Cardiac Tissue Regeneration; The Use of Stem Cells

Chavy Friedlander

1. Introduction

Cardiovascular disease is the leading cause of illness and fatality. Diseases such as coronary artery disease, myocardial infarction and subsequent congestive heart failure pose a serious problem to much of the population in the Unites States and all over the world. The heart's compromised function that results from the above conditions is so influential as the heart has little capacity to repair its lost tissue; once the cardiac myocytes are destroyed its effects are amplified by a drastic decrease in cardiac function.

Until recently with the advent of stem cell technology, researchers believed that once cardiac myocytes are destroyed they could never be regenerated and thus little could be done to reverse the adverse effects of cardiac muscle atrophy (Laflamme and Murry 2005). This dogma, that the heart is a post mitotic non-regenerating organ and that cardiac myocytes are terminally differentiated cells that participate in cardiac function all throughout life, introduced more than 60 years ago, is being challenged as the results of different experimental and clinical work are showing capacity for cardiac tissue hyperplasia This paper will explore the various kinds of stem cells used in an attempt to regenerate cardiac tissue.

2. Stem Cells

Stem Cells are self renewing cells that characteristically can differentiate into other types of cells. Hematopoietic stem cells, adipose-derived stem cells, muscle-derived stem cells, cardiac stem cells and embryonic stem cells are some kinds of stem cells currently being studied in their use as cellular therapy for myocardial regeneration.

A. Hematopoietic Stem Cells

Hematopoietic stem cells (HSCs) are bone marrow derived stem cells. These cells have been the target of tissue engineering and cellular therapy as they are self renewing cells that can differentiate into multiple cell lineages (Wu et al 2006). HSCs exhibit great potential in developing into cardiomyocyte-like cells as blood and cardiac tissues originate in adjacent areas in embryonic development. In early development, blood cells originate in the mesoderm: the hematopoietic precursor cells accumulate in the blood until it finally reaches the bone marrow. The heart muscle is generated within the anterior lateral mesoderm. Originating from adjacent

regions of the mesoderm in the developing embryo is what contributes to these stem cells potency (Leri et al 2005).

Clinical work with the importation of bone marrow cells to the infarcted heart to regenerate cardiac cells has yielded varying results. Orlic and colleagues (2001a) transplanted bone marrow cells into the infarcted hearts of mice to see if the dead myocardium would be restored. The researchers sorted Lineage -, ckit+ bone marrow cells from mice and implanted them into the contracting wall bordering the infarct shortly after coronary ligation. Indeed the Lineage-, ckit+ cells differentiated into myocytes and formed vascular structures occupying 68% of the infarcted portion of the ventricle nine days after transplantation.

Similar to Orlic, Balsam et al (2004) also studied the effects of implanting Lineage - c kit+ hematopoietic stem cells expressing green fluorescent protein (GFP+), isolated from mice into the ischameic myocardium of wild type mice. Abundant GFP+ cells were detected in the myocardium ten days after the implantation, but by 30 days post implantation few GFP+ cells were detected. Balsam's work suggests that the HSCs remain in the infarcted region of the heart for a limited amount of time. Unlike Orlic's findings, Balsam discovered that the GFP+ cells did not express cardiac tissue specific markers, but rather, most of them expressed the hematopoietic specific cell marker CD45, indicating that these cells never differentiated into cardiomyoctes. Murry et al (2004), in his work tracked the fate of hematopoietic stem cells with 145 HSC transplants into normal and injured adult mouse hearts and found that transdifferentiation into cardiomyocytes was undetectable.

Researchers have also been investigating the mechanism for the therapeutic effects of HSC transplantation – primarily via the paracrine and cytokine signaling. Korf-Klingebiel et al's (2008) work with bone marrow stem cells sheds lights on the paracrine signaling between transplanted bone marrow stem cells and resident cardiomyocytes in the hosts's cardiac tissue. In their experiment, this research group shows that HSCs secrete proangiogenic, cytoprotective growth factors and cytokines thus promoting angiogenesis and cardiomyocyte survival via paracrine effects. Orlic et al on a search to find a noninvasive method implementing HSCs, to recover the injured heart found that transdifferentiation of primitive bone marrow stem cells is dependent on tissue damage and the level of pluripotent cells. He suggests that HSCs, mobilized by stem cell factor and granulocyte-colony stimulating factor, would home to the infarcted region, replicate, differentiate and promote myocardial repair. In his experiment, cytokine mediated translocation of bone marrow stem cells resulted in a significant degree of tissue regeneration. Cytokine induced cardiac repair decreased mortality by 68%, infarct size by 40% and diastolic stress by 70%. Cardiac ejection fraction also increased as the consequences of the formation of new myocytes with arterioles and capillaries connected with the circulation of the unaffected ventricles (2001- b).

Jackson et al (2001) tested a side population of CD34- bone marrow stem cells for their capacity to regenerate cardiac myofibrils in ischemically injured cardiac tissue. In his study, the side population cells and their progeny became incorporated into both cardiac muscle and vessel structures where they displayed the characteristics of differentiated cardiomyocytes, suggesting that the stem cells do differentiate into cardiomyocytes. While Jackson's study seems interesting, it cannot be taken as evidence that hematopoietic stem cells take on the cardiac cell phenotype after engraftment as his method used for isolating the side population CD34- bone marrow cells

appears to be lacking and thus there is much possibility that contaminating non hematopoietic stem cells in his experiment is what contributed to the cardiac cell engraftment.

The findings of Orlic and his counterparts have motivated researchers to investigate the effects of HSC injections in the post infarcted human heart. Several clinical trials have explored the hypothesis that an intracoronary infusion of these bona marrow cells may enhance recovery of Left Ventricular (LV) systolic function following Acute Myocardial Infarction (AMI). Meyer et al (2006) studied the specific effects of bone marrow cells on the recovery of left ventricular (LV) function in patients after AMI. His group learned that hematopoietic bone marrow cells did not significantly increase the long term systolic ventricular function of the post infarcted heart. He administered a single dose of HSCs to 30 of 60 patients undergoing post infarction therapy. In the control group LV ejection fraction increased by .7- 3.1 percentage points after 6 and 18 month respectively; LV ejection fraction in the bone marrow cell transfer group increased by 6.7 and 5.9 percentage points. Meyer noted that the difference in LV ejection fraction improvement was significant after six months but not after 18 months. While the single dose of HSCs did not provide long term benefit to left ventricular systolic function, the accelerated ejection fraction recovery rate after AMI due to HSC therapy was beneficial to the patient recovery.

