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Review

Heart muscle engineering: An update on cardiac muscle replacement therapy

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Abstract

Cardiac muscle engineering aims at providing functional myocardium to repair diseased hearts and model cardiac development, physiology, and disease in vitro. Several enabling technologies have been established over the past 10 years to create functional myocardium. Although none of the presently employed technologies yields a perfect match of natural heart muscle, it can be anticipated that human heart muscle equivalents will become available after fine tuning of currently established tissue engineering concepts. This review provides an update on the state of cardiac muscle engineering and its utilization in cardiac regeneration. We discuss the application of stem cells including the allocation of autologous cell material, transgenic technologies that may improve tissue structure as well as in vivo engraftment, and vascularization concepts. We also touch on legal and economic aspects that have to be considered before engineered myocardium may eventually be applied in patients and discuss who may be a potential recipient.

Keywords: Tissue engineering; Myocytes; Stem cells; Cell therapy; Transplantation

1. Introduction

Tissue engineering aims at restoring, maintaining, or enhancing tissue and organ function. Langer and Vacanti were the first to promote the concept that cells may be seeded on artificial matrices to support formation of functional tissue-like structures that may be used to repair diseased organs or to screen for wanted and unwanted effects of drugs [1]. Particularly the perspective to use engineered tissues as organ replacements in patients has intrigued scientists and clinicians alike. Independent of the scientific and intellectual challenge, there is a clear medical necessity to develop alternatives to current organ transplantation given the world wide mismatch between patients waiting for organ transplants (15.995 in 2004 in Eurotransplant countries) and the availability of donor organs (1.793 in 2004 in Eurotransplant countries; www.eurotransplant.nl). Engineering of fully functional and transplantable organs like heart, liver, kidneys, or pancreas has not yet been achieved and is complicated by the structural and functional complexity of the respective organs. Consequently, most tissue engineers focus on developing organ units with a defined function rather than complete organs. Ultimately, these units may be used to restore and enhance a defined organ function in vivo. Examples for this concept are the development of hepatocyte spheroids which are metabolically active and may function as detoxifying units [2] or beta-cell aggregates which may eventually produce insulin in patients with diabetes [3].

Building a heart would require the assembly of multiple functional units which include excitable and contractile

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atrial and ventricular myocardium, epi- and endocardial structures, valves, and vasculature. Eventually, all parts would have to function as a single entity to propel blood through the organism, withstand diastolic loading, and adapt appropriately to immediate physiologic stimuli, e.g. fight-or-flight response, or to a chronically increased circulatory demand, e.g. physiologic hypertrophy. In light of this complexity it is questionable whether it will ever be possible to build a heart. This may in fact not even be desirable if it turns out that heart muscle or valves can be engineered at a size and quality sufficient to serve as surrogates in vivo. Notably, bioartificial valves have already been tested in humans with some encouraging, yet preliminary results [4].

This review focuses exclusively on tissue engineering of heart muscle that may be used to restore or enhance contractile function of failing myocardium in vivo. We discuss different cardiac tissue engineering principles and focus on issues related to the improvement of engineered heart muscle structure in vitro and its applicability in vivo. In addition, legal and economic aspects will be covered. We will not discuss advances in vessel and valve engineering nor do we aim at comparing cell implantation strategies with tissue engineering-based myocardial regeneration and would instead like to refer the interested reader to excellent reviews in the field [5–7]. A detailed discussion of synthetic and biological materials in cardiac tissue engineering is available elsewhere [8–10].

2. Principles of cardiac muscle engineering

Engineered heart muscle must develop systolic force, withstand diastolic load with appropriate compliance, and form an electrical and functional syncytium. So far, three distinguishable strategies have been developed to construct contractile heart muscle equivalents: (I) seeding cardiac myocytes on synthetic or biologic matrices [11–16], (II) supporting the propensity of cardiac myocytes to form contracting aggregates by entrapment in collagen [17–19], and (III) stacking cardiac myocyte monolayers to form multi-layered heart muscle constructs (Fig. 1) [20]. Development of systolic force is the most important feature of artificial myocardium. However, data on contractile properties are only available for (1) Artificial Myocardial Tissue (AMT) which is developed by seeding neonatal rat cardiac myocytes on collagen sponges [12], (2) Engineered Heart Tissue (EHT) which forms after entrapment of embryonic chick or neonatal rat heart cells in an extracellular matrix environment [17–19], and (3) stacked neonatal rat cardiac myocyte monolayers [20]. Maximal force of contraction was at 0.02 mN in AMT and 2–4 mN in stacked monolayers and EHTs [8]. The low contractile force in AMTs points to a conceptual disadvantage of preformed matrices in myocardial tissue engineering. Essentially, cardiac myocytes are “forced” to seed a structurally defined environment if preformed matrices are employed and seem to be limited in their potential to organize into three-dimensional force generating units. Another caveat relates to the limited compressibility and distinct stiffness of presently used materials. Whether new materials including nanomaterials will constitute better matrices for cardiac muscle engineering remains to be shown. Presently, methods supporting the propensity of cardiac myocytes to “freely” form contractile aggregates [21,22] and their organization into complex myocardial structures in the absence of obstructing

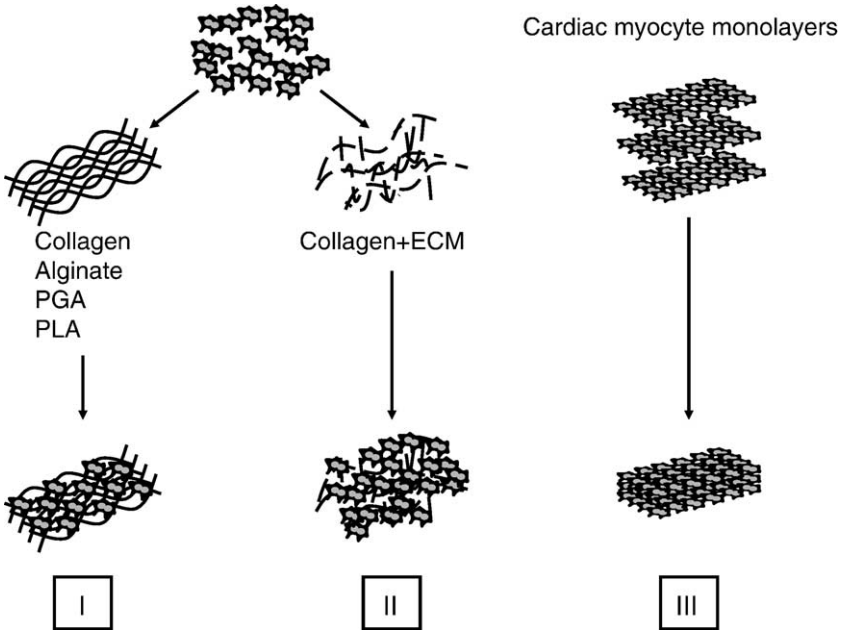

Fig. 1. Different concepts in cardiac muscle engineering using either preformed matrices as scaffold material (I) [11–16], soluble collagen and extracellular matrix (ECM) components to entrap cells (II) [17,18], or monolayer “sandwiches” (III) [20].

materials seem to be advantageous from a functional but also structural point of view.

