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Engineered heart tissue for regeneration of diseased hearts ☆

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Abstract

Cardiac tissue engineering aims at providing contractile heart muscle constructs for replacement therapy in vivo. At present, most cardiac tissue engineering attempts utilize heart cells from embryonic chicken and neonatal rats and scaffold materials. Over the past years our group has developed a novel technique to engineer collagen/matrigel-based cardiac muscle constructs, which we termed engineered heart tissue (EHT). EHT display functional and morphological properties of differentiated heart muscle and can be constructed in different shape and size from collagen type I, extracellular matrix proteins (Matrigel®), and heart cells from neonatal rats and embryonic chicken. First implantation studies in syngeneic Fischer 344 rats provided evidence of EHT survival and integration in vivo. This review will focus on our experience in tissue engineering of cardiac muscle. Mainly, EHT construction, matrix requirements, potential applications of different cell types including stem cells, and our first implantation experiences will be discussed. Despite many critical and unresolved questions, we believe that cardiac tissue engineering in general has an interesting perspective for the replacement of malfunctioning myocardium and reconstruction of congenital malformations.

Keywords: Cardiac tissue engineering; Cardiomyocyte; Collagen; Extracellular matrix; Stem cell; Transplantation

1. Introduction

Myocardial infarction and heart failure generate substantial socioeconomic costs and represent the main cause of death in industrialized countries. Despite improvement of pharmacological and invasive treatment regimens numbers of patients with heart failure are increasing. This is partially due to an increased likelihood to survive otherwise fatal myocardial infarctions with a severe impairment of left ventricular function. A marked reduction of quality of life and a high mortality of heart failure patients aggravate the need for new treatment strategies. Implantation of cells or in vitro reconstituted tissue constructs might offer a novel causal therapy to patients with malfunctioning myocardium [1].

Cardiac tissue engineering approaches rely mainly on the use of synthetic or biological matrix materials and heart cells to reconstitute contractile cardiac muscle-like tissue in vitro that might be utilized for replacement of diseased myocardium in vivo [2–13]. These approaches are in line with the general principles of tissue engineering as proposed by Langer and Vacanti in the early-1990s [14]. The ideal cardiac tissue construct should display functional and morphological properties of native heart muscle and remain viable after implantation. Mechanical, electrical, and functional integration into the organ architecture should result in improved systolic and diastolic function of diseased myocardium. Thus, constructs should be (1) contractile, (2) electrophysiologically stable, (3) mechanically robust yet flexible, (4) vascularized or at least quickly vascularized after implantation, and (5) autologous. Unfortunately, such ideal biomaterial does not exist, yet.

Over the past ten years different groups have contributed to the field of cardiac tissue engineering [2–13]. Approaches in cardiac tissue engineering have been either matrix based or non-matrix based. Matrix-based approaches can be further subdivided into

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Table 1
Overview of applied methods in cardiac tissue engineering

Matrix	Constitution	Bioreactor	Study
Polystyrene beads/collagen threads	Solid	+	[6]
Polyglycolic acid	Solid	+	[7]
Gelatine mesh	Solid	+	[3]
Modified collagen	Solid	±	[5]
Gelatine mesh	Solid	–	[10]
Alginate	Solid	–	[11]
Collagen mesh	Solid	–	[2]
Collagen	Liquid/gel	+	[8]
Collagen/matrigel	Liquid/gel	+	[13]
Collagen	Liquid/gel	–	[9]
Collagen/matrigel	Liquid/gel	–	[12]
No matrix		–	[4]

Matrix-based approaches utilize either solid or liquid matrix material to allow for cell attachment or maintenance of a high-density cell milieu to support spontaneous formation of cardiac tissue in a defined volume, respectively. Bioreactors have been employed to improve oxygen/metabolic supply and/or impose mechanical load on developing tissue constructs. (+) Studies utilize bioreactors; (–) studies do not utilize bioreactors.

methods that apply solid and sometimes preformed matrices or liquid, not formed matrix–cell mixtures. Further, different types of bioreactors have been used to improve metabolic supply [7,15] or impose mechanical stretch [8,13] on engineered heart constructs (overview in Table 1). So far, reconstitution of strongly contracting heart muscle constructs (systolic force development of 1–3 mN) has only been achieved by approaches that avoid solid matrices as a cell attachment substratum [4,8,9,12,13].

Engineered heart tissue (EHT) is made from a liquid cell matrix-mixture composed of neutralized, liquid collagen type I, extracellular matrix proteins (Matrigel®), and freshly isolated heart cells from neonatal rats. This reconstitution mixture can be pipetted into casting molds of different shape and size. So far, we have generated EHTs with a lattice and a circular geometry [9]. In culture, we could observe spontaneous remodeling of the liquid reconstitution mixture and a development of spontaneously and synchronously contracting solid EHTs after 5–7 days. Subsequently, the culture is continued under cyclic mechanical stretch for another 5–10 days. Stretching improves morphological, functional, and mechanical properties of EHT [8]. The suitability of EHTs as graft material for potential replacement therapy has been tested in syngeneic Fischer 344 rats [16]. These studies have provided evidence that EHTs survive in vivo for at least 8 weeks, remain contractile, and become morphologically integrated into the host myocardium. In this review we discuss construction, cell–matrix composition, and functional properties of EHTs and first EHT-implantation studies as well as animal models for replacement therapy approaches and possible applications of stem cells for cardiac tissue engineering.

