WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 7, Issue 3, 425-455. Review Article ISSN 2277 – 7105

SIMPLE CLINICAL REVIEW OF SKELETAL MYOBLASTS IN THE TREATMENT OF ISCHEMIC CARDIOMYOPATHIES

***Mohammed A. Syam, MD Intern, Amin M. Sammour, MD Intern, Ali Abdulghafour, MD Intern, Mohammed M. Aloosh, MD Intern**

Student, Intern, SRC, Medicine.

Article Received on
18 December 2017,

Revised on 08 Jan. 2018,
Accepted on 29 Jan 2018

DOI: 10.20959/wjpr20183-10126

***Corresponding Author**
Mohammed A. Syam, MD Intern
Student, Intern, SRC, Medicine.
dr.ammeen.sm@hotmail.com,
Dr.mas93@hotmail.com,
Kh.haider@sr.edu.sa,

ABSTRACT

Background: Significant recent developments have occurred in the field of cardiac regeneration and stem-cell therapy. Understanding the new technological advances in cell therapy will ultimately allow us to achieve a goal of cell-based cardiac repair. **Objective:** This study aims to: 1) Provide a reference paper analyzing multiple preclinical and clinical studies in the SkMs transplantation, and analyzing the last progression has been reached. 2) Discuss benefits, complications, and limitations encountered in this field. 3) List suggested solutions for common complications being faced. 4) Discuss the future prospective and what can be achieved. **Methods:** PubMed database was used for articles selection, and the following keys used in the mesh {skeletal myoblasts, Cardiomyopathies, Ischemic Heart Disease, Myocardial Infarction}. A total of 174 articles were found, with further restriction by PubMed filter, and reviewing the articles titles and abstracts the final results were 47 articles. **Inclusion criteria:** all relevant articles to our review with the following topics: skeletal myoblasts, Cardiomyopathies, Ischemic Heart Disease, and Myocardial Infarction. **Exclusion criteria:** Other articles not related to this field. The data will be extracted according to specific form in which it's going to be reviewed by group members to weigh the benefits versus complications, last progress have been achieved, and limitations encountered by researchers. **Conclusion:** SKM treatment of IHD have been progressed since its beginning, a lot of obstacles have been solved, but still significant work need to be done to overcome the remaining ones. The recent results are very encouraging, and promising to emphasize more in this filed.

KEYWORDS: (Skeletal myoblasts, Ischemic heart disease, and myocardial infarction).

INTRODUCTION

In the US, an estimated 15.4 million people have coronary heart disease, of whom 7.6 million are affected by myocardial infarction and 2.1 million by congestive heart failure.[1] Actually this dilemma is not restricted to US alone, but it is a global issue where cardiovascular disease is the leading cause of death worldwide accounting for nearly 17 million deaths per annum according to the World Health Organization.[2]

In ischemic cardiomyopathies, the cardiac cells are injured irreversibly in results to reduced blood supply to the heart tissue, which induces ischemic damage, and ultimately leading to the death of cardiomyocytes, and disruption of cardiac function resulting in what is called heart failure. Despite pharmacologic and surgical approaches, heart failure remains one of the significant diseases lacking the complete cure. So far, the only definitive treatment for heart failure is heart transplantation, which can't be depended on as the first line treatment due to the limited availability of donor hearts and complications from immunosuppressive therapies.[3] Therefore, this has pushed the researchers looking for another options of therapy.

In the last two decades, the classical concept of the heart as an organ with extremely limited regenerative capacity has been changed. Different ideas have been developed to induce regeneration of the dead cardiomyocytes by introducing stem cells into the affected area.[4-5] Many types of progenitor cells having the capability to differentiate into heart cells (such as : smooth muscle cells, embryonic stem cells, mesenchymal bone marrow stromal cells, hematopoietic stem cells, and skeletal myoblasts) were tested in vitro, then introduced by different sophisticated methods to determine their possible beneficial effects in vivo.[6] Each one of these stem cells has its own characteristics, which favor some on other. As the skeletal myoblasts proved their capability in multiple experiments to replace the dead cardiac myocytes with maximum benefits and limited complications, they attracted the researchers to proceed in different detailed trials including the use of various animal models and introduction techniques, multiple transfections overexpressing various factors (modified SkMs), and finally landing up into the human clinical trials.[7]

In this article a critical review including some of SkMs journey in the treatment of ischemic heart disease including their beneficial effects, limitations encountered and their possible solutions, and what is the future prospective of their uses.

METHODS AND MATERIALS

❖ **Sample**

PubMed was chosen as the search database for the articles selection, because it's one of the major research databases within the suite of resources that have been developed by the National Center for Biotechnology Information (NCBI). The following keys used for the Mesh **("myoblasts, skeletal"[MeSH Terms] OR ("myoblasts"[All Fields] AND "skeletal"[All Fields]) OR "skeletal myoblasts"[All Fields] OR ("skeletal"[All Fields] AND "myoblasts"[All Fields])) AND (("therapy"[Subheading] OR "therapy"[All Fields] OR "treatment"[All Fields] OR "therapeutics"[MeSH Terms] OR "therapeutics"[All Fields]) AND ("cardiomyopathies"[MeSH Terms] OR "cardiomyopathies"[All Fields])),** a total of 174 articles were found. Further modification by using the filter "restriction to human studies- Last 5 years", and "restriction to animal's studies- Last 10 years" left us with 81 articles, 68 of them were about animals, and 13 articles were about human. Further screening by title, and reviewing the abstracts yielded a 47 articles which were enrolled, of whom 34 articles were about animal studies, and 13 articles were about human studies (fig 1). **Inclusion criteria:** the articles were selected based on the relevance to the project which should include one of the following topics. {Skeletal myoblasts, Cardiomyopathies, Ischemic Heart Disease, Myocardial Infarction}. **Exclusion criteria:** all other articles which didn't have one of these topics as their primary end point, or repeated studies.

❖ **Measures**

Assessment of the benefits versus the complications of SKM in the treatment of IHD, the limitations that encountered the researchers, and analyzing the last progression has been reached.

To ensure absences of bias, the data were extracted according to specific form that were done before the start of the study. Based on this form the data were extracted, filled in the form, and divided between the group members to review it before finalization for analysis.

❖ **Analysis**

No software was used, The data were extracted based on specific form that contain.

(Title of the study, name of the author, Objective, Number of the sample, Method of Intervention, Results, and Outcomes), these data were reviewed by the group members to weigh the benefits against the complications, to know what are the new things in this filed,

and where it stands now. Double revision of each member's outcomes was applied to ensure the validity and minimize the mistakes.

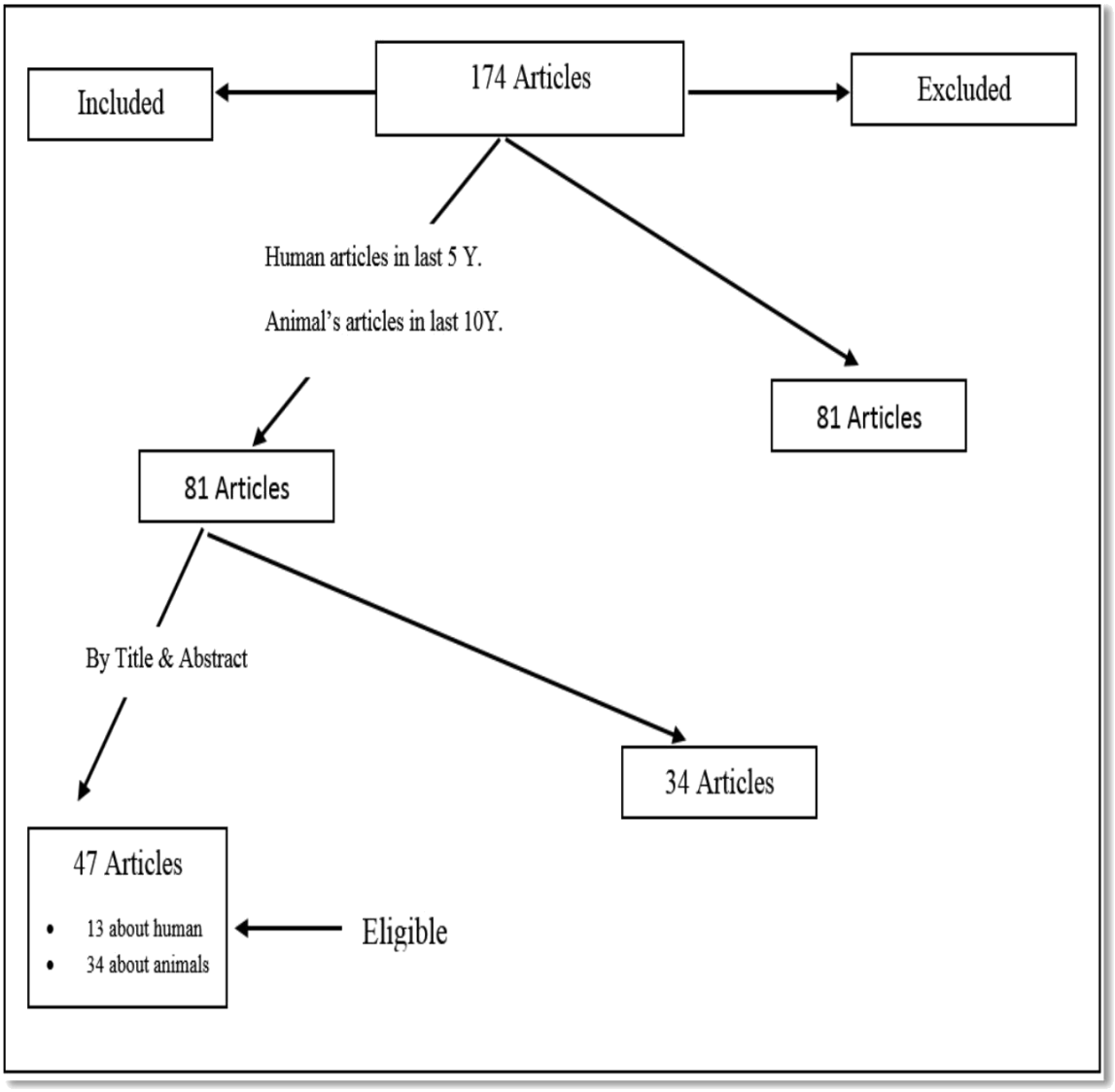

Figure 1: Flow chart for processing the articles included.

