

Review article

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MYOGENIC CELLS APPLICATIONS IN REGENERATION OF POST-INFARCTION CARDIAC TISSUE

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Skeletal myoblasts (SMs) transplantations are the focus of intense investigation due to their therapeutic potential for cardiac repair. Heart diseases are the main problem in humans worldwide, resulting very often in death of patients. Consequently, investigators sought methods which could improve myocardial function in patients with cardiac failure. Stem cells therapy belongs to one of the highly promising and the most realistic methods of treating myocardial infarction. Despite the fact that many cell types were transplanted into damaged myocardium, autologous skeletal myoblasts seem to be the most encouraging cell source for myocardial repair due to their myogenic and contractile phenotype, biochemical and functional similarities to cardiac cells, high proliferative capacity *in vitro* and resistance to ischemia. Moreover, engrafted myoblasts could repopulate post-infarction scar and improve cardiac function. Unfortunately, transplanted SMs do not functionally or electrically integrate with the host myocardium, which could also cause ventricular arrhythmias, therefore, the use of these cells still remains a subject of intensive studies aiming at improvement of this therapeutic method. In this paper we recapitulate the current state of knowledge concerning the use of skeletal myoblasts in the treatment of post-infarction scar tissue, and discuss the problems resulting from their applications.

Key words: *myocardial infarction, post-infarction regeneration, stem cell therapy, myocardium regeneration, skeletal myoblasts*

INTRODUCTION

Stem cells have been under investigations due to their remarkable potential of growth and differentiation into many cell types in the body, with the promise for treating variety of diseases and injuries. Stem cell technology could deliver tissue regeneration after injuries for which natural repair mechanisms are not able to restore functional recovery and for which current therapeutic strategies have minimal effectiveness. Stem cells hold great promise for regenerative medicine due to their potential to regenerate tissues without the production of scar tissue, which is commonly related to healing processes.

Myocardial infarction has become one of the leading causes of death among people in industrialized nations. The percentage of people suffering from heart infarctions is rising every year, which is related to the increasing age of population and with improper lifestyle (bad eating habits, stress *etc.*). Myocardial infarction leads to the loss of cardiomyocytes, which is caused by reduced blood flow triggering a cascade of events including inflammation, formation of non-contracting fibrous scars (leading to a decrease in systolic function and left ventricle remodeling), changes in the workload of the myocardium and, if the damaged area is large sufficiently, it may cause the congestive heart failure (1-3). In the place of cardiomyocytes which underwent apoptosis and necrosis a scar is formed, incapable of contraction. The scar tissue should be replaced by a new, functionally active cardiac tissue in order to restore the

heart functions (4). Rescued cardiomyocytes may attempt to compensate the loss of function and repair the tissue after myocardial infarction. However, myocardial restoration is inadequate due to low regenerative capabilities of mature cardiac cells (they are postmitotic and incapable of replication) to replace dead cells after the process of ischemia (5).

Data mentioned above mobilized physicians to search for methods, which could reshape the dilated left ventricle (LV) through the use of drugs, heart transplantation and cardiac devices. Despite a significant improvement in patients' survival, these therapies have been shown to be insufficient (3, 6, 7). Consequently, investigators are currently exploring the use of stem cells transplantation into myocardium to limit infarct size and reduce the subsequent cardiac failure incidence. At the moment, cell transplantation is a very promising and the most realistic method of treating heart disease (8). The main goal of this therapy is to repopulate post-infarction scar tissue with contractile cells, which could replace dead cardiac myocytes, restore systolic function and prevent the remodeling process (3, 9). Research has been conducted to find the optimal cell type which could be implanted in the post-infarct scar. Investigators have sought the mechanism by which the implanted cells improve cardiac function, and factors allowing their longer survival after transplantation (8, 10, 11).

So far the exact mechanism of stem cells activity in treating myocardial infarction remains unknown. Some reports indicate their differentiation potential into cardiomyocytes, which are

capable of contraction. Others suggest the possible role of factors released from stem cells after transplantation, which act locally to stimulate angiogenesis and recruit other cells to the area of infarction scar, thus improving cardiac function by inhibiting apoptosis and fibrosis (12-14).