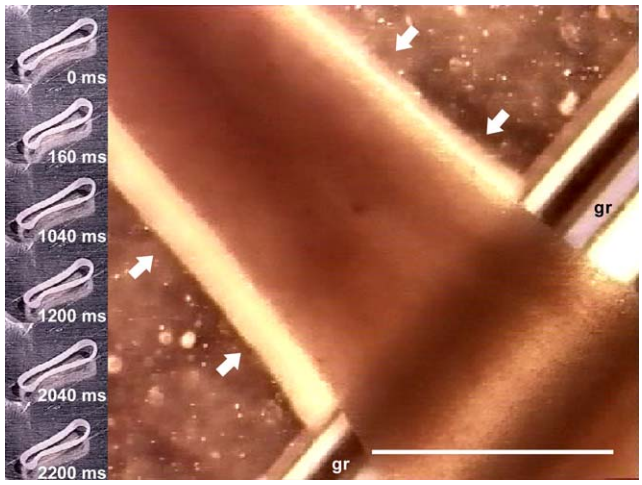

Fig. 1. Macroscopic view of contracting EHTs. Serial recordings of 3 spontaneous contractions of an EHT in Tyrode's solution (left panels). Low power microscopic view of an EHT (arrows) attached to a glass rod (gr; right panel). Online movie clips demonstrating the contractile function of EHTs can be viewed at: www.pharmakologie.uni-erlangen.de or at http://circres.ahajournals.org/cgi/content/full/90/2/223/DC1/. Bar: 1 mm.

2. Construction and matrix properties of engineered heart tissue

Culture of heart cells in a reconstitution mixture consisting of collagen type I, extracellular basement membrane proteins (Matrigel®), chick embryo extract, and horse serum-containing medium yields EHTs with morphological and functional properties of native heart tissue (Fig. 1) [9,12,13]. The method differs in two important aspects from other, matrix-based approaches. First, the soluble collagen type I, in contrast to solid matrices (e.g. polyglycolic acid, alginate, collagen/gelatine meshes), apparently promotes organization of heart cells to a synchronously contracting heart tissue-like construct. The intrinsic property of heart cells to form contractile aggregates has been described many years ago for embryonic chick cardiac myocytes and in a more recent publication for neonatal rat cardiac myocytes [6]. The second important aspect is, in our view, culture under persistent mechanical load. In our early experiments with square cardiac tissue lattices, load was imposed on the constructs by metal spacers that were designed to hold the cardiac muscle construct at a defined distance via two Velcro-coated glass tubes that were attached to opposing sides of the construct [9]. This yielded biconcavally shaped spontaneously and synchronously contracting lattices spanning the gap between the two tubes and prevented fibrotic degeneration of the cardiac muscle construct. More recently circular EHTs have been developed. In this geometry load is imposed on the cells more equally which results in a significantly improved tissue structure [12,13]. Continued culture under cyclic stretch improved

