This Review is part of a thematic series on Cardiovascular Tissue Engineering, which includes the following articles:

Custom Design of the Cardiac Microenvironment with Biomaterials
Heart Valve Tissue Engineering
Engineering Myocardial Tissue
Small-Diameter Artificial Arteries Engineered In Vitro
Regenerative Cardiomyocytes for Cardiovascular Tissue Engineering

Richard T. Lee, Guest Editor

Engineering Myocardial Tissue

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Abstract—To create an artificial heart is one of the most ambitious dreams of the young field of tissue engineering, a dream that, when publicly announced in 1999 (LIFE initiative around M. Sefton), provoked as much compassion as scepticism in the scientific and lay press. Today, it is fair to state that the field is still far away from having built the “bioartificial heart.” Nevertheless, substantial progress has been made over the past 10 years, and a realistic perspective exists to create 3-dimensional heart muscle equivalents that may not only serve as experimental models but could also be useful for cardiac regeneration. (Circ Res. 2005;97:1220-1231.)

Key Words: tissue engineering ■ heart ■ stem cells ■ regeneration

The term “Tissue Engineering” was introduced in 1987 by members of the US National Science Foundation (NSF) in Washington, D.C. and defined a year later at an NSF-organized conference on tissue engineering in Lake Tahoe, California as “Application of principles and methods of engineering and life sciences toward fundamental understanding of structure–function relationship in normal and pathological mammalian tissues and the development of biological substitutes to restore, maintain, or improve functions.”

The “repair” part of tissue engineering overlaps, but is not synonymous with, “cell therapy” which intends to promote the formation of new tissue or to improve the function of an existing tissue by injecting or infusing suspensions of isolated cells. This concept has gained much attraction over the past years and is currently tested in controlled clinical trials (for review see references 1–6). Tissue engineering aims at generating functional 3D tissues outside of the body that can by tailored in size, shape, and function according to the respective needs before implanting them into the body. First clinical experiences have been published using bioengineered skin, cartilage, and vascular grafts,7–9 but the present data are still preliminary (for review of the “bioartificial heart,” see also ref 10). This review will give an overview on the evolution of cardiac tissue engineering and today’s state-of-the art concepts. Tissue engineering heart valves and blood vessels will not be covered here because these are topics of other reviews in this Series.

History of Cardiac Tissue Engineering

The spontaneous beating of heart tissue explants and their cellular outgrowth when placed in culture dishes under suitable conditions have fascinated researchers for generations.11–15 Cardiac tissue engineering has several roots: developmental biology, cardiac muscle cell biology, and material science. The first man-made 3D heart tissues have been generated long before the term tissue engineering was introduced.11–15 In the late 1950s, Moscona generated spheroid aggregates from embryonic chick heart cells by cultivating freshly isolated cells in Erlenmeyer flasks under continuous gyration (Figure 1A).16 After 18 hours in this simple bioreactor aggregates formed containing up to 200 cells. Many researchers adapted Moscona’s model and found that the aggregates were functionally more similar to intact heart

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Reviews
tissue than standard 2D-monolayer cultures (Figure 1A). This early work proved that isolated cells from immature hearts retain the capacity to reform heart-like tissues under cell culture conditions.

Another fascinating finding supports the notion that cardiac myocytes have an inherent preference to aggregate. When cultured at high density for extended time periods in serum-containing culture medium researchers often see that entire rhythmically contracting monolayers start to detach from the culture surface forming spontaneously beating cardiac sheets floating in the dish. Because these sheets, devoid of mechanical load, quickly retract and stop to beat, the detachment has been considered a shortcoming of cell culture experiments rather than an exploitable feature until Shimizu and colleagues have used free floating monolayer sheets to generate essentially exogenous matrix free cardiac tissue constructs. In fact, in the late 1980s, Vandenburgh and colleagues searched for a solution to overcome the detachment problem of differentiated skeletal myotubes (showing a similar contractile behavior as cardiac myocytes) and came up with the idea to cover the cultured myotubes with a layer of freshly neutralized collagen type I to chronically impose load on the cells. This led to an improved differentiation state of the myotubes. Similar observations had actually been made in various other cell types before, either by placing collagen on top of or below cellular monolayers or by embedding cells in collagen gels. For example, epithelial thyroid cells cultured in collagen organized into follicles, and kidney and mammary gland epithelial cell lines formed lumina resembling those of the tissues.
from which they were derived. Collectively, these data nicely demonstrate that the 3D environment in a collagen gel promotes tissue formation and cellular differentiation of various cell types in vitro.

