

Cellular Cardiomyoplasty: Clinical Application

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Myocardial regeneration can be induced with the implantation of a variety of myogenic and angiogenic cell types. More than 150 patients have been treated with cellular cardiomyoplasty worldwide, 18 patients have been treated by our group. Cellular cardiomyoplasty seems to reduce the size and fibrosis of infarct scars, limit postischemic remodelling, and restore regional myocardial contractility. Techniques for skeletal myoblasts culture and *ex vivo* expansion using autologous patient

serum (obtained from plasmapheresis) have been developed by our group. In this article we propose (1) a total autologous cell culture technique and procedures for cell delivery and (2) a clinical trial with appropriate endpoints structured to determine the efficacy of cellular cardiomyoplasty.

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Cellular cardiomyoplasty (CMP) consists of *in situ* cell implantation intended to induce the growth of new muscle fibers and the development of angiogenesis in the damaged myocardium. This potentially may contribute to improve systolic and diastolic ventricular functions, and to reverse the postischemic remodeling process [1–3]. Adult myocardium is unable to effectively repair after infarction due to the lack of stem cells [4–6]. For this reason cell transplantation strategies for heart failure have been designed to replace damaged cells with cells that can perform cardiac work, either in ischemic or idiopathic cardiomyopathies. Current possibilities in myogenic and angiogenic cell therapy for myocardial regeneration are the transplantation into the myocardium of different types of cells as autologous myoblasts (originating from skeletal muscle), bone marrow-derived mesenchymal stem cells, circulating blood-derived progenitor cells, smooth muscle cells, vascular endothelial cells, and embryonic stem cells.

Our 15-year clinical experience with latissimus dorsi dynamic cardiomyoplasty [1, 7] and 6-year work in experimental cellular cardiomyoplasty [1, 3, 8] provide the support for the indication and management of cardiac-assist techniques. The aim of this article is to review the role of cell-based myogenic and angiogenic therapy in myocardial diseases and to present an approach for cell culture and cell delivery. In addition criteria for a structured clinical trial determining the efficacy of cellular cardiomyoplasty are presented.

Cell Selection

One of the major questions remaining concerning cellular therapy for heart failure is which cell type is appropriate for myocardial regeneration?. The following list describes the major cell types for cardiac myogenesis and angiogenesis which have been experimentally demonstrated to consent successful *ex vivo* cell-culture or cell-selection procedures followed by intramyocardial implantation (Table 1).

Myoblasts

Skeletal muscle cells are able to regenerate after injury because of the presence of satellite cells. In postnatal skeletal muscle, precursors (myoblasts) can be derived from satellite cells (reserve cells located on the surface of mature myofibers) or from cells lying beyond the myofiber, eg, interstitial connective tissue or bone marrow. Both of these classes of cells may have stem cell properties. When activated by appropriate stimuli satellite cells, proliferate and differentiate into myotubes becoming in some cases new muscle fibers [2, 3, 9]. The major advantages of this cell type is that myoblasts are highly resistant to ischemia and multiply after injury, presenting a high power for multiple mitosis [10].

When skeletal myoblasts are used for cellular cardiomyoplasty the sequence of actions appears to be the following: cells transplanted into the myocardium first impact on diastolic dysfunction. Subsequently when sufficiently organized in myotubes and myofibers systolic performance improves. Implanted cells orient themselves against cardiac stress preventing thinning and dilatation of the injured region [9, 11]. However it is not certain whether improvement in left ventricular performance is mediated by increased systolic function caused by synchronous contraction of the graft, since skeletal myoblasts are known not to contract spontaneously.

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Table 1. Cellular Cardiomyoplasty

Cells for cardiac myogenesis
Skeletal myoblasts
Smooth muscle cells
Bone marrow multipotent adult progenitor cells (MAPC)
Fetal and neonatal cardiomyocytes
Cells for cardiac angiogenesis
Endothelial cells (collected from the intima of arteries or veins)
Bone marrow-derived stem cells
Circulating blood-derived progenitor cells

Moreover denervated skeletal myoblasts could progressively become atrophic.

Bone Marrow Cells

There are four cell lineages that can be isolated from bone marrow: hematopoietic stem cells, mesenchymal stem cells [12], multipotent adult progenitor cells [13, 14], and progenitor endothelial cells [15]. The mesenchymal stem cells (called also bone marrow stromal cells) are capable of giving rise to multiple cell lines.