Evaluation of muscle force in engineered myocardium is hampered by the fact that the cross-sectional area of most engineered heart muscle constructs is not fully comprised of muscle. Therefore, the classical normalization of force to cross-sectional area expressed as mN/mm^2 is difficult. Isolated adult cardiac myocytes or very thin muscle strips devoid of core necrosis develop a twitch tension of up to 56 mN/mm^2 [23]. None of the so far developed cardiac muscle constructs reaches this level of contractility. For example, the cross-sectional area of an EHT (~1 mm^2) is mainly occupied by extracellular matrix (i.e. collagen type I and laminin) and non-myocytes; cardiac myocytes constitute less than 30% of an EHT cross-section [19]. Thus, the estimated muscle cross-sectional area is 0.3 mm^2, yielding a normalized force of "pure myocardium" in EHT of ~13 mN/mm^2. The remaining difference to native papillary muscle of adult rat hearts is likely explained by the less compact structure of the muscle network and the immaturity of neonatal rat heart derived cardiac myocytes in EHTs.

Thus, should cardiac muscle engineers aim at generating heart muscle that fully resembles adult myocardium? The answer to this question clearly depends on the intended utilization of the heart muscle constructs. In case of in vitro heart muscle modeling it should be ideal to develop constructs that closely simulate adult myocardium in order to derive physiologically relevant information. Conversely, the expected low ischemia tolerance of thick, terminally differentiated cardiac muscle samples is expected to impede its application in cardiac regeneration in vivo. Eventually, "loose", but electrically and functionally interconnected, cardiac myocytes networks with a low degree of differentiation may have a better chance to survive in vitro and after implantation in vivo. A caveat to this hypothesis is that "loose" cardiac muscle networks may constitute slow conducting tissues with the concomitant risk of electrical instability and arrhythmias after implantation.

3. Current limitations and research strategies

Several issues will have to be resolved to improve the complexity and function of engineered myocardium: (1) high numbers of cardiac myocytes need to be made available; (2) the survival rate of cardiac myocytes in a three-dimensional environment needs to be improved; (3) the size of three-dimensional myocardium needs to be increased; (4) engineered myocardium must develop appropriate force. The post-mitotic state and a high sensitivity to hypoxia of differentiated cardiac myocytes constitute major obstacles in cardiac tissue engineering. Thus, it is unlikely that biopsy-derived naïve cardiac myocytes can serve as a reasonable cell source. Cardiogenic stem cells might constitute an alternative, potentially unlimited and autologous cell source for cardiac muscle engineering [24–32]. Although the allocation of sufficient amounts of cardiac myocytes to engineer thick myocardium seems possible with appropriate embryonic or adult stem cells, it is unlikely that this alone will resolve the problems of low cell survival and seeding efficiency. Genetic modifications of the respective cells to induce protection from apoptosis and/or reentry into the cell-cycle might turn out to be powerful means to support development of myocardium in vitro and also engraftment in vivo. Yet, non-transgenic options also exist and include applications of growth factors or growth factor providing cells, culture under hyperbaric oxygen, and induction of vascularization.

3.1. Stem cells in cardiac tissue engineering

Embryonic and adult stem cells can give rise to functional cardiac myocytes [24–28,30,33] and could theoretically serve as reliable cell sources for cardiac tissue engineering. Embryonic stem cells (ES-cells) are derived from the inner cell mass of a blastocyst and can differentiate into derivatives of all three germ layers including mesodermal cardiac myocytes. Yet, available ES-cell lines, be it from mice or humans, do not exhibit equal differentiation capacities. Most experiments on cardiac differentiation have been performed with murine D3 and R1 cell lines or human H1, H7, H9, hES2, and hES3 cell lines and their respective subclones (H9.1, H9.2) [24,26,28,29,31]. Differences in cardiac myocyte yield from ES-cells do not only depend on the employed cell line but may also stem from differences in cell handling. Accordingly, the reported number of spontaneously contracting, cardiac myocyte containing embryoid bodies in culture ranges substantially from 8–70% in similar cell lines (WiCell lines) [28,31], while cardiac differentiation in other cell lines (ESI cell lines) depends on paracrine factors delivered by visceral endodermal cells (END-2) or liver parenchymal cells (HepG2) [29]. Recently, bone morphogenic proteins (BMP2), basic fibroblast growth factor (FGF2), oxytocin, and the chemical compounds 5-aza-2′-deoxycytidine as well as cardiogenol have been reported to improve cardiac myocyte yield from ES-cells [31,34–36]. However, there seems to be no common agreement on the mechanism of induction of cardiogenesis in ES-cells and the effectiveness of compounds varies between laboratories and ES-cell lines. Regardless of these obscurities it is undisputed that cardiac differentiation is a common phenomenon in ES-cell cultures. Recently, several groups have identified adult stem cells with a cardiogenic potential [25,27,30,32,33,37,38]. Most compelling evidence for the capacity of adult stem cells to differentiate into cardiac myocytes stems from histological examinations in animals [25,27,32,39–41]. In vitro differentiation of adult progenitors into functional cardiac myocytes has also been observed by some investigators [25,33,42]. However, the

capacity of circulating stem cells to give rise to cardiac myocytes is controversial [43–46].

Nevertheless, stem cells seem to be the only meaningful cell source to allocate enough myocytes for clinically relevant cardiac muscle engineering in the future. One gram of adult myocardium contains an estimated number of 20–40 million myocytes [47] and a typical myocardial infarction that induces heart failure leads to a loss of approximately 50 g of heart muscle [48]. In order to compensate such a loss, it seems likely that engineered myocardium must not only have a similar size but also contain an equal amount of myocytes (50 g~1–2 billion). Clonally growing embryonic [24,26,28,29,31] and adult stem cells [25,40] may provide the necessary cell quantities. Yet, this seems only feasible if cells have a high rate of proliferation and triggers are identified that commit these cells into the cardiac myocyte lineage. Unfortunately, neither of this is the case at present. In fact, human ES-cells proliferate much slower than mouse ES-cells (doubling time 30–35 vs. 12–15 h) [49]. Doubling time of cardiogenic c-kit cells from rats has been reported to be ~40 h and is unlikely to be faster in human c-kit cells [25]. Ultimately, refinement of growth conditions including the use of bioreactor technologies may increase the cardiac myocyte yield also from human cells [50].

