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Percutaneous transplantation of skeletal myoblast in the treatment of post-infarction injury

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Cell therapy may be a potentially attractive approach to restore myocardial contractile performance after an infarction injury. Multipotent stem cells are currently being studied as a possible cell source for myocardial repair within the first few days after the infarction onset in non-revascularizable areas of the left ventricle having viable myocardium. In the presence of fibrotic post-infarction scar with no detectable myocardial viability, direct myocyte precursors, i.e. myoblasts, are being considered as a potential source of new muscle fibres. We review the current clinical experience with transplantation of the autologous skeletal myoblasts in patients with post-infarction heart failure, focusing on percutaneous cell transplantations performed as a sole procedure.

Introduction

Despite the recent developments in the treatment of coronary artery disease, congestive heart failure caused by myocardial infarction still remains a major health problem, affecting millions of patients worldwide with massive negative economic consequences. Myocardial necrosis and subsequent formation of fibrotic scar that replaces viable myocardium may lead to depressed systolic function, left ventricular (LV) remodelling, aneurysm formation, and ultimately to congestive heart failure. In the last few decades, improved treatments and an ageing population has lead to longer post-myocardial infarction survival resulting in increased prevalence of post-ischaemic heart failure.[1] Consequently, the treatment of heart failure has gained widespread attention.

The possibility of repairing and growing new myocardium within the necrotic tissue as a result of cell transplantation has been widely studied in both experimental and clinical conditions.[2–7]

Among the variety of cells studied, autologous skeletal myoblasts are one of the most encouraging cell sources for cardiac repair. Skeletal myoblasts, or satellite cells, are progenitor cells usually residing in a quiescent state under the basal membrane of skeletal muscle fibres, until recruited to proliferate and differentiate into mature skeletal myocytes in response to injuries (*Figure 1*). They are of their autologous origin, the ability to be amplified *in vitro*, and have high proliferative potential resistance to ischaemia and preclinical efficacy.[8] These characteristics have led clinical investigators to evaluate the effect of transplanted autologous myoblasts in patients with post-infarction heart failure. Myoblasts differentiate into myotubes and maintain muscle properties when transplanted into an infarct area.[9–11]

Electromechanical properties of myocardial and skeletal muscle tissues differ significantly. Cardiac cells act together synchronously due to the presence of special cell-to-cell junctions containing N-cadherin and connexin 43[12,13] (*Figure 2*). The latter is a transmembrane protein playing an important role in mechanical and electrical coupling within cardiac tissue.[14] The lack of gap junction protein expression like connexin 43 on skeletal myotubes prevents them from being physically coupled with host cardiomyocytes, suggesting that these cells do not beat in synchrony with the rest of the heart.[10,15,16]

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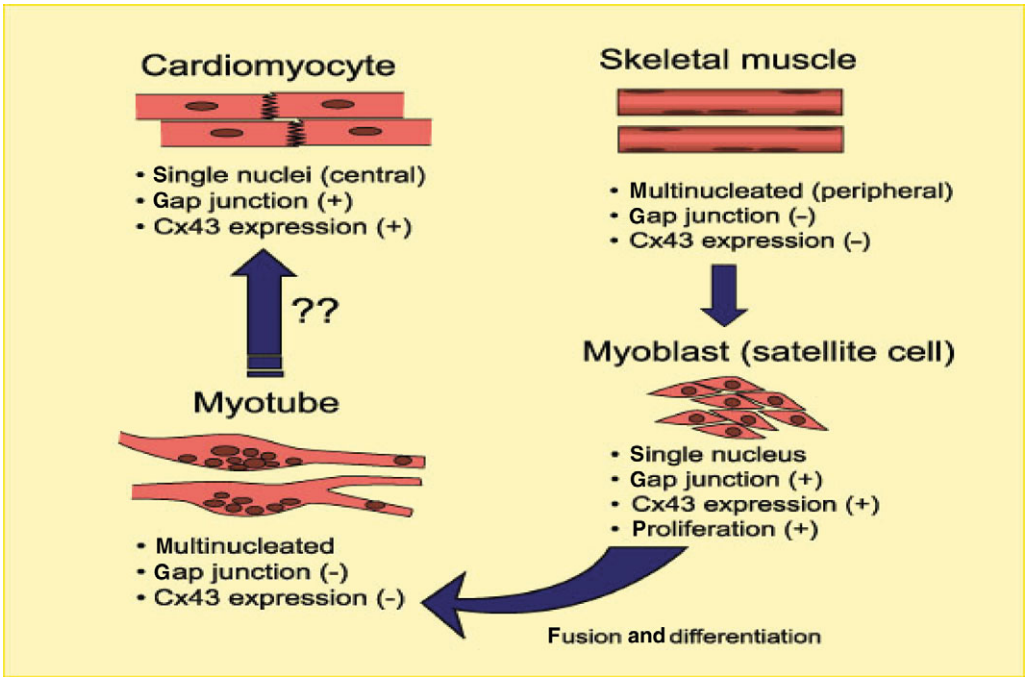