Many different cell types have been transplanted into myocardium in animal models and subsequently in humans (10, 14, 15). However, autologous skeletal myoblasts seem to be one of the best candidates for cardiac repair, due to their biological properties, structural and functional similarities between cardiomyocytes and myocytes, as well as lack of ethical and immunological problems (8, 16).

Therefore, in this paper we have tried to review the progress in skeletal myoblast-based treatment of myocardial infarction, summarize their current state of clinical application in the regeneration of cardiac tissue, as well as, discuss the complications resulting from skeletal myoblast transplantation into the infarcted area.

SKELETAL MYOBLASTS

Skeletal muscle stem cells (SMSC) are quiescent mononucleated cells, which are normally located outside the sarcolemma within the basal lamina of the muscle fiber (8, 17). Their activation is a result of injury, which is manifested by mobilization, proliferation, differentiation and ultimately fusion into new muscle fibers (17-20) (*Fig. 1*). Jankowski *et al.* (21) described four different populations of SMSC: satellite cells (SC), muscle derived stem cells (MDSC), skeletal myoblasts (SMs) and their side population (SP) (8), however myoblasts are progeny of satellite cells obtained upon biopsy of skeletal muscle followed by *in vitro* culture (3).

Due to the high regenerative potential of myoblasts, the ease of isolation, high *in vitro* proliferative capacity, their autologous origin (which solves the problem of rejection by organism) and unipotential characteristic (which prevents tumor formation), they are one of the most encouraging cell sources for clinical therapy including muscle regeneration (muscular dystrophies), heart disease and urinary incontinence (6, 8, 9, 22-24) (*Fig. 2*). Moreover, these cells are resistant to ischemia, which increases their chance of survival in the environment of infarction scar (10, 25).

The transplantation of skeletal myoblasts has been used both experimentally and clinically in the attempts to restore cardiac function. Several clinical trials have been performed in order to evaluate the effectiveness of myoblasts in the treatment of heart failure. Research conducted by Menasche *et al.* (26) based on human autologous skeletal myoblasts transplantation into nonviable heart scar revealed after a 10-month follow-up study an increase in left ventricular ejection fraction (LVEF) and also improvement in systolic shortening in SMs-implanted scar. Moreover, their elastic properties provided the strengthening a scaffold of ventricular wall and reduction in size of post-infarction scar in examined patients. The only adverse event, probably related to the procedure, was the occurrence of sustained, monomorphic ventricular tachycardia (VT) in a short time after surgery in some patients but most were clinically well tolerated (26). Similarly, the trial conducted by Zhang *et al.* (27) also presented an increase in LVEF, improvement of ventricular wall thickness and perfusion in the area where the cells were injected after 4-month follow-up examination. Moreover, decreased left ventricular diastolic diameter was recognized. Positive outcomes were also noted by Dib *et al.* (28) during 24-months post-transplantation period. The group not only described improvement in LVEF and reduction in left ventricular systolic and diastolic volumes, but also observed new areas of viability within the infarction scar. Additionally, histological analysis revealed survival and engraftment of the implanted myoblast. However, in this trial authors also reported some adverse events in patients, such as ventricular fibrillation, atrial fibrillation, ventricular tachycardia, whereas the majority of those cases of arrhythmia was generally well tolerated clinically.

The results obtained in the aforementioned clinical trials have shown that autologous skeletal myoblast transplantation for treating heart failure is feasible and safe with some incidents of cardiac arrhythmia occurring in patients after cell transplantation. Furthermore, these results have demonstrated the survival and engraftment of transplanting skeletal myoblasts into the infarcted myocardium and also early benefit of cellular cardiomyoplasty, suggesting that this method offers a potential therapeutic use for heart failure treatment (26-28). Similar results were obtained by Siminiak *et al.* (29) in the first phase of clinical study with a 12 month follow-up. Researchers observed improvement in myocardial contractility and also an increase in the left ventricular ejection fraction. Hagege *et al.* (30)

