Current perspectives

Cardiac-bio-assists: biological approaches to support or repair cardiac muscle

Ugo Carraro, Gianluca Rigatelli*

*CNR Institute of Neuroscience, Unit for Neuromuscular Biology and Pathophysiology, Laboratory of Applied Myology, Department of Biomedical Sciences, University of Padua Medical School, Padua, *Cardiomyoplasty Project Unit, Legnago General Hospital, Legnago (VR), Italy*

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In contrast to those of other cardiac diseases, the morbility and morbidity of congestive cardiac insufficiency are not on the decrease, in spite of significant progress in pharmacological treatments and due to the increased life expectancy of the population. Cardiac transplant is the therapy of choice when cardiac failure becomes pharmacologically intractable, but all over the developed world (not to mention the situation in the underdeveloped countries) the number of heart transplants has reached the limit set by the availability of donor organs. Sooner or later xenotransplants could solve this problem, but even if our most optimistic hopes regarding their development and reliability are met, xenotransplants would still carry the risk of anthropozoonotic viral infections. Finally, as suggested in a recent overview, the way to the long-lasting clinical use of mechanical circulatory support is a long and winding road.

Other options are related to tissue or cell cardiac bioassistance. Cardiac-bio-assists are biological approaches to the remedy of progressive cardiac failure based on autologous or heterologous tissue or cell transplantation. Some of the work hypotheses are in pre-clinical evaluation (skeletal muscle ventricle), others are under preliminary (cardiomyoplasty, myocardium reduction, implants of myoblasts derived from skeletal muscle satellite cells and implants of embryonic or adult stem cell-derived myocardiocytes, cellular cardiomyoplasty) or advanced clinical testing (dynamic aortomyoplasty and dynamic cardiomyoplasty).

Dynamic cardiomyoplasty is a surgical procedure which could support myocardial function when cardiac insufficiency would become pharmacologically intractable in the mid term. In this procedure a nonessential muscle, the latissimus dorsi (LD), is diverted from its normal role, transferred into the chest, wrapped around the heart (LD wrap), conditioned to fatigue and activated during systole to provide cardiac assistance. The mechanisms of its action are discussed and the risk of myodystrophic lesions of the LD wrap which could reduce the work capability of the pericardial muscle prosthesis remains.

We are now addressing some of these issues by means of clinical research on the group of Italian patients of demand dynamic cardiomyoplasty, and by means of animal experiments aimed at the development and testing of new surgical, clinical and biotechnological approaches. In particular, we will discuss whether the increase in the muscle mass of the distal part of the transposed LD is desirable and feasible or if it is mandatory.

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Address:

Prof. Ugo Carraro

Dipartimento di Scienze Biomediche
Università degli Studi
Viale G. Colombo, 3
35121 Padova
E-mail: ugo.carraro@unipd.it

Surgical options for the treatment of pharmacologically intractable progressive heart failure

In contrast to those of other cardiac diseases, the morbility and morbidity of congestive cardiac insufficiency are not on the decrease, in spite of significant progress in pharmacological treatments and due to the increased life expectancy of the population. Cardiac failure is the most frequent cause of death in Europe, with 500 000 new cases every year and a 50% mortality after 5 years. Cardiac insufficiency heavily limits the autonomy and social relations of the patients who experience an "early functional aging": notwithstanding their chronological age, cardiopathic subjects show performances of 80-year-old persons. Therefore, two are the goals of any therapy: an improved quality of life and an increased survival[1]. Clinical requirements render alternatives to the current pharmacological treatments for advanced heart failure mandatory.

Heart transplant, cardiomyoplasty (myocardium reduction), xenotransplants, and mechanical circulatory supports

Cardiac transplant is the therapy of choice when cardiac failure becomes phar-

macologically intractable, but all over the developed world (not to mention the situation in the underdeveloped countries) the number of heart transplants has reached the limit set by the availability of organ donors. Though admission to the transplant waiting list is becoming evermore selective, 25% of patients still die whilst awaiting a heart. Sooner or later xenotransplants could solve this problem, but even if our most optimistic hopes regarding their development and reliability are met, xenotransplants would still carry the risk of anthropozoonotic viral infections[2].

Ventriculoplasty is attracting the interest of many cardiac surgeons, but because it involves discarding up to 300 g of not necessarily diseased myocardium, it is difficult to view this technique as any other than an extreme remedy to severe dilated cardiomyopathy. It currently carries a high perioperative mortality and an adequate assessment of its benefits awaits the outcome of properly conducted trials[3,4]. Mechanical artificial hearts will continue to be used mainly as a bridge to transplant. Even if the problems of hemocompatibility and infection were solved, the need of an external power supply might, in the long term, pose an unacceptable psychological challenge to the patient[5].

