Diploma Thesis

Cell based therapy after myocardial infarction
The potential of stem cells

submitted by

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Abstract (German)

Der akute Myokardinfarkt stellt eine der führenden Todesursachen der westlichen Welt dar. Auf pathophysiologischer Ebene kommt es im Verlauf zu Umbauprozessen, dem ventricular remodeling, welches zur Einschränkung der ventriukulären Pumpfunktion, sowie in Folge zu chronischer Herzinsuffizienz führt. Zur Zeit besteht die einzige kausale Therapie der Herzinsuffizienz in der Herztransplantation. Während der letzten 20 Jahren wurde an einer neuen Therapieoption, der Stammzelltransplantation, geforscht. Verschiedenen Arten von Stammzellen wurde dabei die Fähigkeit zugeschrieben, an kardialer Regeneration beteiligt zu sein, beziehungsweise das ventrikuläre remodeling aufzuhalten oder sogar umzukehren.

Eine Literaturrecherche wurde durchgeführt, mit dem Ziel Daten über die verschiedenen Arten der Stammzellen und die Ergebnisse der neuesten Forschungen über den Einsatz von Stammzellen nach Herzinfarkt aus Tierversuchen und klinischen Studien zu sammeln. Zudem wurden Daten zur Sicherheit der Anwendung von Stammzellen nach Herzinfarkt ermittelt. Letztlich setzt sich die Arbeit mit der Frage auseinander, ob die Stammzelltransplantation entwickelt und sicher genug ist, um in klinischen therapie für Patienten nach Herzinfarkt eingesetzt werden zu können.

Eine verbesserte Pumpfunktion, sowie eine Reduktion der Infarktgröße konnten durch Einsatz von Stammzelltransplantation nach Herzinfarkt in Tierversuchen nach gewiesen werden. Knochenmarkstammzellen waren die am meisten verwendeten Zellarten in klinischen Studien. Die meisten der mittel- und langfristigen klinischen Studien berichteten über eine verbesserte Herzfunktion, wobei auch Studien existieren, die keinerlei therapeutischen Effekt der Stammzelltransplantation zeigen.

Abstract (English)

Introduction

Acute myocardial infarction is one of the leading causes of morbidity and mortality in developed countries. One of the major complications of myocardial infarction is the process commonly referred to as ventricular remodeling, which causes ventricular dilatation leading to ventricular insufficiency and heart failure. Currently, the only fundamental therapy available for refractory heart failure is heart transplantation. During the last 20 years, stem cell transplantation has evolved as a possible therapy for post-myocardial infarction conditions. Different types of stem cells have been found to assist cardiac regeneration and prevent or even reverse the process of ventricular remodeling.

Methods and aims

A literature research was conducted with the aim of gathering data about the different types of stem cells that have the potential to assist in cardiac regeneration and the results achieved through preclinical animal trials, as well as clinical human trials, using stem cell based therapies to treat post-myocardial infarction conditions. Another aim of this literature research is to gather information concerning the safety of stem cell transplantation. Finally, this research aims at finding out whether or not stem cell therapy is developed enough to be used in clinical treatment of patients that suffered from myocardial infarction.

Results and conclusion

An increased cardiac function and reduced infarct size were reported in the results of many animal trials. Bone marrow derived stem cells were the most common type of cells used in the clinical trials. While most of the mid-term and long-term human trials reported an increased cardiac function as well as other therapeutic effects of stem cells transplantation after myocardial infarction, some human trials reported no therapeutic effects at all. All the human trials reviewed in this paper suggested the safety of stem cells transplantation in cardiac patients. However, it seems that this novel therapy needs to be further developed before it could be applied as a standard therapy. Future research should aim at exploring different aspects of stem cell transplantation after myocardial infarction, such as the mechanism through which stem cell transplantation contributes in cardiac regeneration, the ideal cell type for cardiac regeneration, the most efficient method of cell delivery, the best timing for cell transplantation and the dose of cells that should be injected.

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List of Acronyms

MI: Myocardial infarction

ACE: Angiotensin-converting-enzyme

ADSC: Adipose-derived stem cells

ADSC-CM: Adipose-derived stem cells' conditioned medium

AMI: Acute myocardial

ApoA1: Apolipoprotein A1

ApoB: Apolipoprotein B

BMC: Bone marrow derived stem cell

BMI: Body mass index

CABG: Coronary artery bypass graft.

CM: conditioned medium

CPC: Circulating blood-derived progenitor cells

CSC: Cardiac stem cell

dhUMC: Differentiated human umbilical cord mesenchymal cells

ECG: Electrocardiograph

EF: Ejection fraction

ESC: Embryonic stem cell

G-CSF: Granulocyte colony-stimulating factor

hCSC: Human cardiac stem cell

HDL: High density level

HLA: Human leukocyte antigen

hMSC: Human mesenchymal stem cell

HSC: Hematopoietic stem cell

hUMC: Human umbilical cord mesenchymal cells

IC: Intracoronary

iPS cell: Induced pluripotent stem cell

IV: Intravenous

LAD: Left anterior descending artery

LBBB: Left bundle branch block.

LDH: Lactate dehydrogenase

LV: Left ventricle

LVEDV: Left ventricular end diastolic volume

LVEF: left ventricular ejection fraction

LVESV: Left ventricular end systolic volume

MSC: Mesenchymal stem cell

NSTEMI: non-ST segment elevation myocardial infarction

PAR: population attributable risks

PBC: Peripheral blood cell

PBS: Phosphate buffered saline

PCI: Percutaneos coronary intervention

PTCA: Percutaneous coronary transmural angioplasty.

SCF: Stem cell factor

SPECT: Single-photon-emission computed tomography

STEMI: ST-segment elevation myocardial infarction

SVI: Stroke volume index

TE: Transendocardial

VCF: Velocity of circumferential fiber shortening

WHO: World Health Organization

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1 Introduction

1.1 Myocardial Infarction

Myocardial infarction (MI) is one of the most common presentations of ischemic heart disease, with acute myocardial infarction (AMI) being one of the leading causes of morbidity and mortality in developed countries (1). In 2008, it was estimated by the World Health Organization (WHO) that ischemic heart disease is the leading cause of death in both high and middle income countries, contributing to 12.8% of worldwide deaths, and causing more than 15% of the deaths in the high-income countries (2) (Figure 1).