Schaninger et al (2006) has obtained similar results to Meyer's in his clinical work. His group found that the recovery of left ventricular systolic function in patients after AMI is enhanced by an infusion of bone marrow stem cells into the reperfused coronary artery. Clinical data obtained by Janssen et al (2006), in their controlled experiment of 67 patients, indicate that HSC transfer when applied early after coronary reperfusion may lead to a reduction in infarct size and a better recovery of regional systolic function. Other studies, however, indicate that improvement in left ventricular function after intracoronary injection of bone marrow cells in the AMI is not seen (Lunde et al 2006).

Although the ability for hematopoietic stem cells to differentiate into cardiomyocytes is debated by different researchers, the use of these stem cells for myocardial regeneration nevertheless seems promising. Varying results have been obtained when injecting HSCs into the hearts of humans, yet most studies indicated a short term improvement in systolic function post cardiac trauma. The mechanism for these stem cells, primarily through a cytokine/paracrine signaling pathway can have great implications in the world of non invasive stem cell therapy.

B. Adipose- derived stem cells

These stem cells, derived from mammalian adipose tissue, recently displayed their ability to differentiate in to multiple lineages. In 2001, investigators from UCLA and University of Pittsburg demonstrated that a population of cells derived from collagenase digested human adipose tissue could be induced into multiple cell lineages including cardiomyocyte lineage (Zuk et al 2001). The ease of access to fat and its abundance makes adipose tissue a potentially useful source of stem cells for cardiac tissue regeneration.

Interestingly, adipose -derived stem cells exhibit close associations with bone marrow -derived stem cells. The cell surface phenotype of adipose -derived stem cells is almost identical to that of bone marrow -derived stem cells and thus their patterns of differentiation should seemingly be similar, yet the data of Winter et al (2003) suggests that in vitro differentiation of adipose- derived stem cells in mice is less than observed in bone marrow derived stem cells. According to these findings, the rate of differentiation of adipose- derived stem cells is much

slower than in hematopoietic stem cells. In absolute incongruity to these finding, a comparative study of marrow and adipose derived cells obtained from the same donors, performed by De Ugarte et al (2003) demonstrates that the adipose derived cells require approximately 5% the cell number used for marrow cells in order to reach initial confluence by one week. De Ugarte's work suggests that the proliferative capacity for adipose derived stem cells far exceeds that of hematopoietic stem cells. Their seemingly high proliferative capacity and their similarities to bone marrow derived stem cells signify the potential for adipose tissue to act as an alternate, perhaps preferred cell source for clinical application.

To date there hasn't been much clinical work in the area of using adipose derived stem cells to regenerate myocardium in vivo, yet some studies on the cardiomyocyte differentiation capacity for adipose derived stem cells have been published. Planat-Benard et al (2004) investigated the emergence of cardiomycyte phenotype from adipose derived cells and found that beating cells with cardiomyocyte features were identified after culture of the adipose cells. The cardiomycoyte phenotype was identified with expression of specific cardiac markers, immunocytochemistry staining, and ultrastructural anaylsis. The early culture also displayed a pacemaker activity in the cells. This study is important as it shows that functional cardiomyocyte-like cells could be directly obtained from adipose tissue. The potential for adipose tissue to function as a reservoir of stem cells that can differentiate into cardiomyocyte-like cells must further be investigated.

C. Skeletal muscle- derived stem cells

Skeletal muscle derived stem cells, also known as myoblast or satellite cells are being investigated in their potential to enhance cardiac function post acute myocardial infarction(AMI). These cells that lie under the basal membrane of skeletal muscle fibers, seem promising in the area of cell based therapy due to their preclinical efficacy, availability, ability to be amplified in vitro and their relatively good survival after implantation (Menasche et al 2003). Unlike other stem cells, satellite cells retain their specific characteristics and do not transdifferentiate into cardiomyocte cells when transplanted into cardiac tissue (Reineke et al 1999). Still the myoblast's inherent contractile ability can be a great asset in improving the contractile capacity of scar tissue formed after cardiac injury. Several groups of researchers have investigated the effects of muscle derived stem cells injected into a post injury cardiac scar.

Murry et al (1996) tested the functionality of skeletal myoblasts in injured cardiac tissue via the injection of neonatal skeletal muscle cells into the the hearts of rats directly after coronary injury. At day three after implantation, myotubes expressing fast fiber, easily fatiguable MHCs were present in the injected cells. By week seven, the grafts began to express beta MHCs – a hallmark of the fatigue resistant, slow fiber phenotype. This continued until three months, yet all the while the grafts never expressed cardiomyocyte specific alpha MHCs. Murry reports that even though the grafts did not express a cardiomyocyte phenotype, they were able to contract; upon stimulation ex vivo; wounds containing two week old myoblast grafts contracted. Further, the myoblast grafts could perform a cardiac duty like cycle for six minutes, alternating between relaxation and contraction. Murry's experiment on how myoblast grafts convert to fatigue resistant slow twitch mature myofibrils are one of many such studies and supports the idea that fully developed myofibrils within the cardiac infarct retain their skeletal lineage and do not differentiate into cardiac cells with intercalated discs and other cardiac features.

Taylor et al (1998), in their study found that skeletal myoblasts can differentiate into striated muscle cells within damaged myocardium and these cells augment both systolic and diastolic myocardial performance after transplantation in the damaged myocardium. Taylor's group transplanted skeletal myoblasts into the infarcted myocardium of rabbits, monitored cardiac function in vivo for two to six weeks and examined serial sections of the hearts by light and electron microscopy. Islands of different sizes comprising elongated, striated cells that retained characteristics of both skeletal and cardiac cells were found in the cardiac infarct. Taylor's findings support Murry's claim that the skeletal muscle stem cells retain their skeletal muscle lineage. His study further indicates that in rabbits in which myoblasts were incorporated, myocardial performance was improved.

Similar to Taylor et al, Ghostine et al (2002) studied the effect of skeletal myoblast transplantation on a scar in the infarcted heart of a sheep. Ghostine's group injected myoblasts into the scar and studied subsequent cardiac function at 4 months and 12 months after the injection. His findings show that for up to one year the skeletal myoblast implantation limits post infarction ejection fraction deterioration, and improves systolic scar function by colonization of skeletal muscle cells in the fibrotic tissue. Although Ghostine's results are promising, they can't be validated as this clinical trial involves only one sheep and thus the indicated improvement in cardiac function can stem from various different sources. The true relationship between the skeletal myoblast transfer and greater ejection fraction must be further investigated.