Skeletal muscle assist devices

Against this background, cardiac-bio-assists, a surgical biological alternative to cardiac assists based on the use of autologous transplants of skeletal muscle tissue or of myogenic cells onto or within the myocardium, remain an attractive goal, worth being strenuously pursued[6].

Dynamic cardiomyoplasty and aortomyoplasty. To date, the clinical use of skeletal muscle assistance has been confined to cardiomyoplasty and aortomyoplasty. In these procedures the latissimus dorsi (LD) muscle is wrapped around existing structures, the ventricles of the heart in cardiomyoplasty, or the ascending or descending aorta in aortomyoplasty. At present, experience with aortomyoplasty is limited in terms of the number of patients (still less than 20 worldwide) and of the duration of follow-up[7]. Cardiomyoplasty, on the other hand, has been carried out in over 1000 patients worldwide since its introduction in 1985[8], and there is now a substantial body of follow-up data.

Procedures such as cardiomyoplasty and aortomyoplasty have the considerable advantage of not placing new nonendothelial surfaces in contact with the circulating blood. However, their main limitation lies in the fact that the geometry of the pump is dictated by the size and shape of the existing organs. In some respects the consequences of this limitation are obvious. A grossly hypertrophied heart may be too large to be wrapped effectively by the patient's LD muscle. The branching of major vessels makes it difficult to wrap an appreciable length of the ascending aorta. The small lumen of the descending aorta is a limiting factor to the achievement of an adequate stroke volume when this vessel is compressed. However, just as important as these anatomical considerations are the loading conditions imposed on the muscle wrap. In such a situation, the muscle is constrained to operate far from the peak of its power curve[9].

Dynamic cardiomyoplasty (which sounds similar to, but is different from cardiomyoplasty or ventriculoplasty, that is the partial ablation of the ventricular wall) is one of the surgical options for heart failure and uses a pedicled graft of the LD to wrap the heart (Fig. 1).

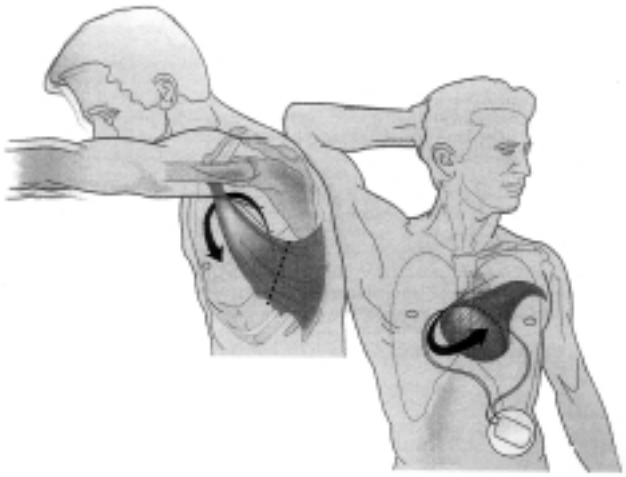

Figure 1. *Surgical transposition of the left latissimus dorsi to wrap the failing heart.*

After 1-2 weeks of healing, the LD is conditioned to fatigue resistance and then permanently activated every second systole[10-12]. Transposition of the LD is not burdened by any functional consequences, and indeed it is a standard procedure used by plastic surgeons to transplant pedicled skin grafts or for breast reconstruction after mastectomy. When pharmacological therapy fails to prevent recurrent episodes of cardiac congestive failure, dynamic cardiomyoplasty offers several potential advantages over alternative options. Unlike mechanical supports, skeletal muscle requires no external power source. The implanted device is a neurostimulator coupled to a pacemaker which usually has to be replaced only every 5 or more years. Each patient serves as his own "donor". For this reason, rejection is not a problem and immunosuppression is not required.

Though the mechanisms of cardiac support are not fully understood, dynamic cardiomyoplasty probably assists the damaged myocardium, thus preventing systolic bulging. Besides, it also girdles the ventricle thereby inhibiting progressive ventricular enlargement. Both mechanisms probably contribute to the subjective decrease in symptoms experienced by patients. More im-

portantly, these mechanisms explain the results of the phase II and III clinical trials, in which an unaltered end-diastolic volume has been reported. This means that progressive left ventricular enlargement is inhibited in spite of the limited or absent evidence of increased systolic support[11-14]. Gealow[15], in a thoughtful and incisive commentary on the topic, has suggested that these changes in our understanding of the mechanism should prompt a reconsideration of the protocols for conditioning and activating the muscle wrap, and she also discusses a series of key issues that need to be resolved. The lack of evidence of systolic augmentation in dynamic cardiomyoplasty may partly be due to a myodystrophic lesion of the LD which may reduce the mass and power of the wrap[16-20]. On the other hand, the clinical improvement is clear in a large majority of subjects and is also appreciated by patients and their relatives[1,10-14].