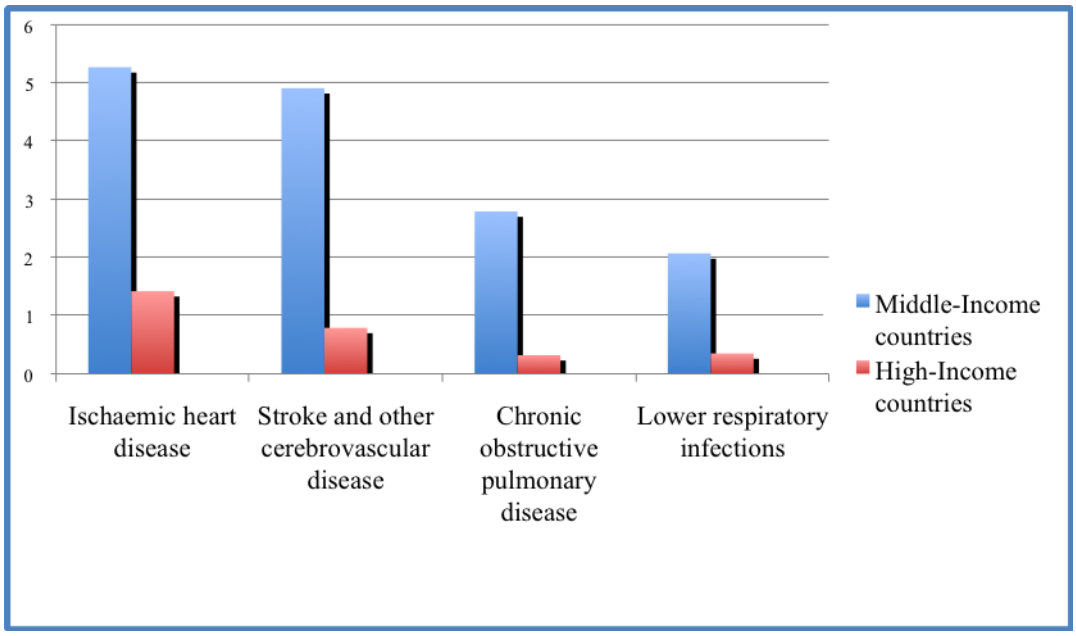

Figure 1: Number of deaths (in millions) and its causes in middle and high-income countries (2)**.**

1.1.1 Definition

Acute myocardial infarction can be defined according to different factors related to clinical, biochemical, electrocardiographic, as well as pathological characteristics. Myocardial infarction as a term indicates the death of cardiac myocytes resulting from ischemia (3). Acute myocardial infarction is a sudden manifestation of insufficient blood supply to the myocardium, caused by a sudden coronary artery occlusion that leads to myocardial ischemia. Ischemia is caused by a perfusion imbalance between blood supply and demand, leading to a shortage of oxygen and other nutrients needed for cellular metabolism and causing necrosis (4). Consequently, the use of the term acute myocardial

infarction should be applied when there is evidence of myocardial necrosis in a clinical setting consistent with acute myocardial ischemia (5).

The WHO initially defined myocardial infarction by the presentation of at least two the following criteria: typical pathological changes in electrocardiograph, increase in the concentration of creatine kinase-MB in blood, and the presence of angina pectoris symptoms (6). However, creatine kinase-MB is not highly specific for myocardial ischemia, which might lead to false diagnosis. More recently, the European society of cardiology, as well as the American College of Cardiology tried to redefine myocardial infarction according to more clinically relevant diagnostic criteria. This criteria includes an increased level of troponin T and I with either ischemic symptoms, pathological Q-waves on electrocardiograph (ECG), or ST-segment elevation or depression on ECG (7).

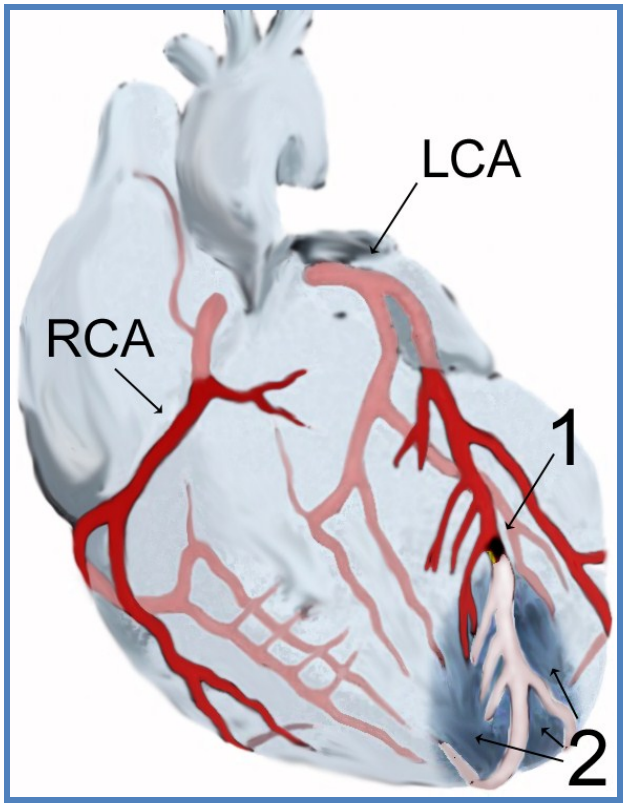

Figure 2: Diagram myocardial infarction: 1) position of stenosis. 2) Ischemic myocardium (137)

1.1.2 Pathopysiology

Myocardial infarction (Figure 2) might occur acutely on top of a chronic atherosclerotic disease of the coronary arteries. The development of atherosclerotic disease could be triggered by many factors, which could directly affect the arterial wall, with a later consequence of developing an atherosclerotic plaque (8, 9). At the early stage of plaque

formation, the coronary arteries undergo a compensatory enlargement of their lumen in the plaque area, preserving an almost normal lumen cross-section area, and delaying any functional manifestation of the disease. Coronary atherosclerosis might therefore remain asymptomatic for years. Later on however, as the stenosis takes over around 40% of the internal lumen, coronary disease might begin to manifest (10). However, acute intraluminal thrombosis could also occur due to disruption of a plaque, causing coronary artery occlusion and leading to an interruption of the blood flow to the myocardium (11). Microembolization and coronary vasoconstriction may also play a role. A thrombus may less commonly result from superficial erosion of the endothelial surface (12). Coronary artery occlusion prevents the oxygen and nutrient supply needed by the myocardium to survive, causing myocardial ischemia. If this ischemic condition lasts for longer than 30 minutes, myocardial cells start to die and myocardial infarction takes place (13). The longer this occlusion continues, the more damage it causes to the myocardium and the bigger the infarct size is. If ischemia continues for more than 6 hours, the whole myocardial tissue supplied by the occluded coronary artery will undergo necrosis. This might cause a decrease in the left ventricular function, and could end up in premature death (14). Different types of myocardial infarction could be classified clinically as shown in table 1.

1.1.3 Symptoms

The most common symptom of myocardial infarction is angina pectoris, a chest pain resulting from myocardial ischemia. This pain is often described by the patients as a sensation of tightness, pressure, or squeezing(4). In up to 70% of the patients, this pain is localized over the sternum, and usually radiates to the left arm, but might also radiate to the epigastrium, lower jaw, right arm, or to the back. An inferior wall infarction could however manifest as an epigastric pain, causing false diagnosis (15). On the other hand, about 20% of myocardial infarctions cause no pain, especially in diabetics and elderly patients (16). Depending on the size and localization of the infarction, patients might also suffer from dyspnea, which indicates a limitation in the left ventricular function, and might be a sign of acute heart failure (15). Other symptoms that might be present in patients suffering a myocardial infarction include diaphoresis, light-headness, weakness, as well as other vegetative symptoms such as palpitations, nausea and vomiting (4).