The main problem remaining with bone marrow cells is that they may differentiate into fibroblasts after implantation in a fibrotic scar, with the risk of becoming a "scar within a scar." Thus the importance of the implantation microenvironment. The apparent transdifferentiation of stem cells may be due to mere cell fusion with parenchymal cells, endowing the stem cell with specialized function [16].

Experimentally bone marrow stromal cells can be induced to differentiate in vitro into myocytes before transplant using a coculture system with cardiomyocytes [17, 18] or by including 5-azacytidine in the cultures [19]. This approach can be compromised for clinical trials in terms of potential cell mutations by 5-azacytidine. In vitro electrostimulation of cell cultures is experimentally used by our group for predifferentiation of stem cells in a myogenic lineage [20].

Peripheral blood stem cells are similar to those obtained from bone marrow aspiration. These cells can be previously mobilized from bone marrow by administration of cytokines in the form of stimulating growth factors, for example granulocyte-colony stimulating factor. Statins can also be used for cell mobilization [21]. The maximum mobilization effect occurs on the fifth day of administration, afterwards a mononuclear cell-rich fraction is isolated. Side effects during cell mobilization should be carefully evaluated, for example leucocytosis and increase of platelet number (responsible of coagulation abnormalities), splenomegaly.

Smooth Muscle Cells

Smooth muscle cells can be obtained from a segment of artery, the vermiform appendix or the uterus during laparoscopy. Experimental studies have demonstrated

successful in vitro cell expansion. After implantation in pathologic myocardium, smooth muscle cells proliferate and hypertrophy in response to the stress of cardiac contractions. Cell engraftment has been demonstrated to be related to the recovery of myocardial elasticity and reduction of fibrotic tissue, improved determinants of diastolic function have been observed [22]. These cells do not contract spontaneously after myocardial implantation.

Cardiomyocytes

Fetal and neonatal cardiomyocytes have been successfully grafted into the myocardium after in vitro expansion. The presence of intercalated disks and connexin 43, a marker of gap junctions required for cell to cell electrical coupling, has been experimentally demonstrated within grafted cardiomyocytes and between grafted cells and host myocytes resulting in improved systolic and diastolic ventricular function. In addition to availability, the clinical application of fetal and neonatal cells raises immunologic and ethical questions [23].

Adult cardiomyocytes present several drawbacks for use in myocardial regeneration owing to the difficulty to expand in the culture medium. In fact adult cardiomyocytes do not divide as they are terminally differentiated cells [24]. Furthermore cardiac cells require adequate vascular supply to survive in infarcted areas, in contrast to skeletal myoblasts which can tolerate an ischemic environment.

Endothelial Cells

Vascular endothelial cells can be harvested from the intima of autologous arteries or veins and be used to induce angiogenesis and neovascularization [25]. Ex-vivo expanded mature endothelial cells had been experimentally transplanted in ischemic myocardium and limbs, this approach presents the advantage of initiate and promote angiogenesis without the limitations of the release of a single protein (vascular endothelial growth factor, basic fibroblast growth factor). Endothelial cells induce an extensive capillary network, but they might not induce the formation of sufficient conduit vessels to regenerate postinfarction myocardial scars. The successive association of angiogenic and myogenic cell therapy should be beneficial, since prevascularization of myocardial scars may improve local conditions for myogenic cell survival (preconditioning).

Embryonic Cells

Embryonic stem cells can be isolated only from the inner cell mass of blastocysts (on day 6 of development), as the external cell mass of blastocyst will become the placenta. These cells are characterized by their capacity to proliferate in an undifferentiated state for a prolonged period in culture. Afterward they can differentiate into every tissue type in the body, forming derivatives of all three germ layers: ecto, meso, and endoderm. Unfortunately their clinical application raises immunologic barriers and bioethical dilemmas [26] and risks of teratoma formation

Table 2. Cellular Cardiomyoplasty: Mechanisms of Beneficial Effects

Ventricular remodeling
Reduces the size and fibrosis of infarct scars
Minimizes global ventricular dilatation
Increases myocardial wall thickness
Induces modulation of extracellular matrix remodeling
Diastolic function
Improves myocardial wall tension and elasticity
Improves of strain and dynamic stiffness
Reverses diastolic creep
Systolic function
Improves regional ventricular wall motion
Increases developed pressures
Improves global ventricular contraction?

because it is difficult to control the cell differentiation process.