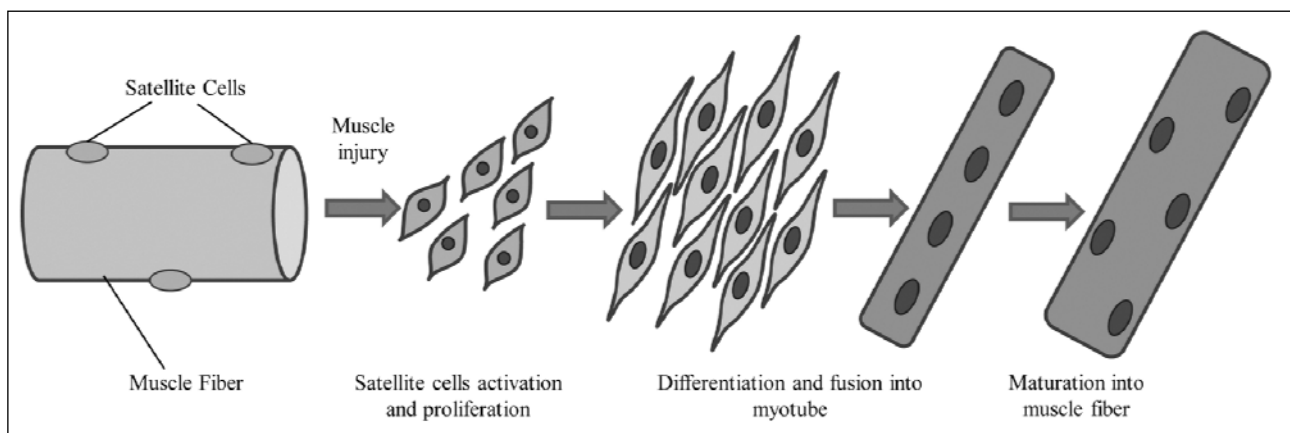


Fig. 1. Stages of muscle fiber regeneration after injury. Satellite cells are quiescent in normal adult muscle and are located outside the sarcolemma within the basal lamina of the muscle fiber. In response to muscle injury, satellite cells become activated and proliferate, prior to differentiation and fusion into myotube, which subsequently mature into muscle fiber. In the regenerated muscle fiber, the nuclei of newly fused satellite cells are initially centralized, whereas, later migrate in order to achieve a more peripheral location.