Type	Clinical features
Type 1	Spontaneous myocardial infarction related to ischemia due to a primary coronary event such as plaque erosion and/or rupture, fissuring, or dissection
Type 2	Myocardial infarction secondary to ischemia due to either increased oxygen demand or decreased supply, e.g. coronary artery spasm, coronary embolism, anemia, arrhythmias, hypertension, or hypotension
Type 3	Sudden unexpected cardiac death, including cardiac arrest, often with symptoms suggestive of myocardial ischemia, accompanied by presumably new ST elevation, or new LBBB, or evidence of fresh thrombus in a coronary artery by angiography and/or at autopsy, but death occurring before blood samples could be obtained, or at a time before the appearance of cardiac biomarkers in the blood
Type 4a	Myocardial infarction associated with PCI
Type 4b	Myocardial infarction associated with stent thrombosis as documented by angiography or at autopsy
Type 5	Myocardial infarction associated with CABG

Table 1: Classifications of myocardial infarction [modified after (3)**]**
LBBB: Left bundle branch block.
CABG: Coronary artery bypass graft.

1.1.4 Electrocardiography

Electrocardiography (ECG) is an important diagnostic device when it comes to cardiac diseases. In case of myocardial infarction, the changes in the different waves on ECG allow a differentiation between old and new events. Moreover, the different leads of ECG could give a clue about the localization of the infarction, suggesting the infarct-related artery, as well as the quantity of myocardium at risk. This might however face a number of limitations, such as the different individual anatomical variations of coronary arteries (17). The initial presentations of myocardial ischemia on ECG are in the form of typical ST-segment and T-wave changes (18). The presence of prominent symmetrical T-waves presenting with increased amplitude in two or more contiguous leads might appear even before any ST-segment changes take place (3). However, this hyper-acute T-wave appears at the very beginning of myocardial ischemia, and is therefore rarely registered. The more common first electrocardiographic manifestation of acute myocardial infarction is ST-segment elevation (Figure 3) with a positive T-wave. This ST-segment elevation is found

on the leads representing the infarct zone. The presence of new ST-segment elevation at the J-point of two contiguous leads with ≥0,1 mV (peripheral leads) or ≥0,2 mV (pericardial leads) indicates a diagnosis of ST-segment elevation myocardial infarction (STEMI). On the other hand, if only primarily ST-segment depressions were found on ECG in the presence of typical myocardial infarction symptoms and biomarkers, then a non-ST segment elevation myocardial infarction (NSTEMI) should be diagnosed (19).

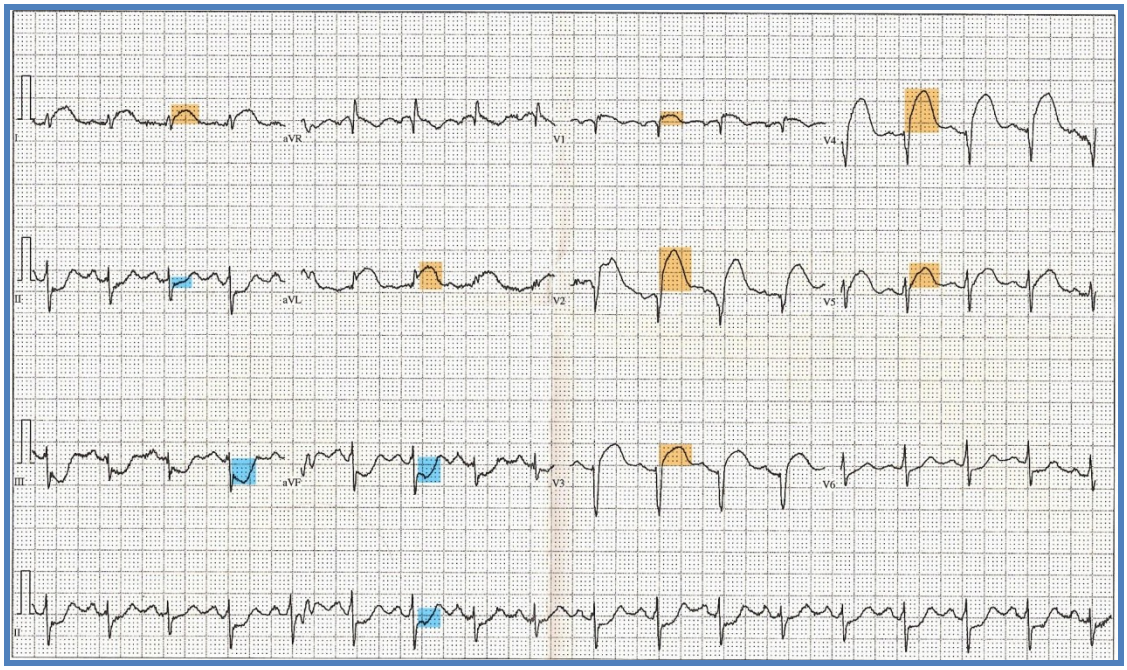

Figure 3: ECG showing anterior wall STEMI, Tachycardia, and Anterior Fascicular Block.
Color Key**: Orange**=ST Elevation (anterior wall), **Blue**=ST Depression (inferior wall) (20)**.**

1.1.5 Biomarkers

During the event of myocardial infarction, myocytes undergo necrosis, releasing a number of proteins and biochemical markers into the blood circulation. One of those biochemical markers is cardiac troponin (T and I), which is currently considered a 'golden standard', representing the preferred biomarker for diagnosing myocardial infarction. Not only is cardiac troponin a highly sensitive marker, but also almost absolutely specific to myocardial damage, making it suitable for diagnosing even microinfarctions in the myocardium (21). The levels of troponin T and I should be tested twice, with at least 6 to 9 hours in between. An increase of the biomarker over the 99th percentile of the upper reference limit, in combination with ischemic symptoms, pathological Q-waves or ST-segment elevation or depression on ECG indicates the presence of myocardial ischemia

(3). Other biochemical markers that indicate cardiac cell necrosis are myoglobin, creatine kinase-MB, lactate dehydrogenase **(**LDH) and many others. (21)

1.1.6 Risk Factors

The most effective method to prevent myocardial infarction is indeed the reduction of the risk factors leading to it. There are many factors that increase the risk of myocardial infarction, with a lot of them being easy to modify. The most important risk factors and their classification are summarized in table 2.