Cell Lines

Cell lines derived from different cell types (stem cells, endothelial cells, and so forth) are commercially produced by cellular biology laboratories. The main drawback of immortalized cultured myogenic or angiogenic cell lines is the potential for tumorigenesis. Unless resolved this will limit the clinical application of this approach.

Atrial Cardiomyocytes as Cardiac Pacemaker

The implantation of cultivated fetal atrial cardiomyocytes into the ventricular wall have been proposed as a biological cardiac pacemaker. Cardiomyocytes with a higher intrinsic rhythmic rate can be implanted into the left ventricle becoming an ectopic pacemaker by functional coupling with host cardiomyocytes. Experimentally dissociated fetal atrial cardiomyocytes (including sinus nodal cells) have been implanted in the left ventricle. Histologic studies showed survival of grafted cells, formation of gap junctions between donor and recipient cells, and spontaneous generation of electrical signals having the morphology of QRS complexes of escape rhythm [27]. This approach may open a new perspective for the treatment of cardiac arrhythmia, principally for infants and premature babies with congenital atrioventricular block.

Mechanisms of Beneficial Effects

The main mechanisms involved determining the beneficial effects of cellular cardiomyoplasty appear to be: reduction of size and fibrosis of infarct scars, limitation of postischemic ventricular remodeling, improvement of left ventricular wall thickening and compliance (diastolic pressure-strain relationship), and increase in regional myocardial contractility (Table 2) [3, 6, 22, 24, 28, 29]. The mechanism explaining the transmission and propagation

of electrical impulses from the native myocardium to the engrafted cells has not been elucidated. Response to a mechanical stimulus exerted by surrounding cardiomyocytes could be responsible for inducing this contraction. Thus functional improvement is obtained from a combination of factors. Beneficial effect of cellular CMP is also based on the regeneration of the collagen matrix [30]. During the cell culture process approximately 20% of fibroblasts remain in mixture with myoblasts. After implantation these fibroblasts could contribute to the regeneration of the myocardial collagen matrix. In order to elucidate the effects of cellular CMP a recent study was performed using a computerized finite element model, with simulations of myocyte transplantation in a failing left ventricle [31].

Patient Selection

ISCHEMIC CARDIOMYOPATHY INCLUSION CRITERIA. Clinical application for cell transplantation is indicated in patients presenting a myocardial infarction of mild extension (between 12 and 18 cm², representing approximately one third of the left ventricle area), without extensive involvement of the ventricular septum (since this portion of the heart is not easily approachable by cell injection). Early cell injection after infarction should be beneficial to prevent a large fibrotic scar. However it appears reasonable to inject cells only after the postischemic inflammatory reaction has subsided [32].

Patients preimplantation clinical status for cellular CMP should be the following: New York Heart Association functional class 2 or 3 or equivalent symptoms, with or without angina; left ventricular wall thickness at echocardiographic evaluation of 4 mm or greater in order to avoid extramyocardial injection and the risk of secondary left ventricle rupture due to the multiple injection points; left ventricular ejection fraction between 20% and 40%.

ISCHEMIC CARDIOMYOPATHY EXCLUSION CRITERIA. All patients with skeletal muscle diseases should be excluded for myoblast implantation. Patients having a history of sustained ventricular tachycardia or fibrillation as well as patients with implantable cardiac defibrillators (ICD) or potential candidates for ICD implantation should be carefully evaluated, as transplant cell-induced arrhythmias are a potential complication. Furthermore subjects with an history of syncope during the previous year, cancer within 5 years, or with an active infectious disease or with positive tests to viral disease should be excluded.

IDIOPATHIC DILATED CARDIOMYOPATHY. Nonischemic cardiomyopathy could benefit from cellular CMP. Cell transplantation have been successfully performed in small cardiomyopathic animals [23], in a canine model of idiopathic dilated cardiomyopathy [15], and in doxorubicin-induced heart failure [33]. On the basis of these experimental results cellular CMP may improve heart function in patients with nonischemic cardiomyopathy. The grafted cells appear to better survive in the host myocardium because myocardial irrigation in this pathology is not significantly impaired.

