Stem Cells And Regenerative Medicine In Cardiovascular Disease, Global Current Status And Future Prospective In Sudan

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Introduction

Stem cells are pluripotent or multipotent cells possessing the ability to develop into specialized cells that ensure proper organ function. Because of their expected major role in regenerative medicine, over the past few years stem cells have been studied extensively as potential tools for the repair or replacement of defective organs in disease[1]. For instance treatment of specific diseases of the nervous system, haematopoetic disorders, diabetes, skin replacement, ophthalmologic diseases, and treatment of cardiovascular diseases. The spectrum of scientific studies involving stem cells in basic, experimental and clinical research is substantial. In some fields it has already brought success, while practical perspectives for treatment of disease are still lacking in others.

Research on human embryonic stem cells represents a special case, being for ethical reasons regulated by strict laws that vary from country to country. In many developed countries with reputations as leaders in biomedical research, stem cell research is carried out in a highly competitive environment, and partially supported by the medical and pharmaceutical industry. However, in the developing countries stem cell research is invisible in an international context.

Regenerative medicine is a game-changing area of medicine with the potential to fully heal damaged tissues and organs, offering solutions and hope for people who have conditions that today are beyond repair. Regenerative medicine could provide an alternative to organ transplantation by using stem cells.

Stem cell is a cell that has the ability to continuously divide and differentiate (develop) into various other kinds of cells/tissues. Stem cells can Self-renew and differentiate into a specialized cell type, can become many types of cells in the body called pluripotent, or become only a few types of cells called multipotent. The sources of stem cells are embryonic stem cells, embryonic germ cells, embryonic carcinoma cells and adult stem cells:

Embryonic Stem Cells (ESCs):

Are derived from donated IVF embryos, grown indefinitely in the laboratory in an unspecialised state, and retain ability to specialise into many different tissue types know as pluripotent. These cells can restore function in animal models following transplantation [2,3]. ESCs can be culture by isolating and transferring the inner cell mass into plastic culture dish that contains culture medium, cells divide and spread over the dish. Inner surface of culture dish is typically coated with mouse embryonic skin cells (called feeder cells) that have been treated so they will not divide. Feeder cells provide ES cells with a sticky surface for attachment and release of nutrients.

Tissue engineers have incorporated cells into three-dimensional structures called scaffolds. Scaffolds are made of biodegradable materials that will slowly degrade in the body after the patch has been transplanted. Scaffold allow cell attachment and migration, deliver and retain cells and biochemical factors, enable diffusion of vital cell nutrients and exert certain mechanical and biological influences to modify cell behaviour. Biomaterials used as scaffold should be easy and reproducible and non-immunogenic. They are either synthetic biomaterials such as polylactic acid (PLA), polyglycolic acid (PGA) and poly(e) caprolactone (PCL) or natural biomaterials such as collagen, fibrin and extracellular matrix.

Adult Stem cells:

In addition to ESCs, stem cells can be generated utilizing mature adult cells through somatic cell nuclear transfer (SCNT)-sometimes called "therapeutic cloning"-or reprogramming to create induced pluripotent stem (IPS) cells. Many animals were cloned after Dolly, cats, pigs, mice, goats, cattle, rabbits using reproductive cloning. The obstacles faced the technology is very inefficient and most clones have deleterious effects and die early, and surviving clones show premature aging signs.

Therapeutic cloning by SCNT in humans use embryo as source for ES cells and then uses ES cells to generate an organ. In this case the organ generated will carry cells with the same genetic markers as the patient (recipient), and it is possible to correct genetic error in ESC in blastula stage. The limitation of technology is that some immune rejection may occur, because about 1% of the DNA in the clone will not be identical to donor cell (patient) and will be identical to egg cell used in SCNT. Another problem is that large number of eggs are needed, which may necessitate excessive hormone treatment of females to induce high rate of ovulation and surgery to retrieve eggs and both can be harmful to human females.

