Tissue engineering and regenerative medicine –
where do we stand?

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Received: March 1, 2012; Accepted: March 13, 2012

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,
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 multidisciplinary 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 biodeling, 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 a 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 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 pre-vascularization [45, 46]. The critical influence of the local recipient environment is minimized with this technique.

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 angiogenic 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

Bleziffer et al. gave an overview on the therapeutic potential of gene transfer strategies in combination with TE and RM [58]. This group...
The T-Rex™ system was integrated into a replication-deficient HSV-1 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 xenogenic sources become an option. Even elimination by the host immune system is the key obstacle to xenotransplantation 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, 25, 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(+)
Bone formation in a heterotopic sheep model using directly auto-transplanted or expanded MSC with 3. A, as in this model, ectopic bone could be generated by 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].

**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).

**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 angiogenic 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 work 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** Telocytes detected in human heart. (a) shows podoms (arrows) with mitochondria in the endoplasmic reticulum and caveolae. (b) shows podomes. (c) shows podomes with mitochondria.
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 neoangiogenesis, 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 neoangiogenesis 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 TE and 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.

Acknowledgements

We thank Prof. L.M. Popescu and his group from Bucharest, Romania for kindly providing us with Figure 4 showing Telocytes (all rights remain with Prof. Popescu).

We thank the Xue Hong and Hanns Georg Geis Foundation for their continuous support of research in TE and RM. Parts of the work were supported by research grants from Baxter Healthcare Corporation.

Conflicts of Interest Statement

The authors confirm that there are no conflicts of interest.
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