RESULTS: (Table 1)

❖ **In vitro**

Study 1

In this study human & murine myoblasts were obtained & cultured in standard in vitro conditions. Myoblasts were transfected with DNA plasmid expressing endothelial nitric oxide synthase (eNOS). Then after; cell proliferation, cycle, apoptosis, and angiogenesis were measured by specific tests. Electroporation appeared to be an efficient transfection method. High amounts of secreted protein were obtained in both cell types. Human myoblasts did not

exhibit any changes in cell cycle; however, eNOS transfected murine myoblasts revealed a significant reduction in cell cycle ratio compared to controls. Transplantation of myoblasts overexpressing eNOS could be promising for cell therapy in regenerating the infarcted heart.

❖ **Animal Studies**

➢ **Small Animals**

- **Rats**

Study 1

In this study the aim was to study the effect of SkMs overexpressing vascular endothelial growth factor (VEGF) on the infarcted myocardium using both scaffold implantation of treated /untreated SkMs, & direct intramyocardial injection of treated / untreated SkMs. Sham's operation group was used as a control. 5 groups were included (n= 10 per group). Transplantation of VEGF-overexpressing SkMs leads to neovascularization of the infarcted hearts, but no functional improvement or reduction of the infarction size were observed.

Study 2

The study hypothesis was usage of genetically engineered myoblasts overexpressing the paracrine factors: vascular endothelial growth factor, hepatocyte growth factor, stromal cell-derived factor-1 (VEGF-A, HGF, SDF-1, or Akt1) is believed to enhance the regenerative capacity of SkMs injected into an infarcted heart. In vitro, a significant increase in expression of VEGF-A, HGF, SDF-1, and Akt1 after transfection was noticed. In vivo, transplantation of growth factor-producing SkMs seeded scaffolds resulted in enhanced angiogenesis (VEGF-A, HGF, and Akt1) or a reduced infarction zone (SDF-1 and Akt1) in the ischemically damaged myocardium. These 4 factors may contribute to the paracrine effects that play an essential role in the regeneration of ischemic myocardium by preventing apoptosis and inducing angiogenesis.

Study 3

In this study it was hypothesized that transplantation of SkMs overexpressing stromal cell-derived factor-1 (SDF-1) might improve cardiac function after MI. 5 groups were included (n=50): sham operation, implantation of scaffold seeded with SDF-1 overexpressing SkMs (PU-SDF-1-SkM)/or untreated SkMs(PU-SkM), intramyocardial injection of SDF-1 overexpressing SkMs(Inj-SDF-1-SkM) /or untreated SkMs (Inj-SkM). Sham animals, showed a clear decrease in systolic function from intervention to study end. In group Inj-SkM and

PU-SkM, all hemodynamic parameters remained unchanged. Systolic function was significantly improved in groups Inj-SDF-1-SkM and PU-SDF-1-SkM at study end without a difference between the two SDF-1 groups. Diastolic function was also increased in group Inj-SDF-1-SkM but not in PU-SDF-1-SkM. Histological analysis revealed a reduced infarction size in all treatment groups at study end but enhanced neovascularization was not observed. Transplantation of myoblasts overexpressing SDF-1 improves cardiac function after MI. The restoration of hemodynamic parameters is accompanied by a reduction in infarction size. The scaffold-based cell application showed no advantages over intramyocardial cell injection.

Study 4

The study aims were to: describe the role of microRNA-21 (miR-21) in interleukin-11 (IL-11) signalling during preconditioning of SkMs, study the long-term fate of preconditioned MY (PCMY) post-transplantation in the infarcted heart.3 groups were included (n=50): intramyocardial injections of basal Dulbecco's Modified Eagle's Medium (DMEM) without cells or containing non-PCMY or PCMY. Two-fold higher survival of male donor PCMY post-engraftment and up to 4 months. PCMY improved angiogenic response. Indices of myocardial contractility including EF and fractional shortening showed significant improvement in PCMY-treated animals. PCMY has a significant role in improving the functionality of the infarcted hearts which persisted up to 4 months.

Study 5

The study aims were to: determine the differential gene expression profile of SkMs and their progeny (myotubes) in comparison with fibroblast-like cells, characterize the cytokines and growth factors released by SkMs, and correlate in vitro and in vivo findings. 2 groups were included (n=11): injection of human SkMs, or culture medium. Gene expression revealed up-regulation of pro-angiogenic (PGF), antiapoptotics (BAG-1, BCL-2), heart development (TNNT2, TNNC1) and extracellular matrix remodelling (MMP-2, MMP-7) genes in SkMs. Culture of smooth muscle cells (SMC), cardiomyocytes (HL-1) and human umbilical vein endothelial cells (HUVECs) with SkMs-released conditioned media demonstrated an increased proliferation of HUVEC, SMC and cardiomyocytes and a decrease in apoptosis of cardiomyocytes. In vivo studies demonstrated expression of human-specific factors similar to those in vitro 1 month after transplantation. SkMs-secreted factors may contribute to the beneficial effects of myogenic cell transplantation in infarcted myocardium.

Study 6

The study aim was to evaluate the long term functional outcomes of SkMs Seeded Patches Implanted on Infarcted hearts. 4 groups were included (n=23): Sham operation, SkMs seeded scaffold, non-seeded scaffold, SkMs intramyocardial injection. Progression toward heart failure was significantly prevented for up to 6 months after injection of SkMs and for up to 9 months following biograft implantation. After 12 months the effect of SkMs vanished & histological studies showed an absence of the transplanted SkMs within the scaffold. Tissue therapy is superior to cell therapy for stabilization of heart function. However beneficial effects are transient.

Study 7

The study aim was to assess whether transplantation of SkMs overexpressing placental growth factor (PlGF) would stimulate angiogenesis and enhance SkMs survival in the failing heart. 2 groups were included (n=95): Intramyocardial injection of autologous SkMs overexpressing PlGF, unmodified SkMs or Ringer solution (control group). Sham-operated rats as an additional non-infarct controls. LV function significantly improved with time, and fractional shortening on day 86 was significantly enhanced in transfected SkMs group relative to control and unmodified myoblast groups. Vascular density and SkMs survival were enhanced in transfected SkMs group relative to other groups. Mean fraction of fibrotic scar tissue was decreased in unmodified and transfected SkMs groups relative to controls on day 86. LV wall thickness was significantly increased in transfected SkMs group relative to other groups. Intramyocardial injections of autologous SkMs overexpressing PIGF improved cardiac function, attenuated cardiac remodeling, induced angiogenesis, and probably enhanced survival of grafted SkMs.

Study 8

In this study it was hypothesized that Hepatocyte Growth Factor (HGF) overexpressing stem cells would restore cardiac function after MI. 5 groups were included (n=50): sham operation, the implantation of scaffold seeded with transfected SkMs (PU-HGF-SkM)or untreated SkMs , or direct intramyocardial injection of a transfected (Inj-HGF-SkM) or untreated SkMs (Inj-SkM). In sham animals a decrease in systolic and diastolic function was observed. Treatment with untreated SkMs did not lead to any significant changes in hemodynamic parameters between the intervention and 6 weeks later. In group PU-HGF-SkM, systolic parameters improved significantly from baseline to study end. Some diastolic parameters were inferior as compared to baseline. In group Inj-HGF-SkM, only pressure half time was

impaired as compared to preinterventional values. Histological analysis showed significantly more capillaries in the infarction border zone in groups PU-HGF-SkM than in sham and Inj-SkM group. The infarction size was not affected by the therapy.Transplanting HGF-transfected myoblasts after MI can limit the development of ventricular dysfunction. Scaffold-based therapy in combination with gene therapy accelerates this capacity. This hemodynamic improvement is accompanied by neovascularization, but not by smaller infarction sizes.