The question of whether skeletal muscles beat in vivo in humans is a newly researched area that is currently being explored. Menasche et al (2008) began a phase I clinical study on the implantation of autologous skeletal myoblasts in post infarction scars in humans. Menasche and his group, veterans in the field of skeletal myoblast implantation, have previously found a linear relationship between the improvement in ejection fraction and the number of injected skeletal myoblasts after transplantation. In 2001, they reported the first human case study of implanting autologous skeletal myoblasts into a post infarction scar during coronary artery bypass grafting. Five months after the implantation, evidence of contraction and viability in the grafted scar by echocardiography and positron emission topography were obtained. (Menasche et al 2001) Later, in 2003, Menasche tested the safety and efficacy of skeletal myoblast implantation in patients with severe ischemic heart disease. Of the ten patients included in his trial, four developed ventricular tachycardia and were implanted with an automatic internal cardioverter- defibrillator, a device to regulate their heart beat (Menasche et al 2003). This could have been predicted with Murry's finding that the satellite cells have an inherent contractile ability While Menasche concludes that his clinical trial demonstrates the feasibility and safety of skeletal myoblast transplantation as his patients had an uncomplicated post operative course, this cannot be viewed uncritically. Although three of the four patients that developed cardiac arrhythmias had preoperative ventricular hyperexcitability the numbers seem very high; almost half the patients involved in this clinical trial developed this severe side effect.

Although in Ghostine et al's experiment (2002), myoblast transplantation has been found to limit post infarction ventricular dilation this was not seen in the Menasche's patients. The probable reason for this is that Menasche's patients that had previous old infarctions had already enlarged ventricular dimensions. In his clinical follow up in 2008, Menasche gathered 98 patients, with either left ventricular dysfunction, or myocardial infarction and an indication for coronary surgery. Each patient received an injection of either 400, or 800 million cells grown from a skeletal muscle biopsy or a placebo solution in and around the cardiac scar. The myoblast

injections did not improve echocardiogroahic heart function. In addition to its failure to improve cardiac function, the myoblast graft increased the number of post operative arrhythmic events (Menasche et al 2008).

Muscle- derived stem cells are readily available, easy to amplify and have a good survival rate after implantation. Myoblasts have the potential to enhance the heart's contractile function as they retain their skeletal muscle cell's contractibility. In humans, the risk vs. benefit ratio of myoblast implantation to increase cardiac function of injured hearts has yet to be determined and must further be investigated.

D. Cardiac Stem Cells

1. Identifying Cardiac Stem Cells

The question of whether there are stem cells derived from cardiac tissue has been thoroughly investigated. Until recently, it was commonly believed that the adult mammalian heart is lacking in the ability to generate new cardiomyocytes: cardiomyocytes do not proliferate. This view is supported in part by clinical observation as primary myocardial tumors are rarely observed in adults (Soonpa and Field 1998). However, recent efforts looking for evidence of cardiomyocyte turnover in the adult mammalian heart have led to varying results.

Studies by Beltrami and colleagues (2001) challenging the dogma that the adult heart is a postmitotic organ, suggest that the human adult heart may have the endogenous capacity to regenerate. By staining the human heart tissue with an antibody specific for Ki-67, a protein closely associated with the cell cycle and cell proliferation, they concluded that there is extensive cardiomyocyte proliferation during normal aging and that the number of cardiomyocyte proliferation is markedly up regulated in and after myocardial infarction. Compared with the number of Ki-67 cells in a series of control hearts from patients who died of noncardiac causes, the number of Ki-67 cells in hearts from patients that have experienced AMI was 84 times higher in regions that bordered the infarct zone, and 24 times higher in regions of the heart distant from the scar. Based on this data, Beltrami calculated myocyte mitotic indexes of .08 and .03 percent in zones adjacent to and distant from the infarcts and suggests that the billions of cardiomyocytes lost in a heart attack are replaced in about 18 days.

Like Beltrami, Bergmann et al (2009), studied the rate of cardiomyocyte turnover in humans. In a search for using an effective method to study cell turnover, Bergmann used a novel approach utilizing a disturbing chapter in the history of nuclear weapons to track cardiomyocyte proliferation. Bergmann took advantage of Spalding et al's (2005) study describing that testing for the radioactive isotope Carbon 14 in cells can reveal the cells age. Bergmann's predecessor discusses how testing of nuclear weapons during the Cold War resulted in a dramatic global increase in the levels of the isotope Carbon 14 in the atmosphere, followed by an exponential decrease after 1963. His work indicates that Carbon 14 in genomic DNA closely parallels atmospheric levels and can be used to establish the time point when the DNA was synthesized and cells were born, thereby measuring the rate of cell turnover. With this effective cardiomyocyte dating method, Bergman' s group determined the age of cardiomyocytes in humans and discovered that cardiomyocytes do indeed renew over the human lifespan albeit at the very slow rate of 1% turning over annually at the age of 25, decreasing to .45% at the age of 75.

Bergman and Beltrami's findings indicate a large discrepancy in the rate of cardiac cell turnover. This may be due to the fact that Bertrami's method of reporting dividing cardiomyocytes with the Ki-67 antigen may have produced drastically exaggerated numbers of reproducing cardiomyocytes. The number of cells with the Ki-67 antigen may be representative of all cells in cardiac tissue in mitosis, not only the cardiomyocytes, and thus Beltrami obtained such a large number in his study on cardiac cells replication. Also, it has been pointed out by Soonpa and Field (1998) that Ki- 67 staining in the heart does not unequivocally establish that cell division has occurred, since a substantial amount of cardiomyocytes are binucleated or polyploidy and in the context of AMI, polyploidy may precede cell death and thus the elevated number of Ki-67 cells during infarction is accounted for.

Research by Kajstura et al (1998) indicates proof of myocyte proliferation. Using confocal microscopy, this investigative group reports that 14 myocytes per million were in mitosis in control human hearts, and a nearly 10- fold increase of this parameter was measured in end stage ischemic heart disease (152 myocytes per million). They also suggest that large numbers of myocytes can be formed with time as mitosis only lasts one hour. Urbanek et al's study (2003)on the myocyte formation from cardiac stem cells in human cardiac hypertrophy indicate that the human heart contains a population of cardiac stem cells that can divide and differentiate into myocytes and that this cardiac stem cell pool is enhanced acutely after infarction.