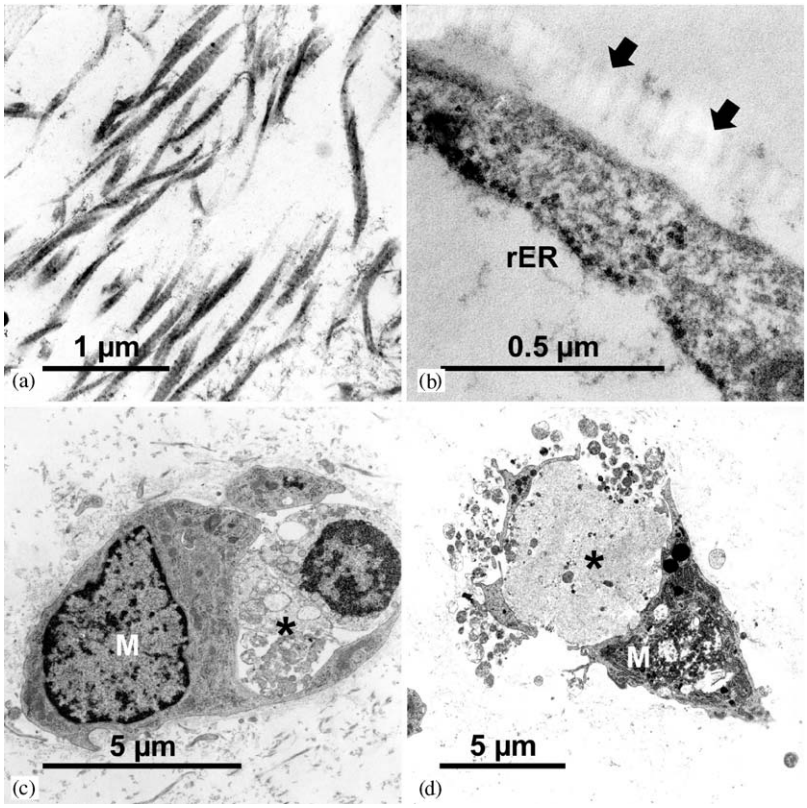

Fig. 2. Transmission electron microscopy demonstrating matrix remodeling processes in EHT. (a) In vitro synthesized collagen within EHT. Note the characteristic cross striation of mature collagen fibrils. Mature collagen is not present in the original EHT reconstitution mixture. (b) Ultra-high resolution imaging of collagen assembly. Arrows indicate extracellular assembly of mature collagen after secretion from a fibroblast within EHT. rER: rough endoplasmatic reticulum inside the fibroblast. (c + d) Phagocytosis of cell debris and matrix components (asterisk) by macrophages (M) within EHT.

morphology and function particularly of lattice-shaped EHTs [8].

During EHT culture (10–14 days) matrix structure changes markedly from mostly randomly oriented collagen fibers without apparent cross striation to a more organized matrix structure with newly synthesized mature collagen with ultrastructurally discernable cross striation (Fig. 2a). We assume that the original, rat tail-derived collagen fibers in the reconstitution mixture provide a provisional 3D environment that is at least partially replaced by newly formed mature collagen and possibly other matrix proteins like laminin and fibronectin to reconstitute extracellular matrix and the basement membrane of cells within EHT [13]. Macrophages and fibroblasts are frequently found throughout EHTs and most likely play a central role in the remodeling process of the EHT matrix (Figs. 2b–d). The change in matrix structure is paralleled by marked changes in MMP and TIMP expression during culture (unpublished data). Eventually, a compact, mechanically stable construct forms that can be manipulated in vitro and implanted in vivo by standard surgical techniques [16].

Various factors have been tested for their role in EHT formation. Increasing the collagen content in the reconstitution mixture yields EHTs with higher stiffness and less contractile force development. In contrast, decreasing the collagen content yields EHTs with a soft matrix structure but improved contractile properties [17]. We chose a collagen content of 0.8 mg/EHT collagen as a compromise between mechanical stability and contractility. Addition of chick embryo extract and horse serum was found to be essential for EHT development [17]. While chick embryo extract could be omitted from the culture medium after an initial culture phase, even short periods of horse serum withdrawal led to cessation of contractions (Fig. 3). Similarly, Matrigel® is an essential factor for EHT formation in rat, but interestingly not in chick EHTs [12]. Geometric shape and size of EHTs can be adjusted by utilization of suitable casting molds [12]. The production of large 3D constructs for replacement of large tissue defects or partial reconstruction of a ventricular wall can be achieved by stacking and merging of multiple EHTs in vitro.

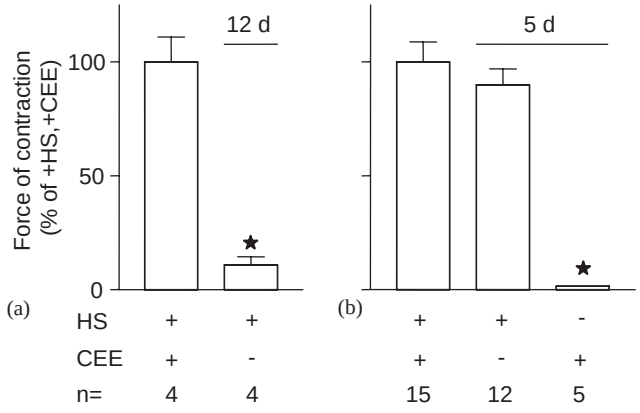

Fig. 3. Effect of horse serum and chick embryo extract on contractile function of EHT. (a) Force development of EHTs was markedly diminished and completely absent (not shown) when culture was performed without chick embryo extract (CEE) and/or horse serum (HS) supplementation, respectively. (b) When CEE or HS was withdrawn after 7 days for the last 5 days in culture contractility was not effected or completely abolished, respectively. Complete culture medium: DMEM, 10% HS, 2% CEE. Force of contraction was measured under isometric conditions at 37°C in Tyrode's solution (2 mmol/l calcium) and pulsed-field stimulation at 2 Hz on culture day 12. When cultured under complete medium conditions EHTs developed (a) 0.68 ± 0.06 and (b) 0.55 ± 0.06 mN $\pm$ SEM at 2 mmol/l calcium. $p < 0.05$ vs. complete culture medium (+HS, +CEE).