Figure 1 Myoblasts are usually in a quiescent state under the basal membrane of skeletal muscle fibres. After being gathered and expanded, myoblasts are transplantated into an infarct area where they differentiate into myotubes and maintain muscle properties. It is still unclear whether myoblasts can transdifferentiate into cardiomyocytes.

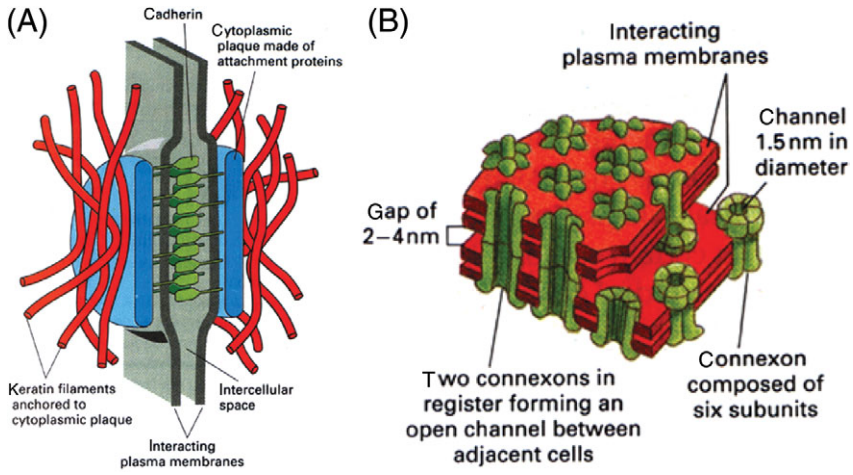

Figure 2 Cell to cell junction. Cadherin (*A*) has an important structural role linking the cytoplasmatic plaque on the inner side and coupling with other catherins on the extracellular side. Connexon 43 (*B*) is composed of six subunits and forms channels between cardiac cells in order to create an electrical coupling.

However, it has been shown that the lack of junctions between grafted cells and host tissue does not preclude improvement in LV contractile function.[17] It has been suggested that transplanted cells can contract synchronously even in the absence of connections between cells, probably by stretching or by direct transmembrane channelling of electric currents.[10,18] The direct contribution of these engrafted cells in improving systolic function was noticed in several studies that indicated a positive effect of skeletal myoblasts on myocardial contractility lasting over time and correlating with the number of implanted cells.[19,20] Although certain *ex vivo* data suggest that skeletal myoblasts may acquire few characteristics of cardiomyocytes or may fuse with them forming *chimeric cells*,[21] it has been assumed that the grafted cells do not transdifferentiate, instead retaining the morphological and electrophysiological properties of skeletal muscle.[9,17]

The capability of myoblasts to improve cardiac function cannot be explained only in terms of electromechanical integration or direct contribution. Other mechanisms were found to play an important role. One

hypothesis proposes that the engrafted cells could affect post-infarction remodelling by limiting the expansion of the post-infarction scar.[22–24] A second possible mechanism is the paracrine effect that myoblasts exert on surrounding myocardial cells. This hypothesis is derived from the observation that these cells are able to release pleiotrophic factors such as vascular endothelial growth factor and insulin growth factor I that could mobilize resident quiescent cardiac cells and promote angiogenesis.[25–27] These factors in association with a marked attenuation of matrix metalloproteinase-2 and -9 up-regulation may work as antifibrotic agents protecting peri-infarction tissues.[28]

Since skeletal myoblasts do not extravasate and may cause microembolizations after intracoronary delivery, their potential application in myocardial regeneration requires direct cell injection into the area of damaged myocardium. Transepicardial cell injection during open-chest surgery and several catheter-based methods have been proposed and studied in clinical trials.[8,29–33] (*Figures 3* and *4*).