3.2. Genetic manipulations to support formation of engineered myocardium

Genetic manipulations of cardiac myocytes may improve their regenerative potential, e.g. by inducing re-entry into the cell cycle or protection from apoptosis [41,51–54]. Either approach would likely effect cardiac myocyte cell number in engineered myocardium in vitro and after engraftment in vivo. Yet, induction of proliferation and cell protection must be controllable to prevent cellular overgrowth and eventually tumor formation. This could be achieved by introduction of conditional transgenes that may not only be activated on demand but also shut off when required [55,56]. First studies to activate the cell cycle in cardiac myocytes were performed in mice overexpressing the large-T oncogene from the Simian Virus 40 under the control of the atrial natriuretic factor (ANF) promoter. These mice developed excessive right atrial tumors as a result of unrestricted cardiac myocyte growth [57]. Eventually, the murine cardiac myocyte cell line HL-1 was derived from these mice [58]. However, HL-1 cells did not allow generation of contractile engineered myocardium (own unpublished observation) and the introduction of a strong viral oncogene into cells that are destined to be used in vivo seems undesirable. Other more specific ways to induce cell cycle activity in cardiac myocytes may be to introduce or block cell cycle regulators and key mediators of apoptosis including the retinoblastoma gene (Rb), cyclins, cyclin dependent kinases, telomerase, p53 or p38 [51–54,59].

Yet, unlimited growth of genetically altered cells and naïve ES-cells goes along with the risk of tumor formation. A possible way to stop uncontrolled growth might be to introduce transgenes that allow targeted activation of suicide genes in cells that have lost growth control. Based on the stem cell hypothesis of tumor formation [60] it would be straightforward to control the expression of such suicide genes by fusion to a regulatory element that controls the expression of pluripotency/stemness genes such as Nanog, Oct 3/4, Rex-1, and Sox 2 [49]. The suicide gene of choice (e.g. thymidine kinase) should only act in cells that express the respective stemness gene and not elicit a significant bystander effect to spare myocytes in the proximity of tumorigenic cells. A caveat to any genetic alteration is the danger of unfavorable transgene integration in regulatory genomic elements which may eventually lead to a loss of function in another gene [61]. Yet, this problem might be controllable by identification of the integration site or targeted knock-in integration.

3.3. Growth promoting and cell survival supporting factors

Various growth factors have been identified to promote hypertrophic growth of cardiac myocytes [62–65]. Among those, IGF-1, insulin, and EGF have improved contractile performance of EHTs [19]. This may not only be the consequence of hypertrophic growth and cardiac myocyte differentiation, but also of improved cell survival. The latter effect could in the case of IGF-1 and insulin be elicited by activation of the anti-apoptotic Akt-kinase pathway [66,67]. An important task for cardiac tissue engineers in the future is certainly to identify more and ultimately all factors including optimal concentrations and time windows in which specific factors have to be present during culture. So far, growth factors are mostly applied by addition of xenogenic sera which is not compatible with human applications. Preliminary data from our own group show that complete serum replacement is possible, at least in the rat EHT model [68]. Other factors that can improve structure and function of engineered myocardium are increased delivery of oxygen and nutrients [69–71] as well as mechanical and electrical stimulation [19,72,73].

3.4. Induction of vascularization in engineered myocardium

The maximal size and thickness of engineered heart muscle will critically depend on oxygen and nutrient supply. Perfusion in vitro may improve the metabolic supply and could be achieved by induction of angiogenesis or vasculogenesis [74,75]. The former may be induced by embedding continuously perfused, functional vessels into engineered heart muscles leading to sprouting of capillaries

from the vessel into the construct. This approach ideally establishes a vascular bed that could subsequently be surgically connected to the recipient vasculature [76]. Vasculogenesis may be achieved by adding endothelial progenitor cells to the engineered heart construct [77,78]. Notably, primitive capillaries form in EHT already under standard culture conditions [19]. This process is further promoted when mixed heart cell populations with a large fraction of non-myocytes are employed [68]. Macrophages appear to play a role in this process possibly by providing angiogenic growth factors and/or by degrading extracellular matrix and thereby "drilling holes" into the respective tissue constructs [79,80]. Whether these "holes" are subsequently lined with endothelial cells to form new capillaries and whether this process can be controlled to establish a defined capillary network remains to be shown. However, macrophages may not only provide factors that support vessel formation but also factors that inhibit this process and even be deleterious for cardiac myocyte development in engineered heart tissue.

4. Cardiac repair with engineered heart muscle

Once myocardium has been engineered in vitro, its tissue structure and function need to be maintained after implantation in vivo, and electrical as well as mechanical contacts have to be established. Optimal oxygen and nutrient supply are essential to support engineered myocardium after engraftment. Finally, tissue grafts must not be rejected and should therefore be made exclusively from non-immunogenic material including autologous cells.

4.1. Mechanical and electrical coupling

Cardiac myocytes couple mechanically through intercalated discs containing adherens junctions which are composed of several molecules including cadherins, desmoplakin, plakoglobins [81]. Cadherin connections develop before formation of gap junctions which mediate electrical contacts in the heart [82]. Loss of cadherin protein leads to a loss of structural integrity of the heart and conduction deficits, the latter being a consequence of subsequent gap junction dysfunction [83]. The presence of adherens junctions and gap junctions have been clearly demonstrated in engineered myocardium by several groups [19,20,73,84,85]. In contrast, histological evidence for coupling of engineered and recipient myocardium is sparse and apparently less organized than cell–cell coupling in healthy myocardium [84]. However, histological identification of cadherin or connexin positive cell–cell contacts cannot be considered as unequivocal proof for mechanical and electrical coupling and additional experiments have to be performed to provide additional evidence for coupling. This may be done by electrical or optical epicardial mapping [86,87], iontophoresis experiments using gap junction permeable dyes [88], or two-photon molecular excitation [89]. Another important issue is whether or not electrical integration, especially of loosely structured tissue grafts, leads to electrical instability of the recipient heart. Presently employed small animal models are not well suited to study this question because baseline heart beat in rodents (e.g. 5–6 Hz in rats) is much higher than the spontaneous beating frequency of all so far developed myocardial tissue constructs (e.g. 1–2 Hz in rat EHT) [19]. Under these conditions, implanted constructs are likely to be overpaced by the recipient heart rate and may thus not elicit substantial arrhythmias. In addition, engineered myocardium may not be capable to excite the bulky recipient myocardium because of the apparent mismatch between the small current source (engineered myocardium) and the large current load of the recipient heart. The underlying electrophysiological concept for the phenomenon of conduction delay or even block as a consequence of a current-to-load mismatch has been nicely demonstrated [90–92]. We could recently identify a similar phenomenon after implantation of EHTs onto infarcted myocardium [71]. In this case, EHTs were activated by the native myocardium without a notable delay. Retrograde activation of the recipient myocardium was rarely observed and, if present, markedly delayed. Consequently, ambulatory long term ECG-measurements did not show an increase in arrhythmic events in animals that received an EHT graft when compared to SHAM-operated animals. Nevertheless, heart muscle grafts devoid of spontaneous electrical activity will likely be advantageous to minimize the risk of arrhythmias especially when tissue grafts are to be implanted in low heart rate animals or humans.

