Cardiomyoplasty: Cellular and Tissue Engineering Approaches

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Abstract
Myocardial infarction is a major cause of morbidity and mortality in the western world. After injury, myocardial repair is limited since cardiomyocytes cannot significantly regenerate in vivo. Current therapeutic options such as medical therapy and whole heart transplantation have either limited clinical benefit or restricted applicability. Over the past years, the shortcomings associated with the available therapies have led to consider cellular-based strategies for the treatment of heart disease. Cellular therapies include the endogenous augmentation of the number of cardiomyocytes, and the transplantation of dissociated cells or cardiac muscle-like tissues in the diseased heart. Herein we review current knowledge on the status, the potential advantages, disadvantages and the clinical applications of the cellular based therapies with an emphasis on cellular and tissue transplantation.
Key words: myocardial infarction, cellular cardiomyoplasty, tissue cardiomyoplasty, tissue engineering.

Basic Appl. Myol. 9 (4): 151-159, 1999

Nearly 1.5 million people in the United States sustain a myocardial infarction each year, and this event proves fatal in approximately one-third of patients [4]. Myocardial infarction is the condition of irreversible necrosis of cardiac muscle that results from prolonged ischemia, when the blood supply to the myocardium is severely reduced or stopped [76]. After an infarction, the myocardium is gradually replaced with fibrous, non contractile scar tissue because myogenic stem cells are absent from the myocardium, and adult cardiomyocytes have been terminally withdrawn from the cell cycle [29, 95]. Complications of an infarction include arrhythmias and congestive heart failure which result from inflammatory, mechanical and electrical abnormalities in regions of the necrotic myocardium.

Standard long term pharmacological therapies, aimed at improving the function of the non-infarcted part of the myocardium, include vasodilators, β-blokers, angiotensin-converting enzyme inhibitors, and antiarrhythmic drugs [76]. Despite these treatments, a significant percentage of patients develop progressive congestive failure and become candidates for a heart transplant. The chronic shortage of organs (one out of ten patients is able to receive a transplant) [4] and immune rejection issues, are major problems associated with heart transplants. Recently, Batista et al have developed the *Batista heart failure procedure*, that involves the excision of a part of the dilated left ventricular free wall, with the rationale that the heart chamber will be able to contract more effectively [6]. Despite the initial encouraging results, the Batista procedure is still being evaluated by many centers around the world and cannot be regarded as a standard surgical therapy for the treatment of heart failure. The total artificial heart, another option for patients with severe symptoms, has been associated with thrombogenicity, infection, power transmission problems and prohibitive operating and maintenance costs [94]. A different approach to augment the function of a failing heart is dynamic cardiomyoplasty, which involves the wrapping of the latissimus dorsi muscle around the ventricles and its stimulation to contract synchronously with the native heart [30, 31, 67]. Although preliminary reports have shown significant improvements in the survival rate of the treated patients, additional research is needed to elucidate the mechanisms of action of dynamic cardiomyoplasty.

From the above, it is apparent that new therapeutic strategies are urgently needed, which would allow for rapid restoration of heart function, without the need of immunosuppression, implanted devices or prohibitive costs [55].

Cellular Based Therapies

Strategies to increase the number of functional cardiomyocytes in the diseased heart, could potentially im-

prove the contractility of the infarct zone and prevent the progression of heart failure [55, 59]. In the following pages, cellular-based strategies, which include endogenous and exogenous augmentation of the number of cardiomyocytes in the diseased heart, are presented.

Endogenous cellular therapies

Although adult cardiomyocytes are considered terminally differentiated [95], recent evidence suggests that they can undergo limited mitosis in some pathophysiological conditions [73] or in the presence of mitogens. Growth factors such as basic fibroblast growth factor (bFGF), insulin-like growth factors (IGF-I or IGF-II), transforming growth factor-β (TGF-β) and platelet derived growth factor (PDGF) have been associated with DNA synthesis and mitosis in cardiomyocytes [11, 16, 64]. When IGF-I was overexpressed in the developing heart, it was showed that the number of cardiomyocytes was doubled in 7 month old mice as compared to non-transgenic control animals [74]. On the contrary, after myocardial injury IGF-I upregulation induced DNA synthesis [75] and hypertrophy [34] in cardiomyocytes, but did not lead to cell division. These results suggest that growth factors may somewhat enhance the ability of adult cardiomyocytes to undergo DNA synthesis and hypertrophy, but that seem unlikely to restore the contractile function of the heart and to replace fibrous tissue.