Study 9

The authors of this study wanted to compare the host immune cell kinetics, survival profile of donor skeletal myoblasts, and skeletal myoblast graft efficacy after autologous and allogeneic skeletal myoblast transplantation into a rat model of myocardial infarction. A 128 animals were divided into four groups: group 1 received medium only, group 2 received medium and cyclosporine, group 3 received autologous skeletal myoblast transplantation, and group 4 received allogeneic skeletal myoblast transplantation with cyclosporine treatment. The muscle biopsy isolated from tibialis anterior muscles of the rat hind limbs. The final results were transplantation of allomyoblasts increased systolic heart function and limited heart dilation after myocardial injury to a similar degree as automyoblasts and the use of allomyoblasts is feasible and effective for cardiac repair with immunosuppressive treatment as compared with automyoblasts.

Study 10

The authors of this study wanted to compare skeletal myoblast (SMB) with bone marrow cell (BMC) injection to highlight donor cell-specific, late-phase arrhythmogenesis and the underlying factors using a rat postinfarction chronic heart failure model. Female wild-type SD rats underwent permanent left coronary artery (LCA) ligation, 3 weeks after LCA ligation, the animals were randomly assigned to three groups: skeletal myoblast group, bone marrow group and control group. Cells were injected into two sites of the periinfarct area using a 31-gauge needle through rethoracotomy, SMBs were isolated from the extensor digitorum longus of male and mononuclear BMCs were collected from femurs and tibias of male. The conclusion of the results were that cell therapy was capable of improving function of the postinfarction chronically failing heart, but there was late-phase arrhythmogenicity specific to donor cell type and downregulation of connexin43 in the host myocardium is an important factor underlying late-phase arrhythmogenicity after SMB transplantation.

Study 11

The objective of this study is evaluating the roles of the factors in the preservation of matrix architecture (in the infarct and remote regions) by varying the timing (postmyocardial infarction) and delivery site of the implanted cells. Selected rats were randomly distributed into 3 groups; infarcted group, non-infarcted group and control group the muscle cells isolated and collected from the hind limb tibialis anterior of the rats. The results shows that regardless of whether the cells were injected into the infarct or the noninfarcted myocardium early after an myocardial infarction or later, skeletal myoblasts improved cardiac function by preventing ventricular dilation and preserving matrix architecture in the remote region, likely mediated by paracrine effects.

Study 12

The authors induced a myocardial infarction in 72 rats to assess the effects of different self-assembling peptides (RAD16-I or RAD16-II) with or without platelet-derived growth factor (PDGF-BB) on survival of transplanted skeletal myoblasts. Two weeks after creation of infarction, rats underwent a baseline echocardiographic assessment of the left ventricle function and only those with an ejection fraction (EF) below 45% were selected for the trial. After a median sternotomy, these animals were randomly allocated to receive intramyocardial injections of culture medium, skeletal myoblasts, self-assembling RAD16-I peptides, or skeletal myoblasts in RAD16-I peptides. The major finding of this study is that self-assembling peptides (either RAD16-I or RAD16-II) were not beneficial for survival of myoblasts when implanted in infarcted rat myocardium.

Study 13

The authors of this study wanted to investigate the feasibility and efficacy of polyethylenimine (PEI) based human vascular endothelial growth factor-165 (hVEGF165) gene transfer into human skeletal myoblasts (HSM) for cell-based delivery to the infarcted myocardium. A total of 48 rats received cyclosporine injection from 3 days before and until 4 weeks after cell transplantation. The cells were isolated in laminin coated 225 mm2 tissue culture flasks using Super-medium. The results showed that PEI nanoparticle mediated hVEGF165 gene transfer into human skeletal myoblasts is feasible and safe. It may serve as a novel and efficient alternative for angiomyogenesis in cardiac repair.

Study 14

The authors' aim of the study was to evaluate the effect of recipient age on the regenerative response to implantation with young skeletal myoblasts (SKMCs) after a coronary artery ligation.

Young and older rats with infarct were divided into 4 groups: young and older recipients of SKMCs isolated from young donors; young and older recipients of culture media (control animals). In each rat, cells or culture media were injected into the central infarcted region. The results showed that in vitro, proliferation and myotube formation were significantly greater in SKMCs derived from young rats than from older rats. In vivo, young and older recipients of SKMCs exhibited increases in cell density, vascular density and collagen preservation relative to age-matched control animals. However, cell therapy produced significantly greater functional improvements in young recipients than in older, along with relative increases in stem cell factor, cell density, cell survival, and angiogenesis.

Study 15

The authors wanted to know wether pharmacologically preconditioned skeletal myoblasts are resistant to oxidative stress and promote angiomyogenesis via release of paracrine factors in the Infarcted Heart. This study included in vitro male and in vivo female rats, SkMs from male Fischer-344 rats (rSkMs) were preconditioned for 30 minutes with 200mol/L diazoxide, and female fischer-344 rats after permanent coronary artery ligation were grouped to receive 80 _L of basal medium without rSkMs (group 1) or containing 1.5_106 non- preconditioned (group 2) or preconditioned (group 3) rSkMs. The muscle biopsy isolated from hindlimbs of male Fischer and was stimulated with intramuscular injection of 0.3 mL mixture of ketamine (10 mg/mL) and xylazine. The results showed that activation of signaling pathways of preconditioning in SkMs promoted their survival by release of paracrine factors to promote angiomyogenesis in the infarcted heart. Echocardiography revealed improved indices of left ventricular function, including ejection fraction and fractional shortening.

Study 16

The authors wanted to evaluate the effects of combined surgery of myoblast sheets (MS) implantation and preventing the impairment of cardiac diastolic function and late remodeling after left ventricular restoration in ischemic cardiomyopathy. Rat myocardial infarction model was established 2 weeks after left anterior descending artery ligation, a total of 45 male rats were randomized into three groups: 15 rats underwent only rethoracotomy (group sham), 15 underwent left ventricular restoration (group LVR), and15 underwent LVR, which

was immediately followed by myoblast sheets (MS) implantation (group LVR+MS). The results showed that MS implantation decreased cardiac fibrosis by suppressing the profibrotic gene expression and attenuated the impairment of diastolic function and the late remodeling after LVR. It is suggesting that MS implantation may improve long-term outcome of LVR for ischemic heart disease.

Study 17

The authors wanted to assess if scaffold-based transfer could overcome injection-associated problems. A total of 93 operated animals included in this study and 47 could be included in this study, the sample was divided into 4 groups, sham operation (group sham, sternotomy, and adhesiolysis only), group underwent implantation of a scaffold seeded with transfected myoblasts, group with untreated myoblasts and group underwent direct intramyocardial injection of transfected myoblasts. The authors found that morbidity and mortality was 28 rats (30%) died during the study mainly of ventricular arrhythmia after the infarction, another 12 rats died between the treatment and the examination period. In the sham group, they noticed a drop of systolic LV function with significantly reduced ejection fraction and left ventricular pressure.

Study 18

The authors hypothesized that adding Mesenchymal Stem Cells (MSCs) to the SMB cell sheets in vitro might enhance their survival and function after transplantation, which might enhance the benefits of SMB cell-sheet transplantation therapy. A total of 40 animals included in this study and underwent left anterior descending artery (LAD) occlusion to induce ischemia, the rats divided into 4 groups as follow: group 1: transplantation of triple-layer of human h-msc cell sheets, group 2: transplantation of triple-layer of rat r-smb cell sheets, group 3: transplantation of triple-layer co-cultured r-smb and h-msc sheets and group 4: control group. The researchers found that co-cultures of h-SMBs and h-MSCs showed significantly enhanced levels of the cytokines. Co- cultures of h-SMBs and h-MSCs showed significantly increases in ejection fraction and anterior wall thickness at 8 week in compare to group 1 & 2. The LV structure was better maintained after the co-culture cell-sheet transplantation at 8 week in compare to group 1 & 2.

- **Mice**

Study 1

The study aim was to prove the positive effect of connexin 43-modified human SkMs on injured hearts. 4 groups were included (n=19): sham operation (Nacl injection), intramyocardial injection of connexin 43-modified SkMs (MbCx), intramyocardial injection of wild-type SkMs (MbWt). An additional control group (mice that were not subjected to LAD ligation). 32-fold higher expression of the connexin 43 gene in the MbCx cell population compared to controls. The susceptibility of the SkMs to oxidative stress conditions and the fusion index were increased in the MbCx cells. Changes in the Myogenin and Myosin Heavy Chain 2 (MYOG and MYH2) gene expression levels in the Gap junction protein α 1 (GJA1) -modified myoblasts. Significant improvement in the post-infarction echocardiographic parameters after intervention using MbCx cells compared with non-transfected myoblasts (MbWt) and the control, wherein a significant decrease in the LV area change in the short axis was observed at 2 month follow-up. Connexin 43-modified human SkMs have shown positive biological and functional effects.

Study 2

The authors wanted to do comparative analysis of the efficacy of different cell candidates for the treatment of heart disease. This study is designed to evaluate the therapeutic efficacy of 4 cell types in a murine model of myocardial infarction. In this study they have done 2 comparisons, one comparing the efficacy of different adult stem cell types, animals were randomized into 5 recipient groups (1) bone marrow derived mononuclear cells (MN), (2) skeletal myoblasts (SkMb), (3) bone marrow derived mesenchymal cells (MSC), (4) fibroblasts (Fibro), and (5) saline (PBS), and one comparing the effects of myocardial milieus on MN survival, animals were randomized into 2 recipient groups (1) injection at the infarct site and (2) injection at the periinfarct site. The researchers found that this is the first study to show that compared to mesenchymal stem cells, skeletal myoblasts and fibroblasts, mononuclear cells exhibit a more favorable survival pattern, which translates into a more strong preservation of cardiac function.