Although these findings are novel in that never before was mitosis seen in cardiac myocytes and that cardiac cells were believed to be incapable of dividing, according to one research group these reports don't provide much clinical use. Leri et al (2005) describes that the problem of compromised cardiac function following an ischemic event isn't the lack of myocytes, it is the accumulation of old poorly contracting cells and the extensive myocardial scarring that occurs following AMI. Leri describes that 12-72 hours following an ischemic induced myocardial necrosis there is an infiltration of neutrophils and an accumulation of macrophages which initiate the removal of necrotic myocardial tissue. Within seven days, scarring, that starts with the deposition of granulation tissue, is initiated and increases over a period of several weeks. It is this scarring that albeit protects the heart form aneurismal dilation contributes to reduced ventricular function in the most ischemic heart. In addition, the non contractile nature of the scar will lead to compensatory ventricular remodeling of nonischaemic myocardium and eventually heart failure.

Leri explains that contrary to the general belief that the heart's restricted regenerative ability is representative of the initial event of impaired cardiac function, it is the newly formed non contractile cells that are responsible for the impaired cardiac function post AMI. The inevitable scar formation that results from cardiac cell aplasia decreases the heart's contractile capacity. The need to surpass the fibrosis had led researchers to develop strategies to replace dead cells with viable cells. Bone marrow derived cells, adipose derived cells, skeletal myoblasts, cardiac cells and embryonic cells are being used experimentally to replace the rigid scar forming tissue in the heart as we are learning. The implanted stem cells most likely form a passive graft thereby decreasing the stiffness of the scarred portion of the ventricular and thus enhancing the heart's contractile function. According to Leri it is the elastic qualities of the new cells that contribute most to enhanced heart function.

2. Cardiac Stem Cells used for therapy

Adult cardiac stem cells(CSCs), isolated on the basis of various stem cells markers such as stem cell antigen c-Kit (Bearzi et al 2007 and Bertrami et al 2003) are believed to be involved in maintaining myocardial homeostasis throughout life but seem to be incapable of counteracting massive degenerative events such as AMI themselves. Application of ex vivo human cardiac stem cells seems to be a promising approach to bolster the hearts inherent repair capacity. Unlike other stem cells, cardiac stem cells should be more effective in making new myocardium as CSCs are programmed to create heart muscle and upon activation can rapidly engender parenchymal cells and coronary vessels possibly rescuing the failing heart (Quaini et al 2002, and Urbanek et al 2005).

Dawn et al (2005) injected CSCs into the coronary arteries of rats four hours after coronary occlusion. Echocardiographical analysis showed that the CSCs attenuated the increase in LV end diastolic dimensions and impairment in LV systolic performance at five weeks after infarction. The CSCs induced myocardial regeneration decreasing the infarct size by 29%. Dawn's study establishes CSC as a possible candidate for cardiac regeneration and indicates that the hearts own stem cells could be collected and stored for therapeutic purposes. Studies by Beltrami et al (2003), Oh et al (2003) and Matsuura et al (2004) support Dawn's findings and reveal that the injection of CSCs locally promotes myocardial regeneration after infarction in rats and mice. Bearzi et al (2007) similarly studied the effect of injecting human cardiac stem cells in the infarcted myocardium of immunodeficient mice and rats and found that the human CSC generate a chimeric heart, which contains human myocardium composed of myocytes , coronary resistance arterioles and capillaries. After isolating the ckit + CSC from eight patients and injecting them into the infarcted myocardium of rats, Bearzi found that the human cardiac cells were structurally and functionally integrated with the rodent myocardium and contributed to the enhanced performance of the infarcted heart.

Aside from transplanting CSCs into infarcted myocardium, some researchers have studied the possibility of activating resident stem cells in cardiac tissue to repair the infarcted heart. Linke et al (2005) studied the dog heart to see if it contains resident cardiac progenitor cells that can be stimulated by the Hepatocyte Growth Factor (HGF)-Insulin like Growth Factor 1 (IGF1) receptor system to regenerate myocardium after infarction. Linke proposed that cardiac stem cells can be activated by this growth factor signaling system. Linke based his clinical trial on a study that describes how HGF enhances vessel growth and favors cell- extracellular matrix interaction, which may be critical during myocardial regeneration. The study further indicates that stimulation on IGF1 receptors prevents cell death and induces differentiation of cardiomyocytes (Reiss et al 1996). Linke's findings were that the dog heart possess a reservoir for cardiac stem cells characterized by undifferentiated cells that are self renewing, and able to differentiate into other cell lineages. In accordance with the previous findings of other researchers, Linke's results are expected. More importantly however, Linke confirmed the idea that the cardiac stem cells posses an HGF and IGF 1 receptor system that when activated induces their migration, proliferation and survival. Linke's group injected HGF and IGF1 intramyocardially to stimulate resident cardiac progenitor cells after infarction. Indeed, the formation of myocytes and coronary vessels within the infarct was stimulated and the newly generated myocytes expressed nuclear and cytoplasmic proteins specific to cardiomyocytes.

With all these findings, we learn that the presence of cardiac stem cells in myocardium seems to be undeniable, yet the origins of the stem cells still remain debatable. The question of whether CSCs are generated within the myocardium or as Urbanek et al (2003) suggests are continuously being supplied to the heart by the bone marrow through the systemic circulation is being investigated, yet for the purpose of this review directed on the implications of stem cells in cardiac injury, this question will not be addressed.

E. Embryonic Stem Cells

These stem cells, derived from the inner mass of the blastocyst, posses unique properties. Embryonic stem cells (ESCs) can be grown in vitro and reproduce indefinitely in their undifferentiated state. They also maintain the property of multilineage commitment having the capacity to differentiate into cells originating from any of the three germ layers of the embryo. Because of their enormous potential, human ESCs have been obtained with the expectation for a future successful application of their broad therapeutic potential to patients. The uses for ESCs can be significant as adult stem cells have certain limitations. In addition to their limited proliferative capacity, the plasticity of adult stem cells has recently been challenged (Amit et al 2000). Recent studies by Terada et al (2002) and Ying et al (2002) indicate that the assumed capacity of transdifferentiation of adult stem cells into other lineages in vivo may simply be the fusing of implanted cells with existing cell types rather than a direct conversion into cardiomyocytes. The aforementioned limitations to adult stem cells and the inherent electrophysiological, structural and contractile properties of ESC derived cardiomyocytes make ESCs an ideal donor cell type for cardiac tissue regeneration.

