Inman E, Spaethe R, Helgerson S.** Fibrin-based biomaterials to deliver human growth factors. *Thromb Haemost.* 2003; 89: 573–82.
56. **Ye Q, Zund G, Benedikt P, *et al.*** Fibrin gel as a three dimensional matrix in cardiovascular tissue engineering. *Eur J Cardiothorac Surg.* 2000; 17: 587–91.
57. **Arkudas A, Pryymachuk G, Beier JP, *et al.*** Combination of extrinsic and intrinsic pathways significantly accelerates axial vascularization of bioartificial tissues. *Plast Reconstr Surg.* 2012; 129: 55e–65e.
58. **Bleiziffer O, Eriksson E, Yao F, *et al.*** Gene transfer strategies in tissue engineering. *J Cell Mol Med.* 2007; 11: 206–23.
59. **Bleiziffer O, Horch RE, Hammon M, *et al.*** T17b murine embryonal endothelial progenitor cells can be induced towards both proliferation and differentiation in a fibrin matrix. *J Cell Mol Med.* 2009; 13: 926–35.
60. **Roizman B.** The function of herpes simplex virus genes: a primer for genetic engineering of novel vectors. *Proc Natl Acad Sci USA.* 1996; 93: 11307–12.
61. **Siemens DR, Austin JC, Hedican SP, *et al.*** Viral vector delivery in solid-state vehicles: gene expression in a murine prostate cancer model. *J Natl Cancer Inst.* 2000; 92: 403–12.
62. **Chandler LA, Doukas J, Gonzalez AM, *et al.*** FGF2-Targeted adenovirus encoding platelet-derived growth factor-B enhances de novo tissue formation. *Mol Ther.* 2000; 2: 153–60.
63. **Svensjo T, Yao F, Pomahac B, *et al.*** Cultured autologous fibroblasts augment epidermal repair. *Transplantation.* 2002; 73: 1033–41.
64. **Andree C, Voigt M, Wenger A, *et al.*** Plasmid gene delivery to human keratinocytes through a fibrin-mediated transfection system. *Tissue Eng.* 2001; 7: 757–66.
65. **Yang KG, Saris DB, Geuze RE, *et al.*** Impact of expansion and redifferentiation conditions on chondrogenic capacity of cultured chondrocytes. *Tissue Eng.* 2006; 12: 2435–47.
66. **Lee RJ, Springer ML, Blanco-Bose WE, *et al.*** VEGF gene delivery to myocardium: deleterious effects of unregulated expression. *Circulation.* 2000; 102: 898–901.
67. **Vacanti JP, Morse MA, Saltzman WM, *et al.*** Selective cell transplantation using bioabsorbable artificial polymers as matrices. *J Pediatr Surg.* 1988; 23: 3–9.
68. **Chandler LA, Gu DL, Ma C, *et al.*** Matrix-enabled gene transfer for cutaneous wound repair. *Wound Repair Regen.* 2000; 8: 473–9.
69. **Tyrone JW, Mogford JE, Chandler LA, *et al.*** Collagen-embedded platelet-derived growth factor DNA plasmid promotes wound healing in a dermal ulcer model. *J Surg Res.* 2000; 93: 230–6.
70. **Erdag G, Medalie DA, Rakhorst H, *et al.*** FGF-7 expression enhances the performance of bioengineered skin. *Mol Ther.* 2004; 10: 76–85.
71. **Berry M, Gonzalez AM, Clarke W, *et al.*** Sustained effects of gene-activated matrices after CNS injury. *Mol Cell Neurosci.* 2001; 17: 706–16.
72. **Lehrman S.** Virus treatment questioned after gene therapy death. *Nature.* 1999; 401: 517–8.
73. **Pannier AK, Shea LD.** Controlled release systems for DNA delivery. *Mol Ther.* 2004; 10: 19–26.
74. **Yao F, Theopold C, Hoeller D, *et al.*** Highly efficient regulation of gene expression by tetracycline in a replication-defective herpes simplex viral vector. *Mol Ther.* 2006; 13: 1133–41.
75. **Vacanti CA.** The history of tissue engineering. *J Cell Mol Med.* 2006; 10: 569–76.
76. **Vacanti CA, Vacanti JP.** The science of tissue engineering. *Orthop Clin North Am.* 2000; 31: 351–6.
77. **Beier JP, Bitto FF, Lange C, *et al.*** Myogenic differentiation of mesenchymal stem cells co-cultured with primary myoblasts. *Cell Biol Int.* 2011; 35: 397–406.
78. **Boos AM, Loew JS, Deschler G, *et al.*** Directly auto-transplanted mesenchymal stem cells induce bone formation in a ceramic bone substitute in an ectopic sheep model. *J Cell Mol Med.* 2011; 15: 1364–78.
79. **Kneser U, Stangenberg L, Ohnolz J, *et al.*** Evaluation of processed bovine cancellous bone matrix seeded with syngenic osteoblasts in a critical size calvarial defect rat model. *J Cell Mol Med.* 2006; 10: 695–707.
80. **Fiegel HC, Pryymachuk G, Rath S, *et al.*** Foetal hepatocyte transplantation in a vascularized AV-Loop transplantation model in the rat. *J Cell Mol Med.* 2010; 14: 267–74.