Induced pluripotent stem cells:

All cells in a person share the same genetic code, or genotype, the microenvironment, influences the switching on and off of different genes. The fusion experiments by Tada and colleagues clearly showed that ES cells and embryonic germ cells contain factors that can induce reprogramming and pluripotency in somatic cells[4]. Before 2006, the prevailing view was that nuclear reprogramming to a pluripotent state is a highly complex process that might entail the cooperation of up to 100 factors[5]. Human IPS cells were similar to human embryonic stem (ES) cells in morphology, proliferation, surface antigens, gene expression, epigenetic status of pluripotent cell-specific genes, and telomerase activity. IPS cells are generated from adult human dermal fibroblasts with the same four factors: Oct3/4, Sox2, Klf4, and c-Myc

The technology consists of isolation and culture of skin cells from a patient. Second introduce three or four pluripotency genes into the skin cells by using an engineered virus carrier. The expression of these genes regenerates the stem cell phenotype. The viruses simply deliver the genes of interest and are themselves engineered not to be harmful. Third the cells are harvested and cultured. Through this process, a subset of the cells generates embryonic-stem-cell-like colonies called induced Pluripotent Stem cells.

The advantage of this technology is that cells would be genetically identical to patient or donor of skin cells (no immune rejection, and no need to use an embryo). However, the disadvantages was that cells would still have genetic defects and mutations and carcinogenic effects may occur especially if the virus mis-placed the carcinogenic gene in other organs tissue.

Adult Stem Cell

This is an undifferentiated cell found throughout the body after embryonic development, multiply by cell division to replenish dying cells and regenerate damaged tissues[6,7]. They are identified in many organs and tissues like the brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin, teeth, heart, gut, liver, ovarian epithelium and testis. Adult stem therapies require a stem cell source of the specific lineage and harvesting or culturing them up to the numbers required is a challenge. The benefit is that patients can use their own stem cells for treatment and therapy. However, the isolation of some types of ASC, for example neural cells from a patient's brain, would be impractical.

Stem Cell therapy in Cardiovascular Disease:

Heart failure is usually the end result of all cardiovascular diseases and regardless of etiology is associated with significant morbidity and mortality reflected by a 5-year mortality of up to 85%, a prognosis that is worse than most cancers. Patients with heart failure can be significantly limited in terms of activity with compromised quality-of-life as well as major socioeconomic burden. Heart transplantation remains the only definitive treatment modality for end-stage heart failure. This is not readily available and carry the lifelong risk of rejection.

In spite of all the advancement and development in therapy and technology, at present there is no cure for the vast majority of cardiac diseases such as coronary artery disease, conductive tissue disorders, valvular heart disease and cardioyopathies. In reality, most therapies and procedures in cardiac diseases are in fact a replacement of a serious disease with a less serious one with no real cure of the disease process.

Heart diseases are eventually fatal since necrotic cells are generally not replaceable, nor repairable. Moreover, the scarred tissue that results from this necrotic process is a precursor for lethal arrhythmias such as ventricular tachycardia and ventricular fibrillation.

The compensatory cardiac remodeling that sets in post myocardial damage is progressive and results in a vicious circle of exacerbation of heart failure and further impairs cardiac function. Device therapy (including stents, balloons, artificial valves and pacemakers) do not in general reverse these pathological changes. For these reasons there is a desperate need for new innovative therapies to address these different pathological processes.

Stem cell therapy is a possible repair/replacement therapy for heart diseases especially heart failure and ischemic heart disease. Stem cell transplantation into myocardial tissue is simple and easy and is usually performed by injecting these cells through an angiography catheter in one or some of the coronary arteries, a procedure that does not take more than 20 minutes. Cells can also be implanted surgically into the myocardium.

Stem cell transplantation either alone or in combination with other therapeutic interventions, has demonstrated promise as a novel curative strategy for treatment of IHD for several years. The prime advantage of the heart cell therapy using stem/progenitor cells is its capability to replace the loss-of-functioning cardiomyocytes, attenuation of infracted site expansion and preservation of the deteriorating left ventricular contractile function.