Class	Risk Factors
I	**Risk factors, that could be modified to surely decrease the incidence of MI** • Smoking • Hypertension • Hypercholesterolemia
II	**Risk factors that could be modified to probably decrease the incidence of MI** • Diabetes mellitus • Left ventricular hypertrophy associated by Hypertension • Pathological glucose tolerance • Low levels of HDL-cholesterol • Physical inactivity • Abdominal obesity
III	**Modifiable risk factors with no sure effect on the incidence of MI** • Hypertriglyceridemia • Alcohol consumption • Lipoprotein (a) • Homocystein • Infections • C-reactive proteins
IV	**Non-modifiable risk factor** • Age and sex • Post menopausal hormone substitution therapy • Family history of MI

Table 2: Risk factors of myocardial Infarction [modified after (22) **]**

Coronary risk factors are multiplicative rather than additive. This is why patients with a combination of more than one risk factor are the most at risk. Smoking is thought to be the most important avoidable risk factor of atherosclerotic vascular diseases, showing a dose-linked relationship between smoking and the incidence of myocardial infarction. It was found that coronary heart disease often runs in families, with more than a family member

being affected. This might be due to factors other than shared genetics, such as lifestyle and environmental factors. Nevertheless, genetic factors have been shown to play an important role when it comes to coronary heart disease, with an eightfold increase in the risk of an identical twin of an affected patient compared to normal population. This risk is also fourfold increased in case of a dizygotic twin (23).

One of the biggest international studies addressing the risk factors of myocardial infarction was published by Yusuf et al. in 2004. The INHEART study (24) aimed to investigate the role of different risk factors that might lead to coronary heart disease. This case-control study enrolled a total of 15152 cases and 14820 controls from 52 different countries, making the outcome results applicable worldwide. The results of this study showed that current smoking and raised apolipoprotein B (ApoB)/ apolipoprotein A1 (ApoA1) ratio represented the most important risk factors, accounting together for almost two-thirds of the population attributable risks (PAR) of an acute myocardial infarction. The effects of those two factors were found to have a graded relation with the incidence of myocardial infarction, showing an increased risk with the increase in the dose response. Smoking even a few cigarettes every day showed an increased risk, suggesting that all levels of smoking are indeed harmful, and that reducing the amount of cigarettes smoked could significantly decrease the risk of myocardial infarction.

The next most important risk factor was the presence of a history of diabetes or hypertension. Psychosocial factors also represented an important risk factor. Although an increased Body Mass Index (BMI) was found to be related to the incidence of myocardial infarction, its effect was insignificant compared to abdominal obesity. Abdominal obesity was found to have doubled the risk of acute myocardial infarction, before multivariate adjustment. However, the effect of abdominal obesity on increasing the risk of AMI showed to be of less significance after adjusting other risk factors, especially apolipoproteins. On the other hand, this study reported that daily consumption of fruits or vegetables and regular physical activity decreased the risk of myocardial infarction. Through modification of these factors and quitting smoking, the risk of acute myocardial infarction could be decreased by more than 75% compared to a smoker with a poor lifestyle. Moreover, moderate consumption of alcohol showed to have a protective effect, reducing as well the risk of myocardial infarction. In sum, these potentially modifiable risk factors were found to account for 90% of the PAR in men, and 94% in women. Furthermore, the effect of these risk factors was even higher in younger patients, contributing to a PAR of 93% in young men, and 96% in young women. This finding

shows that most premature myocardial infarctions could be prevented by limitation of the risk factors.

This study reported that through modification of the lifestyle, and reducing the risk factors, especially smoking and raised ApoB/ApoA1 ratio, the incidence of myocardial infarction worldwide could be significantly decreased.

1.1.7 Management of acute myocardial infarction

Acute myocardial infarction should be treated as soon as possible, as every delay in treatment will cause more damage to the myocardium, dramatically affecting the survival chance of the patient. As soon as acute myocardial infarction is suspected, emergency treatment should be started. Emergency measures should be aiming to increasing the oxygen supply and decreasing the oxygen demand of the heart. This emergency treatment should be in the form of immediate oxygen through nasal prongs, sublingual nitroglycerin (unless contraindicated), and an adequate analgesic. Moreover, 160 to 325 mg of aspirin should be given orally (25). In addition, a beta-blocker could be administrated. Beta-blocking agents are thought to reduce myocardial oxygen demand through decreasing heart rate, systemic arterial pressure as well as myocardial contractility. The reduction in heart rate caused by beta-blockers leads to a prolonged diastole, which might help in the perfusion of ischemic myocardium (26).

The second line of treating myocardial infarction, after conducting emergency measures, is the reperfusion therapy. This aims to restoring the blood supply to the infarct region. This could be done through thrombolytic therapy, percutaneous coronary transmural angioplasty (PTCA), or surgically.

The benefit of fibrinolytic therapy was reported by many studies. A meta-analysis reported an absolute death reduction of around 30 per 1000 patients treated within 6 hours from the onset of symptoms. Moreover, every 1000 patients treated between 7 and 12 hours from the onset of symptoms, about 20 deaths were prevented through fibrinolytic therapy (27). However, fibrinolytic therapy might cause an increase in the incidence of strokes, especially on the first day after treatment (27). The incidence of intracranial hemorrhage is also increased with the use of fibrinolytic therapy, especially in elderly patients, females, patients with a history of cerebrovascular disease and patients with hypertension on admission (28). All this should be taken into consideration before treating a patient with fibrinolytic agents.

The American College of Cardiology and American Heart Association guidelines for treating myocardial infarction in 1999 recommended that primary percutaneos coronary intervention (PCI) should be used as an alternative to fibrinolytic therapy (29), however, this changed shortly afterwards. It was reported in a randomized clinical trial that PCI leads to better clinical outcomes than fibrinolytic therapies (30). A meta-analysis of 23 randomized trials comparing the results of PCI to those of fibrinotylic therapies showed that PCI decreased short-term mortality by 27% (31). These findings, as well as the results of other similar studies, lead to a change in the guidelines of treating myocardial infarction. In 2003, the task force on management of acute myocardial infarction of the European Society of Cardiology presented a guideline update, indicating the use of primary PCI as the therapy of choice, in case it could be performed within 90 minutes of the first medical contact (32).
However, the outcome of PCI depends on many different factors, such as the time needed till PCI is performed and expertise of the performer of the PCI. Whether a later intervention with PCI would be helpful or not, is not very clear. It was reported that PCI performed on stable patients 3 to 28 days after myocardial infarction did not reduce mortality, reinfarction or heart failure, in comparison to patients who received conservative medical treatment only (33). On the other hand, a more recent study examining the efficacy of PCI performed between 12 hours and 28 days from the onset of symptoms of a STEMI reported that this intervention is significantly more effective than conservative therapy alone (34).