Besides cell composition and differentiation, tissues are also defined by cell orientation. In this respect, two factors have been shown to be important: Mechanical strain and orientation of the growth substratum. The groups of Vandenburgh and Terracio independently designed motorized computer-controlled devices to impose stretch on cultured skeletal and cardiac myocytes and showed that mechanical stimulation had a favorable effect on muscle cell orientation and differentiation. When repetitive cycles of stretch and relaxation were imposed on skeletal myotubes cultured at very high density, thin, 3D strips of longitudinally aligned and well developed skeletal myofibers developed. The strips even initiated tendon development and performed work in response to depolarization, and therefore may be considered to be the first engineered 3D skeletal muscle (Figure 1B). An important conclusion from this work was that mechanical load is crucial for the orientation and differentiation of (skeletal) muscle cells and muscle tissue development.

Others showed that cell growth can be influenced by the underlying matrix substrate. For example, when a scraper was drawn across the surface of culture dishes coated with freshly neutralized collagen type I before gelation, Simpson and colleagues observed that neonatal rat cardiac myocytes aligned themselves in parallel with the collagen and adopted a tissue-like organization. High magnetic fields have been used as an alternative means to orient collagen, and Tranquillo and colleagues showed that this method can be used to improve longitudinal orientation of fibroblasts and smooth muscle cells. Others used photolithographic patterning of culture surfaces to govern the growth of cardiac myocytes in specific patterns or biodegradable elastomeric polyurethane films patterned by microcontact printing with laminin lanes.

Another approach to cardiac tissue engineering originated in the search for an improved in vitro heart model for target validation that would allow both the measurement of contractile force and genetic, pharmacological, and mechanical manipulations under controlled conditions in vitro. The solution was an adaptation of a method previously developed for embryonic fibroblasts by Kolodney and Elson in St. Louis (Figure 1C). Essentially, cardiac myocytes were cultured in collagen gels as described above, but between two Velcro covered glass tubes that were positioned in a rectangular well and held at a fixed distance with a metal spacer. Under these culture conditions the cells formed spontaneously contracting biconcave lattices that were anchored on the Velcro covered glass tubes (Figure 1D) and could be attached to mechanical force transducers to record contractile force. Neonatal rat cardiac myocytes initially failed to exhibit differentiation, growth, and tissue formation inside collagen I gels as described previously. Only after addition of extracellular matrix from Engelbrecht Swarm tumors (Matrigel) to the initial reconstitution mix, engineered heart tissue (EHT) could be generated also from rat heart cells. Interestingly, chronic cyclic stretch of the EHTs improved contractile force by a factor of 3 and induced better cardiac tissue development. A further improvement was the introduction of circular casting molds that (1) allow large scale production with minimal handling and are reusable, (2) can be easily miniaturized for high through-put screening, and (3) lead to better tissue formation than the original lattice design because the circular geometry causes a homogeneous force distribution throughout the tissue (Figure 1G). A main conclusion from these observations is that, similar to what has been found before by Vandenburgh and Terracio and colleagues, mechanical load is crucial for a proper heart tissue development.

