Heart Failure Reviews, 8, 259–269, 2003

Cardiac Tissue Engineering for Replacement Therapy

Wolfram-Hubertus Zimmermann and Thomas Eschenhagen

Institute of Experimental and Clinical Pharmacology and Toxicology, Friedrich-Alexander-University of Erlangen-Nuremberg, Germany

Abstract. **Cell therapy is a new concept to repair diseased organs. For patients with myocardial infarction, heart failure, and congenital heart diseases cell based therapies might represent a potential cure. The field can be subdivided into two principally different approaches: (1) Implantation of isolated cells and (2) implantation of *in vitro* engineered tissue constructs. This review will focus on the latter approach. Cardiac tissue engineering comprises the fields of material sciences and cell biology. In general, scaffold materials such as gelatin, collagen, alginate, or synthetic polymers and cardiac cells are utilized to reconstitute tissue-like constructs *in vitro*. Ideally, these constructs display properties of native myocardium such as coherent contractions, low diastolic tension, and syncytial propagation of action potentials. To be applicable for surgical repair of diseased myocardium engineered tissue constructs should have the propensity to integrate and remain contractile *in vivo*. Size and mechanical properties of engineered constructs are critical for surgical repair of large tissue defects. Successful application of tissue engineering in men will depend on the utilization of an autologous or non-immunogeneic cell source and scaffold material to avoid life long immunosuppression. This review will give an overview of recent approaches in cardiac tissue engineering and its first applications *in vivo*. We will discuss materials and cell sources for cardiac tissue engineering. Further, principle obstacles will be addressed. Cardiac tissue engineering for replacement therapy has an intriguing perspective, but is in its early days. Its true value remains to be thoroughly evaluated.**

Key Words. **tissue engineering, replacement therapy, cardiac myocytes, stem cells, engineered heart tissue**

Introduction

Heart disease is the number one cause of death in industrialized nations. Myocardial infarction and heart failure resemble the most prevalent pathologies. In either case, loss of cardiac myocytes accounts for a decrease in myocardial function which can lead to total organ failure or trigger compensatory mechanisms like hypertrophy of the remaining myocardium, activation of neurohumoral systems, and autokrine/parakrine stimulation by various growth factors/cytokines. Conservative treatment of heart failure has focused on reduction of work load (diuretics, nitrates) and protection from humoral factors like catecholamines (β-blockers), angiotensin (ACE-inhibitors, AT1-receptor blockers), and aldosterone (spironolactone). In end stage heart failure heart transplantation remains the last treatment option with good long-term results [1]. Unfortunately, heart transplantation is limited due to an inadequate supply with donor organs. Despite elaboration of pharmacological and surgical treatment, numbers of patients with heart failure are increasing. Thus, there is an obvious need to improve traditional treatment and develop new strategies to cope with heart failure in the future.

Restoration of heart function by replacement of diseased myocardium with functional cardiac myocytes is an intriguing strategy because it offers a potential cure [2]. The principal feasibility of cell implantation in the heart has been confirmed nearly 10 years ago [3–5]. Different groups could reproduce and refine these pioneer experiments and have enlarged our knowledge about the fate of implanted cells of various origin in the myocardium of healthy and diseased hearts [6–18]. Most studies support the notion that cell implantation in models of myocardial infarction can improve contractile, mostly diastolic, function. Presently, clinical studies are under way to investigate the safety and feasibility of cell implantation in patients [14].

An alternative approach to injection or infusion of isolated cells into the heart is the design of artificial cardiac muscle constructs *in vitro* for later implantation *in vivo*. So far, various methods to produce 3D cardiac tissue constructs have been developed [19–34]. First studies indicate that engineered heart muscle constructs can be successfully implanted *in vivo* [23,25,28,30,31,33,34]. This review aims to overview the young field of

Address for correspondence: Wolfram-H. Zimmermann, MD, Department of Clinical Pharmacology and Toxicology, Institute of Experimental and Clinical Pharmacology and Toxicology, Friedrich-Alexander-University of Erlangen-Nuremberg, Fahrstraße 17, 91054 Erlangen. Tel.: +49-9131-852-2780; Fax: +49-9131-852-2773; E-mail: zimmermann@pharmakologie.uni-erlangen.de

cardiac tissue engineering. Scaffold materials, cell sources, and culture conditions as well as major obstacles of the field will be discussed. Finally, we will try to give a necessarily subjective and speculative perspective on the clinical relevance of cardiac tissue engineering based therapies in the future.

State-of-the-Art in Cardiac Tissue Engineering

Early studies from Bader and Oberpriller demonstrated the regenerative capacity of amphibian hearts after autologous implantation of minced ventricular tissue samples into injured newt hearts [35]. In this study a partial regeneration of injured newt ventricles was observed. However, grafted tissue fragments remained morphologically and functionally separate from the native myocardium. In mammals, were the regenerative capacity is lost or at least markedly reduced, Leor et al. [36] could show that tissue fragments from fetal myocardium of rats and humans can be transplanted into rat heart muscle and survived for up to 65 days *in situ* [36]. These early experiments to replace myocardium with cardiac tissue have been extended by true tissue engineering approaches. Different groups including our own could demonstrate that cardiac myocytes from neonatal rats and embryonic chicken can be reconstituted to three-dimensional tissue-like constructs [19–23,25–27,29–32]. Different strategies to engineer cardiac tissue constructs have been employed: (1) Seeding of preformed matrices with cardiac cells, (2) culture of cardiac cells in primarily soluble matrices, and (3) stacking of monolayers of cardiac cells (Fig. 1).

Seeding of preformed matrices resembles the classical tissue engineering approach which has been proposed by Langer and Vacanti [37] in the early 90's. The most prevalent advantage of this approach is that at least in theory matrix constructs can be formed in every shape and size and potentially even as whole organs. These non-vital matrices could then be *vitalized* by cell seeding onto or into the constructs. Successful application of this concept has been reported for reconstitution of cartilage, bone, liver, intestine, and urologic tissues [38]. In cardiovascular tissue engineering the construction of artificial valves and vessels has been quite successful and is on the verge of being introduced into clinical trials [39–42]. In contrast to the aforementioned tissues, utilization of preformed matrices to engineer myocardium has been rather disappointing so far. Mainly, lack of contractile function, poor tissue morphology, and size limitations have been set backs in the field. Several reasons might account for the failure of the classical tissue engineering approach in cardiac tissue engineering: (1) Engineered matrices resemble diffusion barriers and limit nutrition and oxygen supply in thick constructs (>100–200 μm); (2) currently employed scaffolds do not support the organization of cardiac cells in three-dimensional cardiac tissue constructs *in vitro*; (3) differentiation of cardiac myocytes in preformed matrices is not driven to an adult phenotype; (4) contractile function and actively developed forces of tissue constructs are modest or not detectable [20–23,25,26,29].