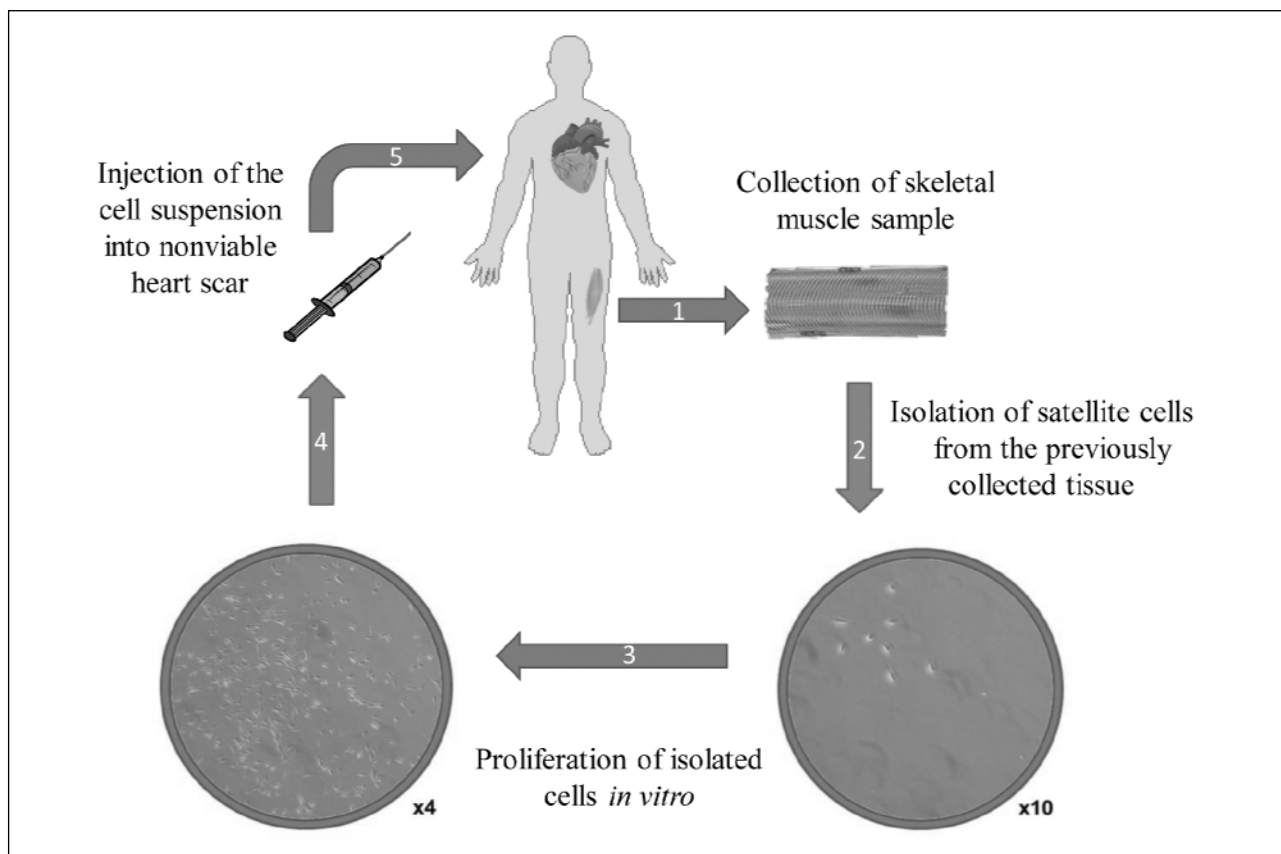


Fig. 2. Schematic representation of procedures leading to autologous transplantation of satellite cells (myoblasts) into post-infarction cardiac tissue.

confirmed in the long-term follow-up study that autologous skeletal myoblast transplantation was safe (absence of tumor development), feasible and relatively straightforward procedure. Additionally, stable improvement of clinical status and ejection fraction was observed in examined patients and eventual arrhythmic risk could be controlled by medical therapy.

On the other hand large multicenter trial (MAGIC) did not confirm the optimistic results showing that myoblast transplantation can be a promising therapy for the treatment of heart failure. In this trial the myoblast-treated patients did not show an incremental improvement in regional or global LV function. Moreover, in MAGIC trial a two times higher number of arrhythmias was noted in each of the myoblast-treated groups in comparison to the placebo group (31).

Obviously, apart from SMs transplantation, there were many studies conducted using other methods that help healing the infarcted area. Authors of these studies confirmed the improvement of cardiac function in patients with heart failure. Steendijk *et al.* (32) reported that implantation of cardiac resynchronization device, used in cardiac resynchronization therapy (CRT), improved LVEF, decreased end-diastolic pressure and reverse remodeling in patients with heart failure and intraventricular conduction delay. Moreover, Witkowski *et al.* (33) revealed that surgical ventricular restoration (SVR) in ischemic heart failure patients improves clinical symptoms and left ventricular systolic function as well as the quality of life. However, SMs transplantation therapy seems to be the most promising because it uses autologous SMs, which are most similar to cardiomyocytes, easy to collect from the patient's muscle and show no immunological conflict. These characteristics of SMs transplantations give hope to complete or

almost complete regression of post-infarction lesions and full heart recovery.

GENETIC MANIPULATIONS ON SKELETAL MYOBLASTS

Manipulations of adherence and gap junction proteins

Successful myoblast therapy depends on several factors, such as: delivery to the target tissue, long-term survival, effectiveness of engraftment, differentiation into cardiomyocytes and integration with the new microenvironment (3).

The mechanism by which implantation of skeletal myoblasts (SMs) may improve heart function is still unclear, in particular, because the transplanted cells are not functionally or electrically integrated with the host myocardium (10). Although, engrafted myoblasts improve post-infarct cardiac function, they do not transdifferentiate into cardiomyocytes and do not appear to express cardiac specific proteins (9, 26). The lack of cardiac-specific genes (N-cadherin and connexin43 (Cx43)), which are required for electro-mechanical coupling with cardiomyocytes, is the reason for inability of engrafted SMs to establish junction with host cardiac cells, which may indicate the post-implantation risk of ventricular arrhythmias (34-37). Studies performed by many investigators, have shown that the major adhesion and gap junction proteins are expressed in undifferentiated skeletal myoblasts, while after differentiation into myotubes the expression of these proteins decreases and is particularly absent in the skeletal muscle grafts in injured rat hearts (34, 35) (Fig. 3). Makkar *et al.* (38) also

