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Cardiac Tissue Engineering: Implications for Pediatric Heart Surgery

Wolfram-Hubertus Zimmermann · Robert Cesnjevar

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Abstract Children with severe congenital malformations, such as single-ventricle anomalies, have a daunting prognosis. Heart transplantation would be a therapeutic option but is restricted due to a lack of suitable donor organs and, even in case of successful heart transplantation, lifelong immune suppression would frequently be associated with a number of serious side effects. As an alternative to heart transplantation and classical cardiac reconstructive surgery, tissue-engineered myocardium might become available to augment hypomorphic hearts and/or provide new muscle material for complex myocardial reconstruction. These potential applications of tissue engineered myocardium will, however, impose major challenges to cardiac tissue engineers as well as heart surgeons. This review will provide an overview of available cardiac tissue-engineering technologies, discuss limitations, and speculate on a potential application of tissue-engineered heart muscle in pediatric heart surgery.

Keywords Congenital heart disease · Tissue engineering · Myocardial repair · Regeneration · Stem cells

W.-H. Zimmermann (✉)
Department of Pharmacology, University Medical Center Goettingen, Robert-Koch-Str. 40, 37075 Goettingen, Germany
e-mail: w.zimmermann@med.uni-goettingen.de

R. Cesnjevar
Department of Pediatric Cardiac Surgery, University Hospital Erlangen, Loschgestr. 15, 91054 Erlangen, Germany

Introduction

The heart is the first major organ that reaches functional competence in the developing embryo and subsequently enables blood flow through the pulmonary and systemic circulation. Its anlage can be distinguished morphologically as early as 7 days and 3 weeks after fertilization in mice and humans, respectively [32]. Heart function can be assessed by ultrasound shortly afterward [41, 48]. Embryonic heart development begins with the formation of the cardiac crescent; this structure will eventually fuse to establish the primitive heart tube; subsequent looping and septation will finally lead to the configuration of the four-chambered heart [32]. These complex processes are commonly disrupted, leading either to premature termination of pregnancy or congenital malformations [50]. Taken together, 4% of developing embryos are affected. Ultimately, structural myocardial defects are diagnosed in approximately 1% of all newborns, making heart malformations the most common pathological congenital condition.

Life-compatible defects, such as atrial and ventricular septum defects (ASDs and VSDs), are frequently associated with other cardiac and extracardiac malformations as well as chromosomal abnormalities (e.g., trisomies). More complex malformations are rare and include, for example, hypoplastic left heart syndrome (HLHS), double inlet left ventricle (DILV), double outlet right ventricle (DORV), tetralogy of Fallot (TOF), and transposition of the great arteries (TGA). Most of these congenital heart defects are generally not compatible with life and require comprehensive as well as early reconstructive surgeries such as a switch operation for TGA and staged Fontan palliation including the Norwood procedure in single-ventricle anomalies [15]. Especially in the latter pathology, surgical repair is generally palliative. Heart transplantation would

be the ultimate therapeutic option but is limited by the scarcity of suitable donor organs. In addition, lifelong immune suppression often causes serious complications, including kidney failure and malignancies. Importantly, long-term outcome of the above-mentioned surgical interventions remains largely unknown and it would clearly be desirable to develop alternative strategies to repair hearts with severe congenital malformations.

One exciting but also highly controversial approach is intrauterine reconstructive surgery [40, 53]. The interested reader is referred to expert reviews in this field for detailed information on this intervention [14, 40]. Alternatively, biological repair using tissue-engineered myocardium might become an option. It will, however, require the generation of functionally competent heart muscle of sufficient size and with in vitro as well as in vivo growth potential.

Several tissue-engineering technologies are already available, enabling the generation of contractile heart muscle in the lab [58]. Moreover, proof-of-concept for an in vivo application of tissue-engineered myocardium to remuscularize a failing heart has already been provided in a rodent model of myocardial infarction [59]. It would, nevertheless, be pretentious to claim that cardiac muscle engineering is on the verge to clinical exploitation. Further refinements to eventually generate force-developing human heart muscle, to provide grafts of relevant size, and to control immunological issues will be necessary to advance the field. This review will discuss the state-of-the-art in myocardial tissue engineering, its limitations, and potential applications in pediatric heart surgery.