4.2. Vascularization in vivo

We and others have demonstrated that engineered myocardium survives if implanted on healthy and infarcted hearts, into the peritoneum, or subcutaneously [15,16,20,84]. Under these conditions, i.e. in the absence of immediate vascularization, engineered heart muscle must be nurtured exclusively by diffusion which is unlikely to sufficiently support thick avascular myocardial constructs. Yet, engineered heart muscle might have obtained some resistance to hypoxia during in vitro culture at ambient oxygen, a condition generally not sufficient to maintain monolayers and certainly not three-dimensional cell cultures under normoxia. Accordingly, we identified elevated VEGF-A transcript concentrations in late stage EHT cultures indicating hypoxia (own unpublished observations). Conversely, elevated VEGF-A concentrations may be useful to support vascularization of EHT grafts in vivo. We did indeed observe that EHTs are quickly vascularized after implantation [84,93] which is in line with observations by others using either alginate-based engineered heart tissues [16] or stacked

monolayer constructs [85]. Despite these observations, "pre-vascularized" heart muscle grafts would be preferable, on the one hand, to allow construction of complex tissues already in vitro and, on the other hand, to connect the vascular bed of such grafts to the recipient circulation at the time of implantation.

4.3. Prevention of rejection

None of the so far developed methods to construct engineered myocardium yields autologous graft material and all implantation studies have been performed in the presence of immune suppressants or in nude rats and mice. One might argue that immune suppression is clinically acceptable given its universal use after allogenic organ transplantation implying that the allogenic character of engineered myocardium may not completely exclude its use in organ restoration. However, autologous tissues would without doubt be preferable. This may in fact be possible with adult stem cell-derived cardiac myocytes or with ES-cells that are derived from the inner cell mass from somatic nuclear transfer derived blastocysts [94]. The former approach would certainly raise less ethical concerns than the latter. Yet, at this point it seems more likely to derive substantial amounts of cardiac myocytes from ES-cells than from adult stem cells. In addition, generation of EHT from murine as well as human ES-cell derived cardiac myocytes has recently been shown to be feasible (own unpublished data). Necessary steps to generate nuclear cloning derived, autologous engineered myocardium are summarized in Fig. 2. Yet, several important questions remain unanswered relating to the source of oocytes (human, animal, or even ES-cell derived) [95], epigenetic instability of clones [96], generation of cells that contain mitochondrial DNA from the oocyte donor and thus being genomically chimeric, and not at last whether the techniques of nuclear transfer can be applied in humans. The latter has been originally suggested in two publications by Hwang and colleagues which have recently been retracted after research misconduct including fabrication of data was proven [97].

5. Legal requirements

Although generation of human engineered heart muscle may become available shortly, we are still far away from a clinical application for following reasons: (1) All present tissue engineering concepts rely on non-human materials including xenogenic matrices and serum supplements; (2) concerns exist regarding the tumorigenic potential of stem cell-containing biomaterials; (3) safety and efficacy of engineered myocardium has not been proven in clinically relevant large animal models; (4) legal regulations limit the use of ES-cells to the NIH registered cell lines in many countries; (5) most available stem cell lines have been cultured on murine fibroblast feeder layers and are irreversibly contaminated by virus or xenogenic DNAs; (6) available ES-cell lines are prone to genetic instability making its applicability in vivo unpredictable. Eventually, all of these issues have to be resolved to allow construction of human engineered myocardium under good manufac-

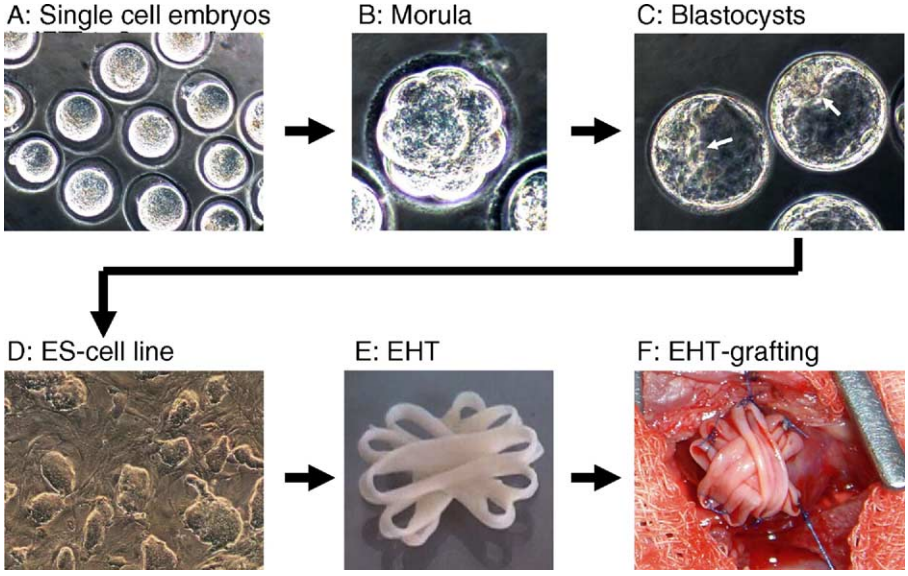

Fig. 2. Concept to engineer myocardium from nuclear transfer derived cells. One-cell embryos may be derived by nuclear transfer of somatic cells from a prospective recipient (A: one-cell mouse embryos); embryonic development may be induced chemically or electrically (B: parthenogenetic mouse morula); blastocyst may be derived with a distinguishable inner cell mass (arrows) containing pluripotent ES-cells (C: parthenogenetic mouse blastocysts); ES-cell lines may be derived from the inner cell mass of a blastocyst (D: ES-cells on mitotically inactivated fibroblasts); ES-cell derived cardiac myocytes may be used to generate contractile engineered myocardium (E: star shaped EHT from rat heart cells); ultimately, engineered myocardium may be implanted into an autologous recipient (F: EHT grafting on an infarcted rat heart).