revealed that the expression of cardiac-specific proteins was decreased during differentiation of myoblasts to more contractile myotubes, which results in the potential for arrhythmia after transplantation (Fig. 3). This lack of cell-to-cell junctions like connexin43 on skeletal myotubes suggests that these cells do not beat in synchrony with the rest of the heart (23) (Fig. 3). The absence of electro-mechanical coupling contrasts with *in vitro* studies carried out by Reinecke *et al.* (39) and Formigli *et al.* (40). Authors demonstrated that myoblasts co-cultured with cardiomyocytes were coupled by functional gap junctions and also showed that cardiomyocytes enhanced gap junction communication and up-regulate expression of Cx43 in myoblasts. However after differentiation into myotubes the expression of N-cadherin and connexin43 was down-regulated (39) (Fig. 3). Many studies were performed in order to increase the expression of gap junction proteins in SMs, which could reduce the arrhythmogenic potential of transplanted cells. A significant enhancement of gap junction communication *via* connexin43, which expression is normally down-regulated during differentiation, was obtained in *in vitro* studies by gene transfer (41) and cyclic stretching (42). Roell *et al.* (43) using a transgenic mouse model have indicated that the transplantation of genetically modified skeletal myoblasts expressing Cx43 in myocardial infarction scar prevents post-infarction arrhythmia. Similar results were obtained by Fernandes *et al.* (44) *via* the cardiac engraftment of autologous connexin43-overexpressing myoblasts in infarcted rats. These results demonstrated that overexpression of Cx43 caused a significant improvement in cardiac function as well as reduction of the risk of post-transplantation arrhythmia. On the basis of the studies done on rat skeletal myoblasts by two research groups: Suzuki *et al.* (45) and Tolmahov *et al.* (46), it has been concluded that transgenic over-expression of Cx43 in skeletal myoblasts improves their electrical coupling with cardiac myocytes *in vitro* and prevents post-infarct arrhythmia. Moreover, these cells demonstrated more rapid differentiation which could also be advantageous in a graft for transplantation to the heart (45).

In order to achieve the best possible results of stem cell therapy, physicians have investigated the non-transgenic manipulation of connexin43 expression. Perumal Srinivasan *et al.* (10) performed an *in vitro* study, in which skeletal myoblasts were integrated into an engineered tissue construct, and demonstrated a long term survival, ordered alignment, as well as, a preserved ability to differentiate into contractile myotubes. Moreover, their studies showed that the passive longitudinal

tensile stress caused maintained or elevated expression of gap junction and adherence proteins during differentiation, which could indicate that the mechanical loading of SMs may improve electromechanical integration with the myocardium. *In vitro* studies carried out by Zhang *et al.* (47) also demonstrated, that the mechanical forces promoted tissue morphogenesis, increased the rigidity of the extracellular matrix and formation of structurally organized cardiac muscle tissue.

Manipulations of growth factors expression

The major problem of the skeletal myoblasts therapy is the high degree of cell death induced by inflammatory process in response to transplanted cells, as well as, inadequate blood supply in the infarcted tissue. Currently, the genetic modifications of skeletal myoblasts seem to be particularly promising in increasing graft survival. The primary purpose of gene therapy is to increase blood circulation in the injured heart, resulting in a better delivery of oxygen and nutrients to the tissue, which could be beneficial in prevention of massive apoptosis of the transplanted cells (8, 9). In order to increase angiogenesis and vasculogenesis, researchers performed a number of studies on rabbit hind limb ischemia model, including the effect of pro-angiogenic factors, with particular reference to vascular endothelial growth factor (VEGF) and the fibroblast growth factor (FGF), due to their ability to stimulate angiogenesis, proliferation and migration of cells involved in organ repair and also their anti-apoptotic potential (48, 49). Recent studies on rat model of ischemic cardiomyopathy performed by Askari *et al.* (50), Yau *et al.* (51, 52), and Xia *et al.* (53) showed that SMs transfected with VEGF improved cardiac function, induced angiogenesis and also increased vascular density in infarcted area. Furthermore, it was demonstrated that cell-based delivery of VEGF suppressed the inflammatory response and limited apoptosis of transplanted cells and cardiomyocytes in the area of damaged tissue (Fig. 4). Similar observations on the same myocardial infarction animal model were made by Tambara *et al.* (54), who showed enhanced neovascularization, vessel density and increased survival of implanted cells, when myoblast expressing fibroblast growth factor were grafted into infarcted scar. *In vitro* research on murine myoblast, using simultaneous overexpression of two potent pro-angiogenic genes encoding the fibroblast growth factor 4 (FGF4) and vascular endothelial growth factor-A (VEGF-A), revealed the tendency to faster proliferation of murine C2C12 cells and increased capillary formation, which