Initial clinical experience: open-chest myoblast transplantation

The first report on autologous skeletal myoblast transplantation during open-chest cardiac surgery was published in the *Lancet* in 2001 by Menasché *et al*.[34] After that case report, two small phase-one clinical trials were started in Paris and Poznan.[8,35] In both trials, 10 patients with severely reduced LVEF undergoing CABG received transepicardial myoblast injection. Five months after the procedure, a significant improvement in symptoms by one NYHA class, an increase of regional wall motion, an increase of global LV ejection fraction (LVEF) as well as an increase in tracer activity on positron emission tomography (PET) were observed, suggesting a new onset of metabolic activity in the previously non-viable scar area. However, 4 years after combined myoblast transplantation and CABG, in almost one-third of the Poznan series the end-diastolic LV diameter was increased (unpublished own observation) generating doubts about the previously reported capability of transplanted cells to reduce ventricular dilation.[23,24] In two other similar phase-one studies published by Herreros *et al*.[30] and Chachques *et al*.,[32] a total of 21 patients received myoblast injection during CABG. Consistent with prior studies, improvements of regional wall motion and global LVEF were noted, suggesting safety and feasibility of the method.

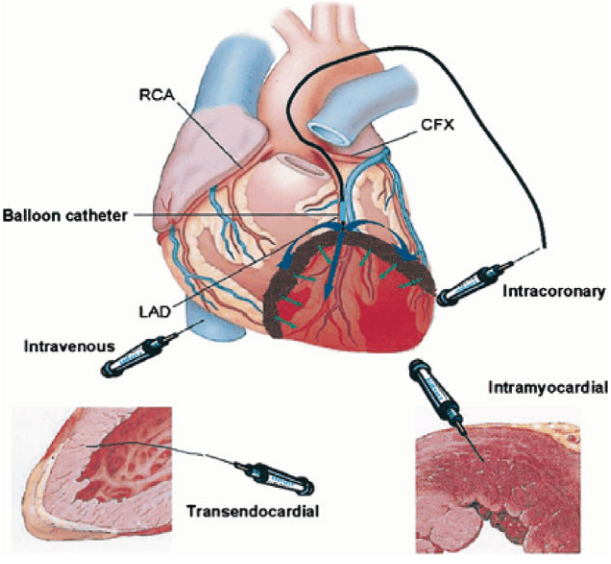

Figure 3 Myoblast-delivering approaches.

In a recent multicentre dose-escalating safety trial conducted in the USA, published by Dib *et al*.,[36,37] 11 patients underwent myoblast transplantation during open-chest surgery. The echocardiographic evaluation as well as PET and magnetic resonance imaging (MRI) scans showed an increased viability of grafted scar, whereas the mean EF improved from 22.7 to 35.9%.

In 2004, Menasché *et al*. started the MAGIC trial, a multicentre, prospective, randomized, double-blind, placebo-controlled trial designed to evaluate the effects of skeletal myoblast transplantation in the context of severe ischaemic heart failure in a population of 300 selected patients. The study was prematurely discontinued in February 2006 after 120 patients were enrolled (97 treated) after the decision of the Steering Committee. The assessment of the risk/benefit ratio is currently under way and the trial could be resumed after the approval of the Data Monitoring Committee.

Cell transplantation during cardiac surgery has certain advantages including easy access to the target area and possible delivery of large numbers of cells per unit. However, direct transepicardial approach may cause additional risk to the patient during surgery, since candidates for cell transplantation often have a history of multiple infarctions and LV dysfunction, and clinical symptoms of severe heart failure. Moreover, the interpretation of clinical outcomes obtained from trials evaluating myoblast injection during CABG is not possible because the effects of the two procedures performed at the same time cannot be easily distinguished and ascertained. However, in light of these limitations and of the trend towards less-invasive, diagnostic, and therapeutic procedures, percutaneous approaches with cell injection as a sole procedure are under investigation.