State-of-the-Art in Cardiac Tissue Engineering

The ultimate goal in therapeutic cardiac tissue engineering is to generate biocompatible, nonimmunogenic heart muscle with morphological and functional properties of natural myocardium. To this end, at least four distinguishable tissue engineering modalities have been established over the past 15 years (reviewed in [11, 58]; Fig. 1). These include the following: (1) the *bioengineering approach,* involving seeding of heart cells on preformed biocompatible scaffolds [4, 5, 27, 29]; (2) the *biological assembly approach*, utilizing hydrogels to entrap heart cells and support their intrinsic capacity to organize into a cardiac syncytium [10, 56, 57]; (3) the *cell sheet approach*, using a unique technique to detach and serially stack monolayer cell cultures to form contractile heart tissue sandwiches [46]; (4) the *decellularization–recellularization approach*, taking advantage of the ability to strip tissues of all cellular components but at the same time retain their extracellular matrix structure to be used as a reseeding substrate for heart or cardiogenic stem cells [39]. In addition to the mentioned techniques, other modalities, such as the microtissue approach [21], have been developed and might indeed provide therapeutic tissue structures. This review will, however, focus only on tissue-engineering modalities enabling the generation of force-generating heart muscle on a macroscopic scale:

(1) The *bioengineering approach* utilizes preformed scaffolds, which are either chemically engineered, such as polylactic acid (PLA), polyglycolic acid (PGA), and polyglycerol sebacate (PGS) [4, 5, 9], or derived from biological sources, such as collagen/gelatin from tendon, alginates from seaweed, and silk from silkworms or spiders [27, 30, 42], and subsequently processed to generate sponges or meshes with various pore sizes. Cells are eventually seeded onto these scaffolds and cultured in a three-dimensional (3D) format. Importantly, most of the above-mentioned materials, with collagen and silk being exceptions, do not support cardiomyocyte attachment or growth in an optimal way. In addition, limited diffusion and, thus, oxygen and nutrient supply have been main caveats of the *bioengineering approach*. Moreover, synthetic polymers commonly have unfavorable degradation properties (e.g., the acidification of surrounding tissue in the case of PLA and PGA) and cause inflammatory responses [52]. This is acceptable or even desirable if the above-mentioned materials are used as suture material, but it is unacceptable if the materials are used as tissue scaffolds in vivo. To overcome this limitation, one might opt to degrade the respective materials before implantation. From the engineering and regulatory point of view, the above-mentioned (bio-)materials would be advantageous because its components are fully chemically defined and have already been approved (e.g., PLA, PGA, alginate, collagen/gelatin sponges) by the US Food and Drug Administration (FDA).

(2) The *biological assembly approach* takes advantage of the intrinsic propensity of cardiomyocytes to generate "cardiac microtissues" if cultured at a high cell density in suspension [1, 21, 22, 34]. Suspending cells in casting molds containing mixtures of extracellular matrix material, such as collagen, laminin, and fibronectin [10, 36, 56, 57], or naturally occurring products from blood, such as fibrin [19], supports self-aggregation in a defined 3D environment. Exposing the developing tissues to biophysical stimuli, such as mechanical strain and electrical stimulation [12, 42], can further be used to guide cardiac tissue formation and will eventually yield strongly