3. Cell composition of engineered heart tissue

As indicated above EHTs are not composed purely from cardiac myocytes, but comprise almost all cell species that are normally found in the heart including cardiac myocytes, fibroblasts, smooth muscle cells, endothelial cells, macrophages, and other cells of leukocytotic origin [13]. Physiologically, the heart consists of approximately 30% cardiac myocytes and 70% non-myocytes [18]. Non-myocytes play a pivotal role in heart and cardiac myocyte development/hypertrophy and function [19,20]. Accordingly, EHTs reconstituted from unpurified heart cell mixtures showed an advanced tissue structure and increased contractile and passive forces when compared to EHTs reconstituted from partly purified cardiac myocyte populations [21]. Thus, we believe that the construction of optimal cardiac tissue constructs depends on a "physiological mix" of non-myocytes and cardiac myocytes. In line with this reasoning, EHTs do not only consist of a cardiac myocyte network embedded in a collagen matrix, but display other organotypic structures such as a well-organized continuous surface completely covered by epithelial cells (Fig. 4) and capillary-like structures that penetrate the matrix and sometimes form large primitive vessel-like structures [13,21].

In the absence of true vascularization or artificial means to supply engineered tissue constructs with

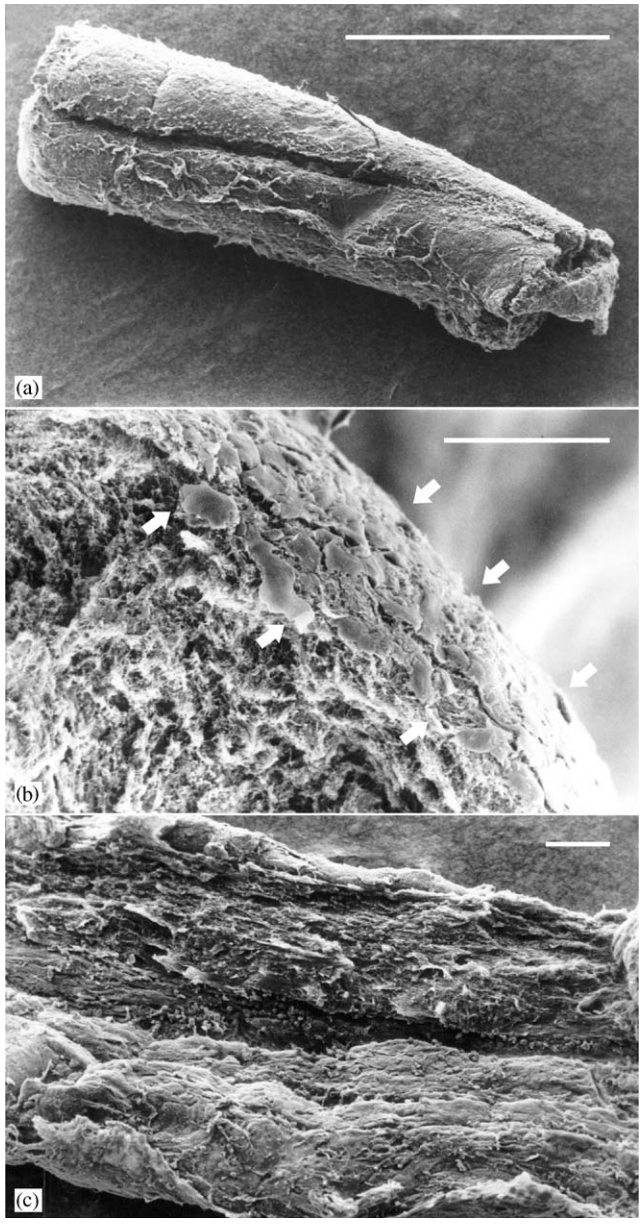

Fig. 4. Scanning electron microscopy of a gold sputtered EHT. (a) EHT fragment at low magnification. (b) Partial cross-section of a fractured EHT. Note the EHT surface which is completely covered by an epithelial cell layer (arrows). (c) Longitudinal view of the interior of an EHT after fracture of the sample. Note the longitudinal orientation of matrix embedded cells within the EHT. Bars: 1 mm (a), 0.1 mm (b + c).

nutrients and oxygen, all tissue engineering, approaches face the problem of critical thickness. Indeed, the maximal thickness of compact muscle strands in EHTs is limited to 100–200 μm. However, in EHTs the majority of cardiac cells form a loose, highly interconnected 3D network consisting of cardiac myocyte strands that are generally less than 20 μm in diameter. This network occupies almost the entire EHT with a

thickness of 1 mm without signs of core necrosis [13]. It is likely that the loose collagen/matrigel matrix does not impose a significant diffusion barrier and that the network of thin cardiac myocyte strands facilitates sufficient oxygen and metabolic supply in EHTs. This is exemplified by the fact that EHTs contract in culture at normal room oxygen for weeks without signs of degeneration. At present, the size of EHTs for implantations is increased by stacking and merging several EHTs. Yet, thicker and larger constructs, necessary for applications in humans, are likely to require advanced methods. One option may be to increase the intrinsic angiogenic activity in EHTs by adding more endothelial cells or angiogenic growth factors. Additionally, bioreactors may allow culture under high oxygen and optimized metabolic supply [6,7,15].