Historically, the muscle contraction is verifiable by palpating the left axillary region for the presence of the muscle twitch. The LD contraction can also be verified by fluoroscopy to note the heart displacement during assisted beats or the shortening of the distance between the intramuscular electrodes or metal clips fixed to the LD wrap. More invasive techniques involving catheterization, such as pressure-volume loop analysis, can also document, though indirectly, the LD contractile activity and provide information about the optimal delay setting. Such invasive techniques are not practical on a routine basis[14]. Proper synchronization is typically achieved by means of M-mode echocardiography. The timing of the LD contraction is tuned on the basis of the observed time of mitral valve closure and of the onset of the electrical impulses delivered by the myostimulator. However, this method is limited to tuning the electrical events rather than the actual mechanical events of LD contraction and relaxation. We developed a new method for the noninvasive, bedside monitoring of the LD function using a standard polygraph previously used for monitoring the cardiac apical motion and heart sounds. The ECG and heart tones are registered simultaneously with the pressure changes due to LD wrap contraction and relaxation that are measured near the rib window by the probe normally used for recording an apicocardiogram. The LD "mechanogram" determines: a) the LD activation threshold; b) the duration of the full LD contraction-relaxation cycle; c) the dynamic contractile characteristics of the LD wrap on the basis of the determination of the tetanic fusion frequency and their changes over time; d) by coupling with echo-Doppler imaging, the optimal synchronization delay between cardiac events and "contraction-relaxation" of the LD wrap, and e) the efficacy of demand stimulation in inducing daily activity-rest stimulation periods (interval stimulation) able to retro-differentiate the LD wrap dynamic characteristics after full slow transformation[21,22].

Tensiomyography, a new noninvasive measuring method for the selective detection of the skeletal muscles' contractile properties *in situ*, is based on the use of a displacement sensor. Tensiomyography, which senses the radial enlargement of the muscle when it contracts, could add to the LD mechanography information on the muscle force and power[23,24].

Skeletal muscle ventricles. The power available for assisting the heart can be harnessed much more effectively if the grafted muscle is molded into a separate auxiliary pump or skeletal muscle ventricle. The skeletal muscle ventricle is not constrained by the existing geometry but is limited only by the size, shape, and fiber orientation of the muscle that is available[6]. Subject only to these limitations, factors such as the volume of the cavity, the wall thickness and the direction of wrap are all within the control of the surgeon and can, in principle, be optimized so as to allow for the maximum pumping performance of which the muscle is capable. Set against this functional potential is the need to ensure that the introduction of such a device into the patient's circulation does not cause thrombus formation with the attendant risks of obstruction of flow through the device and of embolism to vital organs. In this respect, the research to date provides grounds for optimism. In the Detroit laboratories of Dr. L.W. Stephenson, skeletal muscle ventricles have pumped as diastolic counterpulsators in dogs for over 2 years, and some have gone on to function in the circulation for over 4 years.

Demand dynamic cardiomyoplasty. The clinical observations that activity-rest stimulation tunes the dynamic characteristics of the LD wrap are in full agreement with results of long-term training-detraining experiments in rodents, rabbits, goats, sheep and humans[25-27]. The effects of the intermittent stimulation are also corroborated by additional evidence provided using the pattern of stimulation mandatory for dynamic cardiomyoplasty. In these animal experiments, an increased speed of wrap contraction was accompanied by a significant increase in muscle power. It is important to note that in the last experiments measurements of the peak isometric force do not equate to the maximum force-generating capability of these muscles but rather indicate their capacity to perform work under the stimulation conditions generally accepted for clinical use. Under activity-rest pattern of stimulation the LD wrap provides a higher power than a continually stimulated LD. This applies for the left ventricle too. Furthermore, the muscle is fast enough to contract and relax during cardiac systole[10,22,28,29]. We have shown that the slowness of the LD wrap is reversed by the activity-rest regime even after years of standard stimulation (tetanic fusion frequency of 11 ± 2 Hz after standard stimulation vs 30 ± 3 Hz after the demand regime, $p < 0.0001$). No deaths occurred after demand dynamic cardiomyoplasty (8 subjects with a follow-up lasting 42 ± 8 months, of which 14 ± 3 months of demand stimu-

lation). The quality of life was substantially improved with a significant reduction in heart failure symptoms (NYHA class: preoperatively 3.0 ± 0.0, post-demand dynamic cardiomyoplasty 1.5 ± 0.2, $p < 0.0001$). In the subgroup of patients only slightly stimulated by means of LD conditioning, the exercise capacity tended to increase over preoperative values more than 2 years after surgery (maximum oxygen consumption: preoperatively 12.3 ± 0.7 vs 16.6 ± 1.7 post-demand dynamic cardiomyoplasty, $p = 0.05$). In this group of patients the excitation threshold of the LD wrap was not increased throughout the 2 years of follow-up. The conclusions of the Italian Trial of Demand Dynamic Cardiomyoplasty, a phase II study, are that demand stimulation and mechanography of the LD wrap are safe procedures which, in the long term, could offer the benefits of dynamic cardiomyoplasty to patients with pharmacologically intractable heart failure[21].