An alternative to preformed matrices is the utilization of solubilized scaffold material. We could demonstrate that collagen type I and extracellular matrix proteins when mixed with freshly isolated heart cells coalesce to strongly contracting

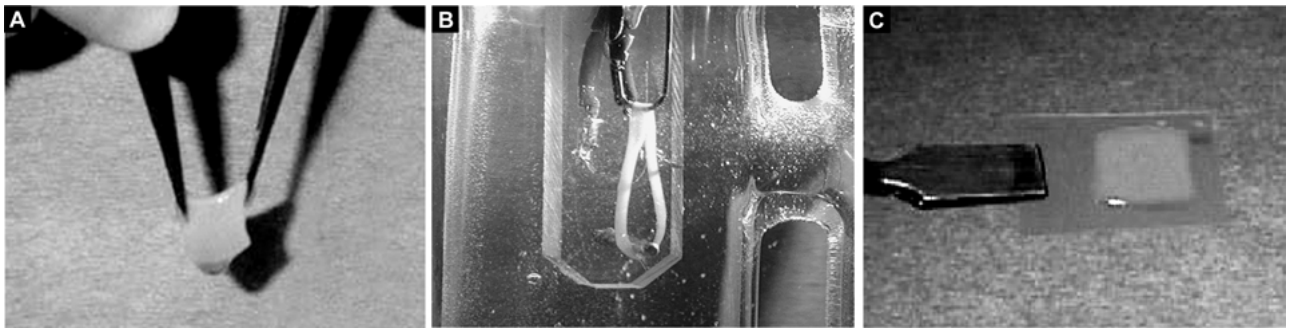

Fig. 1. *Strategies in Cardiac Tissue Engineering. (A) Seeding of preformed collagen fleeces yields* Artificial Myocardial Tissue *[29]. (B) A mixture of solubilized collagen type I, matrigel, and cardiac myocytes coalesces to form* Engineered Heart Tissue *[32]. (C) Stacking of detached cardiac myocyte monolayers yields cardiac tissue sandwich constructs [31]. Figure was adapted from Kofidis et al. In vitro engineering of heart muscle: artificial heart muscle.* J Thorac Cardiovasc Surg *2002;124:63–69 (A), Zimmermann et al. Tissue engineering of a differentiated cardiac muscle contstruct.* Circ Res *2002;90:223–230 (B), and Shimizu et al. Fabrication of pulsatile cardiac tissue grafts using a novel 3-dimensional cell sheet manipulation technique and temperature-responsive cell culture surfaces.* Circ Res *2002;90:e40 (C) with permission.*

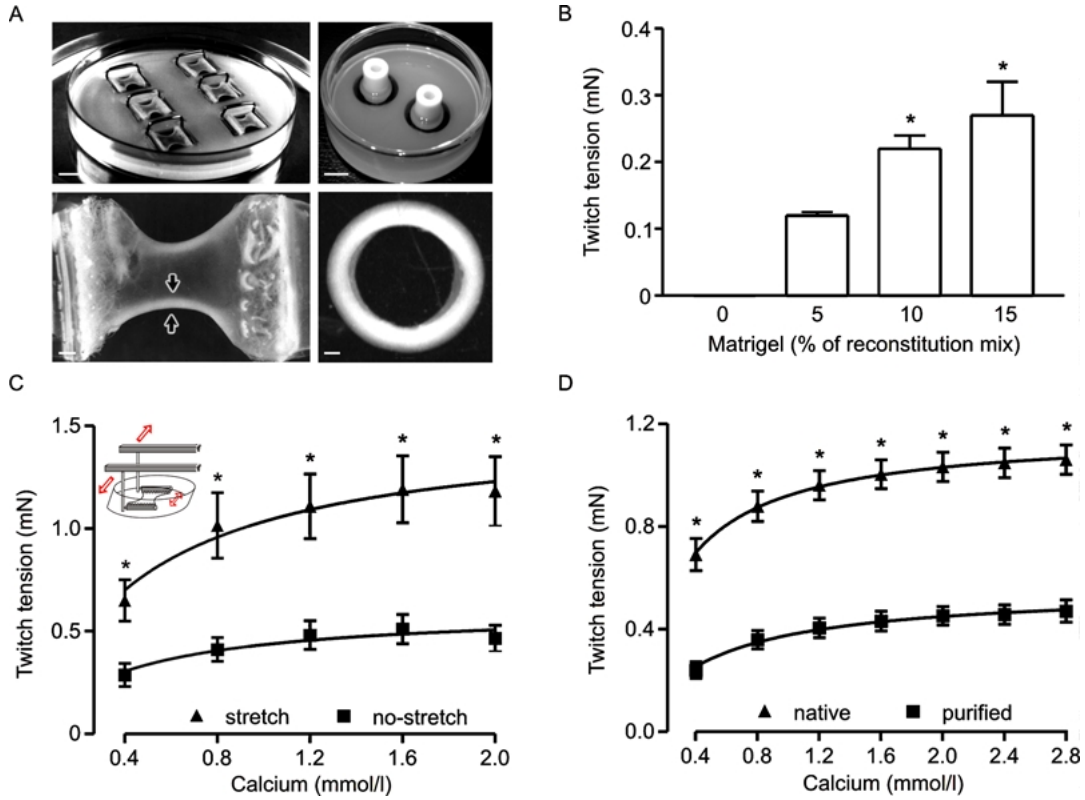

Fig. 2. *Construction of Engineered Heart Tissue. (A) Utilization of casting molds in different shape and size allows construction of EHT with variable geometry. Circular shape was found to be superior to planar EHT constructs. In planar EHTs a high cell density was observed at the concave edges (arrows), whereas the centers were only sparsely populated. In circular EHTs cells are equally contributed throughout the matrix. Addition of matrigel (B; n = 12–24), culture under mechanical load (C; n = 12–16), and utilization of a mixed (native) rather then purified cell population (D; n = 13–17) yields improved contractile function and morphology. Adapted from [26,30,31] and own unpublished data. Bars in (A): 10 mm in upper panels; 1 mm in lower panels.*
** $p < 0.05$ vs. 5% (B), unstretched EHTs (C), and EHTs prepared from purified cell populations (D).*

and highly differentiated *Engineered Heart Tissue* (EHT) [19,24,27,32,34]. The geometric shape of EHT can be altered by utilization of suitable casting molds (square, circular; Fig. 2(a)). We found that 4 factors are important to reconstitute strongly contracting EHT: (1) Addition of matrigel to the reconstitution mixture (only in rat EHT) [27] (Fig. 2(b)), (2) EHT culture under mechanical load [24] (Fig. 2(c)), (3) a circular shape, in contrast to EHT patches [27,32], (4) utilization of cell mixtures rather than purified cardiac myocyte populations [43] (Fig. 2(d)). Under these conditions strongly contracting (up to 3 mN/mm^2) and morphologically highly differentiated muscle constructs can be engineered with sarcomeres in registry, a well organized sarcoplasmic reticulum and T-tubular system, and volume fractions comparable to differentiated cardiac myocytes [32]. Non-myocytes add organoid features like an endo-epicardial surface lining and formation of capillaries by endothelial cells. Recent studies confirmed that EHT can be implanted *in vivo* and remain contractile for up to 8 weeks [34]. *In situ*, enhanced vascularization of EHT was observed. Additionally, implanted EHTs were innervated with nerve bundles containing myelinated and non-myelinated fibers. Formation of capillaries *in vitro* and later vascularization *in vivo* are desirable to prevent ischemic cell damage or death, especially in the core zone of thick engineered constructs. Despite capillarization/vascularization of EHT, thickness of single muscle bundles did hardly increase above the critical diameter of $\sim$100 μm. This is in line with most efforts in cardiac tissue engineering [22,26]. In contrast to the latter approaches, muscle bundles in EHT were not limited to the surface area but could be found throughout EHT where a formation of a highly interconnected muscular network was observed. This appears to indicate that the collagen-based EHT matrix is no significant diffusion barrier but also that beating cardiac muscle aggregates *in vitro* might be generally limited to $\sim$100 μm in diameter. The latter limit is no surprise given a physiological

