

Autologous Cellular Cardiac-implant

Jorge C. Trainini, Daniel Cichero, Noemí Lago, Roberto Giordano, Jorge de Paz, Benjamín Elenchwajg and Juan Carlos Chachques⁽¹⁾

Cardiovascular Surgery, Presidente Peron Hospital, Buenos Aires, Argentina, Brandsen, Buenos Aires and (1) Cardiovascular Surgery Department, Pompidou Hospital, Paris, France

Abstract

Acute myocardial infarction leads to loss of functional myocytes and structural integrity of the heart. Autologous cell transplantation to repair or regenerate injured myocardium is a new direction in the treatment of cardiovascular disease. The clinical advantage of this approach known as cellular cardiac-implant for myocardial repair is obvious because autologous myoblast implantation avoids many of the logistical difficulties associated with cardiac transplant, as well as the need for immunosuppression therapy. Autologous skeletal myoblasts appear to be the most well studied and best first generation cells for cardiac repair. Today more than twenty patients worldwide have been operated with this technique. However the era of cell transplantation has only begun. What type of cell is the best? and What is the efficacy? are major questions that remain to be answered. Nor is it known whether cardiac muscle can form electromechanical junctions with skeletal muscle grafts and induce synchronous contraction.

Autologous cellular cardiac-implant offers the promise of restoring regional function for patients who have had a myocardial infarction. Future studies will need to establish the efficacy of this approach.

Key words: cell transplantation, myoblasts, myocardial infarction.

Basic Appl Myol 13 (1): 39-43, 2003

Hearth failure is an important cause of morbimortality. According to the Framingham study, annual survival for heart failure patient is 57% for males and 64% for females, but at 5 years follow up these rates decrease to 25 and 38% respectively [1].

Congestive heart failure (CHF) is not only a medical problem, but also a social and economic one. Heart transplantation, coronary artery bypass grafting, correction of mitral valve incompetence, cardiac resynchronization therapy, biological and mechanical cardiac assist devices have solved some of the problems, but most of the difficulties remain unsolved [2, 3]. Besides that, all these treatments carry potential complications and high costs [4].

It is well known that necrosed cardiac fibers are substituted by fibrotic tissue. Cellular hypertrophy can partially compensate for diminished contractility, but the remodelling process leads to progressive heart failure.

Since 1992 Marrelli [5] has used cultured undifferentiated myoblasts in experimental studies for repair of non viable cardiac tissue. After this experimental phase [6-7] the way from the investigation to the clinic is taking place progressively.

Repair of cardiac tissue with autologous cells is a fascinating new therapeutic concept [8, 9]. The combination of genetics with cellular biology allowed for the development of cell culture of certain phylogenetic lines for autorepair. This medical paradigm -that could begin a new era in therapy- can not only offer solutions for cardiac problems, but also for neurologic, hepatic, myopathic, endocrinologic and osteoarticular diseases [10-12].

Autologous Cardiac Repair

The classic concept assigns to cardiomyocytes the capability of reproduction until the 3-4 months after birth [13]. That means that shortly after birth there exists a certain number of cardiomyocytes, and they can not be replaced; so, their quantity would gradually decrease. In the event of a myocardial infarction or a chronic process such as hypertensive cardiomyopathy, a certain number of cardiomyocytes die. The necrotic area is replaced by a fibrotic scar, that leads to cardiac remodelling and the progressive development of CHF [14]. However, the results of new research challenge this concept of limited reproduction capability [15-17]. Nadal-Ginard [18]

Autologous cellular cardiac-implant

pointed out the balance that exists between the stimuli involved in myocyte hypertrophy and those that lead to necrosis and apoptosis. Experimental data in mouse showed that the differential rate between the quantity of cardiac cells that die and the total number of cells that make up the ventricular mass, imply the possibility of active replacement of myocytes. Without such a mechanism, the loss of cellular mass would become unsustainable. That implies a new vision on cardiac ageing. Following that concept, the heart would completely regenerate itself in a period of less than 5 years [19].