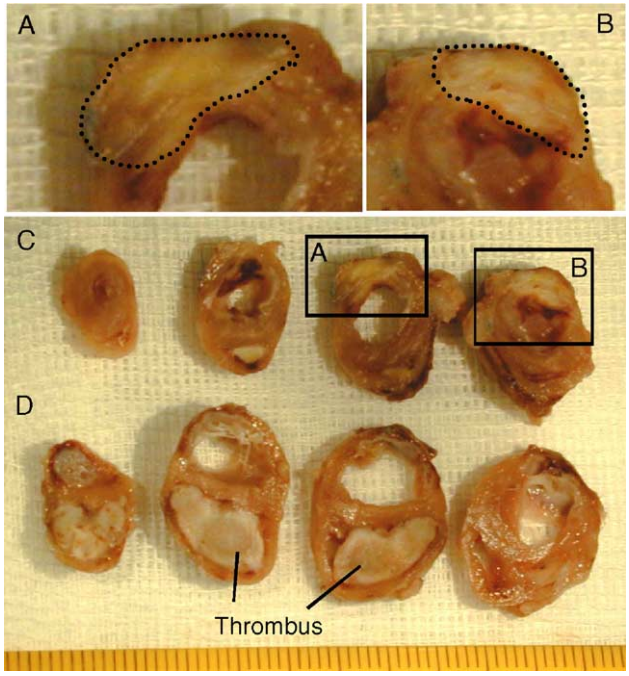

Fig. 3. Replacement of the left ventricular wall with engineered heart tissue in heterotopically implanted rat hearts. Magnification of a left ventricular wall replacement (encircled) 14 days after EHT-implantation (A and B). Parts of the left ventricular free walls of donor hearts were resected. Pleura patches were sutured into the defects with (C) or without (D) subsequent implantation of an EHT patch. Finally, hearts were implanted heterotopically into the abdomen of recipient rats according to standard protocols [102]. Serial sections through the respective hearts from apex to base (left→right) are presented. Note that the formation of a big thrombus in the right ventricle of the control heart (D) was not directly related to the surgical intervention in the left ventricle.

turing practice (GMP) as well as good laboratory practice guidelines (GLP) and eventually test it under good clinical practice (GCP) standards. The latter includes a study design that must compare to the standards of large clinical drug trials to gather conclusive data on the safety, efficacy, and effectiveness of tissue engineering based myocardial repair.

6. Who may benefit from engineered myocardium and who can pay for it?

These questions must be answered in times where pharmacological and interventional therapies including the use of adult stem cell implantations are, on the one hand, improving the perspective of patients with severe cardiac dysfunction and, on the other hand, imposing an increasing economic threat to the health care systems. Donor heart transplantation will likely remain the gold standard in treatment of failing myocardium. As mentioned above, donor organ number does not match the number of patients in need resulting in a one month mortality rate of 2–50% on the waiting list in Eurotransplant countries [98]. In children, the situation is even more dramatic. While building a complete bioartificial heart in vitro seems unlikely in the close future, patches of engineered myocardium may be used to enhance or restore myocardial function in patients who do not qualify for organ transplantation. Here, it may be possible to replace defined regions of the diseased heart with engineered myocardium. Grafting of the latter may be performed in analogy to the Dor procedure where non-functional myocardium is resected and replaced with a non-contractile synthetic patch [99]. Consequently, contractile biografts would be used instead of synthetic materials to not only prevent further bulging of the ventricle but also restore ventricular contractility. Another field of application may be myocardial reconstruction in pediatric patients. Heart defects belong to the most common malformations in newborn children (8 of 1000 newborns) [100]. Not all of them require surgical interventions. However, children born with single ventricles or with atrial and ventricular septum defects may benefit from a surgical reconstruction with engineered myocardium.

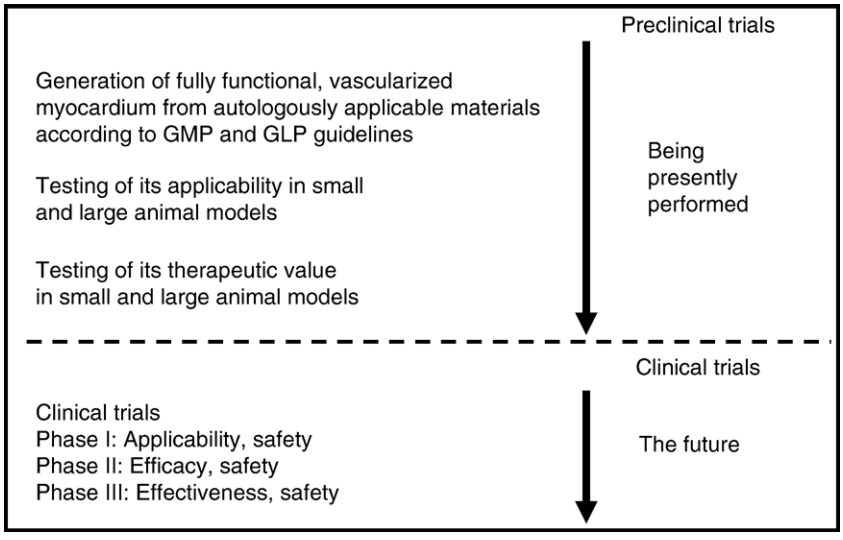

Fig. 4. Summary of important steps towards a clinical utilization of engineered myocardium.

Ideally, this biological patch would grow with physiological development making repeated surgeries dispensable. First trials will most likely test the applicability of artificial heart muscle either to restore myocardial contractility after myocardial infarction or to replace the myocardial wall. Whereas the former experiments may be performed independently of the ventricular pressure, the first wall replacement studies will have to be performed in the low pressure right ventricle or in heterotopically transplanted hearts with lower than physiologic ventricular pressure. Indeed, own preliminary experiments demonstrate that the left ventricular free wall can at least partially be replaced with EHT in heterotopically transplanted rat hearts (Fig. 3).

The perspective of tissue engineering based myocardial regeneration is certainly fascinating, but can we afford it? As in heart transplantation, myocardial restoration by implantation of tissue engineered myocardium will be reserved to industrialized countries and will require close interaction between physicians/surgeons and tissue engineers. Cost may become manageable if autologous graft material will be available. High costs in standard transplantations do not only stem from the surgery alone (estimated at $70.000–$400.000) and hospitalization costs ($200.000–$400.000) but also from the required life long medication including immune suppression ($700–$2.000 per month) [101]. The latter may not be necessary if autologous engineered myocardium becomes available. In addition, engineered myocardium may be custom made by bioengineers directly at heart transplant centers reducing the costs for organ allocation and also the time on the waiting list which in term would reduce hospitalization costs. Nevertheless, thoughts about financial issues should at this early point in time not limit our efforts to strive for developing a clinically feasible cardiac tissue engineering therapy. The clinical and socio-economic relevance of heart failure and the lack of alternatives shall be the driving force in the field which may eventually turn out to not be more expensive than standard but hardly available heart transplantation.

7. Conclusion

The vision to create whole organs in the lab is intriguing. If successful it may offer salvation to patients with so far incurable disabling diseases including heart failure. The latter is not only the leading causes of death in industrialized countries but one of the main reasons for disability in aging populations. Replacing a heart with an engineered heart seems an unlikely option in the near future. However, restoring or at least enhancing heart muscle function by grafting of tissue engineered myocardium seems foreseeable and may not only be applicable in older patients with heart failure but also in children with congenital malformations. The way to a clinically applicable concept to restore heart muscle function with engineered myocardium remains certainly long and several milestones will have to be achieved (Fig. 4). Ultimately, tissue engineering based myocardial regeneration may be an attractive alternative to heart transplantation and other surgical interventions to rebuild the heart.