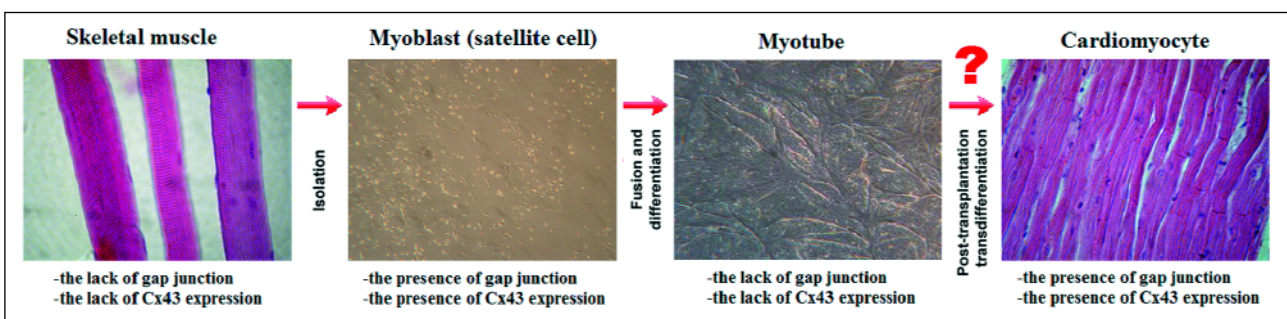


Fig. 3. Scheme of possible post-transplantation transdifferentiation of myoblasts into cardiomyocytes. Myoblasts (satellite cells) are isolated from skeletal muscle and transplanted into post-infarcted heart tissue. In *in vitro* culture myoblasts show the presence of gap junctions and connexin proteins, *e.g.* Cx43. After transplantation into an infarct area of heart tissue they fuse and differentiate into myotubes, losing the characteristic adherence and gap junctions. Due to the lack of cardiac-specific genes (N-cadherin and connexin43) it is still unclear whether myoblasts can transdifferentiate into cardiomyocytes after engraftment. Images from contrast phase microscope presented on the scheme were taken during authors own studies. Based on Siminiak *et al.* (23).