Table 3. Guidelines for Myoblast Culture Technique

All manipulations should be carried out in a laminar flow hood using aseptic technique
Removal of fibrous and adipose tissue from muscle biopsy
Muscle mincing with scissors
Enzymatic (collagenase + trypsin) and mechanical digestion
Multiple centrifugations
Isolation of myoblasts, exclusion of fibroblasts
In vitro myoblasts culture: incubation during 3 weeks in humidified atmosphere, at 37°C, 5% CO ₂
Assessment of percent cell viability (Trypan blue or flow cytometry)
Assessment of myoblasts/fibroblasts rate (CD56-positive and desmin antibodies)
Sterility tests: bacterial, viral, fungal
Cell suspension in 0.5% human albumin for myocardial injection

MUSCLE BIOPSY AND CELL CULTURE TECHNIQUES. To initiate ex-vivo cell culture procedures, the following "virus free tests" should be performed: antihuman immunodeficiency virus (HIV), antihepatitis B-C virus (HBV, HCV), immunoglobulin (Ig) M anticytomegalovirus (CMV), HbsAG, and human T-cell leukemia virus.

The following is a description of the technique used by our group to perform myogenic cellular CMP (Table 3).

Skeletal Muscle Biopsy

Three weeks before cellular CMP, a biopsy sample of the thigh vastus lateralis is taken through a 5-cm incision under local anesthesia. Local anesthetic agents appears to stimulate dormant myogenic cells. A 2- to 3-cm³



Fig 1. A 2 to 3 cm³ biopsy sample of the thigh vastus lateralis (12 to 18 g) is explanted under local anesthesia and high sterile conditions.

skeletal muscle sample (12 to 18 g) is explanted under sterile conditions (Fig 1). Immediate fragmentation of the muscle with scissors is performed and then immersed in complete culture medium or in PBS (phosphate buffer solution, GIBCO, Rockville, MD) and kept at 4°C. The procedure for cell isolation and culture should start as soon as possible so as to guarantee maximum cell survival. However samples can be stored in an appropriate container at low temperature and secondarily transported to the laboratory.

Cell Isolation and In Vitro Expansion

All manipulations should be performed in a laminar flow hood using an aseptic technique. The explanted skeletal muscle pieces are washed in PBS. Adipose tissue and fascia are removed and the muscle is carefully minced with scissors. The muscle fragments are washed again in PBS until the supernatant remains clear. Centrifugation is carried out at 100g for 5 minutes. Tissue dissociation is obtained by two consecutive enzymatic treatments: first, cells are incubated with collagenase IA (1.5 mg/mL for each gram of tissue; Sigma Chemical) and left in the incubator for 1 hour, followed by mechanical dissociation obtained by shaking the tube every 10 minutes. Alternatively, the tube can be placed in a 37°C reciprocating/orbital shaking incubator (ROSI, Thermolyne, Dubuque, IO). A second incubation with 0.25% trypsin 1x ethylenediamine tetraacetic acid (EDTA [2 mL, GIBCO BRL]) is then performed for 20 minutes. Cells are then washed (10 minutes at 300g) and enzymatic reaction stopped by adding 1 mL of the patients own serum. Filtration through a 40- μ sieve (cell strainer nylon, Falcon, Becton Dickinson, Franklin Lakes, NJ) is then performed and a second enzymatic digestion is conducted on the remaining fragments which eventually rest on the sieve. Cells are collected by sedimentation (20 minutes at 300g) and the supernatant is discarded.

Cells pellets are resuspended in fresh complete culture medium: 79% HAM-F12 medium, 20% patient's serum (obtained from blood sample or from plasmapheresis), 1% penicillin/streptomycin (GIBCO) and plated in tissue culture flasks of 300 cm² (TPP, Trasadingen, Switzerland). Amphotericin B (0.25 μ g/mL) and 25 pg/mL basic fibroblast growth factor (human recombinant, Sigma) can be included in the culture medium. Afterwards cell cultures are incubated during 3 weeks at 37°C in a humidified atmosphere containing 5% CO₂. Flasks should be positioned without tilt in the incubator in order to avoid irregular cell proliferation. After a 2- to 3-day incubation time, the medium is changed eliminating dead and blood cells in the supernatant, then fresh complete culture medium is added. Passaging of the cultures (1:5 split) is carried out at subconfluency (50% of confluency) to avoid the occurrence of myogenic differentiation at higher densities. Frequent passaging at 50% confluence is required to prevent the mononucleated cells from differentiating into myotubes. The mean volume of patients' autologous serum prepared for myoblast cultures is 1500 mL. Aliquots of 50 mL are cryopreserved until usage.