Still, while ESCs seem to be the ideal cell type for implantation in the injured heart, the utilization of ESCs in clinical practice is hardly existent because their adult stem cell counterparts present fewer dangers than ESCs. The primary risk associated with the utilization of ESCs is its potential to form tumors, a dangerous side effect stemming from the ESC's capacity to proliferate indefinitely. Human ESCs cultured in suspension form spontaneous cellular aggregates called Embryoid Bodies. These tumors- like structures are found both in vitro and in vivo after ESC implantation (Reubinoff et al 2000, and Thomson et al 1998).

Because of the risks associated with ECS therapy, only several sporadic studies have employed these cells in their uncommitted state to repair the damaged heart. These reports, while indicating an improved ventricular function in the heart, also reveal dramatic side effects including the formation of benign tumors and the engrafting and colonizing of all organs with the possible development of neoplastic regions by the intravenously injected ESCs. In an attempt to avoid these serious complications associated with ESC implantation, researchers utilizing ESC, use methods to partially differentiate the ESCs in vitro before their implantation into the injured heart. This degree of cardiomyogenic commitment enhances the engraftment of cells in the myocardium and attenuates the probability of ESC acquiring undesired cell lineages thereby reducing the risk of tumor formation. To date most studies on the use of ESC exploit this method of differentiation.

Although due to certain ethical issues, there aren't any clinical studies on the implantation of human ESC in the human myocardium, other studies on the cardiomyocyte differentiation capacity of ESCs have been explored. Kehat et al (2001) cultivated human ESCs and plated them to form Embryoid Bodies. Kehat's group found that 8.1% of the cellular aggregates displayed contracting areas and the cells within these spontaneously contracting areas

stained positive for anti-cardiac myosin heavy chain, anti-cardiac troponin and various other cardiac specific features. Under electron microscopy, the areas also displayed varying degrees of myofibril organization consistent with early stage cardiomyocytes.

Clinical trials by Hodgson et al (2004), Min et al (2002) and Min et al (2003) study the effects of transplanting ESC derived cardiomyocytes into the hearts of rats. Hodgson injected infarcted rat hearts with ESCs preexamined for cardiogenicity. The trial found that stem cell delivery generated new cardiomyocytes that integrated into host myocardium and was associated with normalized ventricular architectures, little scar, and decrease in signs of myocardial necrosis. Hodgson's study points out that the ESCs injected were well accepted by the rats as no evidence of graft rejection, or tumor formation was observed. Hodgson's findings conclude that ESCs through differentiation within the host myocardium, can contribute to a stable beneficial outcome on contractile function and ventricular remodeling in the infarcted rat heart

Min et al (2001, injected ESCs into the infarcted hearts of rats intramyocardially. Compared with a control group injected with the equivalent volume of cell free medium, the experimental rat's cardiac function was significantly improved six weeks after cell transplantation. Min's study indicates that embryonic stem cells can be implanted and survive in injured rat myocardium and that these cells improve myocardial function. In a later analysis, Min et al (2003) studied the prolonged effects of ESC transplantation in the infarcted heart. His group injected rats with ESCs stained with GFP and studied the heart and its function 32 weeks after infarction and subsequent injection. After 32 weeks, tissue positive for GFP was found in the myocardium suggesting that the engrafted cells were still present. Echocardiographic data also showed that ESC transplantation significantly improved ventricular function relative to the control group even after four months.

In contrast to the findings of Hodgson and Min, Fijnvandraat's study on embryonic stem cells reveal that ESCs are not capable of restoring cardiac function in the infarcted heart. In 2003, FIjnvandraat in his study on in vitro differentiated embryonic stem cells and its subsequent cardiogenesis in vivo and in vitro found that ESC derived cardiomyocytes maintain a phenotype of the primary heart tube, and not of the chamber myocardium. Fijnvandraat, with very specific regulation of gene expression discovered that the ESC derived cardiomyocytes do not develop into fully mature chamber myocardium, and rather show a phenotype comparable to young embryonic cardiomyocytes in vivo and thus would not be of great importance in the area of restoring cardiac function(Fijnvandraat et al 2003).

The clinical implications of these findings seem somewhat promising as rat ESCs display the potential to restore damaged rat myocardium. Due to certain ethical concerns that lead to complications in obtaining human ESC it may seem plausible to use rat ESC to restore human myocardium. Yet Mcdevitt et al (2005) and Kehat et al (2001)'s studies display that rat and human ESC are very different and thus cannot be used interchangably. Mcdeviit studied proliferation patterns in human and mouse embryonic stem cell derived cardiomyocytes. The study revealed that cardiomyocytes differentiating from human ESCs exhibit a high level of proliferation which progressively decreases as the cells mature in culture. In stark contrast to the cardiomyocytes derived from human ESCs, cardiomyocytes derived from mouse ES cells appeared to be non proliferative using similar experimental techniques.

Kehat, in his work notes that there are some major differences in human and murine ES cell differentiation because there are significant differences in human and murine development.

Kehat compares his results in the differentiation of human ESCs with the rate of differentiation in mouse ESC. He explains that differentiation of human of ES cells proceed at a much slower rate than in mouse ESCs. In mouse cells, cells are grown in suspension for five days and spontaneously contracting areas appear one day after plating and within two- ten days 80-90% of EBs reveals pulsating areas. In contrast to this, human ESCs are grown in suspension for ten days and the spontaneous contracting areas did not appear until day four after plating; and the percentage of pulsating areas is a mere 8.1% Thus to have any really benefit for human myocardial regeneration clinical trials focusing specifically on human ESCs must be utilized .

Min et al (2002), in their experiment also spotted a group of growth factor proteins that seems to be involved in cardiac organogenesis in the early embryonic stages. They noted that different growth factors like vascular endothelial growth factor (VEGF) seem to enhance the functional improvement of post infarcted mouse hearts with transplantation of ESCs. To test whether specific growth factors would assist the ESCs in differentiating into functional cardiac tissue, Kofidis et al (2005) injected undifferentiated and labeled ESC into the infarcted mouse myocardium, added growth factors, and comparatively evaluated the restorative effect of their transplantation. Kofidis's group injected rat hearts with ESC supplemented with fibroblast growth factor (FGF), and ESC supplemented with transforming growth factor (TGF) and compared the subsequent cardiac function to a control group of rats injected only with the growth factors and not the ESC. Each group of growth factors had different effects on the capacity for regeneration. Kofidis found that TGF administration, compared with FGF and VEGF displayed the most restorative and differentiation potential. To further test the effect of supplementing growth factors with ESC, Yang et al (2002) studied VEGF to examine if an improvement in blood flow to ischemic regions can be found. Yang injected ESCs over expressing VEGF into the hearts of mice post infarction and compared the cardiac function to group of mice injected with normal ESCs. The effects of the ESCs and the ESC over expressing VEGF on neovascularization in ischemic were evaluated and compared. Improvement in left ventricular systolic function was significantly greater in post AMI mice transplanted with ESC plus VEGF. In addition, Yang found that the ESC themselves expressed certain amounts of VEGF and thus were able to stimulate the growth of new blood vessels in injured myocardium.