In addition to growth factors, oncogenes and cyclin-dependent cell cycle kinases induced proliferation when introduced into fetal and neonatal cardiomyocytes [20], while they only induced hypertrophy when introduced in adult cardiomyocytes [37, 82]. The association of oncogenes and cell cycle-associated kinases with tumorigenesis will make unlikely their use in a clinical setting.

It has been suggested that another route to muscle regeneration would be to force cardiac fibroblasts, that are 95% of the nonmyocyte fraction of cardiac mass, to differentiate into muscle [19, 62]. In vivo and in vitro studies have shown that fibroblasts can be converted to skeletal muscle cells by tranfection with MyoD, a factor necessary to commit cells to the muscle lineage [13, 62]. In addition, Eghbani et al showed that when cardiac fibroblasts are exposed to TGF-β_1, they acquire certain cardiomyocyte-specific properties [19]. Although these results are encouraging, there is no evidence that the converted fibroblasts will be able to establish functional electrical or mechanical connections to native cardiomyocytes [55].

Induction of angiogenesis in the infarct zone might recruit any surviving cardiomyocytes in the scar and thus might improve regional contractility. A number of studies have already shown that vascular endothelial growth factor (VEGF) and bFGF, administered together or separate, enhanced regional blood flow in the ischemic myocardium [3, 57, 71, 89, 97]. A major drawback of this approach is that there are not any effective techniques to deliver those angiogenic factors to the infarcted myocardium.

Exogenous cellular therapies

Exogenous cellular therapies could offer a new approach to strengthen and rebuild the damaged heart. These involve either the injection of a suspension of dissociated cells (*cellular cardiomyoplasty*) [42, 43, 51, 55, 83, 85], or the implantation of a cardiac-like tissue *(tissue cardiomyoplasty*) [7, 9, 10, 24, 26, 35, 36] into the scarred myocardium (Figure 1). Prerequisites of cellular or tissue transplantation include the improvement of cardiac function without side effects, formation of functional electrical and mechanical connections between grafted and host tissue, and long term survival.

Cellular cardiomyoplastry

Grafting of dissociated cells is an emerging approach for the restoration of host tissue function and delivery of therapeutic proteins to patients. Intracerebral grafting of central nervous system progenitor cells into experimental animals and Parkinson's patients has gained support as an effective means to supplement dopamine levels [17, 84]. In addition, transplanted healthy myoblasts fused with myotubes from Duchenne's patients and the grafted myotubes expressed wild-type dystrophin [32]. More recently the use of genetically engineered cells for the delivery of recombinant proteins to the brain, thymus, and hematopoietic system has also emerged [1, 3, 22, 98]. Possible advantages of cell over tissue transplantation are 1) the dissociated cells can be injected at a specific site without complicated surgery and trauma

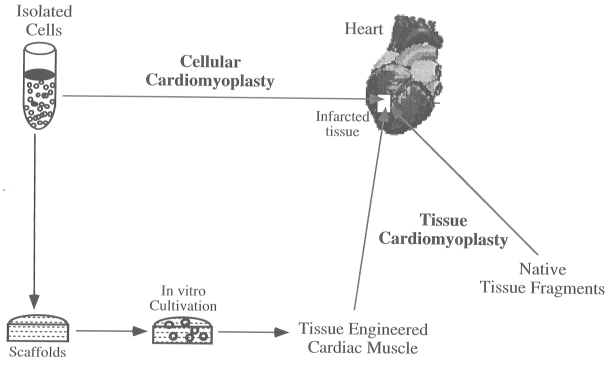

Figure 1. Schematic representation of cellular and tissue cardiomyoplasty. Cellular cardiomyoplasty involves the injection of a suspension of dissociated cells, while tissue cardiomyoplasty involves the implantation of native tissue fragments or engineered tissues in the scarred myocardium. The dissociated cells can be cardiac, skeletal, smooth muscle, fibroblasts, endothelial or embryonic stem cells. Engineered tissues are made by seeding living cells on scaffolds and growing these in vitro before in vivo implantation.

to the surrounding tissue, and 2) the earlier induction of angiogenesis [8].