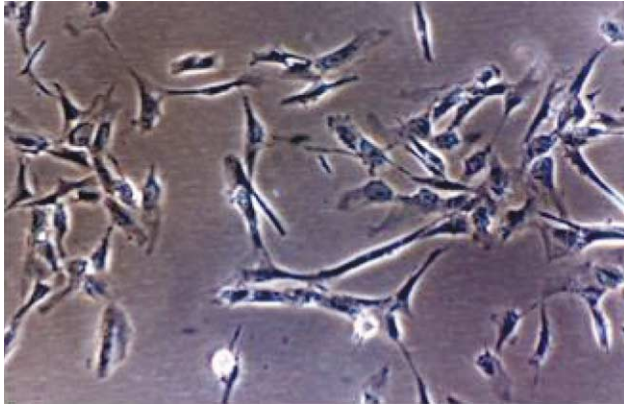


Fig 2. Human skeletal myoblasts after a 3-week in vitro culture period (magnification $\times 40$).

Cell culture flasks are periodically observed using an inverted light microscope with fluorescence (Nikon Eclipse TE 300, Melville, NY). When subconfluency is obtained a first passage is performed. Cells are harvested

by trypsinization (2 mL 0.25% trypsin-EDTA in each flask for 1 to 5 minutes in the incubator). Complete cell detachment is demonstrated by observing floating cells under microscopy. The reaction is then stopped with complete culture medium and the resultant cell suspension is split into another five flasks. Additional passages should be performed in order to obtain the final cells quantity. Commonly, after 3 weeks, more than 200×10^6 cells are obtained (Fig 2). The cell number can be scaled up by repeated passaging in a multiple-tray cell factory or using rotary cell culture systems (Synthecon, Houston, TX). Bacterial (aerobic and anaerobic tests), viral, and fungal controls should be performed at each step of the cell culture procedure.

Fibroblast Removal From Myoblast Culture

In order to reduce the number of fibroblasts and achieve a pure myoblast culture, a preplating step is applied on the first passage. Preplating technique is based on the quicker attachment of fibroblasts compared with satellite cells. Samples in which myoblast purity is below 30% are