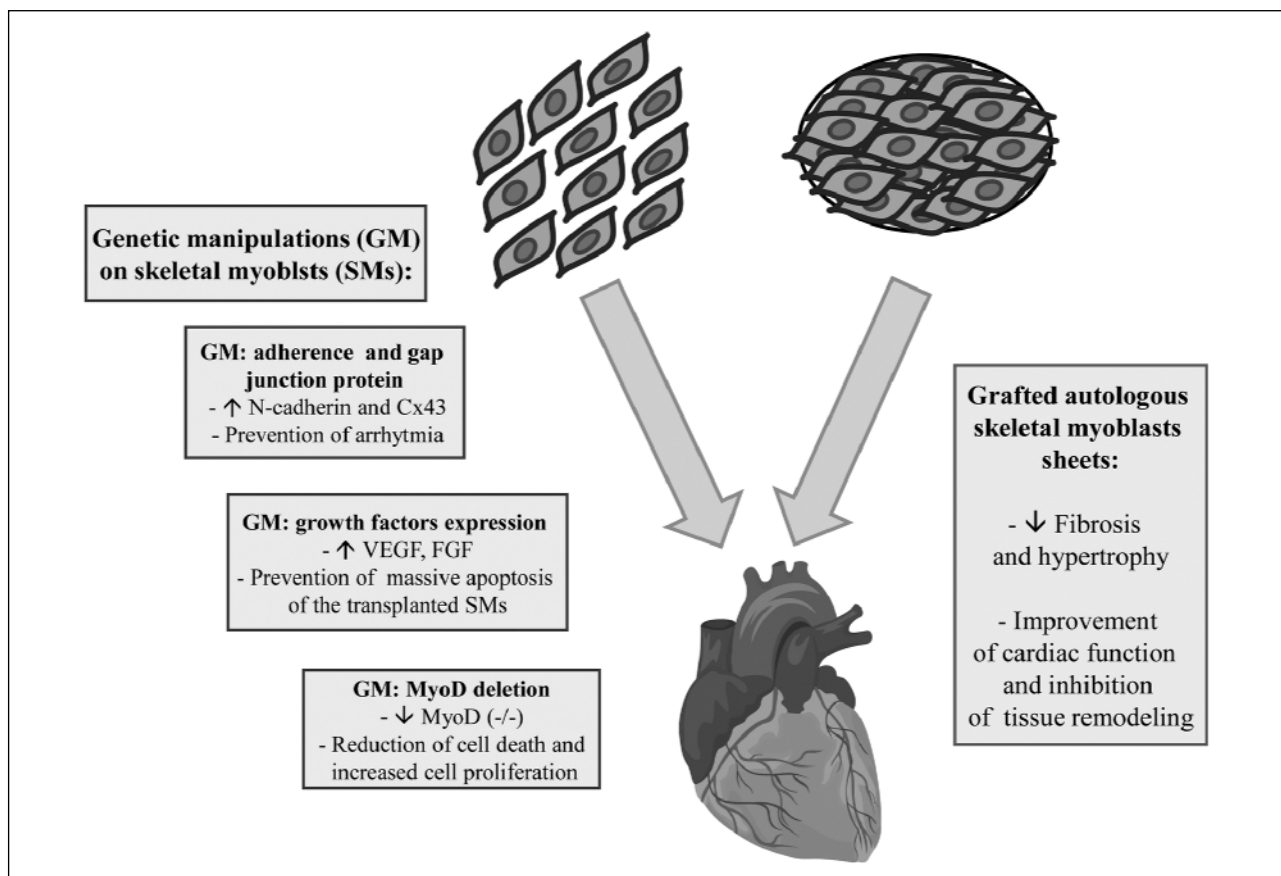


Fig. 4. Methods of skeletal myoblasts (SMs) modifications, used in order to increase their survival after transplantation into infarcted heart, improve cardiac function and to reduce the arrhythmogenic potential of transplanted SMs.

could improve the delivery of oxygen and nutrients to the infarcted zone, and probably prevent massive cell death after stem cells transplantation (8) (Fig. 4).

Additionally, studies by Niagara *et al.* (55) and Suzuki *et al.* (56) demonstrated that pharmacologic preconditioning of myoblasts, leading to increased viability of the cells under oxidative stress *in vitro*, enhanced cell survival after the injection into cardiac tissue (57).

MyoD deletion

Research performed by Nakamura *et al.* (14) on mouse model demonstrated that myoblast lacking the MyoD gene (MyoD^{-/-}) exhibited high resistance to hypoxia, causing a superior efficacy of engraftment, as a large number of MyoD^{-/-} myoblast survived after transplantation. Moreover, the authors observed a reduction of cell death and increased cell proliferation, as well as improvement in systolic cardiac function (there were reductions of left ventricular end-diastolic and end-systolic dimensions, and increased ejection fraction) (Fig. 4). This study showed that transplantation of MyoD^{-/-} myoblast into an infarcted mouse heart induced angiogenesis in the area of injury *via* the secretion of paracrine angiogenic factors including the stromal cell-derived factor-1 (SDF-1) and placental growth factor (PlGF) by MyoD^{-/-} myoblast. The research performed earlier by Hirai *et al.* (58) on mice revealed that MyoD^{-/-} myoblasts acquired a remarkable resistance to apoptosis by up-regulation of anti-apoptotic genes, including: Pax3, Bcl-2, Bcl-xL. The results presented above, suggest that myoblasts with suppressed MyoD function, might be useful in the stem cells-based treatment.