Two other, principally different tissue engineering approaches were developed in parallel. One originated from the material sciences field and can be viewed as an adaptation of “classical” tissue engineering principles to cardiac cells. One of the pioneering groups (Vunjak-Novakovic and colleagues at the MIT in Cambridge, MA) initially used polyglycolic acid as a scaffold in combination with bioreactor cultures (Figure 1E). Alternatively, the group of Li seeded fetal rat ventricular cells onto gelatin scaffolds, cultured them for 7 days in vitro and implanted them onto cryo-infarcted rat hearts. Spontaneous contractile activity, both in vitro and in vivo, was reported, but the histological microphotographs showed mainly cells of unknown identity embedded in the scaffold material and poor sarcomere development in the few cardiac myocytes present in the construct. Leor and colleagues generated alginate scaffolds seeded with fetal cardiac cells and implanted these graft onto rat hearts (Figure 1F). The constructs were highly vascularized, but did not exhibit true integration into the host myocardium. The conceptionally important advantage of the classical tissue engineering approach using preformed matrices as compared with the liquid matrix cell entrapment approach is that technically fabricated, solid materials can be shaped in any 3D geometric form, on a macroscopic, but also, at least in theory, at a microscopic level. Thus, one could fabricate the ideal patch by computer design and then seed it with cardiac myocytes and potentially other cell types, hoping that they would use the matrix as a scaffold to generate a tissue similar to the native heart. In essence, this strategy either uses the natural organ as a blueprint (exemplified in the famous “ear-mouse”) or decellularized grafts that are already successfully utilized in clinical use such as porcine heart valves. One could term this latter approach “biologization” of tissue grafts. The great promise of these strategies with regard to cardiac muscle tissue engineering remains unproven, so far. Limitations include unfavourable matrix properties such as limited diffusion capacity, low mechanical compliance, liberation of potentially toxic substances during degradation, and incompatibility with physiological cell growth. Essentially, cardiac myocytes appear to survive for some time and start to beat inside such artificial scaffolds, but remain largely isolated and do not form coherently beating cardiac tissue. New materials, the molecular refinement of surfaces and creation of microstructures that promote spreading of cells may offer solutions to this problem (review in reference
Alternatively, Vunjak-Novakovic and colleagues successfully developed a somewhat mixed approach by seeding preformed collagen sponges/foam with neonatal rat heart cells suspended in Matrigel together with electrical stimulation for extended times. This led to the generation of cardiac muscle constructs with improved cardiac tissue morphology, contractile function, and molecular marker expression when compared with nonstimulated cultures (Figure 11). Hence, electric stimulation seems to induce a similar degree of cardiac myocyte differentiation as mechanically stimulation. Whether electrical activity per se is a stimulus for advanced differentiation and tissue formation or the resulting contractile activity is not clear and may be difficult to experimentally separate. Yet, the work by Radisic et al also supports the notion that biological extracellular matrices mainly composed of collagen and laminin (present in high concentration in matrigel) are presently the optimal scaffold material in cardiac tissue engineering.

The third approach can be termed tissue engineering without matrix and is a refinement and systematization of the phenomenon of detachment of cellular monolayers after prolonged culture time (see above). Shimizu and colleagues used a thermo-sensitive surface coating for culture dishes which allows cardiac myocytes (or any other cell type) to attach at 37°C as in other normal dishes, but to detach under controlled conditions at room temperature as an intact sheet. These beating cardiac myocyte monolayers can be stacked on one another and grown together to form interconnected 3D tissue sheets of up to 50 to 75 μm thickness (Figure 1H). The advantages of this technique are its relative ease and the independence from potentially immunogenic or pathogenic scaffold materials. Disadvantages are its fragility (leading to handling problems), restriction in terms of geometric form and difficulties to impose mechanical load on the contractile sheets.

Other studies have described variations of the above techniques. One report described generation of coherently beating aggregates from neonatal rat heart cells by exposing cell suspensions with fibronectin coated polystyrene microcarrier beads or oriented collagen fibers to microgravity in bioreactor cultures. Other studies seeded commercially available collagen sponges with neonatal rat heart cells alone and observed cardiac tissue formation. Van Luyn and colleagues experimented with liquid, ‘cardiogenic’ cell-matrix mixtures into hydrogels. These in vitro data are well in line with the established role of the endocardial endothelium for normal cardiac development, where disruption of normal endothelial cell function led to severe cardiac malformation. Collectively, these data suggest that endothelial cells, smooth muscle cells, fibroblasts, macrophages and other noncardiomyocytes play an important or even essential role in the formation of engineered cardiac tissue grafts.

How Far Has Cardiac Tissue Engineering Gone and What Are the Present Limitations?

Tissue Development

It has been questioned whether the term ‘tissue’ may actually be used for engineered myocardial constructs. This argument is based on the classical definition of a ‘tissue’ as being “a part of an organism consisting of an aggregate of cells having a similar structure and function.” Yet other widely used definitions state that a tissue is “a group of similar cells united to perform a specific function” or “a grouping of cells that are similarly characterized by their structure and function.” This definition applies to engineered tissue constructs. In fact, use of stretch or electrical stimulation and mixed populations of heart cells including cardiac myocytes, fibroblasts, smooth muscle cells, endothelial cells, macrophages among others in cardiac tissue engineering leads to the formation of heart muscle constructs with a high degree of organotypic differentiation on the single cell level and also a high native tissue-like cellular complexity. The cellular complexity can be further enhanced by using unpurified heart cell populations instead of purified cardiac myocytes. Cardiac myocytes themselves showed many structural features of terminal differentiation including well-developed sarcomeres, desmosomes, gap junctions and fasciae adherentes. Interestingly, T-tubule-sarcoplasmic reticulum junctions were found rarely that are or not or only rarely observed in neonatal rat hearts. Interestingly, EHTs developed approximately two-fold higher contractile force if generated from an unpurified cell preparation than from a relatively pure cardiac myocyte fraction. Similarly, endothelial cells can promote cardiac myocyte survival and spatial organization when cocultured on hydrogels. These in vitro data are well in line with the established role of the endocardial endothelium for normal cardiac development, where disruption of normal endothelial cell function led to severe cardiac malformation. Collectively, these data suggest that endothelial cells, smooth muscle cells, fibroblasts, macrophages and other noncardiomyocytes play an important or even essential role in the formation of engineered cardiac tissue grafts.