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References

[1] Langer R, Vacanti JP. Tissue engineering. Science 1993;260:920–6.
[2] Wu FJ, Friend JR, Remmel RP, Cerra FB, Hu WS. Enhanced cytochrome P450 IA1 activity of self-assembled rat hepatocyte spheroids. Cell Transplant 1999;8:233–46.
[3] Narushima M, Kobayashi N, Okitsu T, Tanaka Y, Li SA, Chen Y, et al. A human beta-cell line for transplantation therapy to control type 1 diabetes. Nat Biotechnol 2005;23:1274–82.
[4] Shin'oka T, Matsumura G, Hibino N, Naito Y, Watanabe M, Konuma T, et al. Midterm clinical result of tissue-engineered vascular autografts seeded with autologous bone marrow cells. J Thorac Cardiovasc Surg 2005;129:1330–8.
[5] Murry CE, Field LJ, Menasche P. Cell-based cardiac repair: reflections at the 10-year point. Circulation 2005;112:3174–83.
[6] Vesely I. Heart valve tissue engineering. Circ Res 2005;97:743–55.
[7] Isenberg BC, Williams C, Tranquillo RT. Small-diameter artificial arteries engineered in vitro. Circ Res 2006;98:25–35.
[8] Zimmermann WH, Eschenhagen T. Cardiac tissue engineering for replacement therapy. Heart Fail Rev 2003;8:259–69.
[9] Eschenhagen T, Zimmermann WH. Engineering myocardial tissue. Circ Res 2005;97:1220–31.
[10] Zimmermann WH, Melnychenko I, Eschenhagen T. Engineered heart tissue for regeneration of diseased hearts. Biomaterials 2004;25:1639–47.
[11] Bursac N, Papadaki M, Cohen RJ, Schoen FJ, Eisenberg SR, Carrier R, et al. Cardiac muscle tissue engineering: toward an in vitro model for electrophysiological studies. Am J Physiol 1999; 277:H433–44.
[12] Kofidis T, Akhyari P, Boublik J, Theodorou P, Martin U, Ruhparwar A, et al. In vitro engineering of heart muscle: artificial myocardial tissue. J Thorac Cardiovasc Surg 2002;124:63–9.
[13] van Luyn MJ, Tio RA, Gallego y van Seijen XJ, Plantinga JA, de Leij LF, DeJongste MJ, et al. Cardiac tissue engineering: characteristics of in unison contracting two- and three-dimensional neonatal rat ventricle cell (co)-cultures. Biomaterials 2002;23:4793–801.
[14] Carrier RL, Papadaki M, Rupnick M, Schoen FJ, Bursac N, Langer R, et al. Cardiac tissue engineering: cell seeding, cultivation parameters, and tissue construct characterization. Biotechnol Bioeng 1999;64: 580–9.
[15] Li RK, Jia ZQ, Weisel RD, Mickle DA, Choi A, Yau TM. Survival and function of bioengineered cardiac grafts. Circulation 1999; 100:II63–9.
[16] Leor J, Aboulafia-Etzion S, Dar A, Shapiro L, Barbash IM, Battler A, et al. Bioengineered cardiac grafts: a new approach to repair the infarcted myocardium? Circulation 2000;102:III56–61.