So far, a variety of primary and transformed cell types such as skeletal muscle, embryonic stem cells, cardiomyocytes, and smooth muscle cells has been used for cell transplantation into the heart [40, 42, 50, 54, 83], (Figure 1). Important issues with cellular cardiomyoplasty are the selection of the animal model, injury type, the time of implantation after injury, and the selection of cellular markers for the in situ identification of the implanted cells [5, 12, 18, 86].

a) Use of skeletal muscle cells

While injured cardiac muscle cannot effectively regenerate after injury, skeletal muscle has retained an efficient regenerating mechanism. Mature muscle fibers, which are large tubes containing hundreds to thousands of nuclei, although themselves cannot regenerate, they rely on a small percentage of undifferentiated, mononucleated satellite cells (myoblasts) that lies near the basal lamina of muscle fibers [56, 78]. Injury to the muscle fibers activates the myoblasts, which enter the cell cycle and fuse with each other and with the injured myofibers, thus restoring the continuity and function of the skeletal muscle fiber.

Augmentation of cardiac function using myoblasts has many advantages due to the proliferative potential of those cells, and the possibility to use autologous cells, thus eliminating all issues associated with immune rejection. Skeletal myoblasts, used so far, included primary satellite cells isolated from a variety of species, and transformed myoblast lines, such as mouse C2C12 cells. Although transformed cell lines are attractive donor cells because they have high proliferative rates in culture for up to 2 years, their clinical use might be problematic because of their tumorigenic potential [61].

The first evidence that autologous myoblasts could survive when implanted in the acutely cryoinjured myocardium of dogs was by Marelli et al [58]. Koh et al reported that C2C12 cells formed grafts, consisting of differentiated myotubes, which survived in the normal myocardium of syngeneic mice for three months [42]. Functionality studies showed that those grafts did not have any deleterious effects on host heart function. In more recent studies, autologous myoblasts proliferated, differentiated into myotubes and were identified for 4 months in the cryoinjured myocardium of rats, dogs and rabbits [5, 12, 63, 85]. Briefly, Murry et al showed that myoblast grafting in the diseased rat heart generated new contractile tissue, consisting of slow twitch fibers which might be suitable to perform a cardiac-like work load for long term [63]. In other studies, injection of autologous myoblasts into the cryoinjured myocardium of rabbits, which has many properties in common to the human heart, improved myocardial performance [5, 85]. In some of the aforementioned studies, a percentage of the implanted cells displayed a cardiac-like phenotype indicating that myoblasts may be influenced by the cardiac environment to undergo “milieu-dependent” differentiation [12, 85]. In addition, C2C12 grafts genetically engineered to express the angiogenic factor, TGF-β1, grafted into the normal myocardium of mice, showed enhanced neovascularization [41], suggesting that intracardial grafting of genetically engineered cells could be of therapeutic value for individuals with myocardial ischemia or congestive heart failure.

The major drawbacks of skeletal muscle transplantation are: 1) the availability of satellite cells, since their number and mitotic potential decreases with age [27], 2) fibroblast contamination during cell expansion in culture [2, 38], 3) lack of specific markers for satellite cells and 4) the different physiology of skeletal and cardiac muscle [33]. Genetic manipulation of myoblast cultures e.g. to stimulate proliferation in culture, convert fibroblasts to skeletal muscle, add cardiac specific genes before cell transplantation, may overcome some of the aforementioned problems.

b) Use of cardiomyocytes

The feasibility of cardiomyocyte-based grafts was first established with a differentiated tumor line, designated AT-1, derived from trangenic animals expressing the artial nautriuretic factor-simian virus 40 T antigen fusion gene [43]. AT-1 derived grafts survived in the normal hearts of syngeneic mice for at least 4 months without inducing significant damage to the host heart, although no junctions were observed between AT-1 cells and host cardiomyocytes. Soopaa et al reported that fetal cardiomyocytes delivered into the normal heart of syngeneic mice formed stable grafts for as long as two months, and the transplanted cardiomyocytes formed junctions with host cardiomyocytes [83]. Similar results were reported when larger animal models were used [44, 90]. Li et al studied the function of transplanted cardiomyocytes and their capacity to survive in fibrous connective tissue, using fetal cardiomyocytes transplanted into the hearts of syngeneic mice [54]. The transplanted cells formed contractile cardiac tissue that increased in size during the first two weeks of implantation, and contained sarcomeres, desmosomes and fascia adherens, providing some evidence that cardiomyocytes could also be successfully transplanted into myocardial scar tissue. In subsequent studies it was shown that fetal rat cardiomyocytes implanted in and at the peri-infarct borderzone of a cryoinjured myocardium, survived and formed cardiac tissue for up to 8 weeks [54, 79-81]. Although cardiac function was improved at the first 2 months of implantation, at 20 weeks postimplantation it was shown that the graft decreased in size probably due to allograft rejection [53]. In addition, Watanabe et al demonstrated that autologous fetal cardiomyocytes did not survive in the cryoingured myo-