Study 3

The purpose of this study is to find a new way in introduction of vascular endothelial growth factor (VEGF) gene that is cheap and have a good transfection and low cytotoxicity with manipulated SKM into infarcted myocardium, studying the effect of combined VEGF and SKM in treatment of infarcted myocardium, and introduction of Hypoxia inducible factor-1α

d into the promoter region of human VEGF165 (hVEGF165) gene to form hypoxia regulated hVEGF165 (HRE-hVEGF165) gene for controllable VEGF expression. This study was designed to assess the effect of combined vascular endothelial growth factor (VEGF) and skeletal myoblasts (SKM) in treatment of infarcted myocardium. Hypoxia inducible factor-1α was introduced into the promoter region of human VEGF165 gene to form Hypoxia Regulated hVEGF165 (HRE-hVEGF165) gene to induce VEGF expression in the infarcted area). A total of 3 groups were included in this study each group with 3 mice animals underwent introduction of the VEGF gene by synthesis of polyamidoamine (PAMAM) dendrimer by technique called "One pot method" . On synthesis level, the authors reported that the resulting h-PAMAM was structurally analogous to PAMAM and showed excellent DNA protection ability, low cytotoxicity and high gene transfection efficiency in cell lines.

- **Large Animals**

- **Rabbits**

Study 1

In this study the aim was to determine whether autologus SkMs implantation improves the cardiac function after MI & the possible mechanism. 2 groups were included (n=30): Skms intramyocardial injection & medium injection (control). Both maximum rising and falling rate of the left intraventricular pressure were improved in SkMs injected group compared to the control. The positive desmin immunostaining skeletal myofibers in the myocardium were found throughout the infarcted areas & the border zone. Autologous SkMs can establish muscle tissue when transplanted into postinfarction hearts, & this muscle can treat MI effectively.

Study 2

In this study the aim was to determine whether SkMs transfected with connexin43 fused with green fluorescent protein (Cx43EGFP) could improve the functionality & electrical activity of the infarcted heart. 4 groups were included (n=20): untreated, sham, wild type SkMs, and Cx43EGFP transfected SMs.The group injected with SkMs transfected with Cx43EGFP showed improved LVEF, and electrical activities compared to other groups.Cx43EGFP transfected SkMs look promising & have many beneficial effects on the infarcted heart.

- **Sheeps**

Study 1

The purpose of this study is to evaluate myogenic cell transplantation in an ischemic heart model associated with cardiac resynchronization therapy in compare to passive myogenic cell transplantation. A total of 22 animals included in this study and divided into 4 treatment groups, group 1 control, group 2 cell therapy, group 3 underwent cardiac resynchronization therapy (CRT) and group 4 underwent intrainfarct implantation of myoblasts associated with cardiac pacing, then they underwent ligation of 2 coronary arteries and injected autologous cultured myoblasts with or without pacemaker implantation, the atrial synchronized biventricular pacing was performed using epicardial electrodes. The authors did an echo at 4 & 8 week after the intervention and they found significant improvement in the ejection fraction and limitation of left ventricular dilatation in cell therapy with cardiac resynchronization therapy as compared with the other groups. Differentiation of myoblasts into myotubes and enhanced expression of slow myosin heavy chain was observed in the electrostimulated group.

- **Pigs**

Study 1

In this study it was hypothesized that the use of tissue Doppler strain M-mode imaging to assess myocardial layer-specific strain might enable detailed visual evaluation of the regenerative ability of SkMs. 2 groups were included (n=20): SkMs implanted sheets & Sham's operation . Conventional echocardiogram, tissue Doppler strain, & histological study (Ex vivo) were used to assess the anatomical and functional changes. SkMs sheets implantation resulted in the following: 1) Progression of LV remodeling was prevented & EF increased. 2) The subendocardial strain was significantly greater than the subepicardial strain in the treated border region. 3) Vascular density in the subendocardium was significantly higher than in the subepicardium in the treated region. 4) The expression of VEGF was significantly increased. Tissue Doppler strain analysis allows precise evaluation of the effect of cell-sheet implantation on layer-specific myocardial function.

Study 2

In this study it was aimed to assess whether sequential transplantation of autologous SkM by percutaneous delivery is associated with increased cell engraftment and functional benefit. 4 groups were included (n=20): media control, one, two, or three doses of SkM. At the time of sacrifice, cardiac function was significantly better in animals treated with SkM in comparison

with the control group. The group of animals received 3 doses has significant increase in the LVEF & vasculogenesis as well, and decreased fibrosis as compared to the group received a single dose. Repeated injection of SkM in a model of chronic MI is feasible and safe and induces a significant improvement in cardiac function.

Study 3

In this study it was hypothesized that if SkMs implants are arrhythmogenic, they will facilitate the induction of ventricular tachyarrhythmias by promoting heterogeneous propagation of activation wavefronts. 2 groups were included (n=10): subepicardial injection of SkMs & injection of saline (control). Once explanted, epicardial wavefronts over SkMs and adjacent control areas were optically mapped. In SkMs hearts, fibrosis and differentiated cells were found and no tachyarrhythmias were induced. Time taken for the wavefront to depolarize the SkMs and surrounding areas was similar, becoming only slightly longer at SkMs areas after an extra-stimulus. The saline hearts showed similar results. In normal swine hearts, myoblast implants promote localized fibrosis and slightly retard epicardial wavefront propagation only after extra-stimuli. However, SkMs implants are not associated with local re-entry and do not facilitate ventricular tachyarrhythmias in the whole normal heart.

Study 4

The study aim was to in vivo evaluate the effects of autologous SkMs transplantation on myocardial morphology, function, perfusion and scar, and compare percutaneous versus surgical delivery using cardiovascular magnetic resonance (CMR). 2 groups of chronic MI were included (n=10): percutaneous injection of SkMs, and injection via surgical mini-thoracotomy. Decrease in indexed left ventricular end-diastolic volume, thickening of the infarct-related segment (IRS) wall, increase in left ventricle (LV) ejection fraction. Scar tissue within IRS decreased, whereas the number of nonviable segments decreased. Myocardial perfusion of IRS improved. The arrhythmogenic peri-infarct zone increased after SkMs transplantation. Benefits were similar by percutaneous or by surgical delivery. CMR reveals reversed remodeling and improved systolic function, perfusion, and scar after SkMs transplantation. A relative increase in the arrhythmogenic peri-infarct border zone may explain previously reported arrhythmia. Percutaneous and surgical transplantation both lead to comparable improvements in chronic MI.

Study 5

In this study it was hypothesized that autologous SkMs sheets regenerate the infracted myocardium as a preclinical trial. 2 groups were included (n=10): sham operation, autologous SkMs sheet implantation. Cardiac performance was significantly improved in the SkMs group 3 and 6 months after intervention and LV dilatation was well attenuated in the SkMs group. Affected diastolic and systolic functions in infarcted anterior wall were significantly recovered. Vascular density was significantly higher in the SkMs group than the control group. Fibrosis and cell diameter were significantly lower in the SkMs group. Skeletal origin cells and well developed- layered smooth muscle cells were detected in the implanted area. Better myocardial perfusion and more viable myocardial tissue noticed in the distressed myocardium receiving SkMs sheets compared with the myocardium receiving no sheets. SkMs sheet implantation improved cardiac function by attenuating the cardiac remodeling of ischemic myocardium.

Study 6

The authors' aim of this study was to evaluate the effect of myoblast transplantation on left ventricular function, perfusion, and scar formation after compromised coronary flow. A total of 74 pigs included in this study and divided into 2 groups, treatment group and control group, then they underwent a coronary vessel (proximal left circumflex artery) ligation. Skeletal muscle was biopsied for isolation from the porcine vastus lateral muscle. Two weeks after ligation, animals were randomly selected to receive intramyocardial injections of myoblasts. The researchers found that peak-filling rate of the left ventricle improved in the myoblast group but not in the control group, peak ejection rate and duration of diastole improved only in the myoblast group, ejection fraction or local thickening did not change and fibrosis and perfusion were similar in both groups, but more microvessels were present histologically in the myoblast group.

Study 7

The authors wanted to compare the effectiveness of direct adenoviral angiopoietin-1 (Ad-Ang-1) injection with transplantation of skeletal myoblasts (SkMs) over-expressing angiopoietin-1 (Ang-1) for angiogenic response and improvement of heart function in an experimental porcine model of myocardial infarction (MI). A total of 32 female pigs with experimental MI were randomized into 4 treatment groups then they used Ad-Ang-1 for intramyocardial injection of DMEM or transduction of SkMs, after that the animals were immunosuppressed for 6 weeks then they did euthanasia and their heart tissue was processed

for histological studies. The researchers found that SkMs mediated Ang-1 delivery is associated with improved angiogenic response, regional myocardial perfusion and heart function as compared with direct Ad-Ang-1 administration.