Percutaneous myoblast transplantation: cell injection as a sole procedure

Catheter-based transendocardial or transcoronary vein injections, performed as a sole therapy, may allow the evaluation of effect of myoblasts without confounders. It may also enable repeated cell injections in patients with severe myocardial injuries, since excessive number of transplanted cells in a single injection may result in only a small percentage of grafted cells survived. In fact, despite more than 10 years of work in this field,

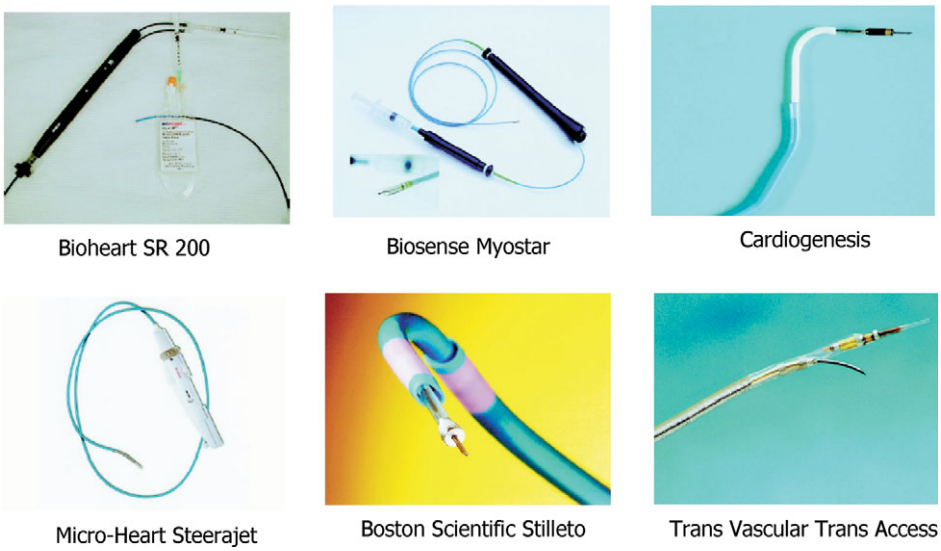

Figure 4 Different *trans*-endocardial and *trans*-coronary-venous cell delivery systems used in recent trials.

the delivering technique has still to be improved; a large percentage of successfully delivered cells leak back out of the injection site or die within the first week.[38] Direct cell injection in the ventricular wall can be achieved both by a transendocardial[29] or a transcoronary vein approach.[33,39]

Catheter-based transendocardial injection is performed using a needle catheter directed perpendicularly to the inner surface of the target area using an electromechanical mapping of the endocardial surface.[40–42] Although this technique has been demonstrated to be feasible, cell delivery by direct injection may be difficult: endoventricular catheter systems currently available have limited stability so that a back-flush of cells from the puncture site is to be expected. In addition, the needle positioned against the endocardial surface does not follow the heart movements making the injection in thinned post-infarction scars or in the border zone of the infarct very challenging.

Catheter-based cell infusion through coronary veins is a relatively new approach recently used in a pilot trial by Siminiak *et al.*[43] and it consists of a catheter-based endovascular system incorporating an IVUS source and an extendable needle (TransAccess, Trans Vascular, Menlo Park, CA, USA). The TransAccess catheter is a monorail, composite catheter system combining both a phased array IVUS and a pre-shaped adjustable nitinol needle. After placing the TransAccess system in the target coronary vein through the coronary sinus, the needle is oriented using IVUS images of the corresponding artery, the pericardium, and the ventricular myocardium as landmarks (*Figure 5*). The nitinol needle is extended into the myocardium and a micro-infusion catheter (IntraLume, TransVascular Inc.) is then advanced through the needle while simultaneously injecting of the therapeutic agent. In contrast to the transendocardial approach, where cells are injected perpendicularly, the TransAccess system delivers cells parallel to the ventricular wall.

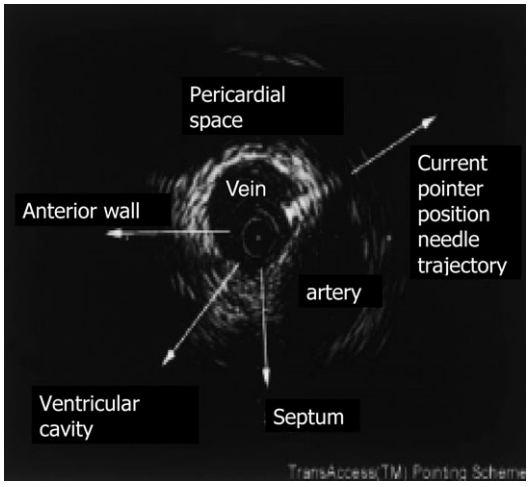

Figure 5 IVUS image of the TransAccess system in a coronary vein. The needle is oriented using IVUS imaging taking the corresponding artery, the pericardium, and the ventricular myocardium as landmarks.