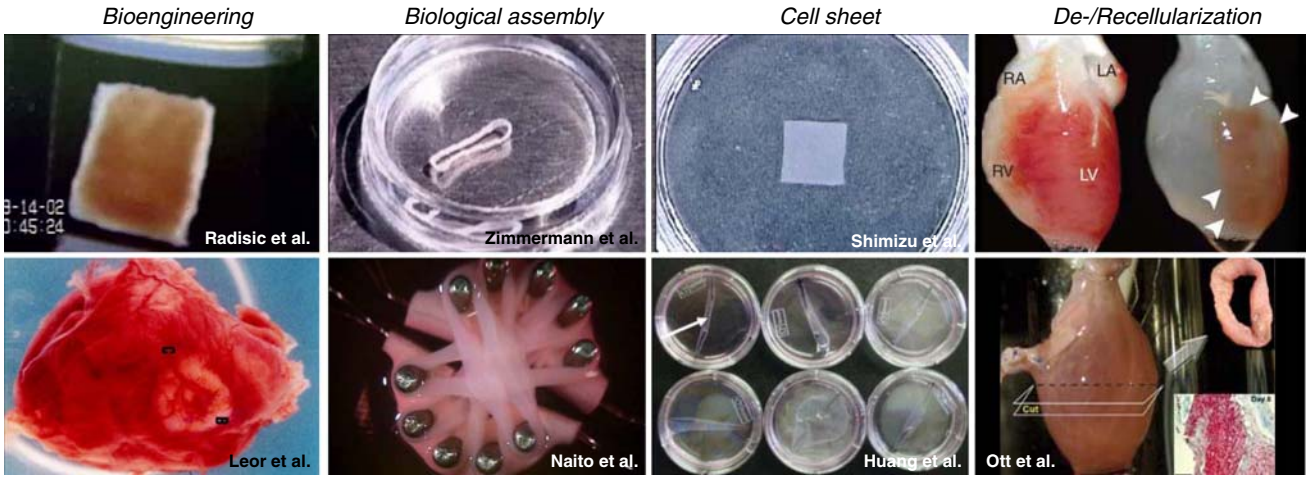

Fig. 1 Tissue engineering modalities enabling the generation of large macroscopically contracting tissue constructs: (1) *bioengineering approach* [27, 42], (2) *biological assembly approach* [36, 57], (3) *cell sheet approach* [19, 46], and (4) *decellularization–recellularization approach* [39]

contracting heart tissue equivalents [57, 59]. In contrast to the *bioengineering approach,* the shape and size of forming tissues are not governed by the matrix material itself, but by the casting molds utilized for initial tissue reconstitution. Although diffusion is also a limitation of the *biological assembly approach*, it appears that hydrogels enable better oxygen and nutrient transport as compared to preformed matrixes. This might be the reason why tissue formation in, for example, engineered heart tissue (EHT) is not restricted to outer layers of the collagen hydrogel-culture format [57]. Instead, muscle within EHTs forms a delicate 3D network, composed of 20–200-µm-thick muscle bundles (Fig. 2). Importantly, single-muscle units generated by the *biological assembly approach* might be fused to form large tissue constructs [36]. This property might in principle even be exploited to generate clinical-scale tissue constructs of various geometries.

(3) The *cell sheet approach* is based on temperature-controlled release of cell monolayers from PIPAAm (polyisopropylacrylamide)-coated culture dishes [38]. PIPAAm has unique hydrophobic or hydrophilic properties depending on the environmental temperature. Physiological temperatures (37°C) facilitate cell attachment, whereas low temperatures (20°C) render the polymeric surface hydrophilic and consequently lead to rapid detachment of cell monolayers. Importantly, this detachment procedure does not disturb cell–cell contacts within the monolayer and apparently maintains all cell surface structures. This might explain why cell sheets can rapidly attach and establish electrical contacts to each other [18, 47] as well as to underlying myocardium if applied in vivo [31]. Yet, the *cell sheet approach* is also diffusion limited. This becomes apparent at a monolayer-stack diameter of ~100 µm (four to six layers) [46]. Employing sequential grafting of thin layers on top of