❖ **Human beings (clinical studies)**

Study 1

The authors wanted to determine the safety and preliminary efficacy of transcatheter intramyocardial administration of myoblasts in patients with heart failure (HF) after myocardial infarction. A total of 21 patients were included in the study, the patients were divided into 3 groups, placebo group with 6 patients, low dose of skeletal myoblasts group, and high dose skeletal myoblasts group. The study shows that an improvement in 6-minute walk test can be seen in a period of 6 month in both groups (low-dose and high-dose myoblast) without significant difference. Also, there was increase in brain natriuretic peptide in the control in compare to (low-dose and high-dose myoblast group) which indicate deterioration in the heart function. The only disadvantage seen in the myoblast groups were increase in the frequency of ventricular tachycardia that require treatment in compare to control group.

Study 2

A total of 12 patients were included in the study, to assess the feasibility and safety of intramyocardial injection of autologous skeletal myoblast (ASKM) obtained from vastus lateralis muscle in patients with old myocardial infarction undergoing coronary artery bypass surgery. The follow up period was 3 month, which show up a significant improvement of left ventricular ejection fraction, increase in the contraction ability of the cardiac segments treated with skeletal myoblast, and increase in the tissue viability in the infarcted area. Also, researchers found that skeletal myoblasts implants are not associated with an increase in adverse events, and no cardiac arrhythmias were detected during early follow-up.

Study 3

A total of 10 patients were included in the study to assess the feasibility and safety of skeletal myoblasts transplantation in conjunction with coronary artery bypass surgery for the treatment of post-infarction myocardial as a primarily goal. Also, as a secondary concern, the left ventricular function was assessed during 12 month follow up period. The autologous skeletal myoblasts biopsy were obtained from vastus latralis muscle, and cultured in specific media before implantation. The study shows an interesting thing in which the use of

amiodrone (anti-arrhythmic drug) infusion before skeletal myoblasts transplantation, and initiation of oral tablets after 24 hour of the surgery for 3 weeks after surgery act as prophylaxis against development of ventricular tachycardia. Also, after assessment of left ventricular function, there was significant increase in the ejection fraction which reach up 40% from the initial measurement, and improvement in the cardiac contractility.

Study 4

This study designed to assess the feasibility and safety of autologous skeletal myoblast transplantation in patients with severe ischemic cardiomyopathy. A total of 10 patients were included in the study, muscle biopsy obtained from vastus latralis, and cultured in a media supplemented with bovine fetal serum, basic fibroblast growth factor, dexamethasone, penicillin, and streptomycin. Finally the cells were injected within and around the scar area. At an average of 10 month follow up, the study shows up that the use of autologous skeletal myoblast transplantation induce a significant improvement in the left ventricular ejection fraction, mean New York Heart Association functional class, and the systolic thickening of the infarcted area. In general there were no adverse complications, except four (out of ten) patients showed delayed episodes of sustained ventricular tachycardia and were managed with an internal defibrillator.

Study 5

The authors of this study wanted to assess the histological analysis of hearts from patients with end-stage heart disease who were transplanted with autologous skeletal myoblasts concurrent with left ventricular assist device (LVAD) implantation. A total of 5 patients were included in the study, a sample were taken from vastus lateralis muscle, and sent into a specific cell isolation facility where myoblasts were isolated and grown. Finally the autologous skeletal myoblasts were injected within and around the scar area.The study show up that skeletal muscle cell survival and differentiation into mature myofibers were demonstrated in the scarred myocardium, with significant increase in the small vessel formation. The only serious adverse effect was brief episodes of ventricular tachycardia which occurred immediately in the postoperative period that resolved with betablockers and amiodarone therapy.

Study 6

A total of 4 patients were included in the study to evaluate the potential application of combined transplantation of autologous skeletal myoblasts cells which were obtained from tibialis anterior and/or medial vastus muscle, with bone marrow mononuclear cells which were aspirated from the ileum just before cell transplantation in patients with severely deteriorated ischemic cardiomyopathy. The study shows up a significant improvement in the left ventricular ejection fraction, with remarkable decrease in the brain natriuretic peptide. Concerning arrhythmia the researchers didn't detect any lethal arrhythmia during 6 month follow up period, and they speculated this to the small amount of implanted myocytes into the scarred area, and the mechanical unloading provided by the pre-implanted left ventricular assist device.

Study 7

The study was designed to assess the safety and feasibility of percutaneous myoblast implantation in heart failure patients with implanted cardioverter-defibrillators (ICD).

A total of 54 patients were included in the study, 40 patients of whom included into skeletal myoblasts (SKM) transplantation group, and 14 included in the control group. A SKM biopsy was obtained from quadriceps or gastrocnemius muscle, and went for culture in specific facility. All of SKM transplantation group patients received prophylactic treatment with amiodarone, four weeks prior to cell transplantation to minimize the risk of ventricular arrhythmias. Myoblasts cells were intra-myocardially injected into akinetic segments under fluoroscopic guidance. At 6 month follow up period there were significant improvement in the 6 minute walk test, and mean New York Heart Association functional class in the treatment group in compare to control group. Unfortunately, therapy didn't show any improvement in the global left ventricular ejection fraction. Concerning arrhythmia, there were 12 sustained arrhythmic events and one death after episodes of ventricular tachycardia (VT) in the treatment group and 14 events in the control group.

Study 8

The study were designed to assess the safety, and efficacy of autologous skeletal myoblasts (ASM) transplantation in addition to coronary artery bypass grafting (CABG) for the treatment of ischemic cardiomyopathy. A total of 7 patients included in the study, 2 patients of whom allocated into high dose ASM group (1), 2 patients into low dose ASM (2) group, and 3 patients into control group (3)) for 12 month follow up period. A skeletal myoblasts (SKM) biopsy were taken from vastus lateralis muscle, and underwent specific culture media

before implantation. To minimize the risk of ventricular arrhythmia all 7 patients received an implanted cardioverter defibrillator (ICD) and treatment with amiodarone as a prophylaxis. The ASM were injected into the infarcted myocardium and the border zone of the infarction during the coronary bypass operation The patients of the group 1, 2 shows one step improvement in New York Heart Association (NYHA) functional class during the follow-up, in compare to group 3 which shows either deterioration (NYHA) functional class or remain the same. Also, the results showed initial improvement in the three groups for both left ventricular end systolic volume and left ventricular end diastolic volume at 6 and 12 months follow-up. This positive tendency continued in the long-term follow-up for the high-dosage group only. There were significant improvement in group 1, and 2 in the left ventricular ejection fraction mostly in the group 1 which reached up to 35% in compare to control group at 6 month follow up. This improvement shows a drop about 3-5 % mostly in group 2, but thereafter remained stable.

Study 9

The authors aimed to investigate the long term hemodynamic effects of intramyocardial injection of autologous skeletal myoblasts in patients with ischemic heart failure. A total of 5 patients were included in the study, muscle biopsy was obtained from quadriceps muscle, and underwent specific culture media. Cell transplantation was done by cardiac catheter injection directly into the infarcted area. During the follow up observation period there were no adverse effect seen. A significant increase in the cardiac output can be obtained by skeletal myoblast transplantation, this increase in cardiac output is achieved by a reduction in end-systolic volume and a slight increase in heart rate, whereas end-diastolic volume remained unchanged. Also, there were no significant changes relative to baseline values in relation to the New York Heart Association functional class.

Study 10

This study were designed to evaluate the safety, feasibility, and efficacy of percutaneous endovascular injection of autologous skeletal myoblasts (ASM) via Biosense 3-dimensional (3D) Mapping and injection system in subjects with congestive heart failure. A total of 23 patients were included in the study, of whom 12 patients allocated into ASM receiving group, and 11 patients into control group. A muscle biopsy were taken from vastus lateralis muscle, and underwent specific culture media before implantation. Myoblasts were injected to the myocardium with the “Biosense intramyocardial injection catheter” directly into the infarcted

area, without any use of antiarrhythmic medications or ICD were administered to patients prophylactically. During the injection procedures there were no complications or any adverse events can be attributed to the skeletal myoblasts injections (SKM). At 12 month follow up period, the treatment group showed a sustained improvements in New York Heart Association and Minnesota Living with Heart Failure Questionnaire (MLHFQ) in compare to the control group.

Study 11

This study were done to evaluate the safety and efficacy of autologous skeletal myoblast sheet (ASM) for the treatment of severe chronic heart failure due to ischemic heart disease. A total of 7 patients were included in the study, a muscle biopsy were taken from vastus medialis and cultured in fetal bovine serum. The ASM sheet transplantation was performed through a left thoracotomy under general anesthesia, and 5 sheets were transplanted onto a large area extending from the anterior wall to the lateral wall of the left ventricle. No other cardiac surgery, such as coronary artery bypass graft or mitral valve repair, were performed. The study shows up that (ASM) sheets implantation induce a significant improvement in the left ventricular ejection fraction, New York Heart Association functional class, and finally in the 6-minute walk test. During the follow up period (26weeks) there were no serious arrhythmia, which required inpatient hospitalization or prolonged existing hospitalization for treatment or monitoring or other adverse effect observed.