The procedure of repair of the fibrotic areas of the heart by cultured phylogenetically related cells is known as “Autologous Cellular Cardioimplant” and its aim is the structural and functional recovery of the damaged area. The cells with potential capability for cardiac reparation may have different origins. We will consider only those of autologous origin, excluding the allogenic as well as the transgenic, as far as we are dealing with the “autoreparation” concept.

Types of Autologous Cells

- 1.- Adult cardiomyocytes. Their reproduction is difficult and they must be obtained by endocardial biopsy. They also require a greater vascular supply than do myoblasts [20].
- 2.- Myoblasts. These cells, also known as satellite cells, originate from the skeletal muscle. They can be easily obtained, cultured and reproduced, and are the most studied, all factors that have allowed their clinical use [21, 22]. These cells lie under the basal membrane in a quiescent state until an appropriate stimulus triggers their proliferation. Myoblasts are in a cellular line that extends from the undifferentiated mesoderm cell to the completely differentiated cardiac, skeletal and smooth muscle cells [23] (Figure 1).

In spite of the fact that those cells are implanted in infarcted areas, those zones are never completely avascular. On the other hand, myoblasts are resistant to ischaemia, allowing for growth and reproduction in a poorly perfused environment.

There are two important differences between myoblasts and cardiomyocytes: the former lack intercellular connections (“gap junctions”) and dihydropyridine membrane receptors.

Intercellular connections

The distinctive junctions between cardiomyocytes are called intercalated discs [24]. They provide both electrical and mechanical cell connections [25]. The discs include specialized structures:

- a.- Gap junctions: establish an electrical connection, and contain a specific protein (connexin-43).
- b.- Fascia adherents and desmosome: account for mechanical junctions through N-cadherin and desmoglein. Cadherin and connexin are classic markers for cellular junctions.

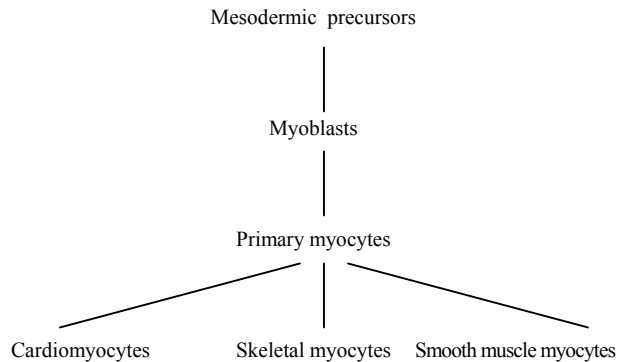


Figure 1. line of descent from undifferentiated to differentiated cells

Dihydropyridine receptors: are required for coupling the electric impulse to kinetic energy. Research has been made in satellite cells in order to integrate this receptor by means of genetic engineering.

- 3.- Smooth muscle cells. Are obtained from arterial walls.
- 4.- Stem cells: Undifferentiated, pluripotent cells, located in the bone marrow [26-27].

Research by Ferrari et al [28], gave rise to the concept of regeneration of the patient's own myocardium as an alternative to heart transplantation. This concept arose from the possibility of utilizing a very undifferentiated cell located at the stroma of the bone marrow, able to differentiate into various types of cells [29, 30]. The type of differentiation is determined by the environmental location. Amazingly, there were found stem cells in the heart, that remain quiescent in the normal heart, but enter in an accelerated process of mitosis when stimulated by growth factors.

In an experimental situation, this potential has been proved, allowing for regeneration of areas of infarcted-fibrosed myocardium, not only by myocytes, but also by endothelial and smooth muscle cells. Experimental work by Wang [31, 32] suggests that in a normal myocardium these cells can generate cardiomyocytes, but in a fibrotic tissue, they developed fibroblasts.

Myoblasts Culture

Culture of skeletal myoblasts is similar to methods used for other types of cells. Aseptic technique is essential to maintain a stable myoblast population.

The tissue used is a fragment of skeletal muscle of approximate 2cmx2cmx2cm, equivalent to 5-8 g. All non muscular tissues of the fragment are removed, and the fragment is treated by enzymatic (collagenase, trypsin) digestion. The cells are then incubated at 37°C, with 5% CO₂ and saturation humidity. When a cell confluence of 75-80% is reached, new cultures are initiated from the original one, until a number of 200-250 million cells is reached. At the end, the implant is made in the fibrosed myocardium, distributing the number and orientation of the implants in relation to the size of the necrosed area.