Earlier on, Kofidis et al (2004) administered a study on growth factors and explicitly studied the effects of Insulin- like growth factor -1 (IGF-1) in enhancing the proliferative capacity of ESC. His group implanted ECSs with IGF- 1 in an attempt to enhance the restorative capability of ESC on injured myocardium. They found that IGF -1 promoted expression of cardiomyocyte phenotype in ESCs in vivo. Their findings indicate that supplementation with IGF -1 enhances a- sarcomeric actin expression, and expression of MHC 1 thus indicating a stronger differentiation potential in the stem cells supplemented with IGF- 1. A trend in better myocardial fractional shortening in the group of mice treated with ESC and IGF-1 was also noted. These studies are significant as they suggest that growth factors may be supplemented with pluripotent cells to drive their differentiation to the desired phenotype and enhance their engraftment, leading to more efficient use of stem cells in cardiac therapy.

Kofidis further describes that contrary to the notion that embryonic stem cell are not susceptible to a host's immune response, ESC transplantation may face similar issues of allorecognition as solid organs transplantation. The idea that ESCs express only small amounts of MHC I, a cell marker foreign to cardiac tissue, in culture and thus are not susceptible to immunorejection (Kaufman and Thomspon 2002)is refuted in Kofidis's experiment as he found

nearly 70% of the injected ESCs in vivo expressing MHC I. In a series of immunological experiments in their lab, Kofidis's group obtained evidence of a humoral response in donor cells. They detected an increased production of Interferon- gamma, Interleukin -2 (IL-2), IL-4, and IL-5 by activated splenocytes of the host in response to ESC injection into the heart. Even so, many clinical trials including that of Hodgson's involving the injection of ESC in the hearts of rats in vivo have displayed no real signs of immunorejection.

Embryonic stem cells posses certain advantageous characteristics in their use for stem cell therapy. Their unlimited proliferative capacity, coupled with their ability to differentiate into any type of cell make them a model contestant for stem cell therapy. In contrast to their adult stem cell counterparts, studies with ESCs have yielded mostly unvarying results in their ability to differentiate into cardiac myocytes and their contribution to improved myocardial performance post myocardial infarction. The dangers associated with transplanting ESC can be circumvented by plating the ESC in vitro to start their cardiomyogenic differentiation before being implanted in vitro. Due to certain human ethical concerns and the subsequent lack of funding, current studies on the implantation of ESC and their functional benefit in damaged human myocardium are lacking. Hopefully this will soon change and therapeutic effects of these stem cells will be taken advantage of.

3. Conclusion

Cellular therapy is the latest in a series of strategies applied in an effort to mitigate the progressive and otherwise irreversible loss of cardiac function that frequently follows a heart attack. The need for an effective therapy to restore cardiac function following a cardiac event in which cardiomyocytes atrophy is essential. Heart failure, the condition that occurs when the heart's contractile reserve is depleted below a critical threshold, is already the most common cause of hospitalization in US citizens over 65, and, as our population ages, some have predicted epidemic proportions of this diseases (Laflamme and Murry 2005). The proposal that heart failure could be reversed or prevented if new myocardium could be grown in diseased hearts is excitedly being explored. This idea has gained widespread attention recently, leading to numerous reports and multiple early stage clinical trials in this field. In this review a summary of current data arising from clinical applications involving hematopoietic, adipose-derived, skeletal muscle-derived, cardiac and embryonic stem cells in cellular therapy for acute myocardial infarction is presented. While studies of cardiac regeneration utilizing these stem cells seem promising and are advancing at a quick rate, perfecting our interventions to repair the heart will take continuous effort and many years.

BIBLIOGRAPHY

Amit M, Carpenter MK, Inokuma MS, Chiu CP, Harris CP, Waknitz MA, Itskovitz-Eldor J, Thomson JA. (2000) Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative   potential for prolonged periods of culture. Dev Biol. 227(2):271-8.

Balsam LB, Wagers AJ, Christensen JL, Kofidis T, Weissman IL, Robbins RC. (2004) Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium Nature. 428:668-73.

Bearzi C, Rota M, Hosoda T, Tillmanns J, Nascimbene A, De Angelis A, Yasuzawa-Amano S, Trofimova I,   Siggins RW, Lecapitaine N, Cascapera S, Beltrami AP, D'Alessandro DA, Zias E, Quaini F, Urbanek   K, Michler RE, Bolli R, Kajstura J, Leri A, Anversa P.(2007) Human cardiac stem cells. Proc. Natl.   Acad. Sci. USA. 35: 14068-73.

Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, Kasahara H, Rota M, Musso E,   Urbanek K, Leri A, Kajstura J, Nadal-Ginard B, Anversa P.(2003) Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell. 114: 763–766.

Beltrami AP, Urbanek K, Kajstura J, Yan SM, Finato N, Bussani R, Nadal-Ginard B, Silvestri F, Leri A,   Beltrami CA, Anversa P (2001). Evidence that human cardiac myocytes divide after myocardial   infarction. N Engl J Med. 334: 1750–1757.

Bergmann O, Bhardwaj RD, Bernard S, Zdunek S, Barnabé-Heider F, Walsh S, Zupicich J, Alkass K,   Buchholz BA, Druid H, Jovinge S, Frisén J.(2009) Evidence for cardiomyocyte renewal in humans.   Science. 324:98-102.

Dawn B, Stein AB, Urbanek K, Rota M, Whang B, Rastaldo R, Torella D, Tang X, Rezazadeh A, Kajstura J, Leri A, Hunt G, Varma J, Prabhu SD, Anversa P, Bolli R. (2005) Cardiac stem cells delivered   intravascularly traverse the vessel barrier, regenerate infarcted myocardium, and improve   cardiac function. Proc. Natl. Acad. Sci. USA. 102; 10:3766-71.

De Ugarte DA, Alfonso Z, Zuk PA, Elbarbary A, Zhu M, Ashjian P, Benhaim P, Hedrick MH, Fraser JK.(2003)   Differential expression of stem cell mobilization-associated molecules on multi-lineage cells   from adipose tissue and bone marrow. Immunol Lett. 89(2-3):267-70.