Different types of stem cells are studied in clinical trials in humans including skeletal myolasts, bone marrow cells, cardiac stem cells and induced pluripotent cells:

Skeletal myoblasts in CVD disease:

Skeletal myoblasts remain the most widely studied stem cells for engraftment into myocardial tissue. Their advantage include less tumorogenecity, autolougos availability and hence less risk of rejection and their resistance to hypoxia and necrosis [8,9]. Moreover, when in contact with cardiac myocytes, myofibrils may contract in a synchronous way with the rest of the myocardial cells.

Skeletal myoblasts proved safety and efficacy in animal models and this has led to an extensive research in humans. The first clinical trial in humans was conducted when an autologous skeletal myoblasts were transplanted into the heart of a 57-year-old male patient, and the transplanted cells survived and differentiated to form islands of skeletal muscle in the infarcted heart[10,11]. The procedure was uncomplicated and was performed as an adjunctive therapy to the routine revascularization by coronary artery bypass grafting surgery(CABG). The result was encouraging with an increase in left ventricular ejection fraction(LVEF) by echocardiography. This has led to several individual attempts and to phase I and II clinical trials.

Most of these studies have used skeletal myoblast engraftment either as an adjunct to CABG, percutaneous transluminal coronary intervention, left ventricular assist device implantation or, in some cases, as a standalone procedure.

The first Phase I study with skeletal myoblasts was reported in 2003 in a group of ten severely ischemic cardiomyopathy patients and assessed the feasibility and safety of autologous skeletal myoblast transplantation[12]. Feasibility was

Defined as the ability of the expansion procedure to yield the target numbers of cells within 2–3 weeks, while safety referred to adverse events related to cell implantation. In this trial myoblast cells were injected in 37 sites throughout the scar area without any immediate postoperative complications. Follow up at 10 months showed improvement in patients' clinical symptoms from the New York Heart Association class 2.7 ± 0.2 preoperatively to 1.6 ± 0.1postoperatively ($p < 0.02$). Transthoracic echocardiography showed up to 63% improved systolic thickening of the scar area and LVEF rose from 23.8 ± 3.9% preoperatively to 32.1 ± 7.5% postoperatively ($p < 0.02$).

Pagani et al investigated the feasibility and safety of autologous skeletal myoblast transplantation in patients with ischemic heart disease undergoing left ventricular assist device implantation as a bridge to orthotropic heart transplantation [13]. Using this protocol, they were able to report the viability of cell transplants histologically in recipient heart after 68, 91, 144 and 191 days of left ventricular assist device support. Histological examination demonstrated that the transplanted skeletal myoblasts survived and differentiated into mature myofibers in three of the four explanted hearts. These results were later duplicated by Dib et al. in five patients

who demonstrated survival, feasibility and safety of autologous skeletal myoblast transplantation, and suggest that this modality offers a potential therapeutic treatment for end-stage heart failure.

Subsequent to the first Phase I study, various groups reported skeletal myoblast engraftment in patients as an adjunct procedure to CABG [14-16].

Since many of the surviving myocardial infarction patients are not candidates for CABG, and it is difficult to access all myocardial segments via epicardial delivery route, an epicardial approach for implantation of stem cells may not be practical in the good number of patients with myocardial infarction who are not suitable for CABG. Transcatheter approach was suggested as an alternative to surgical technique. This delivery method was assessed in large animal models and subsequently used in patients as an adjunctive therapy to percutaneous coronary intervention [17,18]. Initially it was not clear whether transcatheter delivery of cells is comparable to surgical route. A study has reported a head-to-head comparison of percutaneous versus surgical delivery techniques in an experimental animal model of acute myocardial infarction in Yucatan mini-pigs [19]. Magnetic resonance studies revealed that beneficial effects of cell transplantation were independent of the delivery approach. Both delivery approaches led to the reversal of remodelling, and a comparable improvement in systolic function, regional perfusion and scar characteristics 6 months after cell transplantation. There was an observed increase in the arrhythmogenic peri-infarct border zone in both groups of animals.