Mechanisms of Action

In experimental studies, the observed changes imply the organization of implanted myoblasts in myotubes and myofibers, that contribute to the improvement of contractility observed in animal research and clinical studies.

Other observations have been made:

An increase was found in the contractile performance measured by dp/dt “in vitro” and ultrasound and sonomicrometry “in vivo”.

Improvement in diastolic function due to increased elasticity due to fibrotic tissue replacement [33].

Increase of ventricular wall thickness in the infarcted area.

Decrease of ventricular dilation.

Production of angiogenic factors by implanted cells.

Another possible cause for performance improvement is the association of cellular implantation and fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF).

Electromechanical coupling between cardiac muscle and grafted cells must yet be proved “in vivo”; this is not easy to accomplish due to the low expression of N-cadherin and connexin-43 in skeletal muscle fibers. Persistent expression of these proteins must be obtained (according to experimental studies), in order to prove the penetration of the fibrotic tissue barrier by the implanted cells. Evaluation of a specific marker such as troponin could be useful in the assessment of graft state. In experimental studies of myocardial regeneration by satellite cells B-galactosidase expression was used as a marker of viable graft tissue.

Experimental as well as clinical studies deal with models of infarcted areas with fibrotic scars. Experimental protocols for evaluation of this therapy in idiopathic dilated cardiomyopathy and are now in development.

Clinical Experience

Since the first case in June 2000 [7], numerous patients were treated with this technique worldwide (France, Japan, Netherlands, Germany, Italy, USA, Argentina, Spain, China). In the cases of France, Argentina and Spain, cells implantation was performed concomitant with coronary artery bypass surgery, and was made in fibrotic areas non related to the revascularized zones. The first French patient improved from functional class III to class II and from ejection fraction (EF) of 20% to 30%. Contraction was found in the inferior wall, the one implanted with cells [7]. As a group, the 10 patients improved their EF 13% and 11 of the 18 segments treated acquired contractility. In this experience was an early death and some incidence of arrhythmia.

Cellular implants in the patients treated in Netherlands were performed percutaneously by means of special catheters manufactured by “Bioheart” (Bioheart, Inc., Wiston, Florida, USA). This program includes 30 patients in 3 medical centers [34].

Patients treated in the U.S.A. were included in an FDA protocol (Diacrin Inc). The first case was performed in a patient treated with a ventricular assist device as a bridge to transplantation. The cellular implant was performed in fibrotic areas, in order to perform histopathological studies when the heart will be explanted.

In Japan and Germany, cardiac repair was attempted with autologous stem cells from the bone marrow.

The patient treated in Argentina, was a 66 years old male patient. Doppler, Dobutamine and Tissue Ultrasonography, as well as Thallium 201 myocardial perfusion studies, showed posterolateral and inferior akinesia and anterior hypokinesia. Coronary bypass to anterior descending artery was performed and simultaneously 250 million myoblasts were implanted in the posterolateral and inferior walls. At ten months' follow-up the patient is asymptomatic, in functional class I (NYHA), and Dobutamine stress echocardiography showed that myocardium wall width in the area of myoblasts implantation increased from 5 mm to 9 mm, and that regional motility improved in posterior and lateral walls. Motility rate improved from 1.87 to 1.64. To the moment our experience includes three patients, the last two are of recent procedure.

Conclusions

Autologous Cellular Cardioimplant opens a promising era in the therapy of cardiac diseases [35]. Converging knowledge of disciplines derived from genetic and cellular biology will allow to install a new medical paradigm, the autoreparation, not only of the heart, but also of other organs.[36] However, several questions remain to be answered before this possibility becomes a standard procedure. Which is the best cell to use? How should it be implanted? Those issues are of fundamental importance, in spite of the fact that at present cultured myoblasts are the most studied and reliable cells even for clinical studies. After those issues are solved, its actual efficacy should be assessed and compared with the conventional therapies.