cardium of pigs at 4 to 5 weeks of implantation, raising concerns about the feasibility of cardiomyocyte transplantation in large animal models, which have similar physiology and coronary anatomy to humans [93]. Furthermore, Li et al demonstrated that the successful engraftment of cardiomyocytes strongly depends on the age of the donor-cells [54, 55]. Fetal and neonatal rat cardiomyocytes formed contractile cardiac tissue when implanted in fibrotic tissue, but that was not the case for cells isolated from young and adult hearts.

Major drawbacks of cardiomyocyte transplantation are the ethical problems associated with the use of human fetal cells, and the limited availability and proliferation of fetal cells. To address those problems it has recently been suggested: a) to optimize the tissue culture techniques for expansion of neonatal or adult cardiomyocytes [52], b) to use genetically engineered cardiomyocytes [28], and c) to use fetal cardiomyocytes derived from genetically modified pigs with reduced imunogenicity [77].

c) Use of other cell types

Li et al recently demonstrated that fetal smooth muscle cells, transplanted into the cryoinjured myocardium of adult rats, formed smooth muscle tissue, which was shown to improve cardiac function [50]. The rationale for using smooth muscle cells was that these can be easily isolated from autologous sources, expanded in culture, and the grafted tissue can potentially increase wall tension and subsequently improve cardiac function. In addition to smooth muscle, fetal fibroblasts, endothelial and mesenchymal stem cells have been also used (Li et al, unpublished observations).

Recently, there has been a lot of interest on the use of embryonic stem cells which have unlimited proliferative potential, while they can differentiate into every cell in the body under the appropriate stimuli [87, 88]. Cardiomyocytes derived from differentiated embryonic stem cells have physical and molecular characteristics similar to those in the developing myocardium in vivo [39, 65], suggesting that they might be suitable for the formation of intracardial grafts. Klug et al demonstrated that stem cell derived-cardiomyocytes, when implanted into the myocardium of adult mice, formed stable intracardial grafts [40]. Although embryonic stem cell grafts have high potential for success, the ethical concerns associated with embryonic stem cell research are significant drawbacks.

Tissue cardiomyoplasty

Tissue transplantation in the myocardium might improve the efficiency and localization of tissue repair, over injection of dissociated cells [9, 10], (Figure 1). Injected cells have low survival and can migrate through the circulation to other sites [66], thus making difficult the delivery, localization and surgical removal of a predictable amount of cells [85]. In addition, cell injection in the myocardium has not been associated with immediate improvements in cardiac function, since the dissociated cells don't have the structural organization of cardiac muscle [53]. Another advantage of tissue transplantation is the preimplantation monitoring, which allows only the best tissues to be implanted. The tissue origin for tissue cardiomyoplasty can be fragments of native tissue [49] or, if sufficient functional, tissue-engineered cardiac-like tissues [9, 10].

a) Native tissue fragments

Myocardial tissue has been transplanted in a variety of different tissues in the body, in order to investigate the effects of anatomical environment in tissue survival, differentiation and functionality [7, 35, 36]. Bishop et al showed that cells from fetal rat tissue proliferated and obtained a differentiated cardiac phenotype when transplanted into the anterior eye chamber [7]. Furthermore, when tissue fragments of cardiac muscle where implanted into a skeletal muscle bed, they developed into an autonomously beating heart muscle [35, 36]. Leor et al showed that fetal tissue fragments survived in the infarcted myocardium of adult rats [49], suggesting that cardiac tissue allografts or xenografts can be grafted in the infarcted myocardium and might provide a therapeutic strategy to restore cardiac function. Problems associated with the implantation of native tissue fragments include tissue availability, limited ex vivo tissue manipulation, the continuous need of immunosuppression and the difficulty to induce angiogenesis [51, 55].