[17] Eschenhagen T, Fink C, Remmers U, Scholz H, Wattchow J, Weil J, et al. Three-dimensional reconstitution of embryonic cardiomyocytes in a collagen matrix: a new heart muscle model system. FASEB J 1997;11:683–94.
[18] 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.
[19] Zimmermann WH, Schneiderbanger K, Schubert P, Didie M, Munzel F, Heubach JF, et al. Tissue engineering of a differentiated cardiac muscle construct. Circ Res 2002;90:223–30.
[20] Shimizu T, Yamato M, Isoi Y, Akutsu T, Setomaru T, Abe K, 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.
[21] Akins RE, Boyce RA, Madonna ML, Schroedl NA, Gonda SR, McLaughlin TA. Cardiac organogenesis in vitro: reestablishment of three-dimensional tissue architecture by dissociated neonatal rat ventricular cells. Tissue Eng 1999;5:103–18.
[22] Moscona AA. Tissues from dissociated cells. Sci Am 1959;200: 132–4 [passim].
[23] Hasenfuss G, Mulieri LA, Blanchard EM, Holubarsch C, Leavitt BJ, Ittleman F, et al. Energetics of isometric force development in control and volume-overload human myocardium. Comparison with animal species. Circ Res 1991;68:836–46.
[24] 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;48:173–82.
[25] Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, et al. Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell 2003;114:763–76.
[26] 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.
[27] Oh H, Bradfute SB, Gallardo TD, Nakamura T, Gaussin V, Mishina Y, et al. Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. Proc Natl Acad Sci U S A 2003;100:12313–8.
[28] Kehat I, Kenyagin-Karsenti D, Snir M, Segev H, Amit M, Gepstein A, et al. Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. J Clin Invest 2001;108:407–14.
[29] Mummery C, Ward-van Oostwaard D, Doevendans P, Spijker R, van den Brink S, Hassink R, et al. Differentiation of human embryonic stem cells to cardiomyocytes: role of coculture with visceral endoderm-like cells. Circulation 2003;107:2733–40.
[30] Laugwitz KL, Moretti A, Lam J, Gruber P, Chen Y, Woodard S, et al. Postnatal isl1+ cardioblasts enter fully differentiated cardiomyocyte lineages. Nature 2005;433:647–53.
[31] 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.
[32] Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, et al. Bone marrow cells regenerate infarcted myocardium. Nature 2001; 410:701–5.
[33] Badorff C, Brandes RP, Popp R, Rupp S, Urbich C, Aicher A, et al. Transdifferentiation of blood-derived human adult endothelial progenitor cells into functionally active cardiomyocytes. Circulation 2003;107:1024–32.
[34] Wu X, Ding S, Ding Q, Gray NS, Schultz PG. Small molecules that induce cardiomyogenesis in embryonic stem cells. J Am Chem Soc 2004;126:1590–1.
[35] Kawai T, Takahashi T, Esaki M, Ushikoshi H, Nagano S, Fujiwara H, et al. Efficient cardiomyogenic differentiation of embryonic stem cell by fibroblast growth factor 2 and bone morphogenetic protein 2. Circ J 2004;68:691–702.
[36] Jankowski M, Danalache B, Wang D, Bhat P, Hajjar F, Marcinkiewicz M, et al. Oxytocin in cardiac ontogeny. Proc Natl Acad Sci U S A 2004;101:13074–9.
[37] Jackson KA, Majka SM, Wang H, Pocius J, Hartley CJ, Majesky MW, et al. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. J Clin Invest 2001;107:1395–402.
[38] Malouf NN, Coleman WB, Grisham JW, Lininger RA, Madden VJ, Sproul M, et al. Adult-derived stem cells from the liver become myocytes in the heart in vivo. Am J Pathol 2001;158:1929–35.
[39] Urbanek K, Torella D, Sheikh F, De Angelis A, Nurzynska D, Silvestri F, et al. Myocardial regeneration by activation of multipotent cardiac stem cells in ischemic heart failure. Proc Natl Acad Sci U S A 2005;102:8692–7.
[40] Linke A, Muller P, Nurzynska D, Casarsa C, Torella D, Nascimbene A, et al. Stem cells in the dog heart are self-renewing, clonogenic, and multipotent and regenerate infarcted myocardium, improving cardiac function. Proc Natl Acad Sci U S A 2005;102: 8966–71.
[41] Cai CL, Liang X, Shi Y, Chu PH, Pfaff SL, Chen J, et al. Isl1 identifies a cardiac progenitor population that proliferates prior to differentiation and contributes a majority of cells to the heart. Dev Cell 2003;5:877–89.
[42] Messina E, De Angelis L, Frati G, Morrone S, Chimenti S, Fiordaliso F, et al. Isolation and expansion of adult cardiac stem cells from human and murine heart. Circ Res 2004;95:911–21.
[43] Nygren JM, Jovinge S, Breitbach M, Sawen P, Roll W, Hescheler J, et al. Bone marrow-derived hematopoietic cells generate cardiomyocytes at a low frequency through cell fusion, but not transdifferentiation. Nat Med 2004;10:494–501.
[44] Murry CE, Soonpaa MH, Reinecke H, Nakajima H, Nakajima HO, Rubart M, et al. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. Nature 2004; 428:664–8.
[45] Balsam LB, Wagers AJ, Christensen JL, Kofidis T, Weissman IL, Robbins RC. Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. Nature 2004;428:668–73.
[46] Laflamme MA, Murry CE. Regenerating the heart. Nat Biotechnol 2005;23:845–56.
[47] Beltrami CA, Finato N, Rocco M, Feruglio GA, Puricelli C, Cigola E, et al. Structural basis of end-stage failure in ischemic cardiomyopathy in humans. Circulation 1994;89:151–63.
[48] Gepstein L. Derivation and potential applications of human embryonic stem cells. Circ Res 2002;91:866–76.
[49] Wobus AM, Boheler KR. Embryonic stem cells: prospects for developmental biology and cell therapy. Physiol Rev 2005;85: 635–78.
[50] Zandstra PW, Bauwens C, Yin T, Liu Q, Schiller H, Zweigerdt R, et al. Scalable production of embryonic stem cell-derived cardiomyocytes. Tissue Eng 2003;9:767–78.
[51] Pasumarthi KB, Field LJ. Cardiomyocyte cell cycle regulation. Circ Res 2002;90:1044–54.
[52] Pasumarthi KB, Nakajima H, Nakajima HO, Soonpaa MH, Field LJ. Targeted expression of cyclin D2 results in cardiomyocyte DNA synthesis and infarct regression in transgenic mice. Circ Res 2005;96:110–8.
[53] Oh H, Taffet GE, Youker KA, Entman ML, Overbeek PA, Michael LH, et al. Telomerase reverse transcriptase promotes cardiac muscle cell proliferation, hypertrophy, and survival. Proc Natl Acad Sci U S A 2001;98:10308–13.
[54] Engel FB, Schebesta M, Duong MT, Lu G, Ren S, Madwed JB, et al. p38 MAP kinase inhibition enables proliferation of adult mammalian cardiomyocytes. Genes Dev 2005;19:1175–87.
[55] Gossen M, Bujard H. Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. Proc Natl Acad Sci U S A 1992;89:5547–51.
[56] Wang Y, Xu J, Pierson T, O'Malley BW, Tsai SY. Positive and negative regulation of gene expression in eukaryotic cells