From a functional point of view, work will be required to test the possibility of integration of the graft with the remaining myocardium. In spite of the fact that intercalated discs were found in the implants in normal myocardial tissue, those findings were not definitively demonstrated in fibrotic areas.

Regional ventricular function is not easy to assess, as well as the capability of the repaired zone of beating synchronously with the remaining ventricular wall. These difficulties must be solved with the help of different methods (sonomicrometry, positron emission tomography, echocardiography).

In summary, the repair of the heart with autologous cells could have profound medical importance. Clinical feasibility and efficiency rates must be demonstrated with histological and functional evidence. A multidisciplinary effort will be required to bring these techniques into routine clinical practice.

Address correspondence to:

Jorge C. Trainini, Cardiovascular Surgery, Presidente Peron Hospital, Buenos Aires, Argentina, Brandsen 1690- 3o "A", C1287AAJ, Buenos Aires, tel./fax 5411 4302 3810, Email: jctrainini@hotmail.com.

References

- [1] Ho KL, Anderson KM, Kannel WB: Survival after the onset of congestive heart failure in Framingham Heart Study subjects. *Circulation* 1993; 88: 107-115.
- [2] Trainini JC, Barisani JL, Cabrera Fischer EI, Chada S, Christen A: Chronic aortic counterpulsation with latissimus dorsi in heart failure: Clinical follow-up. *J Heart Lung Transplant* 1999; 18: 1120-1128.
- [3] Chachques JC, Grandjean P, Schwartz K, et al: Effect of latissimus dorsi dynamic cardiomyoplasty on ventricular function. *Circulation* 1988; 78 (Suppl): III-203-216.
- [4] Trainini JC. The concept of "guilty" structure in heart failure. *Rev Argent Cardiol* 2001; 69: 128-135.
- [5] Marelli D, Desrosiers C, El-Alfy M, Kao RL, Chiu RC-J: Cell transplantation for myocardial repair: an experimental approach. *Cell Transplant* 1992; 1: 383-390.
- [6] Li R-K, Jia Z-Q, Weisel RD, Merante F, Mickle DAG: Smooth muscle cell transplantation into myocardial scar tissue improves heart function. *J Mol Cell Cardiol* 1999; 31: 513-522.
- [7] Menasche P, Hagege A, Scorsin M, et al: Myoblast transplantation for heart failure. *Lancet* 2001; 357: 279-280.
- [8] Tam SKC, Gu W, Nadal-Guinard B: Molecular cardiomyoplasty: potential cardiac gene therapy for chronic heart failure. *J Thorac Cardiovasc Surg* 1995; 109: 918-924.
- [9] Clarke DL, Johanson CB, Wilbertz J, et al: Generalized potential of adult neural stem cells. *Science* 2000; 288: 1660-1663.
- [10] Rajnoch C, Chachques JC, Berrebi A, Bruneval P, Benoit MO, Carpentier A: Cellular therapy reverses myocardial dysfunction. *J Thorac Cardiovasc Surg* 2001; 121: 871-878.
- [11] Atkins BZ, Lewis CW, Kraus WE, Hutcheson KA, Glower DD, Taylor DA: Intracardiac transplantation of skeletal myoblasts yields two populations of striated cells in situ. *Ann Thorac Surg* 1999; 67: 124-129.
- [12] Murry CE, Kay MA, Bartosek B, Hauschka SD, Schwartz SM: Muscle differentiation during repair of myocardial necrosis in rats via gene transfer with MyoD. *J Clin Invest* 1996; 98: 2209-2217.
- [13] Zak R: Development and proliferative capacity of cardiac muscle cells. *Circ Res* 1974; 35 (Suppl 6): 17-26.
- [14] Oliveri R: Apoptosis in the Heart Failure. *Rev Argent Cardiol* 2000; 68: 603-607.
- [15] Anversa P, Kajstura J: Ventricular myocytes do not terminally differentiate in the adult mammalian heart. *Circ Res* 1996; 78: 536-546.
- [16] Sadoshima J, Izumu S: The cellular and molecular response of cardiac myocytes to mechanical stress. *Annu Rev Physiol* 1997; 59: 551-571.
- [17] Anversa P, Palackal T, Sonnenblick EH, Olivetti G, Meggs LG, Capasso JM: Myocyte cell loss and myocyte cellular hyperplasia on the hypertrophied aging rat heart. *Circ Res* 1990; 67: 871-885.
- [18] Nadal-Ginard B: Inducción de nuevos cardiomiocitos en el corazón adulto: futuro de la regeneración miocárdica conmo alternativa al trasplante. *Rev Esp Cardiol* 2001; 54: 543-550.
- [19] Scholzen T, Gerdes J: The ki-67 protein: from the known and the unknown. *J Cell Physiol* 2000; 182: 311-322.
- [20] Murry CE, Wiseman RW, Schwartz SM, Hauschka SD: Skeletal myoblast transplantation for repair of myocardial necrosis. *J Clin Invest* 1996; 98: 2512-2523.
- [21] Chiu RC-J, Zibaitis A, Kao RL: Cellular cardiomyoplasty: myocardial regeneration with satellite cell implantation. *Ann Thorac Surg* 1995; 60: 12-18.
- [22] Taylor DA, Atkins BZ, Hungspreugs P, et al: Regenerating functional myocardium: improved performance after skeletal myoblast transplantation. *Nat Med* 1998; 4: 929-933.
- [23] Fishman MC, Olson EN: Parsing the heart genetic modules for organ assembly. *Cell* 1997; 91: 153-156.
- [24] Soonpaa MH, Kon GY, Klug MG, Field LJ: Formation of nascent intercalated disks between grafted fetal cardiomyocytes and host myocardium. *Science* 1994; 264: 98-101.
- [25] Reinecke H, MacDonald GH, Hauschka SD, Murry CE: Electromechanical coupling between skeletal and cardiac muscle implications for infarct repair. *J Cell Biol* 2000; 149: 731-740.
- [26] Vogel C: Stem cells: new excitement persistent questions. *Science* 2000; 290: 1672-1674.
- [27] Caplan AI: Mesenchymal stem cells. *J Orthop Res* 1991; 9: 641-650.
- [28] Ferrari G, Cusella-De Angelis G, Coletta, et al: Muscle regeneration by bone marrow derived myogenic progenitors. *Science* 1998; 279: 1528-1530.
- [29] Orlic D, Kajstura J, Chimenti S, et al: Bone marrow cells regenerate infarcted myocardium. *Nature* 2001; 410: 701-705.
- [30] Makino S, Fukuda K, Miyoshi F, et al: Cardiomyocytes can be generated from marrow stromal cells in vitro. *J Clin Invest* 1999; 103: 697-705.
- [31] Wang J-S, Shum-Tim D, Chedrawy E, Eliopoulos N, Galipeau J, Chiu RC-J: Marrow stromal cells for cellular cardiomyoplasty: the importance of microenvi-