Study 12

The study were designed to assess the feasibility, safety, and efficacy of autologous skeletal myoblast transplantation performed via a percutaneous trans-coronary venous approach in patients with post-infarction left ventricular dysfunction. A total of 10 patients were included in the study, muscle biopsy was obtained from vastus latralis and grown in specific cell culture media. Skeletal myoblasts transplantation into infarcted area were done by “trans-access catheter system” under fluoroscopic and intravascular ultrasound guidance trough either anterior inter ventricular vein, or middle cardiac vein. To minimize the risk of ventricular arrhythmia patients were treated with prophylactic amiodarone infusion before and during the procedure.The researchers were able to perform a successful intramyocardial injections into the target area of the left ventricle without any adverse effect related to the procedure. In concern about cardiac arrhythmia, only single ventricular extrasystoles were seen during intramyocardial cell injections in all cases. During 6 month follow up period

there were improvement in both New York Heart Association functional class and the left ventricular ejection fraction.

Study 13

This study were designed to assess the safety, and efficacy of 2 doses of autologous skeletal myoblasts (ASM) compared with a placebo injection in patients undergoing coronary artery bypass grafting (CABG) operations for multi-vessel coronary artery disease and severe cardiac dysfunction. A total of 97 patients were included in the study, of whom 33 patients allocated into low dose ASM group (1), 30 patients into high dose ASM group (2), and 34 patients enrolled into control group (3). A muscle biopsy were taken from the thigh, and grown in specific culture media. ASM injected into an average of 30 sites in and around myocardial akinetic segments identified by echocardiography. To minimize the risk of ventricular arrhythmia an implantable cardioverter-defibrillator (ICD), and amiodarone therapy started at the time of biopsy and continued for 3 months postoperatively. The researchers observed during 6 month follow up period a significant improvement in both left ventricular ejection fraction and New York Heart Association functional class, in group 1, and 2, especially in high dose group (2). In concern to arrhythmia and other adverse effects, a higher number of arrhythmic events in the myoblast-treated patients were observed. Despite this higher rate of arrhythmic events the 6-month rates of major cardiac adverse events and of ventricular arrhythmias did not differ significantly between the treatment and placebo groups.

DISCUSSION

Autologous skeletal myoblasts (SkMs) are the most well-studied type of cells which have been extensively characterized in preclinical experimental animal models as well as in the clinical studies. They have many characteristics that support their superiority over other cell types[7] (fig 2). SkMs implanted into infarcted hearts have shown functional restoration in both systolic and diastolic (increased LVEF, segmental contractility, and compliance of the infarcted myocardium), and structural improvement such as delayed LV remodeling, neovascularization, and increased systolic thickening of the scared area beside their safety and feasibility in vitro, in vivo and in human beings as well.[8-10]

As mentioned above SkMs gained the focus from many of research groups having an interest in this field due to their incredible, valuable and beneficial results achieved until now.[8-10] From this stand, many experimental studies of SkMs have been conducted and still lots of studies are under process. Multiple review papers similar to ours have been published and an

overall similar results were obtained encouraging more effort, and time to be spent in such a field. Following are some of the strengths and limitations of our project:

Strength

The reviewers were supervised by an experienced person who has multiple publications in this field, providing them with the most sensitive and crucial points to be addressed. A double revision of the results of each articles included in this paper was carried out, minimizing the possibilities of mistakes and increasing the reliability of the results. This review includes around 47 articles (34 animals studies, 13 human studies) addressing various points of view in each article, which provide an excellent information in the clinical perspective. Finally, we included recent and modern articles (animal studies: last 10 y, human studies: last 5 y), so the people who will read this review will know the most recent progresses and the obstacles still encountered.

LIMITATIONS

Firstly, we restricted ourselves to one search engine (PubMed), to collect relevant information. Although this is a global, versatile engine, and is extensively used by researchers as it contains a huge number of articles. However, it doesn't include all of the conducted studies especially the clinical studies in this specific field; increasing the chance of missing an important studies and information relating to our review project. Secondly, this review as mentioned previously, consists of 13 clinical studies, and as the main purpose of the majority of researches is the prioritized concern about safety and efficacy of SkMs. This number of human studies is considered low to support strongly the use of SkMs as a treatment of ischemic cardiomyopathies. Finally, restriction of the reviewed clinical studies to the last 5 years is considered a limitation in the sense that we failed to compare the data emanating from earlier studies.

Challenges

SkMs are similar to any type of stem cells in term of their advantages and disadvantages. These disadvantages should not be an obstacle in their progress to patient use, instead they should lead the scientists to make efforts to overcome their limitations and maximize their beneficial effects. Transplanted SkMs show an electromechanical isolation from the host myocardium which disturbs the synchronous contractility, and functional integrity of the myocardium, thus inducing an arrhythmia which is considered a major problem in SkMs transplantation.[11] Medical therapy or automatic cardiac defibrillator implantation treat and

lower the risk of arrhythmias. Genetic modification of SkMs with connexion 43 overexpression improves functional integration and synchronous contractility through increased amplitude of gap junction conductance with the host myocytes.[12-13] Massive attrition rate, especially in the acute phase affecting negatively the overall results of these experiments.[14] As the early loss of SkMs was attributed to oxidative stress, poor availability of nutrients, lack of adherence with the host tissue and the inflammatory/immune responses, many strategies have been suggested to encounter these issues including ischemic preconditioning of the cells by exposure to multiple cycles of hypoxia and re-oxygenation which found to improve the survival rate of the implanted cells.[15-16]

FUTURE PERSPECTIVE

Despite the promising results achieved using this type of cells, still there are some issues which need to be resolved for an optimal prognosis. The dose of the cells, route of delivery, and survival duration play an important role on the final results. Optimization of culture conditions preserve the proliferation and differentiation characteristics. Repeated injections have shown better results (reduction in the size of the infarcted area and higher LVEF) as compared to a single dose injection.[17-18] Intramyocardial injection as a route of cells delivery is widely used, although it provides the preciseness of directing the cells into the infarcted area by direct visualization and shows good results, it is an invasive procedure and considered to be proarrythmogenic. Recently, biological cell-sheet was seeded with SkMs and applied on the infarcted area as a delivery route and found to have an equal positive outcomes, but less traumatic compared to intramyocardial injection. This method allows the adherent cells - seeded sheet on the affected epicardium, by avoiding the needls trauma which lead to less fibrosis and less arrhythmia beside the positive effects.[19-20] Many clinical studies reported the presence of ventricular arrhythmia in patients receiving SkMs, however and interestingly enough, all reported cases were treated pharmacologically.[21] Genetic modified SkMs overexpressing different cytokines and growth factors found to achieve better results when compared to wild type cells, and enhance the paracrine effect along with the direct effect of stem cells.[22-36] Paracrine mechanisms need to undergo an extensive experiments since it can lead to optimal results. Finally, as in this field the two dominating cells are SkMs and bone marrow stem cells have promising results, therefore, it was suggested that their combination with each other might offer a better results.[37]

In conclusion, as the stem cell therapy is still in infancy, the obstacles encountered should not hinder the researchers to exert an extensive efforts to overcome them, having in mind as an

encouragement points all the positive results fulfilled in the clinical experiments until now and that if this therapy achieved its optimal results would reduce the major burden of such a disease. SkMs remain as other types of stem cells which is impossible to be without having disadvantages, but their advantages as previously mentioned overcome their disadvantages leading them to be the most well-studied donor cell type.

REFERENCES

1. Go AS,Mozaffarian D, Roger VL et al. Executive summary: Heart disease and stroke statistics—2013 update: A report fromthe American Heart Association. Circulation, 2013; 127: 143–152.
2. Cleland JG, McGowan J. Heart failure due to ischaemic heart disease: epidemiology, pathophysiology and progression. J Cardiovasc Pharmacol, 1999; 33(Suppl. 3): S17–S29.
3. M. J. Russo, A. Iribarne, R. Easterwood et al., "Post-heart transplant survival is inferior at low-volume centers across all risk strata," Circulation, 2010; 122(11 supplement 1): S85–S91.
4. MacLellan RW. Mending broken hearts one cell at a time. J Mol Cell Cardiol, 2002; 34: 87–89.
5. Sim EK, Jiang S, Ye L et al. Skeletal myoblast transplant in heart failure. J Card Surg, 2003; 18: 319–327.
6. Calvin C. Sheng, Li Zhou, and Jijun Hao. Current Stem Cell Delivery Methods for Myocardial Repair. BioMed Research International, 2013.
7. Shazia Durrani, Mikhail Konoplyannikov, Muhammad Ashraf, and Khawaja Husnain Haider. Skeletal myoblasts for cardiac repair. Regenerative Medicine, 2010; 5(6): 919-932.
8. 8-Menasche P, Hagege AA, Scorsin M, et al. Myoblast transplantation for heart failure. Lancet., 2001; 357: 279–280.
9. Hagege AA, Carrion C, Menasche P, et al. Viability and differentiation of autologous skeletal myoblast grafts in ischaemic cardiomyopathy. Lancet, 2003; 361: 491–492.
10. Menasche P, Hagege AA, Vilquin JT, et al. Autologous skeletal myoblast transplantation for severe postinfarction left ventricular dysfunction. J Am Coll Cardiol, 2003; 41: 1078–1083.
11. Ferreira-Cornwell MC, Luo Y, Narula N, Lenox JM, Lieberman M, Radice GL. Remodeling the intercalated disc leads to cardiomyopathy in mice misexpressing cadherins in the heart. J Cell Sci., 2002; 115: 1623–1634.