These conclusions were further supported by the results of Doppler flow wire analyses in demand dynamic cardiomyoplasty.

As previously reported, in 7 patients with idiopathic left ventricular cardiomyopathy (mean age 57.1 ± 6.2 years; NYHA class 1.43 ± 0.5; time between dynamic cardiomyoplasty and start of demand protocol 32.6 ± 17.6 months), a 0.018 inch peripheral Flex™ Doppler flow wire was advanced under fluoroscopic guidance into the descending thoracic aorta in order to measure the peak aortic flow velocity, a measurement directly correlated with the cardiac output.

A significant increase in the mean peak aortic velocity in the assisted period vs rest and in assisted vs unassisted beats was recorded: the respective increases were 8.42 ± 6.98% and 7.55 ± 3.0% proving the effective systolic assistance produced by the LD contraction. A positive correlation ($r^2 = 0.53$) between the frequency of tetanic fusion (a parameter which is indicative of the speed of muscle contraction) and the percent increase in the mean peak aortic velocity in the assisted period vs rest was found[30-33], suggesting the beneficial effect of the demand stimulation protocol on muscle performance.

Myogenic stem cells in cardiac muscle repair

In post-natal muscle, skeletal muscle precursors (myoblasts) can be derived from satellite cells (reserve cells located beneath the basal lamina on the surface of mature myofibers) or from cells lying beyond the myofiber, i.e., from interstitial connective tissue (in and out of blood vessels) or bone marrow. Both of these classes of cells may have stem cell properties. In addition, the old idea that post-mitotic myonuclei lying within mature myofibers might be able to re-form myoblasts or stem cells is re-examined (Fig. 2), and related to recent heretical observations for similar post-mitotic cardiomyocytes. In adult hearts (which previously were not considered capable of repair), the role of replicating endogenous cardiomyocytes and the recruitment of other (stem) cells into cardiomyocytes for new cardiac muscle formation have recently attracted much attention[34]. The relative contribution of these various sources of precursor cells in post-natal muscles and the factors that may enhance stem cell participation in the formation of new skeletal and cardiac muscle *in vivo* are the focus of this chapter. Although many endogenous cell types can be converted into skeletal muscle, the contribution of non-myogenic cells to the formation of new post-natal skeletal muscle *in vivo* appears to be negligible. Whether the recruitment of such cells to the myogenic lineage can be significantly enhanced by specific inducers and an appropriate microenvironment is now being intensively investigated. Among an ever longer list of options, dermal fibroblasts appear promising as a realistic alternative source of exogenous myoblasts for transplantation purposes. With regard to heart muscle, experiments showing the participation of bone marrow-derived stem cells and of endothelial cells in the repair of damaged cardiac muscle are encouraging.

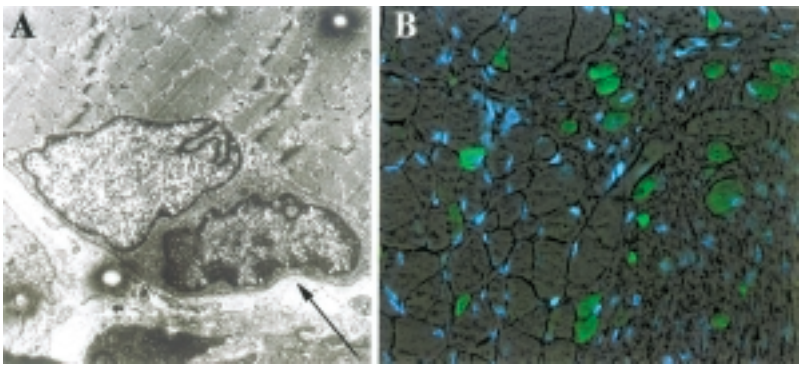

Figure 2. Panel A: *electron micrograph of the myonucleus and satellite cell* (arrow) *in 30 day denervated and regenerated muscle (×7100) after 1 bupivacaine injection.* Panel B: *double exposure anti-myosin heavy chain embryonic (green)/Hoechst (blue staining for nuclei) under bright-field illumination at contrast phase in 30 day denervated and regenerated muscle with many myofibers positive for myosin heavy chain embryonic. Magnification, 40×.*