Clinical trials evaluating percutaneous myoblast transplantation performed as a sole procedure in patients with post-infarction heart failure studied both endoventricular and transcoronary-venous catheter systems.

In 2003, the Rotterdam group[29] injected autologous myoblast suspensions into the area of post-infarction injury of five patients using an endoventricular catheter under electromagnetic guidance (*Figure 6*). Although the small sample size evidently precludes any conclusions about efficacy, this early experience has primarily documented the feasibility of this approach. An increase of LVEF and regional wall motion was observed at 3-month follow-up by angiography, though nuclear radiography and MRI failed to confirm this improvement. At 6 months, a trend towards increased LVEF was observed by both angiography and nuclear scan. A sub-study conducted to evaluate short- and long-term results of

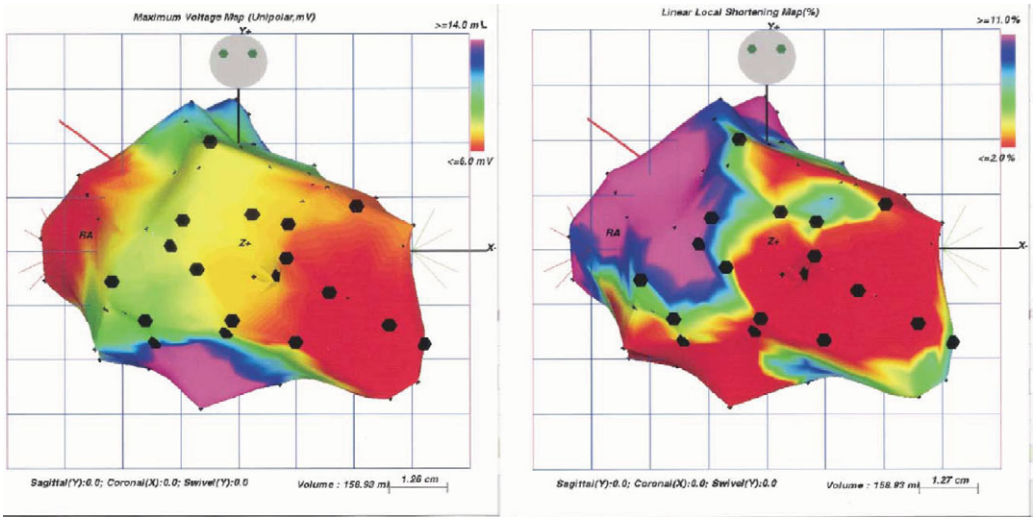

Figure 6 Unipolar voltage (left) and linear local shortening (right) NOGA maps. The myocardial scar is scaled red indicating an area with a <6 mV voltage and a $<2\%$ shortening. Black dots indicate the transendocardial injection sites.

myoblast transplantation on regional and global LV functional by two-dimensional echocardiography with dobutamine infusion and tissue Doppler imaging (TDI) showed an improvement of target wall systolic velocity and of global LV function during low-dose dobutamine infusion, indicating an improvement of contractile reserve.[44] (*Figure 7*).

In a recent study published by the same group, 10 to 15 injections of autologous myoblasts using Myostar™ (Cordis, Warren, NJ, USA) were given using an endoventricular approach. At 6-month follow-up, an increased EF and cardiac output, a reduction of 'systolic volume, and a trend towards improved stroke work were observed. These haemodynamic improvements were confirmed by pressure–volume loops analysis 1 year after percutaneous myoblast transplantation.[45] (*Figure 8*).