The beneficial effects of cell therapy may have been clouded by adjunctive revascularization procedure and standalone cell implantation assessment was needed to delineate the beneficial effects of cell therapy. Smits et al. reported the feasibility and promise of catheter-based cell transplantation using autologous skeletal myoblasts as a standalone therapy for the treatment of ischemic heart disease. In this study LVEF increased from 36 ± 11% to 41 ± 9% in 3months follow up ($p = 0.009$) and 45 ±8% at 6 months ($p = 0.23$). Regional wall analysis by MRI demonstrated significantly increased wall thickness at the target areas compared with the remote areas (wall thickening at target areas after 3-month follow-up: 0.9 ± 2.3 mm, 1.8 ± 2.4 mm, $p = 0.008$). Subsequent studies involving transcatheter delivery of skeletal myoblast demonstrated similar results, but with observed post-procedural arrhythmias in three patients [20].

Menasche et al. Conducted the first Phase II trial – Myoblast Autologous Grafting in Ischemic (MAGIC) trial[21]. This was a multicenter, randomized, placebo-controlled, three-arm, double-blind study included patients indicated for CABG with left ventricular dysfunction of ischemic aetiology (ejection fraction ≤35%). The study was conducted by 24 hospitals in France, Germany, Belgium, the UK and Italy. A total of 120 patients were included in the study, divided into three

groups (high dose, low dose and placebo groups). Of the 120 patients, 97 underwent CABG surgery and received autologous myoblast injections (400 or 800 million). Of these, 34 were assigned to the control group, 33 to the low-dose cell group and 30 to the high-dose cell group. Approximately 97, 93 and 98 initially akinetic myocardial segments were injected with autologous myoblasts in the placebo, low-dose and high-dose cell group, respectively. The 6 months follow-up demonstrated that the number of patients having improved segmental contraction by one grade was not affected by myoblast injections, regardless of the dose (placebo group: 48%; low-dose group: 35%; high-dose group: 40%; p = 0.66). Skeletal myoblast transfer did not improve regional or global left ventricular function beyond that seen in the control patients. The absolute change in ejection fraction between 6 months and baseline was 4.4, 3.4, and 5.2% in the placebo, low-dose and high-dose groups, respectively. However, the high-dose cell group had a significant decrease in left ventricular volumes compared with the placebo group. Although a higher incidence of arrhythmic events were observed in myoblast-treated patients, rates of major cardiac adverse events and ventricular arrhythmias at 6 months did not differ significantly between the pooled treatment and placebo groups. Therefore, the study concluded that skeletal myoblast transplantation, in combination with CABG, did not improve heart function, and further investigations are required to investigate the increased number of early postoperative arrhythmic events after skeletal myoblast transplantation. Several small trials using catheter-based delivery of myoblasts implantation were also conducted, these include the Myogenesis Heart Efficiency and Regeneration Trial (MYOHEART), Safety and Effects of Implanted Skeletal Myoblasts (MyoCell) and Controlled Study Using 3D Guided Catheter-Based Delivery of Autologous Skeletal Myoblasts for Ischemic Cardiomyopathy (CAUSMIC) trial.

Bone Marrow-Derived Cells:

BMC are widely used in clinical trials. They mainly contain mesenchymal stem cells (MSCs), hematopoietic stem cells, border cells and endothelial progenitor cells. Though, the acquisition of BMC induces pain and the number of BMCs decreases significantly with age, its separation is simple and large numbers of cells can be obtained without the need for ex-vivo expansion. It also has the advantage of autologous availability avoiding the risk of rejection and ethical disputes.

The REPAIR-AMI [22] multicenter randomized controlled trial included 204 patients with acute myocardial infarction and received the autologous bone marrow-derived mononuclear cells (BMMNCs) intracoronary or placebo treatment. 4 months later, in the treatment group LVEF improved slightly. However, in one year-follow-up, the cardiac indicators associated with remodelling had no significant changes.

Subgroup analysis showed that only patients with large myocardial infarction had a marked effect.