Only few acute myocardial infarction patients would require an acute coronary artery bypass grafting (CABG), however it might be indicated in case PCI failed to restore blood supply to the infracted myocardium. Other acute indications for CABG are the presence of refractory symptoms after PCI, cardiogenic shock, as well as acute mitral regurgitation or ventricular septal defect (12). This is why it is preferable to perform PCI in a place close to a cardio surgical unit. Even though there has been a major improvement in the intraoperative preservation with cardioplegia and hypothermia, the time and effort needed to perform surgical reperfusion is more than that needed when other methods of reperfusion are used. Thus, patients representing with a myocardial infarction and are candidates for reperfusion would be primarily treated through PCI or thrombolysis (25).

1.2 Cardiac remodeling and myocardial regeneration

1.2.1 Cardiac remodeling

One of the major complications of myocardial infarction is the process commonly referred to as cardiac remodeling. This process, which is more likely to occur after large transmural infarcts, involves a complex alteration of the ventricular architecture, including acute and chronic transformation of not only the necrotic, but also the non-necrotic zones (35). Ventricular remodeling is assumed to occur in more than 50% of cardiac patients that suffered a myocardial infarction (36).

Cardiac remodeling could be defined as changes in genome expression accompanied by other changes of the myocardium on the molecular, cellular and interstitial levels. These events manifest clinically as changes in the architecture (size and shape) and the function of the heart. Cardiac remodeling might occur as a physiologic condition in athletes, in form of compensatory changes in the architecture and function of the cardiac muscle. Pathologically, cardiac remodeling could manifest throughout the progress of some cardiac diseases, such as myocardial infarction, pressure or volume overload, myocarditis and idiopathic dilated cardiomyopathy. On the cellular level, the process of remodeling includes many changes such as myocyte hypertrophy, apoptosis, necrosis and fibrosis (37). Following a myocardial infarction, the number of myocytes is dramatically reduced and the surviving myocytes undergo an initial compensatory process, through elongation and hypertrophy, in order to maintain stroke volume. (38). This leads to stretching cell membranes causing an increased wall stress, which might lead to an energy imbalance, increasing the myocardial oxygen demand and consequently causing further ischemia (39). Neurohormonal activation is also thought to play a major role in the prognosis of cardiac remodeling. Myocyte elongation and stretching cause an increase in local norepinephrine activity, as well as increased release of angiotensin. In culture, angiotensin II was found to increase protein synthesis in fibroblasts and myocytes. Moreover, angiotensin II might cause an increase in coronary artery permeability. This leads to more diffusion of growth factors into the myocardial interstitium. Angiotensin II is also thought to be cytotoxic to cardiac myocytes, leading to necrosis and fibrosis. As a result of increased levels of angiotensin II, aldosterone production is increased, causing an increase in collagen synthesis by myocardial fibroblasts, and playing a role in myocardial necrosis, through their effect on electrolyte balance that leads to myocyte death (37).

In addition to myocytes, other cell types also contribute in remodeling. For instance, it was found that fibroblast and endothelial cells are activated following ischemic conditions. When fibroblasts are stimulated collagen synthesis is increased, followed by fibrosis of infarcted as well as non-infarcted areas of the ventricular myocardium, contributing to the process of remodeling (40). However, it is not yet clear to what extent this contribution affects cardiac remodeling. In addition, apoptosis is also thought to play a role in the process of cardiac remodeling, as it has been found to occur at a higher rate after ischemia, MI and reperfusion (41).

The outcome of all these events is further worsening in cardiac function and further increased neurohormonal activation, stimulating more collagen synthesis, and leading to more fibrosis (37).

Remodeling could be partially prevented through early treatment with an angiotensin-converting-enzyme (ACE) inhibitor (42, 43), which was found to improve the survival rates (44). Beta blockers are also used in the treatment of cardiac remodeling and were as well found to decrease morbidity and mortality rates (45). Although both ACE inhibitors and beta blocking agents are known to slow down, and sometimes even reverse certain parameters of cardiac remodeling (37), they do not resemble a causal treatment. With no current treatment for the underlying causes, remodeling still resembles a major cause of post-infarct heart failure and mortality (46). In the last decade however, regenerative medicine, especially stem cell research, has attracted a lot of attention, with promising results that gave hope for filling the gap needed to achieve myocardial regeneration.

1.2.2 Myocardial regeneration

Until recently, it was thought that the human heart is a postmitotic organ, with the idea that the number of parenchymal cells is established at birth, and that lost cardiomyocytes resulting from a cardiac disease or normal aging process cannot be replaced by newly formed cells (47). Accordingly, it was thought that the only way for cardiac growth is through hypertrophy of the existing myocytes. This theory hasbeen reinvestigated recently. A clinical study done by Beltrami et al. (48) reported the identification of various cell division events occurring after myocardial infarction. Those findings verified the fact that myocyte proliferation took place after the event of myocardial infarction, raising the idea of myocyte contribution in the growth of the muscle mass of the myocardium through proliferation, and providing evidence that cardiomyocyte replication is markedly increased

during acute pathologic states, challenging the dogma that the adult heart is a postmitotic organ. Another study examining the sex-mismatched cardiac transplants in humans rose yet another challenge to that theory. This study reported that Y-chromosome positive myocytes and coronary vessels where identified in female hearts after being transplanted to male recipients, suggesting that these male cells migrated to the transplanted female hearts and differentiated into myocytes and functional cardiac structures (49). Recently, it was reported that the human heart actually has a significant growth reserve, with some groups suggesting that the heart replaces its myocyte as well as non-myocyte compartments multiple times during the course of life (47).
Those findings highlighted the role of stem cells in cardiac regeneration, and confronted the old paradigm with the fact that human heart regeneration is in fact not as static as it was thought to be.

Despite those findings and the intensive research, the rate of myocardial regeneration remains under debate (50), with different groups reporting different rates of myocardial regeneration throughout the normal aging process of the heart. An experimental trial on mice estimated an endogenous cardiomyocytes turnover ranging between 1.3% and 4% per year, predominantly through proliferation of resident cardiomyocytes (51). Another more recent mice study suggests as well that the regeneration process originates from pre-existing cardiomyocytes, however in a rate of only 0.76% per year, with this rate decreasing as the heart ages, and increasing after myocardial injury (50).
In human hearts, it was reported that cardiomyocyte regeneration takes place in a low turnover rate of about 1% annually at the age of 25, and decreases gradually with aging, to reach about 0.45% at the age of 75. This study suggested that about half of the cardiomyocytes are exchanged within the normal life span of the heart (52). Other groups reported completely opposite information concerning cardiomyocytes regeneration. Kajstura et al. (53) suggested an annual myocyte turnover at the rate of 7-10% at 20 years of age, with this rate increasing throughout the aging process to reach up to 40% by the age of 100. The same group suggested that between 20 and 100 years of age, the myocyte compartment is replaced 15 times in women, and 11 times in men. However, compared to other organs, the heart does not possess a high regeneration rate. The liver for example has a very high rate of regeneration, which is clearly seen after a 2/3 partial hepatectomy. After surgical removal of three out of five liver lobes, the residual 2 lobes grow in size to restore the original mass of the liver. In rats and mice, this process is completed within one week

after surgery. This is majorly achieved through hepatocyte proliferation, with more than 95% of hepatocytes undergoing cell proliferation during the first 48 hours (54).
While the rate of cardiac regeneration is not effective enough to overcome the injury caused by myocardial infarction, which can cause the death of about a billion cardiomyocytes (55), the findings indicating cardiac regeneration highlighted the role of stem cells in myocardial regeneration, and gave a new hope to restoring the myocardial loss that follows an infarction. This attracted the attention of researchers, who began to explore the therapeutic effects of transplanting different types of stem cells in patients that suffered a myocardial infarction.