Clinical applications of myoblast transplantation. In addition to their role in regeneration, normal skeletal myoblasts have been isolated, cultured, and then transplanted *in vivo* to replace defective genes in myopathies, e.g., dystrophin in the Duchenne muscular dystrophy (DMD)[35]. The clinical possibilities of myoblast transfer therapy have stimulated much research, although many fundamental immune problems remain to be solved[36,37]. Stem cells as an alternative source of myoblasts have particular clinical merit in the potential treatment of DMD patients by an *ex vivo* gene therapy approach, owing to the fact that the use of autologous cells avoids potential problems related to immune rejection. Because satellite cells from the skeletal muscle of DMD boys probably have a limited capacity for replication, an alternative source of autologous myogenic stem cells (e.g., from dermal fibroblasts or bone marrow stem cells) is ideal for genetic correction and re-implantation into the defective dystrophic muscles.

Transplantation of cultured myoblasts has also been used to replace defective muscles, e.g., for urinary incontinence[38] and in acutely injured myocardium[39], and has been widely used to deliver genes into the bloodstream, brain, or joints[40]. Cultured myoblasts are also required for the rapidly emerging discipline of tissue engineering to construct *ex vivo* potential artificial muscles for transplantation purposes[41]. For all of these purposes, stem cells represent a potential powerful alternative source of myogenic precursor cells.

Cellular cardiomyoplasty by skeletal myoblasts, embryonic cardiomyocytes (cardiomyoblasts) and myocardiogenic stem cells. It is possible that during development circulating stem cells may give rise to many of the stem cells ascribed to specific tissues. In post-natal tissues, it is not clear whether bone marrow-derived stem cells contribute to the cells in the vasculature or whether they become resident in interstitial connective tissue. However, on the basis of data deriving from studies of the clearance of green fluorescent protein-labeled lineage-negative blood cells, it has been calculated that 20 000-100 000 hematopoietic stem cells (HSCs)/progenitors enter the blood every day and that these are rapidly sequestered (within minutes) into tissues. These observations on circulating bone marrow-derived stem cells have attracted great interest because the delivery of muscle precursors through the bloodstream represents an ideal route for their distribution to all skeletal muscles. It should be borne in mind that the use of umbilical cord blood may be an alternative and possibly superior source of pluripotent stem cells. The banking of such cord cells has been proposed for future clinical applications. Because adherent myocardiogenic stem cells (MSCs) obtained from adult bone marrow can expand well in culture (in contrast to HSCs), marrow stromal cells may also be a good source of therapeutic cells for transplantation purposes[42]. A critical question relates to the potential scale of contribution of such nonmuscle stem cells, especially those derived from adult tissues, to *de novo* myocardium formation *in vivo*.

Strategies for repair. When confronted with the problem of heart damage after infarction, myocyte replacement is the ideal scenario. There is increasing interest in cell transplantation as a potential therapy for cardiovascular disease[43]. Cardiomyocyte replacement could be achieved by stimulating the proliferation of endogenous mature cardiomyocytes or stem cells, or by implanting exogenous donor cardiomyocytes possibly derived from stem cells.

It should be emphasized that, to be clinically effective and if the animal is to survive any acute insult that disrupts heart function through damage to the individual cardiac myocytes, the enhancement of cardiomyocyte proliferation after damage must be rapid. In cases in which there is chronic damage to the heart muscle, the proliferation might need to be sustained in such a way as to slowly but continually replace myocytes as necessary during the course of the disease. Whatever the source of the cells and the use to which they are put, a concurrent revascularization must also keep pace with the re-population of the infarct to ensure viability of the repaired region and prevent further scar tissue formation. Finally, the newly formed myocardium must integrate with the existing myocardial wall if it is to assume the function of the tissue it replaces. All of this must occur while the heart continues to beat and perform the essential work of supplying blood throughout the organism. Furthermore, even small areas of imperfectly integrated tissues are likely to severely alter the electrical conduction and syncytial contraction of the heart, with long-term life-threatening consequences. This is in marked contrast to the situation in skeletal muscle where the tissue can rest during repair. Here we discuss the historical use of transplanted skeletal muscle cells and of fetal cardiomyocytes to replace damaged myocardium, to re-initiate mitosis in mature "post-mitotic" cardiomyocytes and to form cardiomyocytes from stem cells (endogenous and exogenous).