intercapillary distance of <20 μm in rat myocardium [44] and the lack of a comparable capillary system in all presently available engineered cardiac muscle constructs. The benefit of EHT innervation *in vivo* is presently unknown but could indicate further integration of EHT into the recipient's organ architecture. Whether true functional integration occurred with syncytial propagation of action potentials from host to graft leading to in unison contractions remains to be elucidated. Despite some indications for graft and host cell couplings via connexin 43 [31] we believe that true electrical coupling needs to be confirmed by electrophysiological means. Another inherent problem to all cell based therapy approaches is the need for unmistakable labeling of implanted cells to allow detection *in vivo*. Electrophysiological studies, labeling experiments, and utilization of EHT to repair infarcted myocardium are presently under investigation.

Recently, Shimizu and coworkers have demonstrated that cardiac muscle constructs can be engineered without scaffold materials [31]. This group developed a technique to detach monolayer cell cultures from a temperature sensitive culture surface substratum. After stacking of up to 4 detached monolayers connexin 43 junctions formed between the cell layers and strong in unison contractions of the *sandwich* constructs could be observed. Additionally, these composed constructs survived after subcutaneous implantation. This technique has the obvious advantage that different cell types could be stacked systematically to yield an organoid tissue culture consisting of all cell species that make up the myocardium physiologically [45]. For example, addition of endothelial cells might facilitate the induction of vascularization and fibroblasts may increase the stability of engineered constructs by producing extracellular matrix. At present it is not clear how much matrix is helpful and the surprisingly strong contractile force (1.2 mN) of the sandwich constructs in the absence of scaffold material [31] may indicate that matrix materials can be counterproductive in the effort to engineer contracting cardiac tissue. Indeed, this is supported by our own experiments. Contractile force of EHTs was found to be inversely correlated with EHT collagen content (Fig. 3). On the other hand, a reduction of collagen content below 0.5 mg/ml deteriorated mechanical stability. Similarly, even a single monolayer of cardiac myocytes, spontaneously detached from the culture dish after prolonged culture in serum-containing medium, developed forces of 0.1 mN, but did not allow prolonged measurements in the organ bath due to mechanical instability (own unpublished observation).

In addition to applications in replacement therapy engineered cardiac tissue constructs may be useful for *in vitro* studies. Bursac and coworkers employed preformed matrix based constructs for electrophysiological studies [21]. In our laboratory EHTs have been utilized as an *in vitro* heart model to investigate consequences of acute and chronic mechanical, pharmacological, or molecular manipulations on cardiac myocyte contractility [24,27,32,46].

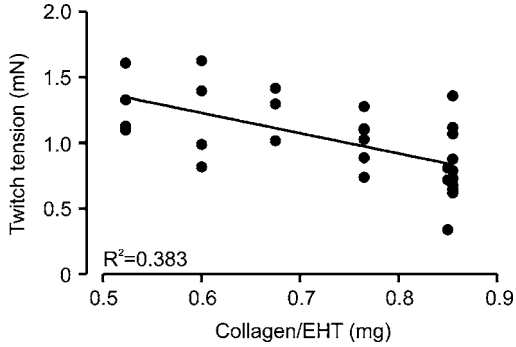

Fig. 3. *Scaffold Material Attenuates Contractile Function of Engineered Heart Tissue. EHTs were reconstituted with increasing collagen content (0.4–0.85 mg/EHT). Twitch tension at maximal calcium (1.8 mmol/l) was reduced at high collagen content and maximal at ~0.5–0.7 mg/EHT. A collagen content <0.5 mg/EHT results in EHT of weak consistency (not shown). To increase mechanical stability and retain strongly contracting EHTs, a collagen content of 0.7–0.8 mg/EHT was found to be optimal. Adapted from [24]. $P < 0.01$ by* Spearman Rank Correlation.

Scaffold Material in Cardiac Tissue Engineering

Different approaches in cardiac tissue engineering can be subdivided by their utilization of scaffold material in (1) methods that use preformed matrices, (2) methods that use solubilized scaffold materials, and (3) methods that do not rely on addition of scaffolds. A broad range of synthetic polymers such as polyglycolic acid, polylactic acid, or polyethylenglycerol and biomaterials such as alginate, collagen, or gelatine have been employed in tissue engineering and have been extensively reviewed elsewhere [38,47–49]. In general scaffold materials should be non-toxic, biodegradable, and biocompatible.

The main advantages of synthetic polymers are (1) defined chemical properties without batch-to-batch variations inherent to the production of biomaterials, (2) reduced or absent immune responses, (3) precise design of mechanical properties and geometric form, and (4) the potential for defined integration and a time-controlled release of bioactive compounds from the matrix. On the other hand, synthetic polymers are necessarily

unphysiological and can only serve as a mechanical scaffold to hold and guide cells in the 3-dimensional space until they produce their own physiological matrix environment. Additionally, biodegradation of synthetic polymers can induce inflammatory responses to the original material or byproducts of its degradation [47]. A drop in local pH upon degradation of polylactic and polyglycolic acid based synthetic tissues has also to be considered as potentially harmful to surrounding cells [50]. By manipulation of chemical and physical properties of synthetic polymers formation of harmful byproducts and inflammatory responses might be reduced [50].

Biomaterials are naturally occurring extracellular matrix components that can be chosen according to the desired application. At least theoretically, they could represent "the right material at the right place". Yet, the physiological complexity of extracellular matrix composition makes it difficult to mimic "an organ-specific environment" experimentally. It is not clear, therefore, whether biomaterials as currently employed, serve a more specific purpose than synthetic scaffolds. Biomaterials may have an advantage of inducing only mild inflammatory responses *in vivo*. On the other hand, the composition of biomaterials strongly depend on the isolation procedure and exhibits batch-to-batch variations. A varying content of other extracellular matrix components and growth factors can exert desired and undesired effects on tissue formation that are difficult to define.