Autologous cellular cardiac-implant

- ronment for milieu dependent differentiation (abstract). *Circulation* 2000; 102 (18 suppl II): II-683.
- [32] Wang JS, Shum-Tim D, Galipeau J, Chedrawy E, Eliopoulos N, Chiu R C-J: Marrow stromal cells for cellular cardiomyoplasty: feasibility and potential clinical advantages. *J Thorac Cardiovasc Surg* 2000; 120: 999-1006.
- [33] Atkins BZ, Hueman MT, Meuchel J, Hutcheson KA, Glower DD, Taylor DA: Cellular cardiomyoplasty improves diastolic properties of injured heart. *J Surg Res* 1999; 85: 234-242.
- [34] Chachques JC, Blanchard D, Shafy A, Argyriadis P, Fabiani JN, Guermontprez JL, Carpentier A: Implantation de myoblastes dans le myocarde par voie endoventriculaire. *Archives Maladies Coeur* 2001; 94: 1428.
- [35] Tam SK, Gu W, Mahdavi V, Nadal-Ginard B: Cardiac myocyte terminal differentiation. Potential for cardiac regeneration. *Ann NY Acad Sci* 1995; 752: 72-79.
- [36] Chachques JC, Carpentier A: Cellular myoplasty: what are we really trying to achieve? *J Thorac Cardiovasc Surg* 2002; 123: 583-584.