2 Stem cells

2.1.1 Definition

Stem cells can be defined as primitive, undifferentiated, undefined pluripotent multi-lineage cells, that possess the ability of self-renewal through mitotic cell division and can create another type of specialized cell that is more differentiated than itself (56). Stem cells are the origin of all other kind of cells in the human body, and currently present an emerging research interest, with the hope of developing stem cell based treatment for many diseases, including as well cardio vascular diseases.

2.1.2 Types of stem cells that have the potential to assist in cardiac regeneration

There are different types of stem cells, with a variety of origins and diverse differentiation potentials. This section however, is concentrating on the types of stem cells that have the potential of supporting myocardial regeneration following an acute myocardial infarction. The major types of stem cells with potential therapeutic effects in cardiac diseases, their advantages and disadvantages, are listed in table 3.

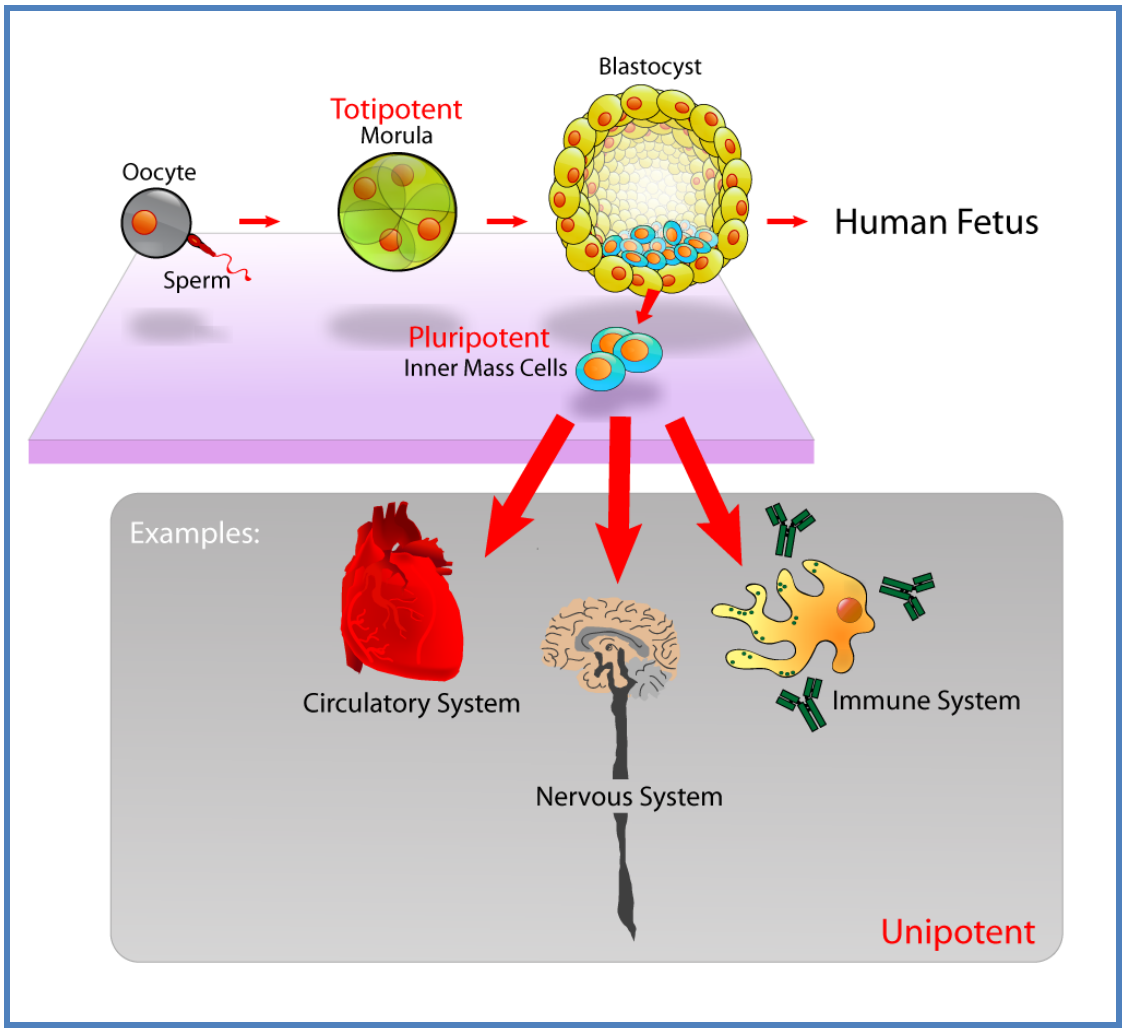

Figure 4: Embryonic stem cell (57)**.**

2.1.2.1 Embryonic stem cell (ESC)

One of the most investigated types of stem cells is in fact the embryonic stem cell. Embryonic stem cells (Figure 3) are pluripotent stem cells, obtained from the blastocyst stage of the embryo (58). ESCs can give rise to all different types of somatic cells in the embryo. One of the major advantages of ESCs lies in their indefinite plasticity and ability of self-renewal, allowing the *in vitro* production of a limitless number of diverse cell types (59). *In vitro*, it is possible to differentiate ESCs into cardiac cells, which possess cardiomyocytic characteristic, such as beating. In just a few days ESCs could yield a mass of these cells in culture (60). This ability to produce a clinically relevant and well defined cell cultures would make it possible to renew and regenerate damaged tissues and organs (61). However, this pluripotency could be in fact a double-edged sword. Due to their pluripotency, ESCs could develop teratomas after their transplantation (62). Another barrier on the way of implementing ESCs based therapy is the fact that ESCs are allogenic. There is evidence of ESCs expressing specific human leukocyte antigen (HLA) subclasses

(63). This is why immune suppression might be needed to decrease the chance of alloimmune rejection of the transplanted cells by the host. Finally, there has been a major debate concerning the ethical and moral issues of using ESCs, as it results in the destruction of fertilized human embryos. It has been debated whether or not a human embryo should be morally considered as a human being (64). All those factors lead to investigating other types of stem cells, one of which is the recently discovered induced pluripotent stem cell.