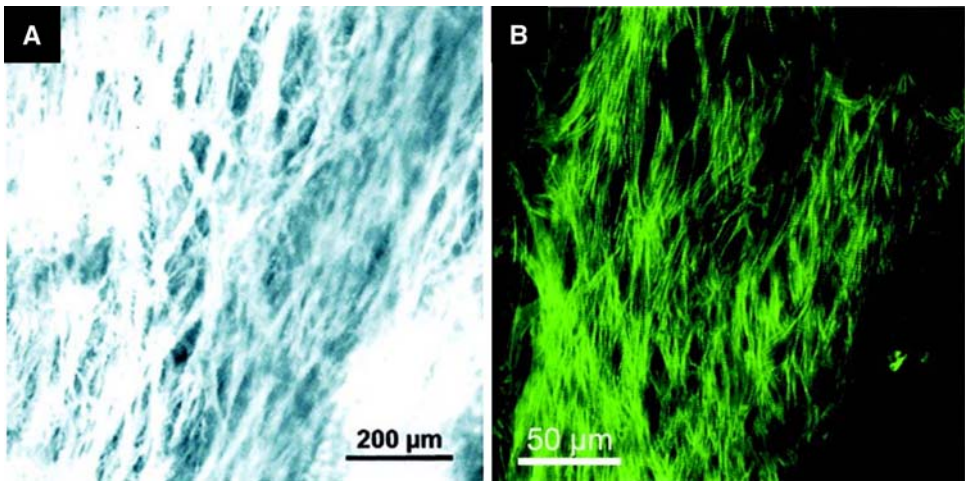

Fig. 2 Muscle formation in EHT. Actin staining (white in [**a**], green in [**b**]) denotes the formation of a dense network of muscle strands, which might in some cases reach a diameter of up to 200 µm. Images from [57] (**a**) and [36] (**b**). (Color figure online)

each other can, however, be exploited to generate compact vascularized tissues with a diameter of at least 1 mm in vivo [47].

(4) The *decellularization-recellularization approach* employs tenside (SDS: sodium dodecyl sulfate) and DNase treatment to dispose of all potentially immunogeneic structures of the heart and subsequent reseeding of the remaining extracellular matrix (ECM) with cells either by direct injection into the "naked" ECM or transfusion through spared vascular channels [39]. The latter approach appears better suited to reestablish a homogenous musculature in the heart but would require that cells migrate into the heart's ECM and form a continuous functional syncytium. Future studies will have to provide evidence that this can indeed be achieved and that the resulting hearts can be transplanted and perform as desired in vivo. To this end, first short-term data from a heterotopic heart transplantation model are encouraging [39].

Limitations

Myocardial tissue engineering still has to go a long way in order to be translated into a clinically exploitable treatment modality, and it is difficult to predict when or even whether it will ever reach its proposed potential. Yet, important recent clinical studies have demonstrated that tissue-engineered products might indeed be applicable to offer mechanical and/or paracrine support to failing hearts ([7]; Sawa et al., personal communication). These studies could clearly be considered pivotal because they describe a first-in-man application of the *bioengineering* [7] and *cell sheet approach* (Sawa et al., personal communication). Yet, the patient number in both of these studies was minimal; therefore, carefully designed follow-up studies need to be conducted to identify the potential of tissue-engineered products in heart repair. However, unearthing the ultimate potential of myocardial tissue engineering will likely require the utilization of contractile muscle elements to remuscularize a failing heart and thereby also functionally compensate for a loss of cardiac muscle. Force-generating myocardium will have to be engineered in vitro and seamlessly integrated in diseased hearts to achieve this goal. Importantly, the potential of electrical and structural integration of heart cells into an allogeneic recipient has already been demonstrated by multiple groups [13, 44, 59] and it is likely that electrical integration will not be an issue if demarcation of implants by scar formation can be controlled [43]. Hence, the most pertinent limitations include (1) the applicability of human heart cells in cardiac tissue engineering, (2) overcoming size limitations, and (3) providing fully biocompatible tissue-engineered muscle. The following list provides a discussion of these issues:

(1) Human heart cells can be isolated from biopsy and autopsy material. These cells will, however, not be suitable for myocardial tissue engineering given their limited number, lack of cell cycle activity, and inability to reestablish a functional syncytium once they have been dispersed into single-cell suspensions. Consequently, cardiogenic stem cells will have to be exploited. Adult stem cells from the bone marrow do not harbor the capacity to generate the substantial amount of myocytes needed for complex myocardial reconstitution [3, 35, 37]. In contrast, embryonic stem cells can, in principle, provide cardiomyocytes in adequate numbers [55]. In addition, embryonic stem-cell-derived myocytes are still capable of developing into complex functional syncytia [20] and, only recently, the use of embryonic stem-cell-derived cardiomyocytes was demonstrated in myocardial tissue engineering [6, 17]. Collectively, these data provide strong evidence that embryonic stem cells might be an appropriate cell source for clinical-scale myocardial tissue engineering. However, cardiomyocyte yield from embryonic stem cells is generally low (1–5%; [23]) and scalability will, consequently, be an important issue to generate relevant cardiomyocyte numbers and eventually "force-generating" human myocardium. The later goal has, despite two recent reports using embryonic stem-cell-derived myocytes in a 3D culture format [6, 45], not been achieved, but it is likely that hydrogel-cultures will be instrumental to generate such tissues [60]. Similarly, nonembryonic pluripotent stem cells, including induced pluripotent stem cells [49], spermatogonial stem cells [16], and parthenogenetic stem cells [51], will be exploitable in cardiac tissue engineering. These cell types will, on the one hand, not require cell harvest from an early embryo and might, in addition, be suitable for the generation of autologous cells; however, other ethically controversial issues remain, including the need for genetic manipulations in the induced pluripotent stem-cell-based technology and the risk of in vitro mutagenesis and teratoma formation after an in vivo application.

(2) It will be essential to generate and implant thick muscle tissues to achieve meaningful contractile support in the setting of heart failure, to adequately replace scarred heart tissue in individuals with myocardial infarction, or to repair tissue deficiencies in pediatric hearts. The need for continuous oxygen and nutrient supply to metabolically highly active myocardium is a key issue

for all tissue-engineering technologies. Although myocardium can already be reconstituted in vivo with a diameter of up to 1 mm [47, 59], this would likely be insufficient to impart a clinically relevant effect. In vivo physiological muscle diameters of at least 10 mm need to be matched. To achieve this goal, "in vitro vascularization" might be a prerequisite. It is unlikely that this will be achieved by simply adding endothelial cells or smooth muscle cells to a tissue reconstitution mixture, although it has been clearly documented that nonmyocytes will have beneficial effects, which include the formation of primitive capillary structures in tissue-engineered heart muscle [28, 36, 57]. This will, however, not likely be sufficient to provide immediate circulatory support to large engineered tissue grafts in vivo. To overcome this limitation, vascularized myocardial tissues could be generated in vivo either by seeding cardiogenic cells in prevascularized chambers [33] or by introducing a macrovasculature with defined inflow and outflow to enable surgical anastomoses to, for example, the coronary arteries [24].

(3) Tissue-engineered myocardium would ideally be generated from biocompatible and autologous material. Biocompatible matrix materials are commonly available (e.g., collagen). Unfortunately, autologous cardiogeneic cells are not readily available. Adult stem cells from the blood or bone marrow would be logistically ideal but do not have the intrinsic capacity to give rise to cardiomyocytes of sufficient quality and quantity [2]. In contrast, embryonic stem cells can give rise to *bona fide* cardiomyocytes [8, 20, 54], which would, however, elicit an immune response in an allogenic recipient [25]. Alternatively, induced pluripotent [49], spermatogonial [16], and parthenogenetic [51] stem cells could be applied autologously. These cell types appear to have the most characteristics of embryonic stem cells, including the capacity to give rise to cardiomyocytes. This makes them an attractive source for cardiac-muscle-engineering and other tissue-engineering applications. Finally, the necessity for an autologous application needs to be carefully considered, given the time needed to generate a therapeutic autologous cell pool (weeks to months) and, subsequently, an autologous tissue (takes roughly 7 days when embryonic stem cells are used to generate EHTs; our own unpublished data). Having this in mind, it might be worthwhile to consider cell banking for tissue-engineering applications. In this scenario, cells with defined immunological features would be banked to be matched with an allogeneic recipient. Under these circumstances, it would be likely that induction of differentiation followed by cardiac muscle engineering could be achieved within 2–3 weeks. This time frame appears clinically acceptable. In addition, palliative procedures could bridge the gap until tissue-engineered products would be ready for a clinical application. Time-frame concerns for early postnatal heart surgery might also be irrelevant if immune-matched heart tissue could be generated to be available at birth.