12. Suzuki K, Brand NJ, Allen S, et al. Overexpression of connexin 43 in skeletal myoblasts: relevance to cell transplantation to the heart. J Thorac Cardiovasc Surg, 2001; 122: 759–766.
13. Roell W, Lewalter T, Sasse P, et al. Engraftment of connexin 43-expressing cells prevents postinfarct arrhythmia. Nature, 2007; 450: 819–824.
14. Suzuki K, Murtuza B, Beauchamp JR, et al. Dynamics and mediators of acute graft attrition after myoblast transplantation to the heart. FASEB J., 2004; 18: 1153–1155.
15. Haider H, Ashraf M. Strategies to promote donor cell survival: combining preconditioning approach with stem cell transplantation. J Mol Cell Cardiol, 2008; 45: 554–566.
16. Kim HW, Haider HK, Jiang S, Ashraf M. Ischemic preconditioning augments survival of stem cells via MIR-210 expression by targeting caspase-8 associated protein 2. J Biol Chem, 2009; 284(48): 33161–33168.
17. Gavira JJ, Nasarre E, Abizanda G, et al. Repeated implantation of skeletal myoblast in a swine model of chronic myocardial infarction. Eur Heart J., 2009; 31(8): 1013–1021.
18. Premaratne GU, Tambara K, Fujita M, et al. Repeated implantation is a more effective cell delivery method in skeletal myoblast transplantation for rat myocardial infarction. Circ J, 2006; 70: 1184– 1189.
19. Hamdi H, Furuta A, Bellamy V, et al. Cell delivery: intramyocardial injections or epicardial deposition? A head-to-head comparison. Ann Thorac Surg. 2009; 87: 1196–1203.
20. Giraud MN, Ayuni E, Cook S, Siepe M, Carrel TP, Tevaearai HT. Hydrogel-based engineered skeletal muscle grafts normalize heart function early after myocardial infarction. Artif Organs, 2008; 32: 692–700.
21. Fouts K, Fernandes B, Mal N, Liu J, Laurita KR. Electrophysiological consequence of skeletal myoblast transplantation in normal and infarcted canine myocardium. Heart Rhythm, 2006; 3: 452–461.
22. Haider H, Ye L, Jiang S, et al. Angiomyogenesis for cardiac repair using human myoblasts as carriers of human vascular endothelial growth factor. J Mol Med, 2004; 82: 539–549.
23. Ye L, Haider H, Jiang S, et al. Reversal of myocardial injury using genetically modulated human skeletal myoblasts in a rodent cryoinjured heart model. Eur J Heart Fail, 2005; 7: 945–952.

24. Khan M, Kutala VK, Vikram DS, et al. Skeletal myoblasts transplanted in the ischemic myocardium enhance in situ oxygenation and recovery of contractile function. Am J Physiol Heart Circ Physiol, 2007; 293: H2129–H2139.
25. Ghostine S, Carrion C, Souza LC, et al. Long-term efficacy of myoblast transplantation on regional structure and function after myocardial infarction. Circulation, 2002; 106: I131–I136.
26. Al Attar N, Carrion C, Ghostine S, et al. Long-term (1 year) functional and histological results of autologous skeletal muscle cells transplantation in rat. Cardiovasc Res, 2003; 58: 142–148.
27. Taylor DA, Atkins BZ, Hungspreugs P, et al. Regenerating functional myocardium: improved performance after skeletal myoblast transplantation. Nat Med, 1998; 4: 929–933.
28. McCue JD, Swingen C, Feldberg T, et al. The real estate of myoblast cardiac transplantation: negative remodeling is associated with location. J Heart Lung Transplant, 2008; 27: 116–123.
29. Brasselet C, Morichetti MC, Messas E, et al. Skeletal myoblast transplantation through a catheterbased coronary sinus approach: an effective means of improving function of infarcted myocardium. Eur Heart J., 2005; 26: 1551–1556.
30. Hata H, Matsumiya G, Miyagawa S, et al. Grafted skeletal myoblast sheets attenuate myocardial remodeling in pacing-induced canine heart failure model. J Thorac Cardiovasc Surg, 2006; 132: 918–924.
31. He KL, Yi GH, Sherman W, et al. Autologous skeletal myoblast transplantation improved hemodynamics and left ventricular function in chronic heart failure dogs. J Heart Lung Transplant, 2005; 24: 1940–1949.
32. Ye L, Haider H, Jiang S, et al. Improved angiogenic response in pig heart following ischaemic injury using human skeletal myoblast simultaneously expressing VEGF165 and angiopoietin-1. Eur J Heart Fail, 2007; 9: 15–22.
33. Chazaud B, Hittinger L, Sonnet C, et al. Endoventricular porcine autologous myoblast transplantation can be successfully achieved with minor mechanical cell damage. Cardiovasc Res, 2003; 58: 444–450.
34. Zhang Z, van den Bos EJ, Wielopolski PA, et al. High-resolution magnetic resonance imaging of iron-labeled myoblasts using a standard 1.5-T clinical scanner. MAGMA, 2004; 17: 201–209.

35. Atkins BZ, Hueman MT, Meuchel J, et al. Cellular cardiomyoplasty improves diastolic properties of injured heart. J Surg Res, 1999; 85: 234–242.
36. Mikhail Konoplyannikov M, Jiang S, Ahmed R, Ashraf M, Haider HK. Reversal of ischemic cardiomyopathy using reprogrammed skeletal myoblasts overexpressing multiple therapeutic growth factor genes. Circulation, 2009; 120: S773–S774.
37. Ye L, Haider H, Sim EK. Adult stem cells for cardiac repair: a choice between skeletal myoblasts and bone marrow stem cells. Exp Biol Med (Maywood), 2006; 231: 8–19.

Appendices

Box 1. Characteristics of skeletal myoblasts

- Skeletal myoblasts are located between the basal lamina and sarcolemma and account for 2–5% of sub-laminar nuclei of mature skeletal muscle.
- Skeletal myoblasts are activated in response to muscle damage or disease-induced muscle degeneration.
- Skeletal myoblasts express desmin, CD56, Pax3, Pax7, c-met, myocyte nuclear factor, M-cadherin, VCAM1, N-CAM, CD34, Leu-19, and syndecan 3 and 4. Activated skeletal myoblasts first express Myf-5 and/or MyoD, and finally myogenin and MRF4 as the cells differentiate into multinucleated myotubes.
- They possess high proliferative potential *in vitro* under appropriate culture conditions and maintain their undifferentiated status.
- Skeletal myoblasts are highly resistant to ischemic stress.
- Commitment to a well-differentiated myogenic lineage.
- Transplantation of skeletal myoblasts has a low risk of tumorgenicity.
- Autologous availability without ethical issues.
- The use of autologous skeletal myoblasts alleviates the need for immunosuppression.

Figure 2: Skeletal Myoblasts Characteristics.

Table 1: List of Included Articles.

No	Articles
1	Matthias Siepe, M.D., Scaffold-Based Transplantation ofAkt1-Overexpressing Skeletal Myoblasts: Functional Regeneration Is Associated with Angiogenesis and Reduced