Important structural characteristics of cardiac myocyte terminal differentiation are sarcomere organization in registry with a cross-striation pattern including M-bands, formation of cell-cell contacts through desmosomes, gap junctions, and fasciae adherentes, as well as development of T-tubule-sarcoplasmic reticulum junctions (dyads) at the Z-band level. These features have been observed in engineered cardiac constructs that have been chronically stretched or electrically stimulated indicating that these conditions promote terminal cardiac differentiation. Interestingly, M-band formation was found to be enhanced after engraftment of EHTs indicating that additional differentiation factors are provided in the intact adult heart that are lacking in vitro.

Thus, critical factors identified today for cardiac tissue development in vitro are (1) a suitable biological matrix such as collagen I, IV and laminin (which, in case of the sandwich
technique of Shimizu, may be entirely cell-derived), (2) mechanical load, (3) a total heart cell mix, and (4) either electrically stimulated or spontaneous contractile activity. In summary, the various techniques presently used generate constructs with a very high degree of cardiac tissue development (Figure 1E, 1H, and 1I).

Contractile Function

Also functionally, artificial heart constructs resemble intact heart tissues in terms of force-frequency behavior, force-length relationship (Frank-Starling mechanism), response to extracellular calcium and the β-adrenergic agonist isoprenaline as well as its antagonism by acetylcholine-derivatives.31,33 These data support the above conclusion of true heart tissue-development in vitro from a functional point of view. However, differences exist as well. For example, EHTs exhibit a higher calcium sensitivity, and absolute forces remain lower than in the intact heart. Initially, the maximal twitch tension was 0.5 to 1 mN, optimized EHTs today reach up to 4 mN. Others reported active forces between 0.05 mN44 and 1 to 2 mN18 (overview in reference 50). Values of 4 mN are similar to the force of intact muscle preparations such as rat papillary muscles, but experiments in the latter underestimates maximal forces developed by adult, mature cardiac myocytes due to core ischemia. Very thin adult rat heart muscle strips develop forces of 56 mN/mm².57 The diameter of mature rat EHTs is 0.8 mm, therefore the diameter of the two sides of the rings together is 1 mm². Thus, the normalized optimal force of EHT is 4 mN/mm². The difference of EHT force generation as compared with mature heart muscles most likely reflects both a quantitative and a qualitative aspect, as for example a lower fractional occupancy of the EHT tissue by cardiac myocytes and the lower degree of sarcomere formation. Both are likely to be important for tissue formation in the absence of perfusion in vitro, but are, at least partly, overcome after implanting onto the heart.56