Another recently published study describes the results of transventricular injections using the fluoroscopy-guided MyoCath™ catheter (Bioheart, Weston, FL, USA) or the NOGA™-guided catheter system (Biosense-Webster, Waterloo, Belgium). The study failed to show improvement in the EF but wall motion score index improved both at rest and under low-dose dobutamine.[46]

A third catheter-based study, the POZNAN trial, was recently published by Siminiak *et al.*[33] This study was performed as a phase-one clinical trial to assess the safety and feasibility of both the TransAccess® catheter system and the percutaneous autologous myoblast transplantation performed as a sole therapy. Two to four intramyocardial injections delivered up to 100 million cells in 0.6–2.5 mL of saline solution to each patient. The trial confirmed the feasibility of intramyocardial injections using the TransAccess® system with an extremely precise advancement of the micro-lumen catheter in the remote target area up to 4 cm deep within the injured myocardium. The procedure was reported to be technically successful in all but one patient and did not cause any periprocedural adverse event.

The use of both the anterior interventricular vein and the middle cardiac vein, parallel to the posterior descending coronary artery, were shown to be feasible. In addition, compared with the anterior interventricular venous approach, in the POZNAN trial, a middle vein approach to advance the TransAccess® system succeeded in getting closer to the apical segments of the left ventricle.[33] The lack of procedural success in one patient, related to the inability to appropriately position the guiding catheter across the venous valve at the bifurcation of the great cardiac vein, suggests the need for a new and refined guiding catheter.

At 6-month follow-up, NYHA class improved in all patients and EF, assessed by echocardiography, significantly increased by 3 to 8 percentage points in six out of nine patients.[12,14]

Again, efficacy data, although considered promising, have to be interpreted cautiously because of the small size of the sample. These results, however, confirm previous laboratory findings in which autologous myoblasts delivered through the coronary sinus route significantly improved regional wall motion and global LV function.[47]

Safety issues related to myoblast transplantation

It may be speculated that the inability of skeletal myoblasts to transdifferentiate to cardiomyocytes and to form junctions with neighbouring cells may be a substrate for ventricular re-entry arrhythmia. Current experimental and clinical data indeed suggest a possibility of increased risk of arrhythmogenicity. In the first clinical series published by Menasché *et al.*,[8] four patients who underwent autologous skeletal myoblast transplantations during CABG received an implanted automatic internal cardioverter-defibrillators (AICD) due to sustained episodes of ventricular tachycardia (VT). In the Poznan CABG phase-one experience,[35] episodes of sustained

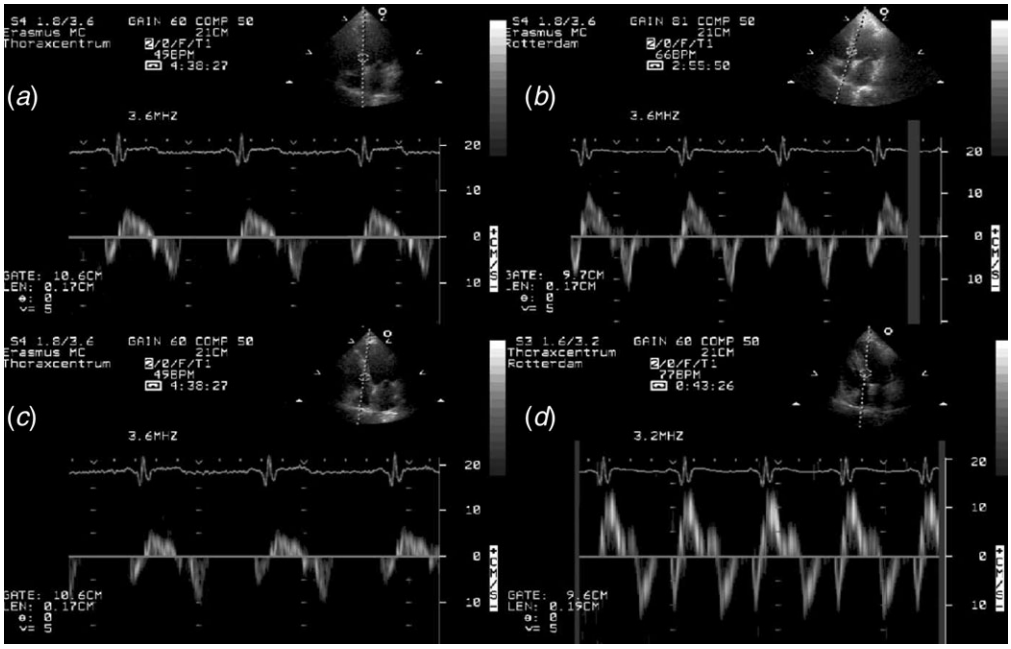

Figure 7 A significant increase of peak systolic velocity measured by TDI is shown at baseline (upper part) and at 1-year follow-up (lower part) both at rest (*A* and *C*) and after a low-dose dobutamine stress echocardiography (*B* and *D*).