Application of Tissue-Engineered Myocardium in Pediatric Heart Surgery

Tissue-engineered-based myocardial repair has been mainly discussed for adult patients, being affected either by global heart failure or localized myocardial damage (e.g., after a myocardial infarct) [58]. Minimal myocardial reconstruction, as sometimes required in isolated ASDs and VSDs, might not be ideal conditions for biological repair with tissue-engineered myocardium. However, children with hypomorphic ventricles as observed in HLHS or DILV could potentially benefit from an augmentation of the affected ventricle or septation of a single ventricle with a fully biological contractile patch (Fig. 3). These procedures would likely require further inflow and outflow track reconstruction as well as seamless electrical and structural integration of the biological graft into the recipient heart. It would, in addition, be likely that such an intervention would have to be paralleled by electrical synchronization of the implant and the recipient heart. Given the prognosis of children with single-ventricle anomalies and the lack of organ donations for

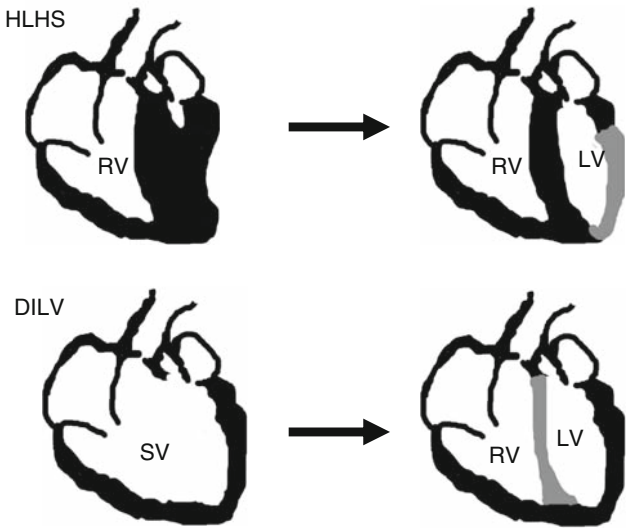

Fig. 3 Schematic drawings of potential applications of tissue-engineered myocardium (*gray*) for left ventricular augmentation (*top*) or single-ventricle septation (*bottom*) in children with HLHS or DILV, respectively. RV: right ventricle; LV: left ventricle; SV: single ventricle

heart transplantation, this additional intervention might be acceptable. Another issue will be to perform the appropriate preclinical and clinical studies to assess safety and feasibility as well as efficacy of such an intervention. Major safety concerns will also depend on the utilized cell type. Especially in the case of embryonic stem cells or non-embryonic pluripotent stem cells and derivatives, teratoma formation cannot be ruled out [26]. This issue has, however, not stopped the FDA from granting approval to a first-in-human trial with embryonic stem-cell-derived oligodendrocytes in spinal cord injury repair (GRNOPC1, Geron Corp.).

Conclusions

Organ repair strategies utilizing cell products are rapidly evolving. Adult stem cells from the bone marrow will be tested shortly in a pivotal phase III trial for efficacy (TOPCARE-AMI). Results from this study are expected in 2011 (Zeiher et al., personal communication). Derivatives from embryonic stem cells will enter a first clinical trial shortly. The outcome of the latter will be of paramount importance not only for cell implantation studies but also for the tissue engineering field. In particular, cardiac tissue engineering will depend crucially on the availability of stem cells capable of providing cardiomyocytes in large numbers. To this end, embryonic stem cells or nonembryonic pluripotent stem cells, including induced pluripotent stem cells, spermatogonial stem cells, and parthenogenetic stem cells, appear to be instrumental. If safety can be demonstrated for embryonic stem cell derivatives in spinal cord injury, it will be logical to also advance tissue-engineering technologies that utilize embryonic stem cells or nonembryonic alternatives into large animals and subsequently human trials.

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