European STAR-heart[23] examined the stem cells therapy in ischemia related chronic heart failure patients. 391 patients with an LVEF < 35% were included. BMMNCs directly infused to the infarct related artery through angioplasty catheter. Effects on the patients were evaluated after treatment of 3 months to 5 years. LVEF, cardiac index, exercise capacity, oxygen uptake, and left ventricular contractility had improved significantly. In addition, in the first assessment after 3-month treatment the beneficial effects observed lasted to 12 and 60 months of follow up. Compared with the control group, long-term mortality of patients treated with stem cells significantly decreased.

Although the results of many experiments show that BMC transplantation can improve LVEF, the long-term effects were not obvious[24]. A meta-analysis of a series of published clinical trials before 2009 found that although coronary artery transplanted stem cells can significantly reduce left ventricular end systolic volume in patients with acute myocardial infarction, they have no significant effect on left ventricular end-diastolic volume[25] . In the randomized controlled BOOST trial [26], patients with ST segment elevation after acute myocardial infarction received an intracoronary BMC infusion. Results showed that LVEF had improved
after 6 months, but this benefit was not sustained at 18 months follow up. And in 5-year follow up there was no difference between the treatment and the control group. However, the safety of the procedure was good and the rate of adverse effects with autologous BMC transplantation is relatively low, and comparable with conventional treatment.

Cardiac Stem Cells (CSCs):

Many types of CSCs which were taken from adult heart tissue can be isolated and characterised including c-kit+ cells and Sca-1+ cells. Regardless of the gender or age of the patient, or of diabetes, David et al. [27] isolate a pool of functional CSCs. They found that these cells have long telomeres, or “caps,” on their chromosomal ends indicating that they retained a significant growth reserve, however, this phenomenon was less demonstrated in diabetic and older patients. CSCs can differentiate into three kinds of major heart cell precursors: myocardial cells, smooth muscle cells and epithelial cells. The isolation of these cells requires direct access to the myocardial tissue that can easily be obtained from right ventricular endomyocardial biopsy [28], a technique that could be used clinically. Some Phase I clinical trials are being conducted to observe the safety and feasibility of using these cells in myocardial repair.

Induced Pluripotent Stem Cells (IPSCs):

IPSCs can be obtained by genetic re-programming of stem cells. In 2006, Takahashi and Yamanaka[29] reported the first successful reprogramming by delivering four stem cell-related genes (Oct3/4, Sox2, Klf4, c-Myc) into skin fibroblasts. IPSCs avoid the moral controversy, and can be patient-specific stem cells avoiding immune rejection. Human IPSCs have been implanted in mouse models of myocardial infarction, and it was indicated that they regenerated myocardium, smooth muscle, and endothelial tissue, restoring post ischemic contractility performance and electric stability [30] . ESCs, IPSCs -derived cells may be contaminated with potentially carcinogenic cells.

Ieda et al. [31] reported that a combination of three developmental transcription factors rapidly and efficiently reprogrammed postnatal cardiac or dermal fibroblasts directly into functional cardiomyocytes. Reprogramming of

Endogenous or explanted adult cells might provide a source of cardiomyocytes for regenerative approaches.

Mechanism of benefit of Stem Cells:

One of the major obstacles in progressing to large-scale clinical trials of cardiac stem cell therapy is the ongoing debate regarding the mechanisms of action by which stem cell therapy leads to cardiac repair. The classical idea that provided the primary motivation for stem cell therapy is that delivery of the appropriate stem cells would repair a damaged heart via active myocardial regeneration resulting from trans-differentiation of the administered stem cells into active and viable cardiomyocytes restoring the lost and necrotic cells[32-34]. New data have led to the recognition of alternative mechanisms of actions. One example is that exogenous stem cells may also stimulate proliferation of endogenous cardiac precursors or stem cells through neovascularization by differentiate into endothelial cells resulting in the promotion of angiogenesis which may turn part of the damaged heart muscle alive and limit scar expansion[35].