b) Tissue engineered cardiac-like tissue

Tissue engineering is an multidisciplinary field with the main goal to generate living tissue equivalents in vitro, in order to replace or enhance tissue function or structure [70]. An impetus for the development of tissue engineered therapies was the donor scarcity, technical difficulties, expense, and complex labor intensive care associated with conventional therapies [46, 48]. Initial efforts have focused on skin tissue equivalents for treating burns, but an increasing number of cell types are now being engineered, such as bone [15], cartilage [25], blood vessels [68], liver [14], skeletal [69] and cardiac [9, 10, 21] muscle, and nerve conduits [96]. Although many of these engineered tissue have survived in vivo, so far only the tissue engineered skin has been approved by FDA for use in humans [70].

A general tissue engineering approach is to use living cells, which are usually associated in one way or another to a three dimensional matrix or scaffolding that can be natural, man made or a composite of both. The scaffold requirements are to provide the surface chemistry needed to guide the reorganization and growth of cells, to be biocompartible, and to be configured such as the attached cells could survive by diffusion [60, 72]. In

addition, the polymer could be biodegradable, so it disappears from the body after it performs its function, thus obviating concerns about long term biocompartibility [45, 47]. In many studies, the engineered tissues are maintained inside tissue culture bioreactors, since it has been shown that different patterns of flow and mixing within the bioreactors can have direct affects on cell shape and function, and can modulate the mass-transfer rates of nutrients and metabolites to the cells [91]. To address the problem of vascularization, the use of prevascularized scaffolds has also been suggested [92].

Cardiac muscle tissue engineering is a relatively new field with few noteworthy publications [9, 10, 21, 24]. Eschenhagen et al demonstrated that embryonic chick cardiac myocytes cultured in three dimensional collagen gels produced a contracting three dimensional heart tissue, which displayed characteristic physiological responses to physical and pharmacological stimuli [21]. Furthermore, the cardiac-like tissue could be used for delivery of therapeutic proteins, since a high percentage of cells was successfully transfected with a reporter gene. Our group reported that cultivation of neonatal rat cardiac myocytes on polymeric meshes in bioreactors resulted in contractile three dimensional cardiac-like tissues [24], which consisted of cardiomyocytes with cardiac-specific structural and electrophysiological properties [9]. The structure and composition of the engineered tissues depended on cultivation conditions [10], suggesting that optimization of cultivation conditions will aid in the development of new three-dimensional cardiac model systems and in identifying limiting factors for scale-up to clinically useful tissues. In addition, we have recently shown that after optimization of key system parameters, certain molecular, structural and electrophysiological properties of the engineered cardiac muscle were comparable to those of native tissues (unpublished observations). Despite the efforts to have uniform cardiac tissue growth throughout the three dimensional scaffolds, all studies so far [9, 10, 21] showed a biphasic tissue organization, with several layers of differentiated cells at the edges and less concentrated cells centrally, due to the diffusional limitations in the construct center [23].

These results suggest that tissue engineered cardiac muscle can provide a model system for basic cardiac research and, if sufficient functional, can potentially be used for in vivo tissue repair. So far, the only attempt to implant engineered tissues in vivo was by Li et al, using fetal rat cardiomyocytes seeded in a gelatine mesh (unpublished observations). The tissue survived implantation in a scarred myocardium and formed junctions with the host tissue. In addition, to cardiomyocytes, other cell types such as skeletal myoblasts, fibroblasts, genetically engineered cells or co-cultures of multi cell types, can be used to engineer tissues for implantation in the diseased heart.

Conclusions

In the last few years, there has been a lot of interest on the use of cellular-based therapies as therapeutic options for patients with severe cardiac dysfunction. It is clear by now that dissociated cells injected in the injured myocardium, can form stable grafts. Future work must focus on the refinement of the engrafting technique and the identification of the best cell type for grafting. Furthermore, a lot of work is needed to optimize the in vitro cultivation parameters, and to investigate the in vivo performance of tissue engineered cardiac grafts. If all the obstacles associated with the cellular based therapies are resolved, then these therapies may be used as healthy alternatives to the current therapeutic options.

Acknowledgments

This work has been supported by a grant from IsoTis BV.

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