When compared to other already quite successful tissue engineering approaches such as artificial cartilage or skin, tissue engineering of cardiac muscle meets a number of additional specific tasks that remain serious hurdles. (1) Cardiac myocytes, in contrast to chondrocytes, are ischemia-sensitive and therefore the matrix should allow unhindered diffusion. It is important to consider that the heart is one of the organs with the highest perfusion and oxygen extraction rate. (2) The dynamic action of the myocardium requires high flexibility, extensibility and at the same time high mechanical stability and endurance of scaffolds. Matrix components should not only serve as a static attachment substratum but provide a dynamic link that transfers load from the surrounding environment to the cells and from the cells to the surrounding environment. (3) The heart exhibits a highly complex architecture in which muscle strands are organized in various inner and outer layers that are interwoven in all three dimensions. Since differentiated cardiac myocytes cannot migrate an optimal matrix should allow complex 3D design.

It is apparent that none of the current materials or approaches fulfills these criteria. Whereas classical tissue engineering approaches aim at optimizing scaffold materials towards these requirements, alternative approaches exist. One is to use scaffolds that are derived from intact organs of donor animals that are decellularized and repopulated with host-derived cells. This approach has been proposed for engineering cardiac valves [51]. Another alternative is the sandwich technique described above which allows the construction of tissue patches of various size [31]. Omission of scaffold material minimizes material-derived problems, but may compromise mechanical stability. The latter limitation could be potentially overcome by addition of fibroblast layers.

Our own approach follows a different concept. Here the initially liquid matrix (collagen I and matrigel) quickly forms a gel that "traps" the cells in the 3D space and appears to provide a 3D environment that is rapidly remodeled by the cardiac cells. Recent experiments have shown that several matrix metalloproteases are dramatically induced (up to 1000-fold) in EHT (own unpublished data). This process is rapid and transient over the first days after casting of the collagen/cell mix. We also found significant formation of new extracellular matrix such as organized collagen fibrils and a complete basal membran around cardiac myocytes [32]. Thus, we believe that cardiac cells from neonatal rats have the intrinsic capacity to form new intact cardiac tissue and that this process is merely stimulated and directed by the collagen/matrigel matrix. It is difficult to directly follow the fate of the original collagen I, but it is likely to be degraded to a large extent and partially resynthesized during EHT formation. Others have also found that neonatal rat cardiac cells form 3D tissue-like structures, simply by cultivating them in rotating culture vessels with polystyrene beads [20]. The additional advantage of our protocol is that EHT production can be easily controlled and directed in terms of geometric form, size, and direction of load. The latter is of tremendous importance. By subjecting EHT to phasic stretch contractile force was increased >twofold (Fig. 2(c)). In contrast, when EHTs are left unloaded during culture, retraction and macroscopically evident thickening of the matrix can be observed and active force development (twitch tension) ceases. In parallel, passive force (resting tension) increases dramatically.

The balance of twitch (TT) and resting tension (RT) is an important property of healthy cardiac muscle [52]. Physiologically, the ratio of active force to passive force (TT/RT) is >1 [52–54]. For EHT we reported a maximal TT of 2–3 mN and a TT/RT ratio of 1.33, 3.29, and 14.02 under basal conditions, maximal calcium, and maximal isoprenaline concentrations, respectively [32]. Others determined maximal TT of 0.02 mN and a TT/RT ratio of 0.08 in cardiac tissue constructs

Table 1. *Contractile properties of engineered cardiac constructs reported in the literature*

Study	Construct	TT (mN)	RT (mN)	TT/RT
Eschenhagen et al. [19]	EHT planar (c)	0.34 ± 0.05	2.81 ± 0.11	0.12
Akins et al. [20]	Polystyrene/Collagen (r)	nd	nd	nd
Carrier et al. [22]	Polyglycolic acid (c + r)	nd	nd	nd
Fink et al. [24]	EHT planar (c + r)	1.91 ± 0.16	nd	nd
Leor et al. [25]	Alginate (r)	nd	nd	nd
Li et al. [26]	Gelatin (r)	nd	nd	nd
Zimmermann et al. [27]	EHT planar (r)	0.51 ± 0.13	0.63	0.81
Kofidis et al. [29]	Collagen (r)	0.019 ± 0.004	0.23	0.08
Shimizu et al. [31]	Stacked monolayers (r)	1.18 ± 0.26	nd	nd
Zimmermann et al. [32]	EHT circular (r)	0.75 ± 0.11	0.15 ± 0.02	5

TT: maximal twitch tension, i.e. systolic force; RT: resting tension, i.e. diastolic force; TT/RT: ratio of TT and RT; nd: not determined; c: embryonic chick cardiac myocytes; r: neonatal rat cardiac myocytes.

engineered by seeding cardiac myocytes onto a collagen fleece [55]. The difference in contractile parameters is most likely due to a higher rigidity of the preformed matrix and the lack of mechanical load on the construct during culture. Interestingly stacked monolayer constructs developed TT comparable to EHT [31]. Diastolic forces were not reported in this study. We observed a TT/RT ratio of 0.12 when single cardiac myocyte monolayers were submitted to contraction experiments (own unpublished data). Table 1 summarizes the contractile properties of currently employed engineered cardiac muscle constructs.

Factors that contribute to the load-induced increase in contractile function are not only an improved cell morphology and differentiation within tissue constructs but also alignment of cells along the axis of stretch [32]. Unloaded culture conditions result in random orientation of cells and a low degree of cardiac cell differentiation. The latter appearance has been observed in most tissue engineering approaches when preformed matrices were employed. Recently, McDevitt et al. reported that patterned scaffold material might allow for guided cell growth and induce cardiac myocyte differentiation [56]. This approach has not yet been introduced into cardiac tissue engineering, but might be feasible to improve cell morphology and possibly function of cardiac muscle constructs.

Cells for Cardiac Tissue Engineering

Another crucial aspect in cardiac tissue engineering is the choice and the composition of cells in engineered heart constructs. Clearly, cardiac myocytes have to be the main cellular component. However, can the heart function without non-cardiac myocytes? Endothelial cells, fibroblasts, smooth muscle cells, neural cells, and leukocytes comprise about 70% of the total cell number in the working myocardium [45] and undoubtedly play an important role in cardiac development and function [57–59]. Endothelial cells and smooth muscle cells, the main components of the vasculature, are not only necessary for transport of nutrition and oxygen but also secrete growth factors and cytokines that are important for heart function. Similarly, fibroblasts and leukocytes permanently secrete growth factors and cytokines. When EHT were constructed with a physiologic cell mixture consisting of cardiac myocytes and non-myocytes rather than a purified cardiac myocyte population contractile function was markedly improved [43] (Fig. 2(d)). The exact contribution of each single cell type to tissue-formation has not been thoroughly analyzed yet, but conceptionally the data strongly suggest that formation of a true cardiac tissue-like 3D construct requires the presence of cardiac myocytes and non-myocytes, ideally in a physiological mix. If correct, this requirement imposes a major conceptional hurdle to the whole field. Possible solutions may come from stem cells that bear at least the potential to differentiate into various cell types (see below). Alternatively, it might be possible to generate relatively simple constructs from pure cardiac myocyte populations and hope that fibroblasts, smooth muscles and endothelial cells and others invade the construct after implantation.