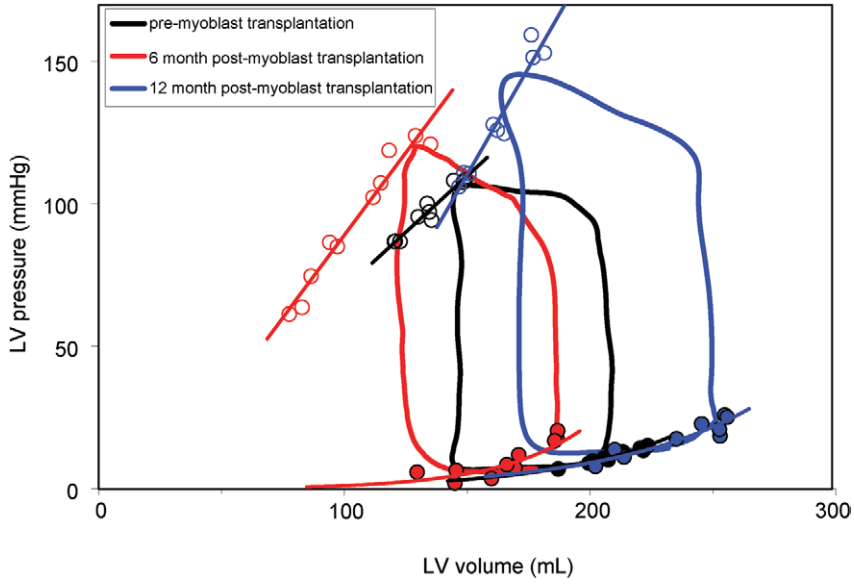

Figure 8 PV loops at baseline, 6 months and 12 months after myoblast transplantation. A significant increase in stroke volume, contractility (represented by ESPVR increased slope and leftward shift) and of diastolic stiffness (represented by upward shift and steeper EDPVR) is shown both at 6 and 12 months.

ventricular tachycardia (VT) were observed in first two patients during early post-operative period, but prophylactic amiodarone administration in the other patients prevented VT episodes so that no anti-arrhythmic treatment was continued later then 6 weeks during follow-up. In the MAGIC trial, designed by Menasché *et al.*, all the 97 treated patients received an AICD after cell transplantation; the trial was suspended in February 2006 after 120 patients were enrolled and the assessment of the risk/benefit ratio is currently under way.

The possible arrhythmogenic effect has been also noticed in trials using an endoventricular catheter-based approach. In the study conducted by Smits *et al.*,[46] one patient received an AICD 6 weeks after the myoblast injection and more seriously, two sudden deaths occurred, triggering the study steering committee to

consult the independent data safety monitoring board. The trial was temporarily suspended and resumed after having implemented the safety measures. Observations from percutaneous series in the POZNAN trial[43] indicate successful prevention of cell transplantation-related ventricular arrhythmias by prophylactic amiodarone administration, suggesting that AICD implantations are not necessarily needed in all patients who undergo myoblast transplantations.[48]

In the absence of electromechanical coupling, the arrhythmogenic mechanisms remain unclear. One possible explanation is that myoblasts, having the ability to generate burst of action potentials, may induce ventricular extrasystoles through electrotonic interactions.[49] Moreover, an arrhythmogenic role could be related to the procedure in itself, including myocardial puncture, inflammatory response to transplanted cells and immune reactions[49] rather than to possible problems with electromechanical coupling between newly developed myocytes and cardiomyocytes.

At the current stage, with only a small number of patients having undergone autologous skeletal myoblast transplantations, it is difficult to predict whether skeletal myoblasts are really arrhythmogenic, especially because patients with ischaemic LV dysfunction easily develop ventricular arrhythmia. Nevertheless, future studies on cell transplantation in patients with post-infarction heart failure will have to focus on potential arrhythmogenic effect. Similarly, large phase-two/three clinical trials are needed to assess the efficacy of myoblast transplantation in chronic post-infarction myocardial injury.

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Conflict of interest: none declared.

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