Fijnvandraat AC, van Ginneken AC, de Boer PA, Ruijter JM, Christoffels VM, Moorman AF, Lekanne   Deprez RH.(2003) Cardiomyocytes derived from embryonic stem cells resemble cardiomyocytes   of the embryonic heart tube. Cardiovasc Res. 58(2):399-409.

Ghostine S, Carrion C, Guarita Souza LC, (2002) Long-term efficacy of myoblast transplantation on   regional structure and function after myocardial infarction. Circulation. 106 :131–6.

Hodgson DM, Behfar A, Zingman LV, Kane GC, Perez-Terzic C, Alekseev AE, Pucéat M, Terzic A. (2004)   Stable benefit of embryonic stem cell therapy in myocardial infarction. Am JPhysiol Heart Circ   Physiol. 287(2):471-9.

Jackson KA, Majka SM, Wang H, Pocius J, Hartley CJ, Majesky MW, Entman ML, Michael LH, Hirschi KK,   Godell MA.(2001) Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. J Clin Invest. 107: 1395–1402.

Janssens S, Dubois C, Bogaert J, Theunissen K, Deroose C, Desmet W, Kalantzi M, Herbots L, Sinnaeve P,   Dens J, Maertens J, Rademakers F, Dymarkowski S, Gheysens O, Van Cleemput J, Bormans G, Nuyts J, Belmans A, Mortelmans L, Boogaerts M, Van de Werf F(2006). Autologous bone   marrow-derivedstem-cell transfer in patients with ST-segment elevation myocardial   infarction:double-blind, randomised controlled trial. Lancet. 367:113–121.

Kajstura J, Leri A, Finato N, Di Loreto C, Beltrami CA, Anversa P. (1998) Myocyte proliferation in end-   stage cardiac failure in humans. Proc Natl Acad Sci USA.95: 8801–8805.

Kaufman DS, Thomson JA. (2002) Human ES cells--haematopoiesis and transplantation strategies. J Anat.   200:243-8.

Kehat I, Kenyagin KD, Snir M, Segev H, Amit M, Gepstein A, Livne E, Binah O, Eldor JI, and Gepstein L.   (2001) Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. J Clin Invest. 108: 407–414.

Kofidis T, de Bruin JL, Yamane T, Balsam LB, Lebl DR, Swijnenburg RJ, Tanaka M, Weissman IL, Robbins   RC. (2004)Insulin-like growth factor promotes engraftment, differentiation, and functional   improvement after transfer of embryonic stem cells for myocardial restoration. Stem Cells.   22(7):1239-45.

Kofidis T, de Bruin JL, Yamane T, Tanaka M, Lebl DR, Swijnenburg RJ, Weissman IL, Robbins RC. (2005)   Stimulation of paracrine pathways with growth factors enhances embryonic stem cell   engraftment and host-specific differentiation in the heart after ischemic myocardial injury. Circulation. 17;111(19):2486-93.

Korf-Klingebiel M, Kempf T, Sauer T, Brinkmann E, FischerP, Meyer GP, Ganser A, Drexler H, Wollert   KC.(2008) Bone marrow cells are a rich source of growthfactors and cytokines: implications for   cell therapy trials after myocardial infarction. European Heart Journal. 29; 2851–2858.

Laflamme MA, Murry CE.(2005) Regenerating the heart. Nat Biotechnol. 23: 845–856.

Leri A, Kajstura J, Anversa P. (2005) Cardiac stem cells and mechanisms of myocardial regeneration.   Physiol Rev. 85: 1373–1416.

Linke A, Muller P, Nurzynska D, Casarsa C, Torella D, Nascimbene A, Castaldo C, Cascapera S, Bohm M,   Quaini F, Urbanek K, Leri A, Hintze TH, Kajstura J, Anversa P.(2005) Stem cells in the dog heart   are self-renewing, clonogenic, and multipotent and regenerate infarcted myocardium,   improving cardiac function. Proc Natl Acad Sci USA 102: 8966–8971.

Lunde K, Solheim S, Aakhus S, Arnesen H, Abdelnoor M, Egeland T, Endresen K, Ilebekk A, Mangschau A,   Fjeld JG, Smith HJ, Taraldsrud E, Grøgaard HK, Bjørnerheim R, Brekke M, Müller C, Hopp E,   Ragnarsson A, Brinchmann JE, Forfang K. (2006) Intracoronary injection of mononuclear bone   marrow cells in acute myocardial infarction. N Engl J Med.355 (12):1199-209.

Matsuura K, Nagai T, Nishigaki N, Oyama T, Nishi J, Wada H, Sano M, Toko H, Akazawa H, Sato T, Nakaya   H, Kasanuki H, Komuro I.(2004) Adult cardiac Sca-1-positive cells differentiate into beating   cardiomycytes. J Biol Chem. 279: 11384–11391.

McDevitt TC, Laflamme MA, Murry CE. (2005) Proliferation of cardiomyocytes derived from human embryonic stem cells is mediated via the IGF/PI 3-kinase/Akt signaling pathway. J Mol Cell   Cardiol. 39(6):865-73.

Menasché P, Alfieri O, Janssens S, McKenna W, Reichenspurner H, Trinquart L, Vilquin JT, Marolleau JP, Seymour B, Larghero J, Lake S, Chatellier G, Solomon S, Desnos M, Hagège AA. (2008)The   Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial: first randomized placebo-controlled study of myoblast transplantation. Circulation. 117(9):1189-200.

Menasché P, Hagège AA, Vilquin JT, Desnos M, Abergel E, Pouzet B, Bel A, Sarateanu S, Scorsin M,   Schwartz K, Bruneval P, Benbunan M, Marolleau JP, Duboc D.(2003)

Autologous skeletal   myoblast transplantation for severe postinfarction left ventricular dysfunction**. J Am Coll   Cardiol.**41(7):1078-83.

Menasche´ P, Hagege AA, Scorsin M, Pouzet B, Desnos M, Duboc D, Schwartz K, Vilquin JT, Marolleau   JP.(2001) Myoblast transplantation for heart failure. Lancet. 357:279–80.

Meyer GP, Wollert KC, Lotz J, Steffens J, Lippolt P, Fichtner S, Hecker H, Schaefer A, Arseniev L,   Hertenstein B, Ganser A, Drexler H.(2006) Intracoronary bone marrow cell transfer after   myocardial infarction: eighteen months' follow-up data from the randomized, controlled BOOST   (Bone marrow transfer to enhance ST-elevation infarct regeneration) trial. Circulation.   113(10):1287-94.