Transplantation of skeletal or fetal cardiac muscle cells into hearts. *Skeletal muscle.* Attempts to use autologous skeletal muscle to repair damaged hearts have included the relocation (from their normal position) of wraps of whole sheets of the LD muscle to supplement myocardial dysfunction[44]. This process, called cardiomyoplasty, was pioneered in the 1980s[45] and has been applied clinically with variable success. Alternatively, transplantation of isolated cultured myoblasts has been used to try to restore muscle function in hearts both in experimental as well as in clinical settings[46]. This requires that the myoblasts be extracted from skeletal muscle, expanded in tissue culture, and then usually injected into the heart muscle. Delivery via the circulation is an alternative option for the supply of donor myoblasts to the whole heart. In all cases, how-

ever, one of the critical goals must be electromechanical coupling through gap junction communication between the engrafted skeletal myoblasts and the surrounding cardiac myocytes[47,48]. Recent studies have improved the survival of transplanted myoblasts by heat shock pretreatment, modified their immunoprotection and confirmed that the benefits of skeletal myoblast transfer are additive with those of conventional therapies such as angiotensin-converting enzyme inhibition[49-51]. Autologous myoblast transplantation has now been reported in human patients, with promising improvements in cardiac function lasting up to 5 months.

Cardiomyocytes. Attention has recently shifted from such studies using skeletal muscle to the use of cardiomyocytes, and especially stem cells, as a source of replacement muscle. Because mature cardiomyocytes are traditionally considered to be incapable of cell replication, to date the donor cardiomyocytes have largely been of embryonic/fetal origin. Some experiments in animal models report successful grafting and maturation of embryonic cardiomyocytes in normal and injured hearts[52]. Other studies show that most of the donor cardiomyocytes (engrafted into mature rat hearts after infarction) retained their embryonic phenotype and that after 4 weeks they did not form junctions with mature heart cells.

Although neonatal donor cells could form junctions with host myocardium, there was massive initial death of donor cells and later the grafts often became surrounded by scar tissue. This problem is a direct result of the inflammation and scarring which occur after infarction, and it may be that the use of cardiomyocyte transplantation therapy could be more effectively developed to address the achievement of functional improvement in myopathic heart diseases[53]. Alternatively, possible engineering of cardiac grafts within three-dimensional scaffolds or aggregates might provide a superior source of donor cells for the re-population of the infarcted heart. The use of skeletal muscle cells to repair damaged heart muscle has had some success, and experiments are ongoing. A more attractive approach is the use of donor cardiomyocytes, but mixed results have been obtained with donor fetal/embryonic/neonatal cardiomyocytes and the use of such donor cells in humans raises major ethical issues.

Cardiomyocytes from stem cells.

• Bone marrow-derived stem cells. Since the 1999 demonstration of cardiac muscle formation from circulating bone marrow cells[53], there have been many reports related to the stem cell contribution to cardiac muscle formation in rodents and, more recently, in humans.

In experimental infarcted hearts in adult mice, cardiomyocytes and vascular cells can be formed *in vivo* from circulating mouse bone marrow stem cells[54] and these stem cells also give rise to cardiomyocytes after direct injection into damaged heart tissue[55]. It appears that cardiomyocytes can be formed from bone marrow-derived HSCs, MSCs, or endothelial stem cells. Clearly, a key issue is the extent to which this can occur *in vivo*. The systemic delivery of highly purified HSCs in lethally irradiated mice contributed to the formation of cardiomyocytes and endothelial cells in ischemic hearts. In rats, the transplantation of bone marrow cells directly into cryo-damaged heart tissue improved heart function with an increase in cardiac myocyte cells and angiogenesis within the scar[56], and related experiments after ischemic heart injury in mice showed many cardiomyocytes and endothelial and vascular smooth muscle cells derived from injected (green fluorescent protein-labeled) bone marrow cell donors.

Bone marrow-derived mesenchymal (stromal) stem cells directly injected into rat hearts also gave rise to cardiomyocytes, and this has also been demonstrated for human bone marrow-derived MSCs[57]. Therefore, both bone marrow-derived HSCs and MSCs can give rise to cardiomyocytes. However, it should be noted that the bone marrow-derived MSCs are probably not normally present in the bloodstream.

Human bone marrow-derived endothelial stem cells transplanted into rat hearts formed new vasculature but not cardiomyocytes, and improved the cardiac function[58]. Because there appears to be a close relationship between endothelial cells and cardiomyocytes (see below), it remains to be clarified whether such bone marrow-derived endothelial stem cells can indeed form cardiomyocytes in some situations. As already discussed for skeletal muscle, there is a close and complex relationship between bone marrow-derived HSCs and endothelial stem cells, and also between MSCs and endothelial stem cells.