2.1.2.2 Induced pluripotent stem (iPS) cells

The recent discovery of induced pluripotent stem cells opened a new door in the field of regenerative medicine, overcoming some of the problems facing the use of ESCs. Through the application of some reprogramming factors, such as OCT4, SOX2, NANOG, and LIN28, it is possible to reprogram human somatic cells into iPS cells (Figure 4), having the required characteristics of embryonic stem cells (65). These similar characteristics include the proliferation capacity, as well as the ability of iPS cells to differentiate into cells of the three germ layers, both in vitro and in vivo (66). iPS cells offer all this without the need to destroy any embryos, solving the ethical issues of ESCs. Moreover, iPS could be produced from patient's own cells, putting an end to the risk of immune rejection. This however should be further investigated and reevaluated before any clinical application of such cells in human trials takes place (67). On the other hand, the risk of tumor formation due to iPS cells transplantation is relatively high (68). This might be due to oncogenic transgene reactivation after transplantation of the iPS cells (69). All this indicates that an iPS cell reprogramming is still a young technique, one that needs to become more efficient before clinical application could be started.

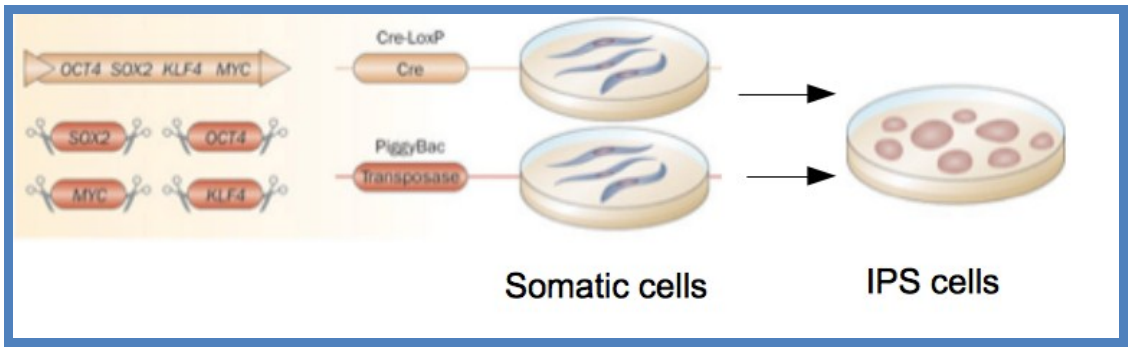

Figure 5: Reprogramming adult cells into iPS [modified after (70) **]**

Type of stem cells	Advantages	Disadvantages
Embryonic stem cells	Totipotent Highly expandable	Immunosuppressant required Ethical debate Lack of availability Tumor potential
Induced Pluripotent stem cells	Pluripotent Indistinguishable from ESCs epigenetically and functionally Embryonic stem cell like autologous adult cells for cell therapy	Oncogenesis
Cardiac stem cells	Multipotent Cardiomyocyte phenotype Electro-physiologically compatible	Immune suppression required Ethical debate Short survival Limited supply
Hematopoietic stem cells Bone marrow/peripheral blood	Multipotent Lack of immunogenicity Autologous transplantation Different lineage of cells	Quantum of cell population not adequate
Mesenchymal Stem Cells Bone marrow Stromal/muscle, skin, and adipose tissue	Allogenic/autologous transplantation Lack of immunogenicity Pluripotent Cryopreservable for future use	Requires expansion

Table 3: Major types of stem cells with a potential for cardiac regeneration. [modified after (56) **]**

2.1.2.3 Mesenchymal stem cells (MSCs)

Another source of stem cells is the adult body. Adult stem cells are undifferentiated progenitor cells that are found in various body parts after complete embryonic development (56). One type of adult stem cells commonly used in clinical trials is the bone marrow derived stem cells (BMCs). Bone marrow contains different types of stem cells that can migrate and differentiate into various phenotypes. Those stem cell populations include mesenchymal stem cells (56). Yet, there are some concerns that complicate the process of developing a cardiovascular therapy based on BMCs. Bone marrow requires an

invasive procedure to be obtained. In addition, it is not exactly known yet, which active cellular constituent of bone marrow is responsible for the therapeutic repair effect (71). Furthermore, it is thought that patients who previously suffered a MI show a functional fatigue of BMCs able to form hematopoietic colonies in comparison to healthy controls (72). Cells with MSCs phenotypes have been, however, also found in tissues other than bone marrow, such as adipose tissue and skeletal muscle (73). Such tissues resemble an abundant easily accessible source of stem cells. In addition, MSCs are thought to have a low immunogenicity, allowing their usage in the future as a source of allogenic cell transplantation (74). However, MSCs resemble relatively large and adhesive cells, raising the fear of causing a vascular obstruction if infused in systematic arteries. It is rather administrated through intracoronary infusion, or by transepicardial injections during coronary artery bypass grafting surgery (75).

2.1.2.4 Hematopoietic stem cells (HSCs)

Hematopoietic stem cell is one of the oldest stem cells ever investigated by researchers. Already in 1960, HSC was analyzed by Till and McCulloch, who found out that it has the ability of both self-renewal and multi-potency, producing not only undifferentiated HSCs, but also cells that give rise to all different types of blood cells (76). From all cell types within the hematopoietic system, HSCs are the only cells with such abilities of self-renewal and multi-potency (77).

HSCs can be harvested from bone marrow, peripheral blood, as well as other sources, including umbilical cord blood (78). More than 3 decades ago, Prindull et al. documented that human umbilical cord blood contains hematopoietic progenitor cells that can produce hematopoietic colonies in vitro (79). Recently, HSCs have also been shown to be present in extra-embryonic tissues, such as chorion and placenta (80).

A number of studies showed that HSCs could differentiate into cardiomyocytes in culture (81-83), which rapidly attracted attention to the possible role HSCs might play in treating cardiovascular diseases, as they represent an abundant source of progenitor cells. In 2001, the study published by Orlic et al. (84) showing differentiation of bone marrow derived cells into cardiomyocytes in vivo caused a burst of interest in bone marrow cells for treating cardiac diseases.

2.1.2.5 Cardiac stem cells (CSCs)

A number of laboratories were able to detect resident cardiac stem cells in developing and adult hearts of animals and humans (85, 86). These findings suggested that myocardial regeneration might be more profound than it earlier was thought to be. It has also been shown that CSCs are effective in treatment of myocardial infarction. In rat models, CSCs isolated from heart ventricles induced myocardial regeneration and reduced the infarct size. Those results set up the heart as a candidate source for stem cells that could contribute in treatments enhancing myocardial repair (87). The cardiac stem cells are however relatively little in amount compared to mature resident cardiomyocytes (88). Moreover, it is thought that the cardiac stem cell pool shrinks with ageing, which might be the reason behind the lack of efficient myocardial regeneration in elderly individuals (88). In case of myocardial infarction, the rate of regeneration is not big enough to overcome the damage caused by the ischemia. The damage caused by a large myocardial infarction can cause the death of up to a billion cardiomyocytes and leads to inflammation, forming a granulation tissue and finally leaving a fibrous scar (55). This injury however is detected by distant stem cells, that in turn migrate to the damaged site and differentiate into more developed cells (89), slightly helping in the regeneration process.