Current tissue engineering approaches utilize cardiac cells from neonatal rats or chicken. These experiments are necessary as proof of principle, but it is obvious that primary cardiac cells will never be a cell source for cardiac tissue engineering in patients. The field relies therefore on stem cells. Most experience has been accumulated with skeletal myoblasts, i.e. muscle-resident satellite cells. This cell type allows large scale propagation *in vitro* and survives after injection *in vivo*. Skeletal myoblasts have been successfully implanted in animal models and in patients [8,14]. Despite concerns regarding the induction of arrhythmias clinical trails are being conducted which will eventually clarify if such treatment is feasible, safe,

and of benefit for the patient. So far, active contractions of implanted myoblasts and true cell-cell coupling has not been demonstrated. In fact, *in vivo* injected myoblasts differentiate in myotubes and not in cardiac myocytes and remain isolated in the host myocardium [60]. The myotube phenotype precludes electrical coupling to the native myocytes because they do not express connexin 43, the main gap junction protein in cardiac myocytes. Whether stable transgene expression of connexin 43 proteins in myoblasts can circumvent the naturally occurring electrical isolation of myotubes and cardiac myocytes remains to be demonstrated *in vivo* [61]. Utilization of myoblast in cardiac tissue engineering might be possible but has not been performed so far.

The most promising cell sources for regenerative medicine are embryonic and adult stem cells. The enthusiasm about this approach has been spurred by recent publications that demonstrated the versatility of embryonic and adult stem cells [62–65]. The capacity of murine embryonic stem (ES) cells to differentiate into cardiac myocytes has been demonstrated earlier [66,67]. The subsequent demonstration of spontaneous differentiation of human ES cells into the three germ layers [68] including the development of functional cardiac myocytes [64] has opened the avenue to a human cell source for cell based therapies including cardiac tissue engineering. Unlimited differentiation capacity and indefinite propagation represent the strongest advantages of ES cells. On the other hand, their allogeneic nature, the potential for tumor formation, and the low efficiency of differentiation into cardiac myocytes are limiting aspects. During the past 20 years, significant progress with regard to the fundamental aspects of ES-derived cardiac cell differentiation, culture conditions, and methods to select for differentiated cardiac myocytes has been made [68–74] and shall be useful to define suitable culture conditions for embryonic stem cells. However, in view of the authors, the need for life-long immunosuppression will finally preclude their clinical use. A potential solution would come from nuclear transfer experiments ("therapeutic cloning"), but beside unresolved ethical issues, fundamental biological and technical questions remain unanswered.

Adult stem cells would be theoretically the ideal choice. They can be derived from the patient and are therefore autologous in nature, no ethical issues exist and tumor formation is unlikely. Potential sources are bone marrow, peripheral blood, skeletal muscle and on the longterm, placental cord blood. Recent evidence for an hitherto unexpected plasticity of adult stem cells [63] strongly favor their use in tissue engineering and gained much excitement. Yet, the true potential of adult stem cells is far from being clear and recent methodologically careful studies raised serious doubts about the data and their interpretation of some enthusiastic studies. For example, whereas most investigators agree that transdifferentiation of bone marror-derived stem cells into cardiac myocytes prinicipally occurs, this event has only been clearly observed after myocardial injury and remains very rare (<0.02%) [75]. Similarly, in contrast to one study that reported a frequency of 18% [76], three studies found either no [77] or only marginal [78,79] chimerism of cardiac myocytes in the human heart after non-sex-matched heart transplantations. Thus, at present the crucial question remains whether it will ever be possible to induce transdifferentiation of adult stem cells at the necessary efficiency. It should also be noted that most types of adult stem cells have not been propagated *in vitro* with the exception of mesenchymal stem cells and skeletal myoblasts. Recent research effort focuses on growth factors and culture conditions that promote cardiac cell lineage commitment of stem cells [68,74] and genes that may be introduced to induce cardiac differentiation [70,72,73].

In this respect the observation that injured myocardium appears to attract adult stem cells to home to the myocardium and to transdifferentiate in cardiac myocytes [12,15] is of significant interest because it opens the way to mimic those conditions *in vitro*. Recent *in vivo* studies have also demonstrated that the local cardiac environment might drive differentiation of implanted immature cardiac myocytes [9,17,34]. These studies suggest that it is not a single factor but a whole concert of soluble factors and cell-cell-interactions that is necessary for (trans)differentiation, making a pure pharmacological approach unlikely to be efficient. Engineered heart tissue might offer an attractive environment to trigger (trans)differentiation. Coculture models of undifferentiated stem cells with non-myocytes of cardiac origin or rodent cardiac myocytes in a 3D culture format are presently under investigation to test this hypothesis directly.

Culture Conditions

One of the principal advantages of tissue engineering is that viable and ischemia-tolerant cells are being selected *in vitro* before implantation, whereas injection of isolated cells leads to destruction of up to 95% of the injected cells during implantation or shortly afterwards. Cell debris at the injection site will trigger inflammatory responses which have biological effects by itself. Indeed, factors released during inflammation may affect scar remodeling, have direct effects on contractile function, and induce angiogenesis. Yet, it is unlikely that these transient effects will contribute to

improvement of contractile function in the long run.

Loss in cell number is also observed during the initial reconstitution of EHTs, but accounts only for 50–70% and then cell number stabilizes during culture (own unpublished data). Surviving cells in tissue constructs most likely adapt to the new environment of low oxygen tension and possibly malnutrition *in vitro* and might be more robust if implanted. Malnutrition might be caused by compromised diffusion into thick tissue constructs and competition for nutrients among the cells in tissue constructs. We could demonstrate earlier that cell number is critical for construction of planar EHT [27]. Whereas an increase in cell number between 1 and 2.5×10^6 cells/ml reconstitution mix resulted in improved tissue-formation and force development, EHTs did not beat when cell number was increased to 3×10^6 and more. Thus, a critical cell density exists. Similar observations have been made when geometric shape of EHT was altered. This problem may be solved by composing several EHTs of optimal size to a larger network. Other strategies to improve tissue formation, cellular differentiation, and contractile function of EHTs that are currently under investigation are different oxygen concentrations, medium compositions, growth factor supplements as well as systematic addition of various cardiac cell types. A factor already noted as being very critical is mechanical load and here modifications of current stretch protocols are being tested.

Immunological Barriers

An important obstacle in all types of cell based therapies might be immune responses. Present approaches will most likely fail in the clinic unless they are truly autologous in nature. Despite a primary autologous approach, i.e. the donor is also the recipient, the necessary cell propagation is usually performed under *in vitro* conditions with non-autologous culture supplements. For example, serum from horse and calf could alter the expression of self antigens and lead to impregnation. The immunologically altered cells would induce immune responses and be amenable to rejection. Indeed, when EHT reconstituted from Fischer 344 cells and Fischer 344 collagen were implanted in syngenic Fischer 344 rats rejection was observed [34]. Prolonged washes prior to implantation did not reduce the immune response. Survival of implants was achieved by sustained immunosuppression with parenteral application of ciclosporine, azathioprine, and prednisolone. Unresolved immunological problems have recently diminished the enthusiasm about xenotransplantation and will be an important aspect in all cell based therapies that needs to be taken into consideration.