Although the formation of vascular cells such as endothelial cells may not appear directly relevant to the attempts to repair damaged hearts, it should be noted that an adequate blood and oxygen supply for newly seeded cardiomyocytes is critical for their survival. Therefore, the ability of transplanted bone marrow stem cells to form the cells of the vasculature is another important advantage in favor of the use of a totipotent cell type[59,60], and in some instances it has been shown that the establishment of new endothelial cells is more common than the formation of new cardiomyocytes. In the popular press there have already been reports of autologous bone marrow stem cells of a patient being directly injected into his heart during coronary bypass surgery. However, details of the outcome/benefits of this procedure are lacking.

Female hearts transplanted into male recipients provide the opportunity to assess the contribution of the host male (Y-chromosome-positive) cells to the undamaged myocardium of the donor heart in a clinical context. In contrast with earlier studies, Quaini et al.[61] reported a significant (7-10%) and rapid (within 4 days) contribution of host (male) cells to myocytes and blood

vessels in such human female heart transplants. One potential criticism to these observations is the possibility that the female donor hearts may have already contained male cells before transplantation. These male fetal cells can be derived from trophoblasts or from nucleated erythrocytes during gestation. They are detected in maternal blood within 6 weeks of conception, a large fetomaternal transfusion occurs at the time of labor and delivery, and microchimerism in the mother has been documented since at least 27 years. Therefore, it would be of considerable interest to know whether the donor females and control females whose hearts were used in the study by Quaini et al. had indeed conceived a male pregnancy (even if it did not go to term). In addition, careful examination of female hearts (in the absence of transplantation) could reveal the potential contribution of such male cells to cardiac chimerism in these mothers[61].

• Endothelial cells. The interesting demonstration of a satisfactory conversion (trans-differentiation) of murine and human endothelial cells into cardiomyocytes in tissue co-culture and *in vivo*[62] raises the real possibility of using human endothelial cells for cardiac repair. This study emphasized the importance of cell contact between the endothelial cells and existing cardiomyocytes for such a conversion. Whereas embryonic and neonatal endothelial cells are capable of forming cardiomyocytes, endothelial cells isolated from mature animals are not, indicating a marked loss of plasticity during development. However, relatively well-differentiated endothelial cells derived from human umbilical veins did form cardiomyocytes. Alternatively, it may be possible to expand populations of circulating human endothelial progenitor cells[63,64].

• Embryonic stem cells. It is well established that murine embryonic stem cells can give rise to cardiomyocytes *in vitro* and *in vivo*[65]. It has now been shown in tissue culture that human embryonic stem cells can also differentiate into cardiomyocytes. However, human embryonic stem cells have a very low efficiency of conversion into cardiomyocytes compared with those of mice. As Kehat et al.[66] have noted, there are striking differences in the human and murine stem cell models, and this must be borne in mind when evaluating experiments in mice for the development of new strategies to improve human heart function. For example, human embryonic stem cells differentiate in the absence of a feeder layer and in the presence of leukemic inhibitory factor. Other notable differences include the lower number of human embryonic stem cells able to undergo differentiation and spontaneous contraction (< 10% compared with > 80% of murine embryonic stem cells) and the slower time course of differentiation (a median of 11 days compared with 2 days for murine cells). Whether these differences reflect true species-related differences, or simply our current understanding of these cell systems, or else different microenvironment requirements, remains to be determined. The use of embryonic stem cells as a source of cardiomyocytes (and many other cell types) is an attractive therapeutic possibility that needs to be fully explored.

• Neural and hepatocyte stem cells. Other studies have shown that the clonal hepatocyte stem cell line WB-F344, when transplanted into the hearts of mice *in vivo* was able to differentiate into cardiac myocytes. Furthermore, although cardiomyocytes can be derived from stem cells of neural tissues during development and in experimental settings, the efficacy is relatively low[63].

Given the time constraints for repair after acute myocardial infarction, the delivery of pre-differentiated cells (cardiomyocyte and vascular cells possibly derived from stem cells) appears desirable. Local delivery of these cells results in direct seeding of the damaged zone, but we need to understand more about how the microenvironment promotes cell differentiation so that this can be exploited. Local delivery might be improved if such cells were engineered into three-dimensional grafts on appropriate matrix/biomaterials. Systemic delivery of stem cells is relatively noninvasive and remains an attractive option. This heavily relies on the ability of cells to home to damaged tissue. However, to date little is known about the factors responsible for such specific tissue targeting. Furthermore, the expansion of the stem cell population in the damaged heart and differentiation into functional cardiomyocytes are both required, and the local conditions that dictate this need to be elucidated. For heart tissue in the past 3 years there have been some rather promising results with bone marrow and endothelial stem cell replacement of cardiomyocytes and vascular cells. These studies need to be firmly substantiated *in vivo* and carefully evaluated for humans. We are still in the early days and, in the absence of solid *in vivo* data, it seems premature to extend such studies to the clinical setting.