with an inducible transcriptional regulator. Gene Ther 1997;4: 432–41.
[57] Field LJ. Atrial natriuretic factor-SV40 T antigen transgenes produce tumors and cardiac arrhythmias in mice. Science 1988;239:1029–33.
[58] Claycomb WC, Lanson NA Jr, Stallworth BS, Egeland DB, Delcarpio JB, Bahinski A, et al. HL-1 cells: a cardiac muscle cell line that contracts and retains phenotypic characteristics of the adult cardiomyocyte. Proc Natl Acad Sci U S A 1998;95:2979–84.
[59] Engel FB, Hauck L, Cardoso MC, Leonhardt H, Dietz R, von Harsdorf R. A mammalian myocardial cell-free system to study cell cycle reentry in terminally differentiated cardiomyocytes. Circ Res 1999;85:294–301.
[60] Wicha MS, Liu S, Dontu G. Cancer stem cells: an old idea—a paradigm shift. Cancer Res 2006;66:1883–90.
[61] Kustikova O, Fehse B, Modlich U, Yang M, Dullmann J, Kamino K, et al. Clonal dominance of hematopoietic stem cells triggered by retroviral gene marking. Science 2005;308:1171–4.
[62] Schaub MC, Hefti MA, Harder BA, Eppenberger HM. Various hypertrophic stimuli induce distinct phenotypes in cardiomyocytes. J Mol Med 1997;75:901–20.
[63] Brutsaert DL. Cardiac endothelial-myocardial signaling: its role in cardiac growth, contractile performance, and rhythmicity. Physiol Rev 2003;83:59–115.
[64] Mohamed SN, Holmes R, Hartzell CR. A serum-free, chemically-defined medium for function and growth of primary neonatal rat heart cell cultures. In Vitro 1983;19:471–8.
[65] Suzuki T, Ohta M, Hoshi H. Serum-free, chemically defined medium to evaluate the direct effects of growth factors and inhibitors on proliferation and function of neonatal rat cardiac muscle cells in culture. In Vitro Cell Dev Biol 1989;25:601–6.
[66] Fujio Y, Nguyen T, Wencker D, Kitsis RN, Walsh K. Akt promotes survival of cardiomyocytes in vitro and protects against ischemia–reperfusion injury in mouse heart. Circulation 2000;101:660–7.
[67] Matsui T, Li L, del Monte F, Fukui Y, Franke TF, Hajjar RJ, et al. Adenoviral gene transfer of activated phosphatidylinositol 3′-kinase and Akt inhibits apoptosis of hypoxic cardiomyocytes in vitro. Circulation 1999;100:2373–9.
[68] Naito H, Melnychenko I, Didie M, Schneiderbanger K, Schubert P, Jaser M, et al. Optimizing Engineered Heart Tissue for Therapeutic Applications as Surrogate Heart Muscle. Circulation in press.
[69] Radisic M, Malda J, Epping E, Geng W, Langer R, Vunjak-Novakovic G. Oxygen gradients correlate with cell density and cell viability in engineered cardiac tissue. Biotechnol Bioeng 2006;93: 332–43.
[70] Radisic M, Yang L, Boublik J, Cohen RJ, Langer R, Freed LE, et al. Medium perfusion enables engineering of compact and contractile cardiac tissue. Am J Physiol Heart Circ Physiol 2004;286:H507–16.
[71] Zimmermann WH, Melnychenko I., Wasmeier GH, Didie M, Naito N, Nixdorff U, et al. Engineered Heart Tissue Grafts Improve Systolic and Diastolic Function in Infarcted Rat Hearts. Nat Med 2006;12:452–8.
[72] 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.
[73] Radisic M, Park H, Shing H, Consi T, Schoen FJ, Langer R, et al. Functional assembly of engineered myocardium by electrical stimulation of cardiac myocytes cultured on scaffolds. Proc Natl Acad Sci U S A 2004;101:18129–34.
[74] Risau W, Flamme I. Vasculogenesis. Annu Rev Cell Dev Biol 1995;11:73–91.
[75] Risau W. Mechanisms of angiogenesis. Nature 1997;386:671–4.
[76] Kofidis T, Lenz A, Boublik J, Akhyari P, Wachsmann B, Mueller-Stahl K, et al. Pulsatile perfusion and cardiomyocyte viability in a solid three-dimensional matrix. Biomaterials 2003;24:5009–14.
[77] Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997;275:964–7.
[78] Levenberg S, Rouwkema J, Macdonald M, Garfein ES, Kohane DS, Darland DC, et al. Engineering vascularized skeletal muscle tissue. Nat Biotechnol 2005;23:879–84.
[79] Khmelewski E, Becker A, Meinertz T, Ito WD. Tissue resident cells play a dominant role in arteriogenesis and concomitant macrophage accumulation. Circ Res 2004;95:E56–64.
[80] Moldovan NI, Goldschmidt-Clermont PJ, Parker-Thornburg J, Shapiro SD, Kolattukudy PE. Contribution of monocytes/macrophages to compensatory neovascularization: the drilling of metalloelastase-positive tunnels in ischemic myocardium. Circ Res 2000;87:378–84.
[81] Franke WW, Borrmann CM, Grund C, Pieperhoff S. The area composita of adhering junctions connecting heart muscle cells of vertebrates: I. Molecular definition in intercalated disks of cardiomyocytes by immunoelectron microscopy of desmosomal proteins. Eur J Cell Biol 2006;85:69–82.
[82] Kostin S, Hein S, Bauer EP, Schaper J. Spatiotemporal development and distribution of intercellular junctions in adult rat cardiomyocytes in culture. Circ Res 1999;85:154–67.
[83] Kostetskii I, Li J, Xiong Y, Zhou R, Ferrari VA, Patel VV, et al. Induced deletion of the *N*-cadherin gene in the heart leads to dissolution of the intercalated disc structure. Circ Res 2005; 96:346–54.
[84] Zimmermann WH, Didie M, Wasmeier GH, Nixdorff U, Hess A, Melnychenko I, et al. Cardiac grafting of engineered heart tissue in syngenic rats. Circulation 2002;106:I151.
[85] Shimizu T, Sekine H, Yang J, Isoi Y, Yamato M, Kikuchi A, et al. Polysurgery of cell sheet grafts overcomes diffusion limits to produce thick, vascularized myocardial tissues. FASEB J 2006.
[86] Dhein S, Muller A, Gerwin R, Klaus W. Comparative study on the proarrhythmic effects of some antiarrhythmic agents. Circulation 1993;87:617–30.
[87] Mironov SF, Vetter FJ, Pertsov AM. Fluorescence imaging of cardiac propagation: spectral properties and filtering of optical action potentials. Am J Physiol Heart Circ Physiol in press. doi:10.1152/ajpheart.01003.2005.
[88] Ruiz-Meana M, Garcia-Dorado D, Hofstaetter B, Piper HM, Soler-Soler J. Propagation of cardiomyocyte hypercontracture by passage of Na(+) through gap junctions. Circ Res 1999;85:280–7.
[89] Rubart M. Two-photon microscopy of cells and tissue. Circ Res 2004;95:1154–66.
[90] Rohr S, Kucera JP, Fast VG, Kleber AG. Paradoxical improvement of impulse conduction in cardiac tissue by partial cellular uncoupling. Science 1997;275:841–4.
[91] Rohr S, Salzberg BM. Characterization of impulse propagation at the microscopic level across geometrically defined expansions of excitable tissue: multiple site optical recording of transmembrane voltage (MSORTV) in patterned growth heart cell cultures. J Gen Physiol 1994;104:287–309.
[92] Fast VG, Kleber AG. Role of wavefront curvature in propagation of cardiac impulse. Cardiovasc Res 1997;33:258–71.
[93] Eschenhagen T, Didie M, Munzel F, Schubert P, Schneiderbanger K, Zimmermann WH. 3D engineered heart tissue for replacement therapy. Basic Res Cardiol 2002;97(Suppl 1):I146–52.
[94] Wakayama T, Tabar V, Rodriguez I, Perry AC, Studer L, Mombaerts P. Differentiation of embryonic stem cell lines generated from adult somatic cells by nuclear transfer. Science 2001;292:740–3.
[95] Hubner K, Fuhrmann G, Christenson LK, Kehler J, Reinbold R, De La Fuente R, et al. Derivation of oocytes from mouse embryonic stem cells. Science 2003;300:1251–6.
[96] Humpherys D, Eggan K, Akutsu H, Hochedlinger K, Rideout III WM, Biniszkiewicz D, et al. Epigenetic instability in ES cells and cloned mice. Science 2001;293:95–7.
[97] Kennedy D. Editorial retraction. Science 2006;311:335.
[98] Smits JM, Vanhaecke J, Haverich A, Vries E, Roels L, Persijn G, et al. Waiting for a thoracic transplant in Eurotransplant*. Transpl Int 2006;19:54–66.

[99] Dor V, Sabatier M, Montiglio F, Civaia F, DiDonato M. Endoventricular patch reconstruction of ischemic failing ventricle. a single center with 20 years experience. advantages of magnetic resonance imaging assessment. Heart Fail Rev 2004;9:269–86.

[100] Jacobs JP, Maruszewski B. Computerized outcomes analysis for congenital heart disease. Curr Opin Pediatr 2005;17:586–91.

[101] Emory Health Care. Heart Transplant Program. http://www.emoryhealthcare.org/departments/heart_transplant/patient_info/faqs.html 2006.

[102] Krupnick AS, Kreisel D, Engels FH, Szeto WY, Plappert T, Popma SH, et al. A novel small animal model of left ventricular tissue engineering. J Heart Lung Transplant 2002;21:233–43.