81. **Bach AD, Arkudas A, Tjiawi J, *et al.*** A new approach to tissue engineering of vascularized skeletal muscle. *J Cell Mol Med.* 2006; 10: 716–26.
82. **Rath SN, Strobel LA, Arkudas A, *et al.*** Osteoinduction and survival of osteoblasts and bone-marrow stromal cells in 3D biphasic calcium phosphate scaffolds

under static and dynamic culture conditions. *J Cell Mol Med.* 2012; in press: doi: 10.1111/j.1582-4934.2012.01545.x

83. **Yang J, Weinberg RA.** Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. *Dev Cell.* 2008; 14: 818–29.
84. **Hutmacher DW, Horch RE, Loessner D, *et al.*** Translating tissue engineering technology platforms into cancer research. *J Cell Mol Med.* 2009; 13: 1417–27.
85. **Polykandriotis E, Euler S, Arkudas A, *et al.*** Regression and persistence: remodelling in a tissue engineered axial vascular assembly. *J Cell Mol Med.* 2009; 13: 4166–75.
86. **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: 729–40.
87. **Kostin S.** Myocardial telocytes: a specific new cellular entity. *J Cell Mol Med.* 2010; 14: 1917–21.
88. **Suciu L, Nicolescu MI, Popescu LM.** Cardiac telocytes: serial dynamic images in cell culture. *J Cell Mol Med.* 2010; 14: 2687–92.
89. **Suciu L, Popescu LM, Gherghiceanu M, *et al.*** Telocytes in human term placenta: morphology and phenotype. *Cells Tissues Organs.* 2010; 192: 325–39.
90. **Cantarero Carmona I, Luesma Bartolome MJ, Junquera Escribano C.** Identification of telocytes in the lamina propria of rat duodenum: transmission electron microscopy. *J Cell Mol Med.* 2011; 15: 26–30.
91. **Zheng Y, Bai C, Wang X.** Telocyte morphologies and potential roles in diseases. *J Cell Physiol.* 2012; 227: 2311–7.
92. **Zheng Y, Li H, Manole CG, *et al.*** Telocytes in trachea and lungs. *J Cell Mol Med.* 2011; 15: 2262–8.
93. **Gherghiceanu M, Popescu LM.** Heterocellular communication in the heart: electron tomography of telocyte-myocyte junctions. *J Cell Mol Med.* 2011; 15: 1005–11.
94. **Gherghiceanu M, Popescu LM.** Cardiac telocytes - their junctions and functional implications. *Cell Tissue Res.* 2012; in press: doi: 10.1007/s00441-012-1333-8
95. **Hinescu ME, Gherghiceanu M, Suciu L, Popescu LM.** Telocytes in pleura: two- and three-dimensional imaging by transmission electron microscopy. *Cell Tissue Res.* 2011; 343: 389–97.
96. **Gevaert T, De Vos R, Van Der Aa F, *et al.*** Identification of telocytes in the upper lamina propria of the human urinary tract. *J Cell Mol Med.* 2011; in press: doi: 10.1111/j.1582-4934.2011.01504.x
97. **Popescu LM, Manole E, Serboiu CS, *et al.*** Identification of telocytes in skeletal muscle interstitium: implication for muscle regeneration. *J Cell Mol Med.* 2011; 15: 1379–92.
98. **Cantarero I, Luesma MJ, Junquera C.** The primary cilium of telocytes in the vasculature: electron microscope imaging. *J Cell Mol Med.* 2011; 15: 2594–600.
99. **Popescu LM, Gherghiceanu M, Suciu LC, *et al.*** Telocytes and putative stem cells in the lungs: electron microscopy, electron tomography and laser scanning microscopy. *Cell Tissue Res.* 2011; 345: 391–403.
100. **Cismasiu VB, Radu E, Popescu LM.** miR-193 expression differentiates telocytes from other stromal cells. *J Cell Mol Med.* 2011; 15: 1071–4.
101. **Nicolescu MI, Bucur A, Dinca O, *et al.*** Telocytes in Parotid Glands. *Anat Rec (Hoboken).* 2011; 295: 378–85.
102. **Nicolescu MI, Popescu LM.** Telocytes in the Interstitium of Human Exocrine Pancreas: ultrastructural Evidence. *Pancreas.* 2012; in press: doi: 10.1097/MPA.0-b013e31823fbded
103. **Rusu MC, Jianu AM, Mirancea N, *et al.*** Tracheal telocytes. *J Cell Mol Med.* 2012; 16: 401–5.
104. **Popescu LM, Manole CG, Gherghiceanu M, *et al.*** Telocytes in human epicardium. *J Cell Mol Med.* 2010; 14: 2085–93.
105. **Gherghiceanu M, Manole CG, Popescu LM.** Telocytes in endocardium: electron microscope evidence. *J Cell Mol Med.* 2010; 14: 2330–4.
106. **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: 866–86.
107. **Gherghiceanu M, Popescu LM.** Cardiomyocyte precursors and telocytes in epicardial stem cell niche: electron microscope images. *J Cell Mol Med.* 2010; 14: 871–7.
108. **Manole CG, Cismasiu V, Gherghiceanu M, Popescu LM.** Experimental acute myocardial infarction: telocytes involvement in neo-angiogenesis. *J Cell Mol Med.* 2011; 15: 2284–96.