4. Functional properties of engineered heart tissue

Contractile parameters of EHTs can be assessed by isometric force measurements in standard organ baths [9,12]. EHTs respond to mechanical (Frank-Starling mechanism) and pharmacological (positive inotropic responses to calcium and isoprenaline) interventions in an organotypic manner (Fig. 5) [9,12,13]. Maximal force of contraction of EHTs has been recorded at $\sim 3\,\mathrm{mN/mm^2}$ which is less, but already comparable to isolated muscle strips from rat hearts ($\sim 20\,\mathrm{mN/mm^2}$). Another important aspect is the ratio of active (systolic) and passive (diastolic) force (see [13] for discussion). The ratio should be above 1 in healthy myocardium from rats and humans [22,23] and amounts to 1.33 in EHTs at basal calcium concentrations (0.4 mmol/l) [13].

Stimulated action potential recordings of cardiac myocytes within EHTs exhibit a negative resting membrane potential ($-73 \pm 2\,\mathrm{mV}$), a steep upstroke (dV/dt: $66 \pm 8\,\mathrm{V/s}$), a plateau phase (action potential duration: $148 \pm 3\,\mathrm{ms}$), and no spontaneous depolarization in phase 4 indicating a relatively mature ventricular myocyte-like electrophysiological phenotype [13]. These findings were somewhat unexpected since EHTs contract spontaneously and rhythmically (~ 1–$2\,\mathrm{Hz}$). During culture the spontaneous beating behavior of EHTs undergoes extensive changes. 1–3 days after casting multiple areas within EHTs contract independently from each other. Coherent contractions of the complete EHT start 3–4 days after casting and, over time of culturing, become increasingly regular and robust, faster in terms of single twitch kinetics, and slower in terms of beating rate. Interestingly, small fragments cut from coherently contracting EHTs retain their propensity to beat spontaneously. This observation may indicate that spontaneous EHT contractions are not the result of a reentry activation but controlled by predominant pacemakers in complete and fragmented EHTs.

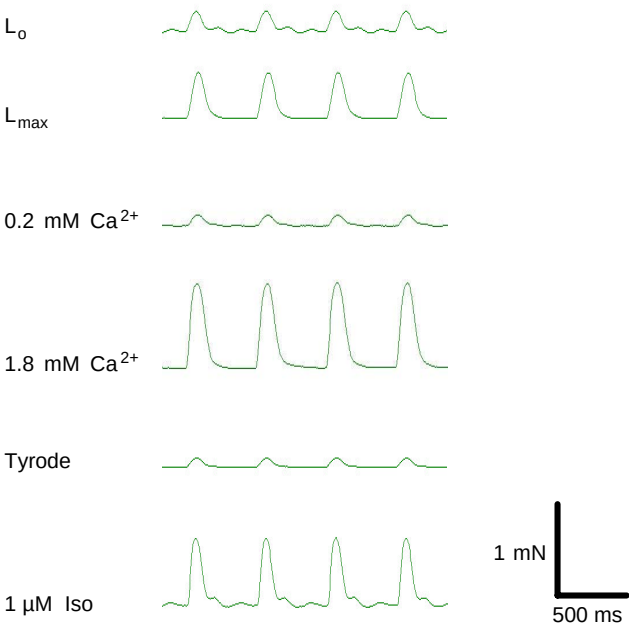

Fig. 5. Contractile properties of EHT. Representative tracings of an isometric contraction experiment (37°C, 2 Hz pulsed field stimulation). Force of contraction recorded at L_0 (slack length) and L_{max} (optimal preload) in modified Tyrode's solution (0.4 mmol/l calcium), at low (0.2 mmol/l) and high (1.8 mmol/l) calcium, and before (Tyrode) and after stimulation with isoprenaline (1 µmol/l) at 0.2 mmol/l calcium. Extended wash out phases were performed in between interventions.

5. Implantation of engineered heart tissue

EHT-implantation studies were performed in syngeneic Fischer 344 rats and served to investigate in vivo survival, integration, and maintenance of EHT contractile function [16,17]. EHTs were implanted either into the peritoneal cavity or on the beating heart of healthy rats (Fig. 6). First implantation studies failed due to immune rejection that was most prevalent after cardiac EHT implantation [16]. Taking into consideration our syngeneic approach, cells and collagen were prepared from syngeneic donors, this finding was not anticipated but could be explained by residual immunogenic matrix components (Matrigel®) or strongly adherent or impregnated culture medium components (chick embryo extract, horse serum). The necessity of matrigel and serum components did not allow for withdrawal of these potentially immunogenic EHT components neither did prolonged washing in serum-free Tyrode's solution reduce the immune response. Thus, all EHT implantation studies had to be performed under immunosuppression (cyclosporine A, azathioprine, methylprednisolone) [16]. Under this treatment regimen EHTs remained viable and contractile for at least 8 weeks in vivo and became strongly vascularized and innervated. Another important observation was the advanced degree of maturation obtained by implanted cardiac myocytes in vivo. This indicates the importance of a physiological