Perspectives of Cardiac Tissue Engineering

Cardiac tissue engineering is a young field, many important obstacles exist and any prediction remain speculative at present. We believe that culture conditions and many technical aspects are likely to be resolved and that the perspective to create true cardiac tissue patches that can be surgically applied is realistic and represents a clear conceptional advantage over injections of isolated cells. The main hurdle, however, is shared by all cell-based therapies and this is the unresolved question of optimal cell sourcing. Here a long way has to be gone and it is difficult to make any reliable prediction. In our view, it is not yet the time for clinical experiments but for careful evaluation of cardiac cell biology, stem cell biology, and the conditions that promote (trans)differentiation into the various cardiac cells. In cardiology, where traditional pharmacological and surgical therapies have significantly reduced mortality, improved quality of life, and survival of patients with heart disease, any cell-based therapy has to be evaluated rigorously in comparison to classical treatment options. The place for cardiac tissue engineering could be corrections of heart defects in children and terminal stages of ischemic heart diesease and heart failure rather than mild forms of heart disease where other safe treatment options exist. Consequently, the aim would be the repair of rather large tissue defects. In the view of the authors, this can only be accomplished by tissue engineering and appropriate surgical attachment of engineered muscle constructs to the diseased myocardium.

Conclusion

Despite major barriers there is reason for some optimism that cell based therapies and especially tissue engineering could find their place in the treatment of malignant cardiovascular diseases. The following years will bring about new insights into this fascinating field and hopefully answers regarding the optimal scaffold, a cell source that is autologous and unlimited, optimized methods to generate large tissue constructs with relevant contractile properties, and eventually surgical techniques to replace or substitute diseased myocardium with engineered cardiac muscle constructs. An interdisciplinary effort of basic scientists and clinicians will be necessary to achieve this goal.

Acknowledgment

This study was supported by the German Research Foundation (Deutsche Forschungsgemeinschaft) to T.E. (Es 88/8-2) and the German Ministry for Education and Research to T.E. and W.H.Z. (BMBF FKZ 01GN 0124).

References

1. Miniati DN, Robbins RC. Heart transplantation: A thirty-year perspective. *Annu Rev Med* 2002;53:189–205.
2. Reinlib L, Field L. Cell transplantation as future therapy for cardiovascular disease?: A workshop of the National Heart, Lung, and Blood Institute. *Circulation* 2000;101:E182–187.
3. Koh GY, Klug MG, Soonpaa MH, Field LJ. Differentiation and long-term survival of C2C12 myoblast grafts in heart. *J Clin Invest* 1993;92:1548–1554.
4. Koh GY, Soonpaa MH, Klug MG, Field LJ. Long-term survival of AT-1 cardiomyocyte grafts in syngeneic myocardium. *Am J Physiol* 1993;264:H1727–1733.
5. Soonpaa MH, Koh GY, Klug MG, Field LJ. Formation of nascent intercalated disks between grafted fetal cardiomyocytes and host myocardium. *Science* 1994;264:98–101.
6. Li RK, Mickle DA, Weisel RD, Zhang J, Mohabeer MK. *In vivo* survival and function of transplanted rat cardiomyocytes. *Circ Res* 1996;78:283–288.
7. Scorsin M, Hagege AA, Marotte F, Mirochnik N, Copin H, Barnoux M, Sabri A, Samuel JL, Rappaport L, Menasche P. Does transplantation of cardiomyocytes improve function of infarcted myocardium? *Circulation* 1997;96:II-188–193.
8. Taylor DA, Atkins BZ, Hungspreugs P, Jones TR, Reedy MC, Hutcheson KA, Glower DD, Kraus WE. Regenerating functional myocardium: Improved performance after skeletal myoblast transplantation. *Nat Med* 1998;4:929–933.
9. Reinecke H, Zhang M, Bartosek T, Murry CE. Survival, integration, and differentiation of cardiomyocyte grafts: A study in normal and injured rat hearts. *Circulation* 1999;100:193–202.
10. Sakai T, Li RK, Weisel RD, Mickle DA, Jia ZQ, Tomita S, Kim EJ, Yau TM. Fetal cell transplantation: A comparison of three cell types. *J Thorac Cardiovasc Surg* 1999;118:715–724.
11. Tomita S, Li RK, Weisel RD, Mickle DA, Kim EJ, Sakai T, Jia ZQ. Autologous transplantation of bone marrow cells improves damaged heart function. *Circulation* 1999;100:II247–256.
12. Condorelli G, Borello U, De Angelis L, Latronico M, Sirabella D, Coletta M, Galli R, Balconi G, Follenzi A, Frati G, Cusella De Angelis MG, Gioglio L, Amuchastegui S, Adorini L, Naldini L, Vescovi A, Dejana E, Cossu G. Cardiomyocytes induce endothelial cells to trans-differentiate into cardiac muscle: Implications for myocardium regeneration. *Proc Natl Acad Sci USA* 2001;98:10733–10738.
13. Etzion S, Battler A, Barbash IM, Cagnano E, Zarin P, Granot Y, Kedes LH, Kloner RA, Leor J. Influence of embryonic cardiomyocyte transplantation on the progression of heart failure in a rat model of extensive myocardial infarction. *J Mol Cell Cardiol* 2001;33:1321–1330.
14. Menasche P, Hagege AA, Scorsin M, Pouzet B, Desnos M, Duboc D, Schwartz K, Vilquin JT, Marolleau JP. Myoblast transplantation for heart failure. *Lancet* 2001;357:279–280.
15. Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine DM, Leri A, Anversa P. Bone marrow cells regenerate infarcted myocardium. *Nature* 2001;410:701–705.
16. Müller-Ehmsen J, Peterson KL, Kedes L, Whittaker P, Dow JS, Long TI, Laird PW, Kloner RA. Rebuilding a damaged heart: Long-term survival of transplanted neonatal rat cardiomyocytes after myocardial infarction and effect on cardiac function. *Circulation* 2002;105:1720–1726.
17. Müller-Ehmsen J, Whittaker P, Kloner RA, Dow JS, Sakoda T, Long TI, Laird PW, Kedes L. Survival and development of neonatal rat cardiomyocytes transplanted into adult myocardium. *J Mol Cell Cardiol* 2002;34:107–116.
18. Roell W, Lu ZJ, Bloch W, Siedner S, Tiemann K, Xia Y, Stoecker E, Fleischmann M, Bohlen H, Stehle R, Kolossov E, Brem G, Addicks K, Pfitzer G, Welz A, Hescheler J, Fleischmann BK. Cellular cardiomyoplasty improves survival after myocardial injury. *Circulation* 2002;105:2435–2441.
19. Eschenhagen T, Fink C, Remmers U, Scholz H, Wattchow J, Weil J, Zimmermann W, Dohmen HH, Schafer H, Bishopric N, Wakatsuki T, Elson EL. Three-dimensional reconstitution of embryonic cardiomyocytes in a collagen matrix: A new heart muscle model system. *Faseb J* 1997;11:683–694.
20. Akins RE, Boyce RA, Madonna ML, Schroedl NA, Gonda SR, McLaughlin TA, Hartzell CR. Cardiac organogenesis *in vitro*: Reestablishment of three-dimensional tissue architecture by dissociated neonatal rat ventricular cells. *Tissue Eng* 1999;5:103–118.
21. Bursac N, Papadaki M, Cohen RJ, Schoen FJ, Eisenberg SR, Carrier R, Vunjak-Novakovic G, Freed LE. Cardiac muscle tissue engineering: Toward an *in vitro* model for electrophysiological studies. *Am J Physiol* 1999;277:H433–444.
22. Carrier RL, Papadaki M, Rupnick M, Schoen FJ, Bursac N, Langer R, Freed LE, Vunjak-Novakovic G. Cardiac tissue engineering: Cell seeding, cultivation parameters, and tissue construct characterization. *Biotechnol Bioeng* 1999;64:580–589.
23. Li RK, Jia ZQ, Weisel RD, Mickle DA, Choi A, Yau TM. Survival and function of bioengineered cardiac grafts. *Circulation* 1999;100:II63–69.
24. Fink C, Ergun S, Kralisch D, Remmers U, Weil J, Eschenhagen T. Chronic stretch of engineered heart tissue induces hypertrophy and functional improvement. *Faseb J* 2000;14:669–679.
25. Leor J, Aboulafia-Etzion S, Dar A, Shapiro L, Barbash IM, Battler A, Granot Y, Cohen S. Bioengineered cardiac grafts: A new approach to repair the infarcted myocardium? *Circulation* 2000;102:III56–61.
26. Li RK, Yau TM, Weisel RD, Mickle DA, Sakai T, Choi A, Jia ZQ. Construction of a bioengineered cardiac graft. *J Thorac Cardiovasc Surg* 2000;119:368–375.
27. 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.
28. Eschenhagen T, Didie M, Heubach J, Ravens U, Zimmermann WH. Cardiac tissue engineering. *Transpl Immunol* 2002;9:315–321.
29. Kofidis T, Akhyari P, Boublik J, Theodorou P, Martin U, Ruhparwar A, Fischer S, Eschenhagen T, Kubis HP, Kraft