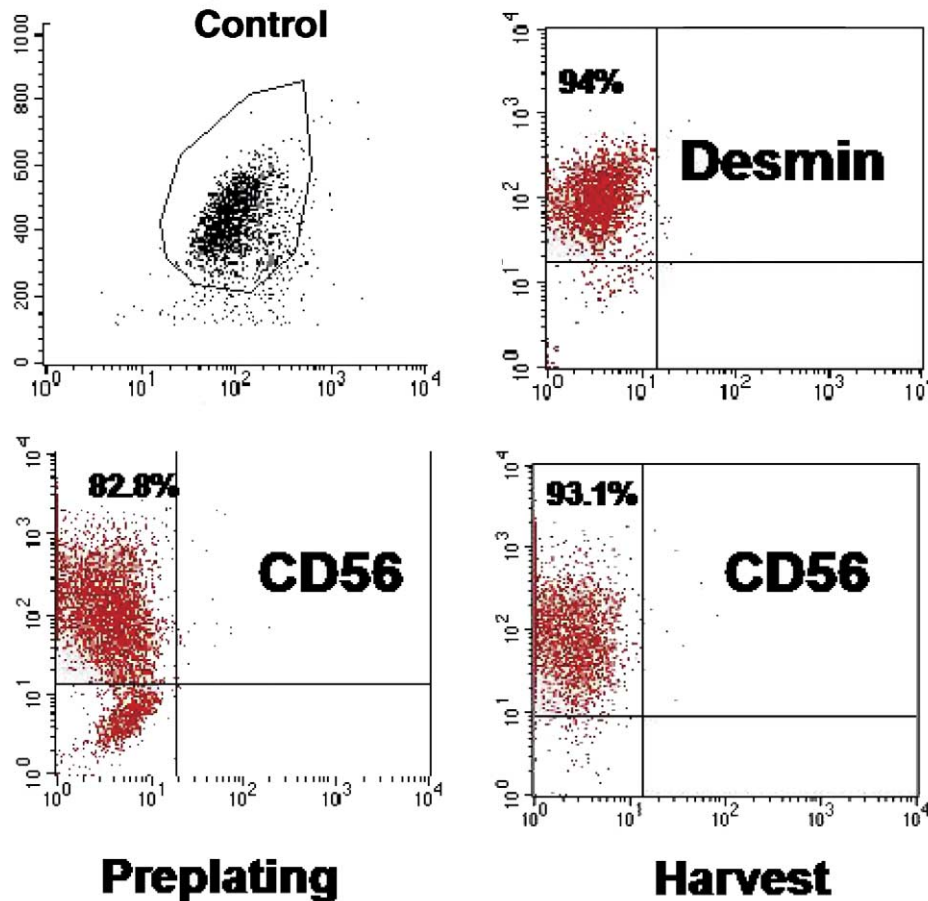


Fig 3. Quantification of myoblasts by flow cytometry. Muscle progenitor cells were harvested and labeled with mouse antihuman CD56 (before preplating step and after 3 weeks in culture). Alternatively cells were labeled with a mouse antibody against human desmin. Isotype antibodies were used as negative controls.

Table 4. Cell Implantation Technologies

Epicardial approach
Surgical: conventional or minimally invasive
Thorascopic
Endoventricular
Catheter-based cell delivery. Guidance:
Three dimensional electromechanical mapping
Biplane fluoroscopy and ultrasound guidance
Magnetic resonance imaging compatible catheter
Intravascular
Catheter-based intracoronary
Coronary venous route or intravenous systemic

subjected to a positive CD56 cell selection using micro-magnetic immunobeads (Anti-Fibroblast MicroBeads; Miltenyi Biotec, Bergisch-Gladbach, Germany). After this selection myoblast purity is generally greater than 90%. The magnetic technique for removing fibroblasts consists in the use of antibodies recognizing muscle progenitor specific cell surface antigens.

Injection Medium

On the day of transplantation, cells are harvested and washed in the injection medium (human albumin 0.5% plus complete culture medium) and kept in ice before implantation. A sample is performed to assess final myoblast rate, by flow cytometry test: percentage of myoblasts CD56-positive (Miltenyi Biotec), and desmin antibody positive cells (Sigma-Aldrich, France; Fig 3). Cell concentration and viability are determined with Trypan blue using a Malassez cytometer or FACS (flow cytometry). Sterility of cell culture is also assessed before implantation (Gram tests).

Cell Implantation

Cellular implantation can be performed by an epicardial or an endovascular delivery approach. In the surgical approach (conventional or minithoracotomy/sternotomy) the ischemic area is well exposed permitting approximately 10 injections of the cell suspension with a 24G to 26G curved needle (for example 25G \times 40 mm retrobulbar ophthalmic needle). The use of a long needle avoids multiple traumatic injection points.

Recommended density of implanted cells is between 50 to 70 million cells per mL. The cell injection procedure should be performed slowly, taking approximately 15 minutes. Cells should be delivered when the implanted needle is progressively removed from the myocardium. The needle injection sites needs finger compression (1 to 2 minutes) after every injection, in order to avoid regurgitation of the cell solution (channel leakage). The number of injection points depends both on the size and configuration of the myocardial infarcted area. Our approach consists in performing the main implantation in

the peri-infarct area (70% of cells), since residual irrigation and collateral myocardial revascularization in this intermediate area allows for a better survival of the implanted cells. The remaining 30% of cells are implanted in the central portion of the scar. The effects of this cell implantation procedure will be the centripetal reduction of the infarct area. For idiopathic dilated cardiomyopathies, multiple cell injections between the coronary artery branches should be performed in both ventricles.

Catheter-Based Cell Implantation

INTRACORONARY. The intravascular delivery is based on the potential migratory properties of some cells which retain their ability to cross the basal lamina (Table 4). This approach could be reserved for nonischemic cardiomyopathies, since intracoronary cell delivery constitutes microemboli that could potentially decrease blood supply in ischemic patients [33-36].

ENDOVENTRICULAR. Intramyocardial cell injection can be performed by a percutaneous transfemoral endoventricular approach. Several catheters are available: MyoStar catheter (Johnson and Johnson, Diamond Bar, CA) [8, 37], Myocath delivery system (Bioheart Inc, Weston, FL) [38], Stiletto (Boston Scientific, Maple Grove, MN) [38], and a helical catheter (Biocardia Inc, San Francisco, CA) [39]. Magnetic resonance imaging compatible catheters are also in development [39, 40].