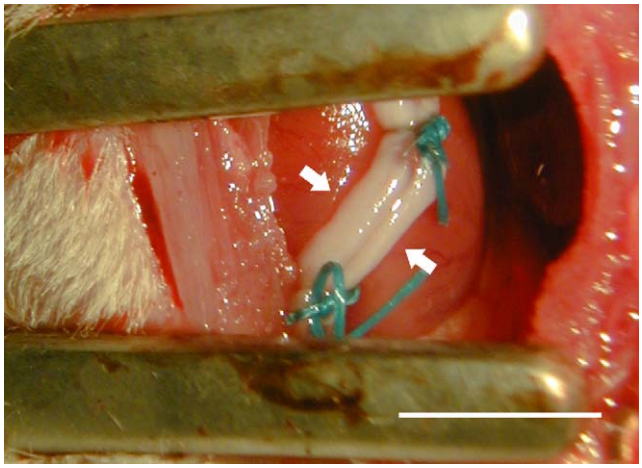

Fig. 6. Implantation of EHT onto the heart of a Fischer 344 rat after left lateral thoracotomy. Rats were anesthetized with isoflurane and ventilated. The third intercoastal space is held open by a retractor. A circular EHT (arrows) was fixed to the myocardium by two single sutures. Bar: 5 mm.

milieu for terminal cardiac myocyte maturation. Presently, EHT implantation studies are carried out in outbred Wistar rats under immunosuppression to investigate the applicability of EHT to regenerate viable myocardium after myocardial infarction.

6. Animal models for experimental replacement therapy

Different rat models have been employed to study the value of tissue engineered heart constructs for replacement therapy [4,10,11,16,17,24]. First implantation studies aimed to investigate whether or not in vitro constructed cardiac muscle grafts survive in vivo, maintain their electric activity, and remain contractile [4,10,16,17]. To assess these basic questions cardiac constructs were implanted subcutaneously [4,10], into the peritoneal cavity [17] or on beating hearts of healthy rats [16]. The question of in vivo survival is not trivial since implanted muscle constructs are not immediately connected to the host vasculature. Thus, diffusion barriers that are present in vitro (see discussion above) are most likely to become even more deleterious in vivo. Despite this apparent obstacle different groups have shown that engineered cardiac muscle constructs do survive in vivo and retain their electromechanical activity [4,10,11,16,17,24]. As other important issues, inflammation and immune response have to be considered in cardiac regeneration with tissue engineered heart muscle. While inflammation caused by the surgical intervention cannot be completely avoided, an additional inflammatory response to scaffold materials or its degradation products might be minimized by chemically altering scaffold materials, utilization of autologous scaffolds or avoiding scaffold material at all. To avoid immunorejection studies have been performed either in nude rats [4] or under immunosuppression (see above) [16].

To investigate whether or not cardiac muscle constructs can be utilized in cardiac regeneration models of myocardial infarction or ventricular replacement have been employed [10,11,24]. To generate myocardial infarctions in rats ligation of the left descending coronary artery (LAD) or freezing of the myocardium with a cryo-probe are widely employed. Both methods have advantages and disadvantages. Ligation of the LAD resembles the natural development of a myocardial infarction more closely. On the other hand, infarct size is more variable when compared to cryo-infarctions. So far, Leor et al. and Li et al. have reported implantation of cardiac grafts on infarcts after coronary ligation and cryo-injury, respectively [10,11]. Implantation of alginate-based cardiac constructs resulted in improved diastolic function which could be explained by an improvement of scar tissue stability and a prevention of aneurysm formation [11]. Eventually, improvement of systolic function has to be achieved to fulfill the promise of cardiac muscle regeneration.

To evaluate the impact of tissue graft implantation on systolic and diastolic heart function and on cardiac remodeling in the infarct model, several critical issues have to be taken into account. (1) Given the variability of infarct sizes particularly in the ligation model, extensive training is necessary to increase the reproducibility of the model to an acceptable level. (2) Infarct size and hemodynamic consequences of an infarction should be investigated prior to grafting in each animal, e.g. by high-resolution echocardiography or magnetic resonance imaging. (3) This allows for a longitudinal study design with repeated non-invasive measurements which provides more valid conclusions at reasonable numbers of animals than a cross-sectional comparison of different treatment groups. (4) Controls such as grafting of tissue constructs from non-contracting cell types are essential to differentiate between effects that are brought about by the contractile activity of new cardiac myocytes and other indirect effects that include improvement of scar elasticity, prevention of aneurysm formation, stimulation of angiogenesis, or cardiac myocyte-growth-promoting paracrine effects.

As an alternative approach Krupnick and colleagues have developed a heterotopic transplantation model to investigate the suitability of cardiac tissue engineering for replacement of the ventricular wall [24]. Full replacement of the ventricular wall of the heart in orthotopic position is presently not possible due to a lack of cardio-pulmonary bypass systems for rats. Hence, heterotopic transplantation allows convenient ex vivo handling of the host heart and implant material but is surgically challenging especially when the

heterotopically implanted heart is to be maintained under loaded conditions.