USE OF AUTOLOGOUS SKELETAL MYOBLAST SHEETS

To overcome problems related to the intramyocardial injection of cells, including cell loss and a limited graft area, a cell delivery system was described, which uses tissue-engineered myoblast grafts grown as sheets (59). Research conducted on canine (59), hamster (60) and rat heart failure model (61, 62) have shown, that transplantation of autologous skeletal myoblast sheets allowed better improvement of the global cardiac function compared with direct myocardial injection (Fig. 4). Skeletal myoblast sheet transplantation repaired the impaired myocardium, suppressed the ventricular fibrosis and increased capillary density, which may indicate that this method might become a novel therapeutic strategy for patients with severe heart failure (Fig. 4). It is considered, that the improved therapeutic effect of the myoblast sheet results from their production of paracrine factors, which locally stimulate the injured myocardium (61). Sekiya *et al.* (63) demonstrated, using rat model, that layered implementation of myoblast sheets attenuates adverse cardiac remodeling of infarcted heart. A substantial improvement of cardiac function was manifested by fewer fibrosis and less hypertrophy (Fig. 4). Moreover, after transplantation of layered myoblast sheets into the injured area the amount of elastic fibers significantly increased. This data suggests that the expression of elastin is one of the main factors inducing therapeutic effect of myoblast sheets engraftment and the overexpression of elastin could enhance these effects. Uchinaka *et al.* (11) has recently revealed that the elastin gene modification in the implanted myoblast sheets improves cardiac function and inhibits tissue remodeling

and dilation of the left ventricular chamber. Researchers suppose that the improvement in the left ventricular performance may be due to the formation of elastin fibers in infarcted area. Moreover, elastin gene-transfected myoblast sheets improved the long term effects of cardiac function, caused by the secretion of paracrine growth factors in the early stage of treatment, and later by the formation of elastin fibers, which generate elasticity in the infarcted zone, as their lifespan is extremely high.

Another problem that needs to be approached in the field of autologous skeletal myoblasts transplantations is the number of implanted cells and the time of implantation after myocardial infarction. Simultaneous injection of large number of cells causes massive cell death and a substantial risk of tissue overgrowth (3). Research conducted on rat models (54, 64) have shown that repetitive injection of myoblast improved the function and contractility of left ventricular, and also increased the engraftment area, as compared to the single implantation. These results suggest that the repeated administration of myoblasts may reduce the risk of massive cell death more effectively than a single injection of their higher number and this method is also a safe therapeutic strategy for the infarcted myocardium treatment.

CONCLUSIONS

Skeletal myoblast-based therapy for heart failure is one of the most promising methods of treatment due to the fact that the implantation of SMs increases cardiac contractility, improves post-infarct cardiac function, limits infarct expansion and heart remodeling. Moreover, skeletal myoblasts seems to be one of the best candidates for cardiac repair because of their myogenic and contractile phenotype, high regenerative potential and proliferative capacity *in vitro*, ease of isolation, availability for autologous transplantation as well as resistance to tissue ischemia.

Although skeletal myoblasts are significantly tolerant to poor graft environment, a large number of cells transplanted into myocardium do not survive due to inadequate blood supply in infarcted tissue. Nowadays, the genetic modifications of SMs seem to be a very effective method of increasing their survival, thereby resulting in a better delivery of oxygen and nutrients in the damaged tissue, which could be beneficial in prevention of massive apoptosis of engrafted cells (Fig. 4).

Despite encouraging data related to clinical applications of SMs into post-infarction scar tissue, the mechanism by which these cells improve cardiac function is still unknown. Studies have shown that SMs do not form electromechanical connections with host cardiomyocytes, which results in ventricular arrhythmias. In order to reduce the arrhythmogenic potential of transplanted cells the transgenic and non-transgenic manipulations were conducted, leading to significant improvement of heart function and reduction of post-transplantation arrhythmia (Fig. 4).

It is undeniable that stem cells therapy brings hope to many patients with myocardial infarction who, otherwise, may suffer from the continuing progress of heart disease, finally leading to death.

Acknowledgements: This study was supported by National Science Centre Grants No. N N311 123538 and UMO-2011/03/B/NZ9/03987.

The authors thank Dr. Malgorzata Gajewska for help with manuscript preparation.

Conflict of interests: None declared.

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Received: March 19, 2013

Accepted: July 19, 2013

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