Critical Size

One of the central unresolved problems of all tissue engineering approaches is the limitation of maximum size which is defined by maximum diffusion distances for nutrients and oxygen. Indeed, none of the various tissue engineering approaches developed today generate cardiac tissue-like, contracting constructs in which compact muscle strands exceed 50 to 100 μm. Early tumor angiogenesis studies have shown that, in the absence of capillarization and perfusion, tumors implanted into nude mice did not reach a size of more than 2 to 3 mm.58 These values, however, depend both on the metabolic demand and the cellular density of the respective tissue. Beating cardiac myocytes obviously have a very high metabolic activity. Accordingly, the density of capillaries in the adult heart is very high, amounting to approximately 2400 to 3300/mm² in rat and human at different postnatal stages (intercapillary distance ≈19 to 20 μm).59,60 On the other hand, the early embryonic rat heart (until ED 16) as well as the adult frog heart are avascular and nourished exclusively from the lumen by blood circulating within the trabecular system.61,62 Thus, large heart muscles either develop physiologically through vascularization or through intense trabecularization with single strands smaller than 50 to 75 μm. The latter pattern is recapitulated in EHTs, in which the network of cardiac myocytes strands is loose in most parts and consists of muscle bundles with a diameter of 30 to 50 μm (see Figure 2 in reference 55). Only in some parts compact muscle strands form that reach up to 100 μm. Similarly, 3 to 4 cardiac myocyte monolayers can be successfully stacked, more did not improve the thickness of tissue constructs.18 Some groups increase oxygen and nutrient delivery by cultivating constructs in bioreactors, increasing ambient oxygen concentrations and/or by adding oxygen carriers such as perfluorocarbon.63,64 Indeed, such optimization of culture conditions has had beneficial effects on cell density and metabolic activity.64 and has been modeled to allow construction of cardiac tissues with a clinically meaningful thickness of up to 500 μm.64 Another strategy is to incorporate a perfused natural vessel into engineered heart muscle constructs55 or to stimulate angiogenesis in cardiac tissue constructs. In EHTs, primitive capillaries and larger vessel-like structures lined with CD31 positive endothelial cells develop spontaneously.55 It seems unlikely that these vessel structures play a significant role in oxygen and nutrient exchange under in vitro conditions, but they may facilitate perfusion after implantation. Levenberg and colleagues have recently mixed endothelial cells and fibroblasts to a culture of skeletal myoblasts on a synthetic polymer and observed generation of skeletal muscle constructs with increased density of vascular structures.66 A third strategy to overcome size limitations in vitro is to weave several EHTs or comparable constructs together, thus forming a network in which each individual construct remains accessible for unlimited diffusion and exchange of nutrients. Large networks could be useful as an improvement of the “ACORN” technology (CorCap Cardiac Support Device), a therapeutic device currently tested in humans with terminal heart failure with predominant ventricular dilatation.67 Such a “chain mail” biological network (Figure 2) could have the advantage over the synthetic CorCap Cardiac Support Device in that it could support both diastolic and systolic function of a failing heart. Taken together, several potential strategies have been developed to overcome size limitations, both in vitro and after implantation in vivo. Yet, critical size remains an unresolved problem and its solution is one of the most important tasks in the field.

Implantation Studies

The early studies by Li and Leor indicated that cardiac myocyte-populated gelatin and alginate-grafts, respectively, were visible for extended time after implantation as a patch onto the heart and contained cells, despite the absence of immunosuppression.38,39 No definite proof for the development of new cardiac tissue was provided. Shimizu et al. demonstrated survival and vascularization of their sandwich constructs and recorded beating activity after implantation into nude rats.18 Survival, vascularization and terminal cardiac differentiation of cardiac myocytes was observed in EHTs after implantation onto rat hearts in the presence of immunosuppression.56 Even though these data collectively
suggest that constructs are rapidly vascularized after implantation and that hypoxia during and after implantation is not a major problem, several important questions remain unanswered: Is vascularization sufficient? How many of the cardiac myocytes initially implanted survive over time? Do the constructs couple electrically and mechanically to the host myocardium? Does the implantation of cardiac constructs beneficially affect cardiac performance? The early studies reported improved function as evidenced by enhanced developed pressure in isolated Langendorff-perfused hearts and improved left ventricular dimensions as well as fractional shortening. However, the effects were generally small and similar effects have been reported in numerous cell injection studies, apparently independent of the cell type injected. Thus, the specific functional contribution of grafting 3D constructs as compared with cell injection remains unclear at present. Thorough evaluation of this issue is indispensable for the further development of the field.

Future Developments

Matrix Materials
As outlined above, the optimal scaffold for engineering cardiac muscle tissue has not yet been found. Alginate, gelatin, polyglycolic or polylactic acid, as presently used for engineering cartilage, bone, ear, skin, blood vessels, or heart valves, will not likely be the materials of choice. The reason lies in the physiological properties of cardiac muscle tissue, particularly the need for high mechanical stability coupled with great compliance. Moreover, muscle fibers and bundles in the heart form layers with different orientation to provide optimal pump function. At present it is difficult to imagine that any technically fabricated material will ever mimic such sophisticated structure and even if it would do so, the cardiac cells, stem cells or stem cell-derived cardiac myocytes still have to grow into the free spaces, interconnect and thereby form an electrical and mechanical syncytium. On the other hand, material sciences and particularly nanotechnology are necessary to mimic the structure and function of the cardiac tissue.
progressing rapidly and it may be that in a collaboration between engineers, physicists, biotechnologists, biologists and morphologists the ideal scaffold material for creating truly “artificial” heart tissues will be designed.88,89 Recent examples are tubular scaffolds for the engineering of blood vessels that were generated by electrospinning of collagen and elastin70 and collagen tubes for cardiac tissue engineering.71 Another innovative approach is to use MRI-derived 3D images of an organ as a blueprint for the fully automated fabrication of a synthetic copy.72 It will be interesting to see whether these sophisticated methods in nanotechnology and imaging will prove useful in creating optimized cardiac constructs.