INTRAVENOUS. Systemic intravenous cell delivery can be performed for myocardial ischemia [41]. The disadvantage of this approach is the nonselective distribution pattern of injected cells.

Another approach consists in a percutaneous selective coronary venous cannulation and intramyocardial cell injection (TransAccess MicroLume Delivery System, Transvascular, Inc, Menlo Park, CA). The coronary sinus is cannulated percutaneously and a balloon-tipped catheter advanced to the anterior interventricular vein or middle cardiac vein. A microinfusion catheter is then advanced through a sheathed extendable nitinol needle, deep into remote myocardium [42].

Patient Follow-Up

Patient hospital discharge should be carefully evaluated, as ventricular arrhythmias can be observed during the first 15 postimplantation days. They are probably due to the incorporation of cells and the culture medium into the ventricular wall, representing a risk of ectopic generation of electrical disorders. For this reason electrocardiographic monitoring and postoperative antiarrhythmic medication is justified (for example amiodarone). Furthermore corticosteroids can be administered after cell implantation in order to reduce the inflammatory response due to inoculation. Our approach consisting of cell cultures in human autologous serum demonstrated the absence of postoperative cardiac arrhythmias.

Patients are studied every 3 months during the first year of follow-up and every 6 months thereafter. Heart failure neurohormonal factors, for example brain natriuretic peptide (BNP) should be included in the follow-up. Ventricular function is evaluated by basal-dobutamine stress echocardiography and radionuclide ventriculography (MIBI-gated single-position emission computed tomography [SPECT]). Myocardial viability is assessed with fluorodeoxyglucose (18-FDG) positron emission tomography (PET), uptake of gadolinium by magnetic resonance imaging, and stress-redistribution-reinjection ²⁰¹thallium scintigraphy.

Clinical Studies

Three clinical trials on cellular CMP have been initiated by our group.

Myoblast Trial

Autologous cultivated skeletal myoblasts have been implanted in postinfarction myocardial scars during coronary artery bypass graft surgery. Procedures were performed in 18 patients. Myoblasts were cultivated during 3 weeks in autologous patient's serum obtained by plasmapheresis or from blood samples. Patients treated with autologous-serum cultivated cells were free of cardiac arrhythmia; this obviates the need for the implantation of a defibrillator [8, 43].

Cells CD133+

Mobilized mononuclear bone marrow cells have been implanted into postinfarction myocardial scars during CABG. This protocol is based in the utilization of a subpopulation of bone marrow cells, the CD133+ progenitors, which have a tendency to differentiate in true angioblasts and muscle cells. Cells are obtained from peripheral blood after mobilization with granulocyte-colony stimulating factor. Cell selection is performed using a isolation kit including a magnetic separation column (CliniMACS, Miltenyi Biotec). This approach avoids cell culture procedures [15].

Cells for Ischemic Mitral Valve Regurgitation

The MIRAGE clinical trial (Mitral-valve Ischemic Repair Associated with Graft of Endogenous-cells) includes randomly patients presenting left ventricle postischemic scars (akinetic and metabolically nonviable) and surgical indication for mitral valve repair. Cells CD133+ are implanted during open-heart surgery in the posterior left ventricle wall and the papillary muscle, using a simultaneous endoventricular and epicardial injection approach. Ischemic mitral regurgitation is a distinctive valve disease in that, unlike with organic valvulopathies, abnormalities of the left ventricle are not the consequence but the cause of the valve disease. Ischemic mitral regurgitation is more a pathology of the myocardium than the valve [44].

International Clinical Trials

Since June 2000 more than 150 patients with ischemic myocardial disease and some with dilated cardiomyopathy have been treated worldwide in various cellular therapy clinical trials. The number of patients treated with autologous skeletal myoblasts was equivalent to those treated with bone marrow cells (BMC) and the number of surgical implantations was equivalent to those of percutaneous catheter-based procedures. The geographical distributions was as follows:

Europe

Clinical trials have been performed in the following countries. France: [15, 45]. Spain: Pamplona [46], Salamanca, Coruna, Valladolid. Germany: Dusseldorf [35]; Rostock [47], Frankfurt [36]. United Kingdom: Leicester [48]. Netherlands: Rotterdam [49]. Italy: Milan, Padova [49]. Poland: Poznan. Russia: Tomsk.