Perspectives of future research

A major issue of cardiac-bio-assists remains muscle damage induced either by transplantation of *in vitro* expanded myogenic cell clones (see above) or by the chronic abnormal stimulation of skeletal muscle tissue transposed around the heart, in particular when a continuous muscle-to-heart contraction ratio is applied. Sport scientists and physiatrists are well aware that spontaneous exercise *per se* could constitute a trauma for muscle fibers[66-72]. The unusual work performed in cardiomyoplasty by the wrap may damage the LD muscle tissue. Direct histological evidence of muscle damage had been collected in sheep and goat experiments[72]. There are reports explaining the long-term cessation of the effects of the procedure with indirect[73] or direct[18,74]

evidence of major muscle atrophy, fibrosis and fat infiltration[75]. On the contrary, autoptic cases directly show that this may not necessarily be the case. Fifteen months and even 8 years after cardiomyoplasty morphological and molecular analyses of the LD wrap showed a preserved muscle mass and patent vessels with normal endothelial and smooth muscle walls[19,76]. Interestingly, in these two cases the LD wrap was activated every second or fourth sensed QRS, and the clinical results were excellent.

Although the majority of patients who undergo cardiomyoplasty enjoy an improvement in their quality of life, between 15 and 20 derived no benefit from the procedure[77]. Furthermore, in a review of 127 patients who underwent cardiomyoplasty over a 10-year period, it was reported that less than 60 of patients survived for more than 2 years after the operation[73]. On the basis of evidence from animal and human studies, this somewhat disappointing outcome has been attributed to the functional deterioration of the muscle wrap. Graft damage involving replacement of muscle by fibrous tissue and fat appears to be caused by a combination of factors including changes in the vascular conformation, the loss of resting tension, and chronic electrical stimulation[73,78], but ischemia, particularly of the distal part of the muscle involved in the wrap, is increasingly regarded as the most important factor of all. The sacrifice of the so-called collateral vessels has been shown to cause damage in both sheeps and goats. This damage is exacerbated by the increased metabolic demand associated with the stimulation needed to condition and to activate the graft[6,79].

Mannion et al.[80,81] tried to overcome this problem by enforcing a vascular delay of 3 weeks after reconfiguring the muscle and before initiating stimulation. Such a period is not a vascular delay in the true sense because the muscle is reconfigured in the same procedure. The clinical cardiomyoplasty protocol includes a delay of 2 weeks. The idea is that this provides time for neovascularization, thus increasing the area effectively perfused by the thoracodorsal artery. The problem is that it also delays the benefit that the patient might otherwise derive from the operation. Moreover, it is by no means sure that the delay is effective. Even with a delay, it is still possible to demonstrate the additive damaging effects of stimulation and division of collateral vessels[81,82]. Better results have been obtained by implementing a true vascular delay in which the collateral vessels are divided but the LD muscle is left *in situ* for 10 days before elevating it as a graft[83].

On the other hand, even these studies left several questions open. We hope to find an answer in incoming research plans. We hope to:

• optimize surgical procedures and electrical stimulation protocols of the LD wrap in order to reduce the risk of myodystrophic lesions of the muscle wrap;

• sustain Italian industrial research by investing needed resources to study and develop a new demand myo&cardio stimulator (demand LD/Cardio Pacer), a new device for the activation of the dynamic wrap and for the measurement, by noninvasive analyses, of the dynamic characteristics of the muscle tissue transposed around the heart (mechanography of the LD wrap);

• explore the potentials of myoblast implantation to augment the muscle mass of the distal part of LD, which is the one wrapped around the heart and the most damage-prone section of the transposed tissue.

These objectives will be pursued with clinical research on patients of demand dynamic cardiomyoplasty, and by animal experiments to develop and test new surgical, clinical and biotechnological approaches. We will test pre-stimulation of the LD[84,85], with or without combined vascular delay surgery[86,87]. We plan studies to take advantage of the recent developments of cell therapy approaches[34,54,88]. Recently in a dog model of dynamic cardiomyoplasty, injected myoblasts from masseter muscle fully colonized the electrostimulated LD. Consequently, power and fatigue resistance of the engineered muscle increased to clinically-relevant values[89]. In particular, we will explore, in rodent models, the feasibility of increasing the muscle mass of the LD wrap by injecting autologous myoblasts derived from muscle satellite cells[89] or myogenic stem cells either within the blood circulation or derived from bone marrow.

Addressing the issues related to the mechanisms which control the muscle dynamic characteristics in the peculiar conditions dictated by clinical constraints, and to the prevention of myodystrophic changes secondary to the peculiar use of the LD wrap will give the final push to demand dynamic cardiomyoplasty among the surgical options available for the treatment of pharmacologically intractable heart failure.

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