Eventually, findings from rat models will have to be evaluated in larger animals like pigs, sheep, and dogs. Despite the obvious advantage of genetically modified mice, which would in principle allow for a utilization of available growth promoting and otherwise favorable transgenics in cardiac tissue engineering, it remains to be demonstrated that comprehensive implantation studies with tissue engineered heart constructs can be performed reproducibly in a mouse model.

7. Stem cells in cardiac tissue engineering

For clinically relevant applications of tissue engineering it will be crucial to identify a suitable cell source. The ideal cell should be autologous and available at large quantities, and should exhibit phenotypic cardiac qualities. This cell source has not been identified, yet. A major limitation of cardiac tissue engineering is the inability of cardiac myocytes to proliferate [25]. This precludes in vitro propagation and therefore application of primary cardiac myocytes as an autologous cell source for cardiac tissue engineering. In contrast, embryonic and certain adult stem cells demonstrate unlimited proliferation capacity and provide great hope for cardiac replacement therapy [26,27]. Previous studies have demonstrated the potential of embryonic and adult stem cells to (trans-)differentiate into cardiac myocytes [28–36]. Yet, quantities of stem cell derived cardiac myocytes are low and replacement of a large myocardial infarction (25% of the left ventricle infarcted) would require approximately 1×10^9 myocytes [37] which indicates that the major challenge for stem cell-based cardiac tissue engineering will be to increase the efficiency of stem cells (trans-)differentiation into cardiac myocytes [38,39]. Additionally, concepts have to be developed that take into consideration that the heart consists of multiple different cell types that most likely have to work in concert for an optimized function and thus would be required in tissue engineering of heart muscle (see above). The usefulness of embryonic stem cells might be limited due to their allogeneic character which would require either induction of immune tolerance [40] or life long immunosuppression [41]. Recently, non-immunogenic cardiac grafts have been prepared from recipient derived cardiac myocytes generated by somatic cell nuclear transfer [42]. Besides serious ethical concerns related to this technique the issues of quantity (generation of enough cardiac myocytes) and quality (generation of differentiated and functional cardiac myocytes) pertain to therapeutic cloning as to other stem cell-based approaches.

8. A long way to clinical applications

Despite exciting achievements in cardiac tissue engineering and stem cell research many crucial questions remain unanswered and preclude a valid prediction of its clinical value. (1) Most importantly an ideal cell source has not been identified yet and methods for large scale production of differentiated cardiac cells are lacking. (2) Whether implantation of engineered cardiac tissue constructs can improve systolic and diastolic function of malfunctioning myocardium remains unclear. (3) Whether or not the technically more demanding tissue engineering approach offers clear advantages over cell injection or infusion remains to be elucidated. (4) Finally, even if all requirements are being fulfilled, it remains to be answered if the best cell-based therapy approach is more efficacious than traditional or stem cell directed pharmacological treatment.

9. Conclusion

Cardiac tissue engineering is a promising field aiming at replacement of diseased myocardium with prefabricated cardiac tissue-like constructs. As such, it might be an alternative to the direct injection or infusion of isolated cells [35,43–49]. Various groups including our own have developed methods to fabricate 3D cardiac tissue-like constructs from neonatal rat heart cells in vitro. Functional properties, tissue integration and immunological toleration of the engineered constructs are currently investigated mostly in rat models with first exciting results. Nevertheless, the potential clinical value of cardiac tissue engineering approaches will critically depend on the availability of a suitable cell source and the proof of concept in larger animal models.

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References

[1] 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:E182–7.

[2] 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;124:63–9.

[3] Akhyari P, Fedak PW, Weisel RD, Lee TY, Verma S, Mickle DA, Li RK. Mechanical stretch regimen enhances the formation of

bioengineered autologous cardiac muscle grafts. Circulation 2002;106:I137–42.

[4] 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.

[5] van Luyn MJ, Tio RA, Gallego y van Seijen XJ, Plantinga JA, de Leij LF, DeJongste MJ, van Wachem PB. Cardiac tissue engineering: characteristics of in unison contracting two- and three-dimensional neonatal rat ventricle cell (co)-cultures. Biomaterials 2002;23:4793–801.

[6] 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;5:103–18.

[7] 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;64:580–9.

[8] 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;14:669–79.

[9] 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;11:683–94.

[10] Li RK, Jia ZQ, Weisel RD, Mickle DA, Choi A, Yau TM. Survival and function of bioengineered cardiac grafts. Circulation 1999;100:II63–9.

[11] 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.

[12] 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;68:106–14.

[13] Zimmermann WH, Schneiderbanger K, Schubert P, Didie M, Munzel F, Heubach JF, Kostin S, Neuhuber WL, Eschenhagen T. Tissue engineering of a differentiated cardiac muscle construct. Circ Res 2002;90:223–30.