Paracrine signaling actions is another proposed beneficial mechanism [36]. These paracrine and autocrine effects of stem cells include the production of vascular endothelial growth factor (VEGF)). Several studies have confirmed that stem cell therapy of heart failure may benefit from this effect by the promotion of angiogenesis, regulation of myocardial apoptosis and immune modulation and other paracrine and autocrine effects. VEGF may also promote reconstruction of myocardial vascular network and may increase permeability of capillary walls, activate matrix metalloproteinase, and promote endothelial cell proliferation and migration.

Challenges of SC therapy in CVD:

The outcome and benefit of STEM Cell therapy is facing many challenges. We have to choose the best method of cell delivery to the target site without contamination of the rest of body tissue.

The best timing of treatment is not yet well known and clinical trials should address this problem to identify the best timing of therapy.

It was quite obvious that there are a good proportion of non responders to the proposed therapy and this indicates the need to identify those patients who will benefit more from those with expected modest or no positive effect.

Another major problem is that these cells are implanted into a non viable poorly vascularied and hypoxic tissue, and the rate of survival of these cells may be poor and not optimum. Most stem cells cannot survive in such a hostile environment. However, these challenges can be addressed by prey conditioning of cells prior to transplantation, the use of appropriate drug therapy and the use of gene modified stem cells that are more resistant to this hostile environment and improve the long term survival of cells.

The future of SC therapy in Sudan:

Not unlike the rest of the world, CVD disease is quite prevalent in Sudan and associated with major socioeconomic burden. For instance Rheumatic heart disease is common and affects children at very young age and most patients eventually require mechanical valves implantation [37]. IHD is also common and most patients with AMI are left with significant myocardial damage and suffer from end stage heart failure and non-revascularable coronary arteries[38]. Idiopathic cardiomyopathy with terminal heart failure is also quite prevalent and resulted in disabling heart failure and eventually fatal disease. Cardiac transplantation with all its limitations and dis-advantages is not available and patients are desperate for new and more effective therapy than the available therapeutic options of valvular and bypass surgeries and percutaneous coronary interventions.

Stem Cell is a hope for these patients and is probably cheaper than conventional therapy and can easily be performed either per-cutaneously or surgically. Research in this area needs collaboration of cardiac hospitals and biomedical laboratories and genetic scientists. From the cardiac point of view, the cardiac part in this game is easy with the readily available catheterization laboratories and cardiac surgery theaters. Transplantation of cells is simple and monitoring of therapy is easy and reproducible which can be achieved by clinical symptoms imrovement and assessment of myocardial function by echocardiography, CMRI and nuclear scanning. The most difficult part is preparation, isolation and culture of the

appropriate stem cells which require a well equipped genetic laboratory and skilled genetic scientists.

The first collaboration between cardiac and laboratory scientist in this field in Sudan was witnessed through a recent workshop held in Soba university hospital, the hospital affiliated with Khartoum University. In February 19, 2015, the Faculty of Medical Laboratory Science, University of Khartoum, hosted the first workshop on stem cells and regenerative medicine as part of (The 6th Annual Conference of Postgraduate Studies and Scientific Research) activities organized by the University of Khartoum.The workshop focused on stem cell technology, ethical and religious perspectives on stem cell research and stem cell therapy. Specific topics on stem cell therapy in cardiovascular disease, neurological diseases and dental disorders were debated. About 100 scientists and students attended the workshop and opened up a dialogue on different aspects related to stem cell therapy and isolation technology.

The biomedical laboratory of the faculty of Medical laboratory is well established both on equipment facilities and technical scientist. This capacity was built through collaboration with other international institutes such as National Institute of Genetic Engineering and Biotechnology, Tehran, Iran, and the Center of Stem Cells at Soochow University, China. This collaboration facilitated training of postgraduate students and established a robust research activity. Sudan Cardiac Centre is in collaboration with the laboratory to establish research in this area. This, hopefully, will materialize in the near future an extensive research on regenerative therapy in patients with cardiovascular disease in Sudan.

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