Cell Sources for Cardiac Tissue Engineering

The greatest conceptual problem of cardiac tissue engineering (as for all cell-based therapies) relates to a suitable cell source. It has been estimated that the adult human left ventricle contains $5 \times 10^7$ cardiac myocytes,73 i.e., 40 million cardiac myocytes per gram of native myocardium. Thus, even the creation of a small tissue patch would require tens of millions of cardiac myocytes, a number impossible to obtain from a primary human cardiac cell source, not to speak of the ethical aspect. Thus, the primary cells used today for cardiac tissue engineering can only serve as a proof-of-principle. Great hope has been created by recent findings indicating that stem cells exist in the adult organism that could give rise to the formation of autologous cardiac myocytes as well as endothelial and smooth muscle cells. Such cells have been found in various tissues, including the bone marrow,74,75 peripheral blood,76,77 umbilical cord,78 and adipose tissue.79 Initial studies in mice showed that lin−, c-kit− cells can be isolated from bone marrow and regenerate new myocardium when injected into the heart of recipient mice after myocardial infarction.79 These data prompted the initiation of clinical studies in patients with acute myocardial infarction indicating that such treatment is safe and may provide functional benefit (review in reference 8). The first results of larger, randomized and placebo-controlled studies are eagerly awaited in late 2005. Yet, recent studies in mice using similar cells as the original study75 reported no cardiac regeneration at all.80–82 Thus, the capacity of bone marrow stem cells to regenerate the heart is currently under intensive debate and a detailed discussion is beyond the scope of this article (for review see references 5 and 7).

New hope was raised by the demonstration of progenitor cells resident in the heart that have the capacity to differentiate into functional cardiac myocytes and repair the heart.47–49,75,83 The first report by Beltrami and colleagues described small, round c-kit+ cells organized in niches in the adult rat heart that could be clonally expanded and, when injected into infarcted hearts, generated new viable myocardium.47 This study has been subsequently supported by data showing that a c-kit+ cell population can be propagated from murine and human heart specimens and, in case of murine cells, form spontaneously contracting cardiospheres even in the absence of coculture with primary heart cells.84 Moreover, c-kit+ cells could be mobilized to regenerate new myocardium in infarcted dog hearts by injecting IGF-1 and HGF-1.83 Another study isolated sca-1+ cells from a total adult murine heart cell population and found that these cells can acquire a cardiac phenotype after treatment with 5′-azacytidine, a compound known to induce muscle cell differentiation.85 By using an extensive genetic labeling strategy it was also shown that sca-1+ cells home to the injured myocardium when injected intravenously and have the capacity to differentiate into cardiac myocytes as well as to fuse with host cells. Subsequent studies extended these findings showing that sca-1+ expressing cells can be slowly expanded in vitro and, in the absence of primary heart cells, differentiate to beating cardiac myocytes when treated with oxytocine.86 Moreover, data were presented showing that cardiac differentiation of sca-1+ cardiac stem cells depends on FGF-286 and that only a CD31−, isl-1− subset of sca-1+ cells may actually represent cardiac precursors.87 Finally, the LIM domain transcription factor isl-1 has been identified by genetic means to mark a cardiac progenitor population of which few cells appear to remain in the postnatal heart.49 Isl-1+ cells have also been expanded and differentiated into contracting cardiac myocytes under coculture with neonatal rat cardiac myocytes. Fusion was made unlikely by pretreatment of myocytes with formaldehyde. It is not clear at present whether c-kit, sca-1 and isl-1 indeed label three different cardiac progenitor populations as indicated by some of the above studies89,90 or whether expression of these markers reflects the dynamic phenotype of the same one. In any case, these exciting findings open the door not only to an autologous cell therapy approach but also to the engineering of autologous cardiac tissues. Such tissues would be ideal for cardiac repair as being likely devoid of tumorigenic and immunological problems. However, tissue engineering from autologous stem cells has yet to be shown.