[14] Langer R, Vacanti JP. Tissue engineering. Science 1993;260:920–6.

[15] Carrier RL, Rupnick M, Langer R, Schoen FJ, Freed LE, Vunjak-Novakovic G. Effects of oxygen on engineered cardiac muscle. Biotechnol Bioeng 2002;78:617–25.

[16] Zimmermann WH, Didie M, Wasmeier GH, 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:I151–7.

[17] Eschenhagen T, Didie M, Münzel F, Schubert P, Schneiderbanger K, Zimmermann W. 3D engineered heart tissue for tissue replacement therapy. Basic Res Cardiol 2002;97(Suppl. 1):I146–52.

[18] Nag AC, Zak R. Dissociation of adult mammalian heart into single cell suspension: an ultrastructural study. J Anat 1979;129:541–59.

[19] Brutsaert DL, De Keulenaer GW, Fransen P, Mohan P, Kaluza GL, Andries LJ, Rouleau JL, Sys SU. The cardiac endothelium: functional morphology, development, and physiology. Prog Cardiovasc Dis 1996;39:239–62.

[20] Harada M, Itoh H, Nakagawa O, Ogawa Y, Miyamoto Y, Kuwahara K, Ogawa E, Igaki T, Yamashita J, Masuda I, Yoshimasa T, Tanaka I, Saito Y, Nakao K. Significance of ventricular myocytes and nonmyocytes interaction during cardiocyte hypertrophy: evidence for endothelin-1 as a paracrine hypertrophic factor from cardiac nonmyocytes. Circulation 1997;96:3737–44.

[21] 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 [abstract].

[22] 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;37:541–8.

[23] 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;94:683–9.

[24] 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;21:233–43.

[25] Pasumarthi KB, Field LJ. Cardiomyocyte cell cycle regulation. Circ Res 2002;90:1044–54.

[26] Blau HM, Brazelton TR, Weimann JM. The evolving concept of a stem cell: entity or function? Cell 2001;105:829–41.

[27] 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;282:1145–7.

[28] 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 Ca^{2+} channel blockers. Differentiation 1991;48:173–82.

[29] 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.

[30] 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;108:407–14.

[31] Malouf NN, Coleman WB, Grisham JW, Lininger RA, Madden VJ, Sproul M, Anderson PA. Adult-derived stem cells from the liver become myocytes in the heart in vivo. Am J Pathol 2001;158:1929–35.

[32] 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;98:10733–8.

[33] Minasi MG, Riminucci M, De Angelis L, Borello U, Berarducci B, Innocenzi A, Caprioli A, Sirabella D, Baiocchi M, De Maria R, Boratto R, Jaffredo T, Broccoli V, Bianco P, Cossu G. The mesoangioblast: a multipotent, self-renewing cell that originates from the dorsal aorta and differentiates into most mesodermal tissues. Development 2002;129:2773–83.

[34] 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;107:1395–402.

[35] Orlic D, Kajstura J, Chimenti S, Bodine DM, Leri A, Anversa P. Transplanted adult bone marrow cells repair myocardial infarcts in mice. Ann NY Acad Sci 2001;938:221–9.

[36] 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;418:41–9.

[37] Gepstein L. Derivation and potential applications of human embryonic stem cells. Circ Res 2002;91:866–76.

[38] 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;97:11307–12.

[39] Xu C, Police S, Rao N, Carpenter MK. Characterization and enrichment of cardiomyocytes derived from human embryonic stem cells. Circ Res 2002;91:501–8.

[40] Fandrich F, Lin X, Chai GX, Schulze M, Ganten D, Bader M, Holle J, Huang DS, Parwaresch R, Zavazava N, Binas B. Preimplantation-stage stem cells induce long-term allogeneic graft acceptance without supplementary host conditioning. Nat Med 2002;8:171–8.

[41] Drukker M, Katz G, Urbach A, Schuldiner M, Markel G, Itskovitz-Eldor J, Reubinoff B, Mandelboim O, Benvenisty N. Characterization of the expression of MHC proteins in human embryonic stem cells. Proc Natl Acad Sci USA 2002;99:9864–9.

[42] Lanza RP, Chung HY, Yoo JJ, Wettstein PJ, Blackwell C, Borson N, Hofmeister E, Schuch G, Soker S, Moraes CT, West MD, Atala A. Generation of histocompatible tissues using nuclear transplantation. Nat Biotechnol 2002;20:689–96.

[43] 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;105:1720–6.

[44] 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;4:929–33.

[45] 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;357:279–80.

[46] Li RK, Mickle DA, Weisel RD, Zhang J, Mohabeer MK. In vivo survival and function of transplanted rat cardiomyocytes. Circ Res 1996;78:283–8.

[47] 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;410:701–5.

[48] 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;105:2435–41.

[49] Strauer BE, Brehm M, Zeus T, Kostering M, Hernandez A, Sorg RV, Kogler G, Wernet P. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. Circulation 2002; 106:1913–8.