T, Leyh R, Haverich A. *In vitro* engineering of heart muscle: Artificial myocardial tissue. *J Thorac Cardiovasc Surg* 2002;124:63–69.
30. Krupnick AS, Kreisel D, Engels FH, Szeto WY, Plappert T, Popma SH, Flake AW, Rosengard BR. A novel small animal model of left ventricular tissue engineering. *J Heart Lung Transplant* 2002;21:233–243.
31. Shimizu T, Yamato M, Isoi Y, Akutsu T, Setomaru T, Abe K, Kikuchi A, Umezu M, Okano T. Fabrication of pulsatile cardiac tissue grafts using a novel 3-dimensional cell sheet manipulation technique and temperature-responsive cell culture surfaces. *Circ Res* 2002;90:e40.
32. Zimmermann WH, Schneiderbanger K, Schubert P, Didie 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.
33. Eschenhagen T, Didie M, Münzel F, Schubert P, Schneiderbanger K, Zimmermann WH. 3D engineered heart tissue for tissue replacement therapy. *Basic Res Cardiol* 2002;97:I146–152.
34. Zimmermann WH, Didie M, Wasmeier G, Nixdorff U, Hess A, Melnychenko I, Boy O, Neuhuber WL, Weyand M, Eschenhagen T. Cardiac grafting of engineered heart tissue in syngenic rats. *Circulation* 2002;106:I151–157.
35. Bader D, Oberpriller JO. Repair and reorganization of minced cardiac muscle in the adult newt (Notophthalmus viridescens). *J Morphol* 1978;155:349–357.
36. Leor J, Patterson M, Quinones MJ, Kedes LH, Kloner RA. Transplantation of fetal myocardial tissue into the infarcted myocardium of rat. A potential method for repair of infarcted myocardium? *Circulation* 1996;94:II332–336.
37. Langer R, Vacanti JP. Tissue engineering. *Science* 1993;260:920–926.
38. Vacanti JP, Langer R, Upton J, Marler JJ. Transplantation of cells in matrices for tissue regeneration. *Adv Drug Deliv Rev* 1998;33:165–182.
39. Hoerstrup SP, Sodian R, Daebritz S, Wang J, Bacha EA, Martin DP, Moran AM, Guleserian KJ, Sperling JS, Kaushal S, Vacanti JP, Schoen FJ, Mayer JE, Jr. Functional living trileaflet heart valves grown *in vitro*. *Circulation* 2000;102:III44–49.
40. Sodian R, Hoerstrup SP, Sperling JS, Daebritz S, Martin DP, Moran AM, Kim BS, Schoen FJ, Vacanti JP, Mayer JE, Jr. Early *in vivo* experience with tissue-engineered trileaflet heart valves. *Circulation* 2000;102:III22–29.
41. Steinhoff G, Stock U, Karim N, Mertsching H, Timke A, Meliss RR, Pethig K, Haverich A, Bader A. Tissue engineering of pulmonary heart valves on allogenic acellular matrix conduits: *In vivo* restoration of valve tissue. *Circulation* 2000;102:III50–55.
42. Teebken OE, Haverich A. Tissue engineering of small diameter vascular grafts. *Eur J Vasc Endovasc Surg* 2002;23:475–485.
43. Zimmermann WH, Schneiderbanger K, Schubert P, Didié M, El-Armouche A, Eschenhagen T. Engineering of an organoid cardiac tissue equivalent *in vitro*. *Circulation* 2001;104:II–129.
44. Korecky B, Hai CM, Rakusan K. Functional capillary density in normal and transplanted rat hearts. *Can J Physiol Pharmacol* 1982;60:23–32.
45. Nag AC, Zak R. Dissociation of adult mammalian heart into single cell suspension: An ultrastructural study. *J Anat* 1979;129:541–559.
46. El-Armouche A, Rau T, Zolk O, Ditz D, Pamminger T, Zimmermann WH, Jäckel E, Harding SE, Boknik P, Neumann J, Eschenhagen T. Evidence for protein phosphatase inhibitor-1 playing an amplifier role in beta-adrenergic signaling in cardiac myocytes. *FASEB J* 2003;17:437–439.
47. Mikos AG, McIntire LV, Anderson JM, Babensee JE. Host response to tissue engineered devices. *Adv Drug Deliv Rev* 1998;33:111–139.
48. Chaikof EL, Matthew H, Kohn J, Mikos AG, Prestwich GD, Yip CM. Biomaterials and scaffolds in reparative medicine. *Ann NY Acad Sci* 2002;961:96–105.
49. Hench LL, Polak JM. Third-generation biomedical materials. *Science* 2002;295:1014–1017.
50. Agrawal CM, Athanasiou KA. Technique to control pH in vicinity of biodegrading PLA-PGA implants. *J Biomed Mater Res* 1997;38:105–114.
51. Booth C, Korossis SA, Wilcox HE, Watterson KG, Kearney JN, Fisher J, Ingham E. Tissue engineering of cardiac valve prostheses I: Development and histological characterization of an acellular porcine scaffold. *J Heart Valve Dis* 2002;11:457–462.
52. Mellors LJ, Barclay CJ. The energetics of rat papillary muscles undergoing realistic strain patterns. *J Exp Biol* 2001;204:3765–3777.
53. Holubarsch C, Ruf T, Goldstein DJ, Ashton RC, Nickl W, Pieske B, Pioch K, Ludemann J, Wiesner S, Hasenfuss G, Posival H, Just H, Burkhoff D. Existence of the Frank-Starling mechanism in the failing human heart. Investigations on the organ, tissue, and sarcomere levels. *Circulation* 1996;94:683–689.
54. Weil J, Eschenhagen T, Hirt S, Magnussen O, Mittmann C, Remmers U, Scholz H. Preserved Frank-Starling mechanism in human end stage heart failure. *Cardiovasc Res* 1998;37:541–548.
55. Kofidis T, Akhyari P, Wachsmann B, Boublik J, Mueller-Stahl K, Leyh R, Fischer S, Haverich A. A novel bioartificial myocardial tissue and its prospective use in cardiac surgery. *Eur J Cardiothorac Surg* 2002;22:238–243.
56. McDevitt TC, Angello JC, Whitney ML, Reinecke H, Hauschka SD, Murry CE, Stayton PS. *In vitro* generation of differentiated cardiac myofibers on micropatterned laminin surfaces. *J Biomed Mater Res* 2002;60:472–479.
57. Long CS, Henrich CJ, Simpson PC. A growth factor for cardiac myocytes is produced by cardiac nonmyocytes. *Cell Regul* 1991;2:1081–1095.
58. Shah AM, Grocott-Mason RM, Pepper CB, Mebazaa A, Henderson AH, Lewis MJ, Paulus WJ. The cardiac endothelium: Cardioactive mediators. *Prog Cardiovasc Dis* 1996;39:263–284.
59. Gray MO, Long CS, Kalinyak JE, Li HT, Karliner JS. Angiotensin II stimulates cardiac myocyte hypertrophy via paracrine release of TGF-beta 1 and endothelin-1 from fibroblasts. *Cardiovasc Res* 1998;40:352–363.
60. Reinecke H, Poppa V, Murry CE. Skeletal muscle stem cells do not transdifferentiate into cardiomyocytes after cardiac grafting. *J Mol Cell Cardiol* 2002;34:241–249.
61. Suzuki K, Brand NJ, Allen S, Khan MA, Farrell AO, Murtuza B, Oakley RE, Yacoub MH. Overexpression of connexin 43 in skeletal myoblasts: Relevance to cell transplantation to the heart. *J Thorac Cardiovasc Surg* 2001;122:759–766.
62. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic

stem cell lines derived from human blastocysts. *Science* 1998;282:1145–1147.
63. Blau HM, Brazelton TR, Weimann JM. The evolving concept of a stem cell: Entity or function? *Cell* 2001;105:829–841.
64. Kehat I, Kenyagin-Karsenti D, Snir M, Segev H, Amit M, Gepstein A, Livne E, Binah O, Itskovitz-Eldor J, Gepstein L. Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. *J Clin Invest* 2001;108:407–414.
65. Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA, Verfaillie CM. Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* 2002;418:41–49.
66. Doetschman TC, Eistetter H, Katz M, Schmidt W, Kemler R. The *in vitro* development of blastocyst-derived embryonic stem cell lines: Formation of visceral yolk sac, blood islands and myocardium. *J Embryol Exp Morphol* 1985;87:27–45.
67. Wobus AM, Wallukat G, Hescheler J. Pluripotent mouse embryonic stem cells are able to differentiate into cardiomyocytes expressing chronotropic responses to adrenergic and cholinergic agents and Ca2+ channel blockers. *Differentiation* 1991;48:173–182.
68. Schuldiner M, Yanuka O, Itskovitz-Eldor J, Melton DA, Benvenisty N. From the cover: Effects of eight growth factors on the differentiation of cells derived from human embryonic stem cells. *Proc Natl Acad Sci USA* 2000;97:11307–11312.
69. Klug MG, Soonpaa MH, Koh GY, Field LJ. Genetically selected cardiomyocytes from differentiating embronic stem cells form stable intracardiac grafts. *J Clin Invest* 1996;98:216–224.
70. Bodmer R, Venkatesh TV. Heart development in Drosophila and vertebrates: Conservation of molecular mechanisms. *Dev Genet* 1998;22:181–186.
71. Müller M, Fleischmann BK, Selbert S, Ji GJ, Endl E, Middeler G, Müller OJ, Schlenke P, Frese S, Wobus AM, Hescheler J, Katus HA, Franz WM. Selection of ventricular-like cardiomyocytes from ES cells *in vitro*. *Faseb J* 2000;14:2540–2548.
72. Jamali M, Rogerson PJ, Wilton S, Skerjanc IS. Nkx2-5 activity is essential for cardiomyogenesis. *J Biol Chem* 2001;276:42252–42258.
73. Wang D, Chang PS, Wang Z, Sutherland L, Richardson JA, Small E, Krieg PA, Olson EN. Activation of cardiac gene expression by myocardin, a transcriptional cofactor for serum response factor. *Cell* 2001;105:851–862.
74. Boheler KR, Czyz J, Tweedie D, Yang HT, Anisimov SV, Wobus AM. Differentiation of pluripotent embryonic stem cells into cardiomyocytes. *Circ Res* 2002;91:189–201.
75. Jackson KA, Majka SM, Wang H, Pocius J, Hartley CJ, Majesky MW, Entman ML, Michael LH, Hirschi KK, Goodell MA. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *J Clin Invest* 2001;107:1395–1402.
76. Quaini F, Urbanek K, Beltrami AP, Finato N, Beltrami CA, Nadal-Ginard B, Kajstura J, Leri A, Anversa P. Chimerism of the transplanted heart. *N Engl J Med* 2002;346:5–15.
77. Glaser R, Lu MM, Narula N, Epstein JA. Smooth muscle cells, but not myocytes, of host origin in transplanted human hearts. *Circulation* 2002;106:17–19.
78. Laflamme MA, Myerson D, Saffitz JE, Murry CE. Evidence for cardiomyocyte repopulation by extracardiac progenitors in transplanted human hearts. *Circ Res* 2002;90:634–640.
79. Müller P, Pfeiffer P, Koglin J, Schafers HJ, Seeland U, Janzen I, Urbschat S, Böhm M. Cardiomyocytes of noncardiac origin in myocardial biopsies of human transplanted hearts. *Circulation* 2002;106:31–35.