The alternative to adult stem cells or cardiac progenitors are embryonic stem cells which are able to form virtually any cell type of the body in vitro under appropriate conditions.88–90 Genetically selected ES cell-derived cardiac myocyte form electrical connections with the host myocardium when injected into the heart.91 The pluripotency of ES cells may be of particular importance for cardiac tissue engineering when considering the role of noncardiomyocytes in EHT formation (see above). Indeed, recent experiments showed that spontaneously contracting EHTs can be generated from mouse ES cells and produce forces similar to EHTs from primary cardiac cells (own unpublished data). Human ES cells possess principally the same features as mouse ES cells including the propensity to spontaneously differentiate into cardiac myocytes92–94 and a recent study showed that transplanted human ES cell-derived cardiomyocytes survived in the swine hearts under immunosuppression and acted as a rate-responsive biological pacemaker.95 The potential of cardiac differentiation apparently strongly depends on the cell line, but also on the quantification algorithm. Some lines seem to have a very limited or basically no potential for cardiac differentiation at all. This raises questions with regard to the present federal restrictions to a limited number of already existing human ES cell lines (listed by the NIH at http://stemcells.nih.gov/research/registry/). Moreover, it calls for studies deciphering the mechanisms of cardiac differentiation from ES cells. On this basis pharmacological strategies could be developed to direct cardiomyogenic differentiation. Because ES cells can be easily and quickly propagated in
unlimited quantities, even the immense cell number theoretically needed to produce cardiac muscle patches to cure human heart diseases appears a realistic option.

Major problems of ES cells are their immunogenicity and their potential to form tumors. Data exist that ES cells at least partly escape the normal immune responses and some groups have even injected mouse ES cells into rat hearts and found large numbers of surviving cardiac myocytes up to 32 weeks thereafter without evidence for tumor formation. In contrast, significant immune responses were seen after injecting GFP-labeled mouse ES cells into allogenic mouse hearts. The reasons for these discrepancies are not clear at present, but it is evident that immune responses have to be anticipated. Options to deal with this problem could be to create ES cell banks containing immunologically diverse phenotypes from which the best fit for the respective patient could be chosen. And finally the option exists to derive potentially autologous ES cells by nuclear transfer.

The most significant problem of ES cells in regenerative approaches is the problem of tumor formation as noted in careful studies. Even the risk can be minimized by various means, it is likely that even 1 tumorigenic cell in 1 million can create problems in a cardiac repair approach in which 100 million cells are needed. Thus, injection of undifferentiated ES cells for cardiac regeneration must be viewed with scepticism. Two principal strategies are currently used to reduce this risk. One is to positively select cells that acquire a cardiac phenotype, for example by introducing into the genome of an ES cell line a neomycin resistance gene under control of a cardiac specific promotor. With this strategy noncardiac myocytes can be eliminated before implantation of the cells/tissue by adding neomycin-derivatives into the culture medium. Alternatively or in addition, it may be necessary to introduce a suicide gene into the ES cell clone such as herpes simplex virus thymidine kinase which makes all undifferentiated potentially tumorigenic ES cell-derived cells sensitive to drugs such as ganciclovir. This strategy could be used as an emergency treatment if tumor formation occurs. However, none of these genetic approaches is 100% efficient or safe. In fact, any approach to alter a stem cells’ genome may be affected by genetic and epigenetic instability. Moreover, the genetic manipulation itself could create new tumorigenic problems by deleterious integration of the vector into the genome. It will therefore be essential to apply rigorous tests to exclude such problems before any application, because safety will be crucial for the success of the entire ES cell concept. Other unresolved issues relate to insufficient vascularization, one of the likely reasons for graft size reduction after ES cell injection.

**Immune Response**

All allogenic cell-based therapies face the problem of immunorejection and it is questionable whether patients and doctors would accept lifelong immunosuppression with potentially fatal consequences such as tumor development, cyclosporine-induced renal failure, Cushing syndrome, and osteoporosis. This issue is in fact a strong argument for autologous stem cell therapies, most likely with resident cardiac stem cells. Alternatively, ES cell-based approaches, will require either a way to circumvent immunorejection or the opportunity to do therapeutic cloning, either with oocytes derived from volunteers or from ES cells.