	Infarction Size. Tissue Engineering Part A., October 2010; 17(1-2): 205-212.
2	Yasuhiro Shudo, MD, Addition of Mesenchymal Stem Cells Enhances the Therapeutic Effects of Skeletal Myoblast Cell-Sheet Transplantation in a Rat Ischemic Cardiomyopathy Model. Tissue Eng Part A., 2014 Feb; 20(3-4): 728-39.
3	Abdel Shafy, MD, Association of electrostimulation with cell transplantation in ischemic heart disease. J Thorac Cardiovasc Surg., 2009 Oct; 138(4): 994-1001.
4	Kai Zhu, Transplantation of novel vascular endothelial growth factor gene delivery systemmanipulated skeletal myoblasts promote myocardial repair. Int J Cardiol., 2013 Oct 3; 168(3): 2622-31.
5	Mikhail Konoplyannikov, Activation of Diverse Signaling Pathways by Ex-Vivo Delivery of Multiple Cytokines for Myocardial Repair. Stem Cells Dev, 2013 Jan 15; 22(2): 204-15.
6	Thomas J. Povsic, A double-blind, randomized, controlled, multicenter study to assess the safety and cardiovascularneffects of skeletal myoblast implantation by catheter delivery in patients with chronic heart failure after myocardial infarction. Am Heart J., 2011 Oct; 162(4): 654-662.e1.
7	Jesu ´s Herreros, Autologous intramyocardial injection of cultured skeletal muscle-derived stem cells in patients with non-acute myocardial infarction. Eur Heart J., 2003 Nov; 24(22): 2012-20.
8	Tomasz Siminiak, MD, Autologous skeletal myoblast transplantation for the treatment of postinfarction myocardial injury: Phase I clinical study with 12 months of follow-up. Am Heart J., 2004 Sep; 148(3): 531-7.
9	Philippe Menasche´, MD, Autologous Skeletal Myoblast Transplantation for Severe Postinfarction Left Ventricular Dysfunction. J Am Coll Cardiol, 2003 Apr 2; 41(7): 1078-83.
10	Francis D. Pagani, MD, Autologous skeletal myoblasts transplanted to ischemia-damaged myocardium in humans. Histological analysis of cell survival and differentiation. J Am Coll Cardiol, 2003 Mar 5; 41(5): 879-88.
11	TOMOYUKIFUJITA, Clinical Impact of Combined Transplantation of Autologous Skeletal Myoblasts and Bone Marrow Mononuclear Cells in Patients with Severely Deteriorated Ischemic Cardiomyopathy. T Fujita et al. Surg Today, 2011 Jul 20; 41(8): 1029-1036.
12	Henricus J. Duckers MD, Final results of a phase IIa, randomised, open-label trial to evaluate the percutaneous intramyocardial transplantation of autologous skeletal myoblasts in congestive heart failure patients: the SEISMIC trial. EuroIntervention, 2011 Feb; 6(7): 805-12.
13	Jens Brickwede, Long-term follow-up after autologous skeletal myoblast transplantation in ischaemic heart disease. Interact Cardiovasc Thorac Surg, 2014 Jan; 18(1): 61-6.
14	Paul Steendijk, Intramyocardial injection of skeletal myoblasts: long-term follow-up with pressure–volume loops. Nat Clin Pract Cardiovasc Med, 2006 Mar; 3 Suppl 1: S94-100.
15	Nabil Dib, MD, One-Year Follow-Up of Feasibility and Safety of the First U.S., Randomized, Controlled Study Using 3-Dimensional Guided Catheter-Based Delivery of Autologous Skeletal Myoblasts for Ischemic Cardiomyopathy (CAuSMIC Study). JACC Cardiovasc Interv, 2009 Jan; 2(1): 9-16.
16	Yoshiki Sawa, MD, Safety and Efficacy of Autologous Skeletal Myoblast Sheets (TCD-51073) for the Treatment of Severe Chronic Heart Failure Due to Ischemic Heart Disease. Circ J., 2015; 79(5): 991-9.

17	Tomasz Siminiak, Percutaneous trans-coronary-venous transplantation of autologous skeletal myoblasts in the treatment of post-infarction myocardial contractility impairment: the POZNAN trial. Eur Heart J., 2005 Jun; 26(12): 1188-95.
18	Philippe Menasché, MD, The Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) Trial First Randomized Placebo-Controlled Study of Myoblast Transplantation. Circulation, 2008 Mar 4; 117(9): 1189-200.
19	Agnieszka Janeczek, Genetically modified human myoblasts with eNOS may improve regenerative ability of myogenic stem cells to infarcted heart, Kardiol Pol, 2013; 71(10): 1048-58.
20	Robert von Wattenwyl, Scaffold-Based Transplantation of Vascular Endothelial Growth Factor–Overexpressing Stem Cells Leads to Neovascularization in Ischemic Myocardium but Did Not Show a Functional Regenerative Effect, ASAIO J., 2012 May-Jun; 58(3): 268-74.
21	Britta Blumenthal, Polyurethane Scaffolds Seeded With Genetically Engineered Skeletal Myoblasts: A Promising Tool to Regenerate Myocardial Function, Artif Organs, 2010 Feb; 34(2): E46-54.
22	Britta Blumenthal, Functional regeneration of ischemic myocardium by transplanted cells overexpressing stromal cell-derived factor-1 (SDF-1): intramyocardial injection versus scaffold-based application, Eur J Cardiothorac Surg, 10, 2011; 40(4): e135-e141.
23	Khawaja Husnain Haider, MicroRNA-21 is a key determinant in IL-11/Stat3 anti-apoptotic signalling pathway in preconditioning of SkMs, Cardiovascular Research, 2010; 88: 168–178.
24	Maitane Perez-Ilzarbe, Characterization of the paracrine effects of human SkMs transplanted in infarcted myocardium, European Journal of Heart Failure, 10(2008): 1065–1072.
25	Marie-Noëlle Giraud, Long-Term Evaluation of Myoblast Seeded Patches Implanted on Infarcted Rat Hearts, Artif Organs, 2010 Jun; 34(6): E184-92.
26	Matthias Gmeiner, Improvement of cardiac function in the failing rat heart after transfer of SkMs engineered to overexpress placental growth factor, J Thorac Cardiovasc Surg, 2011 May; 141(5): 1238-45.
27	Annika Poppe, Hepatocyte Growth Factor-Transfected SkMs to Limit the Development of Postinfarction Heart Failure, Artif Organs, 2012 Mar; 36(3): 238-46.
28	T.J. Kolanowski, In vitro and in vivo characteristics of connexin 43-modified human SkMs as candidates for prospective stem cell therapy for the failing heart, Int J Cardiol, 2014 Apr 15; 173(1): 55-64.
29	Yasuhiro Shudo, Myocardial Layer-Specific Effect of Myoblast Cell-Sheet Implantation Evaluated by Tissue Strain Imaging, Circ J., 2013; 77(4): 1063-72.
30	Juan Jose´ Gavira, Repeated implantation of skeletal myoblast in a swine model of chronic myocardial infarction, Eur Heart J., 2010 Apr; 31(8): 1013-21.
31	Javier Moreno, Skeletal myoblast implants induce minor propagation delays, but do not promote arrhythmias in the normal swine heart, Europace, 2010 Nov; 12(11): 1637-44.
32	Eric Larose, Percutaneous Versus Surgical Delivery of Autologous Myoblasts After Chronic MI: An In Vivo Cardiovascular Magnetic Resonance Study, Catheter Cardiovasc Interv, 2010 Jan 1; 75(1): 120-7.
33	Shigeru Miyagawa, Impaired Myocardium Regeneration with Skeletal Cell Sheets—A Preclinical Trial for Tissue-Engineered Regeneration Therapy, Transplantation, 2010 Aug 27; 90(4): 364-72.
34	Zhuang Wei, Autologus myoblasts transplantation improves heart function after MI, J.

	of central south university, 2011 Apr; 36(4): 286-93.
35	Ieva Antanavi_ci_ut_e, Exogenous connexin43-expressing autologous SkMs ameliorate mechanical function and electrical activity of the rabbit heart after experimental infarction, Int. J. Exp. Path., 2015; 96: 42–53.
36	Changfa Guo, MD, Myoblast Transplantation for Cardiac Repair: From Automyoblast to Allomyoblast Transplantation. Ann Thorac Surg, 2008 Dec; 86(6): 1841-8.
37	TOMMI PA¨ TILA, Improved diastolic function after myoblast transplantation in a model of ischemia-infarction. Scand Cardiovasc J., 2009 Apr; 43(2): 100-9.
38	Steven R. Coppen, PhD, A Factor Underlying Late-Phase Arrhythmogenicity After Cell Therapy to the Heart Global Downregulation of Connexin43 in the Host Myocardium After Skeletal Myoblast Transplantation. Circulation, 2008 Sep 30; 118(14 Suppl): S138-44.
39	Patrick Farahmand, MD, Skeletal Myoblasts Preserve Remote Matrix Architecture and Global Function When Implanted Early or Late After Coronary Ligation Into Infarcted or Remote Myocardium. Circulation, 2008 Sep 30; 118(14 Suppl): S130-S137.
40	Koen E.A. van der Bogt, MD, Comparison of Different Adult Stem Cell Types for Treatment of Myocardial Ischemia Circulation, 2008 Sep 30; 118(140): S121–S129.
41	Gilbert Dubois, Self-Assembling Peptide Nanofibers and Skeletal Myoblast Transplantation in Infarcted Myocardium. J Biomed Mater Res B Appl Biomater, 2008 Oct; 87(1): 222-8.
42	Husnain K. Haider, Skeletal Muscle Derived Stem Cells for Myocardial Repair. Recent Pat Cardiovasc Drug Discov, 2007 Nov; 2(3): 205-13.
43	Lei Ye, MD, Transplantation of Nanoparticle Transfected Skeletal Myoblasts Overexpressing Vascular Endothelial Growth Factor-165 for Cardiac Repair. Circulation, 2007 Sep 11; 116(11 Suppl): I113-20.
44	Chung-Dann Kan, MD, Recipient Age Determines the Cardiac Functional Improvement Achieved by Skeletal Myoblast Transplantation. J Am Coll Cardiol, 2007 Sep 11; 50(11): 1086-92.
45	Muhammad Idris Niagara, Pharmacologically Preconditioned Skeletal Myoblasts Are Resistant to Oxidative Stress and Promote Angiomyogenesis via Release of Paracrine Factors in the Infarcted Heart. Circ Res., 2007 Mar 2; 100(4): 545-55.
46	Lei Ye a, Angiopoietin-1 for myocardial angiogenesis: A comparison between delivery strategies. Eur J Heart Fail, 2007 May; 9(5): 458-65.
47	Shunsuke Saito, Myoblast Sheet Can Prevent the Impairment of Cardiac Diastolic Function and Late Remodeling After Left Ventricular Restoration in Ischemic Cardiomyopathy. Transplantation, 2012 Jun 15; 93(11): 1108-15.