Min JY, Yang Y, Converso KL, Liu L, Huang Q, Morgan JP, and Xiao YF.(2002) Transplantation of   embryonic stem cells improves cardiac function in post-infarcted rats. J Appl Physiol. 92: 288–   296.

Min JY, Yang Y, Sullivan MF, Ke Q, Converso KL, Chen Y,Morgan JP, and Xiao YF.(2003) Long-term   improvement of cardiac function in rats after infarction by transplantation of embryonic stem   cells. J Thorac Cardiovasc Surg. 125: 361–369.

Murry CE, Soonpaa MH, Reinecke H, Nakajima H, Nakajima HO, Rubart M, Pasumarthi KB, Virag JI,   Bartelmez SH, Poppa V, Bradford G, Dowell JD, Williams DA, Field LJ.(2004) Haematopoietic   stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. Nature.   428(6983):664-8.

Murry CE, Wiseman RW, Schwartz SM, Hauschka SD.(1996) Skeletal myoblast transplantation for repair   of myocardial necrosis. J Clin Invest. 98:2512–23.

Oh H, Bradfute SB, Gallardo TD, Nakamura T, Gaussin V, Mishina Y, Pocius J, Michael LH, Behringer RR,   Garry DJ, Entman NL, Schneider MD.(2003) Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. Proc Natl Acad Sci USA. 100: 12313–12318.

Oh H, Taffet GE, Youker KA, Entman ML, Overbeek PA, Michael LH, Schneider MD. (2001)Telomerase   reverse transcriptase promotes cardiac muscle cell proliferation, hypertrophy, and survival. Proc   Natl Acad Sci USA. 98: 10308–10313.

Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine   DM, Leri A, Anversa P.(2001a) Bone marrow cells regenerate infarcted myocardium. Nature. 420: 701–705.

Orlic D, Kajstura J, Chimenti S, F, I, Quaini F,B, Bodine DM, Leri A Anversa P.(2001b) Mobilized bone   marrow cells repair the infarcted heart, improving function and survival Proc Natl Acad Sci USA.   98(18): 10344–10349.

Planat-Bénard V, Menard C, André M, Puceat M, Perez A, Garcia-Verdugo JM, Pénicaud L and Casteilla   L.(2004) Spontaneous Cardiomyocyte Differentiation From Adipose Tissue Stroma Cells Circ. Res.   94;223-229.

Quaini F, Urbanek K. Beltrami AP, Finato, N. Beltrami CA, Nadal-Ginard B, Kajstura J. Leri A, Anversa P.   (2002) Chimerism of the transplanted heart. N Engl J Med. 346: 5–15.

Reinecke H, Zhang M, Bartosek T, Murry CE. (1999) Survival, integration, and differentiation of   cardiomyocyte grafts: a study in normal and injured rat hearts. Circulation. 100:193–202.

Reiss K, Cheng W, Ferber A, Kajstura J, Li P, Li B, Olivetti G, Homcy CJ, Baserga R,   Anversa P. (1996) Overexpression of insulin-like growth factor-1 in the heart is coupled with   myocyte proliferation in transgenic mice. Proc Natl Acad Sci USA. 93:8630–8635.

Reubinoff BE, Pera M, Fong CY, Trounson A, and Bongso A. (2000) Embryonic stem cell lines from human   blastocysts: somatic differentiationin vitro. Nat Biotech. 18: 399–404.

Schachinger V, Erbs S, Elsasser A, Haberbosch W, Hambrecht R, Holschermann H, Yu J, Corti R, Mathey   DG, Hamm CW, Suselbeck T, Assmus B, Tonn T, Dimmeler S, Zeiher AM. (2006) Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. N Engl J Med. 355:1210–   1221.

Soonpaa MH, Field LJ. (1998) Survey of studies examining mammalian cardiomyocyte DNA synthesis.   Circ Res. 83: 15–26.

Spalding KL, Buchholz BA, Bergman LE, Druid H, Frisén J. (2005) Forensics Age written in teeth by   nuclear tests. Nature. 437, 333-334.

Taylor DA, Atkins BZ, Hungspreugs P, Jones TR, Reedy MC, Hutcheson KA, Glower DD, Kraus WE. (1998)   Regenerating functional myocardium: improved performance after skeletal myoblast   transplantation. Nat Med. 4:929–33.

Terada N, Hamazaki T, Oka M, Hoki M, Mastalerz DM, Nakano Y, Meyer EM, Morel L, Petersen BE, Scott   EW. (2002) Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion.   Nature. 416(6880):542-5.

Thomson JA, Eldor JI, Shapiro SS, Waknitz MA, Swiergiel JJ,Marshall VS, and Jones JM.(1998) Embryonic   stem cell lines derivedfrom human blastocysts. Science 282: 1145–1147.

Urbanek K, Quaini F, Tasca G, Torella D, Castaldo C, Nadal-Ginard B, Leri A, Kajstura J, Quaini E, Anversa   P.(2003) Intense myocyte formation from cardiac stem cells in human cardiac hypertrophy. Proc   Natl Acad Sci U S A. 100: 10440–10445.

 Urbanek K, Torella D, Sheikh F, De Angelis A, Nurzynska D, Silvestri F, Beltrami CA, Bussani R, Beltrami   AP, Quaini F, Bolli R, Leri A, Kajstura J, Anversa P.(2005) Myocardial regeneration by activation of multipotent cardiac stem cells in ischemic heart failure. Proc Natl Acad Sci U S A. 102: 8692– 8697.

Winter A, Breit S, Parsch D, Benz K, Steck E, Hauner H, Weber RM, Ewerbeck V, Richter W.(2003)   Cartilage-like gene expression in differentiated human stem cell spheroids: a comparison of bone marrow-derived and adipose tissue-derived stromal cells. Arthritis Rheum.48(2):418-29.

Wu KH, Liu YL, Zhou B, Han ZC.(2006) Cellular therapy and myocardial tissue engineering: the role of   adult stem and progenitor cells. Eur J Cardiothorac Surg. 30(5):770-81.

Yang Y, Min JY, Rana JS, Ke Q, Cai J, Chen Y, Morgan JP, Xiao YF.(2002) VEGF enhances functional   improvement of postinfarcted hearts by transplantation of ESC-differentiated cells. J Appl   Physiol. 93(3):1140-51.

Ying QL, Nichols J, Evans EP, Smith AG. (2002) Changing potency by spontaneous fusion. Nature.   416(6880):545-8.

Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick   MH.(2002) Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell. 13:4279–   4295.