Another somewhat neglected problem is that most of the commonly used media supplements are xenogenic (eg, fetal calf serum, horse serum, chick embryo extract, matrigel, collagen). This results in several problems: Infectious risks, nonadherence with standards of good laboratory and manufacturers practice (GLP, GMP), and the generation of immune responses even with autologous or syngenic cells. For example, complete immune rejection was seen when EHTs made from neonatal Fischer rats and Fischer rat tail collagen I were implanted into adult syngenic Fischer rats. The most likely explanation is that, despite extensive washing before implantation, remnants of the chick embryo extract and horse serum, or Matrigel had impregnated the genetically autologous cells or the extracellular matrix and provoked an immune response. Another explanation could be induction of self-antigens during prolonged culture. It will be necessary therefore to identify suitable culture conditions for engineered heart tissues that are devoid of xenogenic growth supplements. Some critical factors for cardiac myocyte cultures including epidermal growth factor, hydrocortisone, L-thyroxine, albumin, selenium, and transferrin have already been identified.

**Realistic Perspectives of Cardiac Tissue Engineering in the Next 10 Years**

Statements on the perspectives of science share the limitations of stock market predictions and remain essentially speculations, founded on facts and spiced with personal convictions. With this in mind, some predictions can be made. The total artificial heart will likely remain fiction for a while, but some important milestones toward this ambitious goal have already been achieved as outlined above. The following summarizes realistic accomplishable achievements:

**Drug Screening Assay Based on Engineered Cardiac Constructs**

Engineered cardiac tissues will be useful for drug development and target validation in a format that allows for medium-to-high throughput assays (Figure 2). Experiments are on the way to construct a 96-well plate ES-cell EHT format to eventually generate a humanized experimental model for drug research.

**Serum-Free Culture Conditions for Cardiac Tissue Grafts**

Experiments to define such culture conditions are currently being pursued in several laboratories. High throughput screening systems as mentioned above will be helpful to identify cardiogenic or cardiac growth supportive factors in the right concentration and combination as well as the right timing. Given the availability of serum-free media for many other cell types there is little reason to assume that a similar condition cannot be established for cardiac cells and tissues.
Prevention/Slowing of Remodeling After Myocardial Infarction by Tissue Grafts in Large Animal Models

A realistic perspective is that large networks composed of several engineered cardiac grafts can be generated and sutured around the heart. Such “biological chain-mail” as exemplified with the EHT-technology in Figure 2 could be designed both from primary cardiac and ES cells and tested in rats, mice and pigs after myocardial infarction.

Correction of Heart Defects by a Contracting Patch in a Large Animal Model

Congenital heart defects such as complex Tetralogy of Fallot, and aplastic right and left ventricles would profit from the implantation of actively contracting engineered tissue patches. This approach will likely be tested first in the low pressure system of the right heart with patches made from ES cell-derived myocytes.

Autologous Cardiac Graft From an Autologous ES Cell Line Generated by Nuclear Transfer

Techniques to generate autologous blastocysts by nuclear transfer and to produce autologous ES cell lines (ntES cell) from these early embryos have been successfully established over the past 5 years, in various animal species and recently also in human.104 Thus, despite the present formal hurdles and the ongoing ethical debate, it seems realistic to create autologous artificial heart muscle from ntES cell lines and test their applicability in an animal model in the next years.

Autologous Cardiac Graft From Resident Cardiac Precursors

Given the discovery of cardiac progenitors it should also be possible to isolate such cells from a cardiac biopsy, amplify and differentiate them into functional cardiac myocytes and create a 3D tissue construct.

Conclusions

Cardiac tissue engineering is a relatively young, actively developing field. Its great attraction, both for researchers and the public, lies, first of all, in the fascinating natural capacity of heart cells to form spontaneously beating, well organized heart-like tissues in the culture dish—a finding that was made more than 50 years ago. Apart from the naive enthusiasm this phenomenon excites, there are good reasons to assume that engineered cardiac tissues will be of practical use in the near future, as an improved experimental model, as a model system for cardiac development and, much later, as a therapeutic option for cardiac repair. The latter optimism is mainly based on the enormous progress in stem cell research. What has been pure fantasy 10 years ago now becomes a realistic prospect—to create contracting heart muscles from the patients own stem cells and to use them to alleviate heart disease. Many critical caveats remain, however, and intense and stringent scientific work is mandatory, before such strategies should head into the clinic. And finally, future developments in mechanic devices, xenotransplantation, cell therapy and not at last pharmacology may decide whether or not cardiac tissue engineering will indeed find its place in the practical therapy of heart disease.

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