Americas

United States: Arizona Heart Institute [50], Mount Sinai NY Hospital, Temple University Hospital, UCLA, Cleveland Clinic, University of Michigan [51], Washington Hospital Center [52]. Bioheart Inc and Genzyme Corp announced myoblast trials to be performed in America and Europe (MyoHeart and MAGIC Trials: Myoblast Autologous Grafting in Ischemic Cardiomyopathy); in these trials implantable cardioverter defibrillators should be associated. Argentina: Avellaneda Hospital Buenos Aires [53], Rosario, La Plata. Brazil: Incor San Pablo, Rio de Janeiro [54].

Asia

Japan: Yamaguchi University, Ube [55]. China: Hong Kong University [56], Nanjing Medical University [57]. Singapore: National University Hospital [58].

Comment

Cell transplantation is being recognized as a viable strategy to improve myocardial viability and limit infarct growth. Encouraging experimental results have permitted the clinical application of cellular CMP. In our approach a total autologous myoblast culture procedure was used. The main benefits of human-autologous-serum cell expansion is that it can be performed without risk of prion, viral, or zoonoses contamination. Traditional cell cultures techniques involve the use of fetal bovine serum (FBS) for cell growth. Contact of human cells with fetal bovine serum results after 3-week in fixation of animal proteins on the cell surface, representing an antigenic substrate for immunologic adverse events. After cell implantation an inflammatory reaction occurs with subsequent fibrosis. Clinical-pathologic studies performed after cellular CMP showed the transplanted cells were embedded within fibrosis and without neovascularization [51, 59]. This histologic configuration represents a risk for micro-reentry circuits that can in-

duce ectopic generation of severe ventricular arrhythmias. When *in vitro* myoblasts culture is produced using autologous blood serum the risk of arrhythmia is reduced [60]. This obviates the need for the implantation of defibrillators [8, 43, 46, 53].

The technical approach used to implant the cells could influence the efficacy of cellular CMP. In fact cell mortality after transplantation appears to be more important when grafted in the center of high-fibrotic ischemic scars (decreased oxygen and nutrients supply to the chronic ischemic myocardium) [32]. Implanting the cells mainly in peri-infarct areas and the association with therapeutic angiogenesis may improve cell survival and the results of cellular CMP [2, 61]. The best functional results seems to be obtained in patients presenting a heterogeneous infarct area (patchy appearance), namely a mixture of viable myocardial tissue and multiple small scars. Therefore a "vascularized fibrosis" seems to be a better indication for cellular CMP than a "nonvascularized" postinfarct scar [43]. It is possible that periodically repeated cell injections should be necessary to progressively reduce the size of infarct scars in ischemic cardiomyopathies or to gradually improve diseased myocardium in nonischemic cardiomyopathies. This approach should be simplified by the development of percutaneous catheter-based cell implantation procedures.

Combined cellular transplantation with multisite cardiac pacing is actually under investigation in our department. After skeletal myoblast implantation in a experimental myocardial infarction model, atrial synchronized biventricular pacing was performed using epicardial electrodes. These studies showed improved cell distribution, development of myotubes and increased expression of slow myosin heavy chain isoforms (better adapted at performing cardiac work). In addition, this combined approach should be promoted in patients with indication of atrio-biventricular resynchronization [8, 62].

Perspectives

Cell implantation to treat patients with ischemic or dilated cardiomyopathy is a new concept. Data from well-designed clinical studies are needed to confirm the beneficial effects observed in feasibility studies. Directly injecting skeletal myoblasts-derived cells into ischemic myocardium seems to provide the substrate for electrical instability leading to malignant arrhythmia. A number of clinical difficulties remain to be solved, for example concerning the choice of the best cell type and the best cell dose for each cell type. Also the most optimal method to improve cell engraftment after implantation remains to be identified. Future randomized studies should provide convincing evidence that cellular cardiomyoplasty itself has any beneficial effects as most of the studies have been performed while associating surgical or percutaneous coronary artery revascularization procedures.

The major challenges for future research programs are the preconditioning for predifferentiation of stem cells before transplantation [63, 64], the improvement of host-

cell interactions (mechanical and electrical coupling), and the optimization of the rate of surviving cells after myocardial implantation [65, 66]. The association of cell-based therapeutic angiogenesis before cellular myogenesis seems to be justified in order to induce prevascularization of postinfarct scars. Electrostimulated cellular CMP should play an important role in transforming a passive cell-based procedure to a dynamic cellular support.

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