The unique characteristics of stem cells , and their ability to proliferate and differentiate into other cell types were rapidly translated into a massive amount of research experiments. A lot of different groups of researchers examined the beneficial effects of stem cell transplantation on post-myocardial infarction conditions. In the last 15 years, a lot of success was reached in that field, with promising results from not only animal experimental trials, but also human clinical trials.

3 Materials and methods

3.1 Aims

This work aimed to gather data about the different types of stem cells that have the potential to assist in cardiac regeneration, and identify the types of stem cells used in preclinical and clinical trials for treating patients after a myocardial infarction. Moreover, the therapeutic effects of stem cell transplantation after myocardial infarction were to be validated through the results of preclinical animal trials, as well as clinical human trials, using stem cell based therapies to treat post-myocardial infarction conditions. Another aim of this literature research was to gather information concerning the safety of stem cell transplantation. Finally, this research aimed as well to find out whether or not stem cell transplantation therapy is developed enough to be used in clinical treatment of patients that suffered from myocardial infarction.

3.2 Methods

An intensive literature research was conducted using the platforms Pubmed, Medline, as well as different electronic medical Journals. As for Pubmed and Medline, the search queries that were performed included the keywords and corresponding MeSH terms for ‘stem cells’ , ‘stem cell transplantation’ , ‘embryonic stem cells’, ‘induced pluripotent stem cells’, ‘bone marrow stem cells’, ‘mesenchymal stem cells’, ‘cardiac stem cells’, ‘hematopoietic stem cells’, ‘myocardial infarction’, ‘ stem cell therapy’, ‘randomized (randomised) controlled trial’, ‘human trials’, ‘animal trials’, and others.

Manual reference checking of the bibliographies of the retrieved articles was also performed, and relevant reviews were retrieved and included in the search.

Further electronic search included other World Wide Web platforms such as “Stemcells.nih.gov’’.

Moreover, five books were also used to gather information about myocardial infarction and its treatment.

No language restriction was applied throughout the search, however, the reviewed articles were mostly written in English, while most of the reviewed books were written in German.

4 Results

4.1 Animal Trials

Stem cells transplantation as a post-MI treatment gained a lot of attention over the last 15 years, with a big amount of experimental trials performed. A few of the important animal trials conducted between 2000 and 2011 are summarized in table 4. One of the most important animal model trials was published by Orlic et al. (84) in 2001, and counts as a milestone in this field. This group examined the effect of bone marrow cells injection in mice after inducing MI through coronary ligation. BMCs were injected directly into the border-line of the infarct. They reported that 9 days after BMCs transplantation, 68% of the infarcted ventricular zone was occupied with newly formed myocardium. This de novo myocardium consisted mostly of proliferating myocytes, and to a less degree of endothelial and smooth muscle cells. The results of this study attracted great attention, opening the door for more trials that followed.

In a later study, the same group examined whether the mobilization of animal's own bone marrow cells, using cytokines, would lead to homing of those cells in the infarct region and to promoting myocardial repair (90). This was done through injecting recombinant rat stem cell factor (SCF) as well as recombinant human granulocyte colony-stimulating factor (G-CSF) in mice 2 weeks after performing splenectomy on them. This was done once a day for 5 days, and was followed by coronary artery ligation to induce MI. SCF and G-CSF injections were continued for another 3 days. Another group of mice, that were as well splenectomised and infarcted, were injected with saline at the same rate, and acted as controls. Ejection fraction (EF) was significantly higher in the cytokine-treated group than the control group, and hemodynamics were significantly increased in the cytokine-treated mice. Mice were killed at 27 days, and anatomical and histological analysis was carried on. Till that point of time, most of the surviving mice were from the cytokine-treated group, indicating a cytokine-induced cardiac repair that reduced mortality by 68%. It was also reported in this study that BMCs mobilization promoted myocardial regeneration in cytokine-treated mice, decreasing the infarct size by 40%. A band of newly formed myocardium took over most of the infarcted area in cytokine-treated mice, while healing process with scar formation was the case in the control group, with absence of any myocardial regeneration. These results suggested that cytokine administration, which leads to mobilizing BMCs into the circulation and their homing into the infarcted area of the myocardium, significantly increased the magnitude of myocardial repair. Such a therapy

has multiple benefits over stem cells transplantation, as it could be performed in a noninvasive way, and does not require foreign cell transfer, which might cause immunological reactions as well as other adverse effects.

Author (year) Study	**Animal model**	**Outcome**
Orlic el al (2001) (84) The effect of BMC injection on ischemic myocardial proliferation	Mouse	68% of infarct zone reoccupied by new myocardium, consisting majorly of proliferating myocytes
Orlic et al (2001) (90). The effect of mobilizing animal's own bone marrow cells through cytokine injection	Mouse	Ejection fraction increased. Hemodynamics increased. Reduced mortality by 68% 40% reduction in the infarct size
Dai et al (2005) (91) Long term effects of MSC transplantation after MI	Rat	Improved global LV function at 4 weeks No significant long-term effect on LV function
Krause et al (2007) (92). The effect of IV MSC injection on improving cardiac function after MI	Pig	Improve in systolic and diastolic LV function Increase in myocardial thickness
Hatzistergos et al (2010) (93) The effect of transplantation of a combination: MSC and CSC	Swine	Significant reduction of infarct scar size Ventricular compliance and contractility significantly increased 7-fold enhanced engraftment of stem cells
Latifpour et al (2011) (94) Therapeutic effects of hUMC on post-MI conditions	Rabbit	Significant reduction in the size of myocardial scar Significant increase in LVEF

Table 4: Animal trials using stem cell based therapies to treat myocardial infarction.

The long-term effects of cell based therapy on MI were also examined by Dai et al. (91). Either labeled MSCs or saline were injected into the hearts of Fischer rats 1 week after inducing MI. The transplanted MSCs did not fully differentiate into cardiomyocytes, and failed to significantly replace the scar tissue. An assessment of the MSCs presence and differentiation in the myocardium as well as their effect on left ventricular (LV) ejection

fraction was performed 4 weeks after treatment. Although labeled MSCs could be detected at this point of time, they did not show muscle specific marker proteins, nor did they manage to replace the scar with muscle tissue. However, at this point of time LV stroke volumes as well as LV ejection fraction were significantly higher in the MSCs-treated group than in the saline group, highlighting the beneficial effects of MSCs transplantation. Reassessment at 6 months showed a loss of these benefits, and although MSCs were still present in the myocardium of the MSCs-treated group, with about 40% of the present MSCs expressing muscle-specific markers, no significant differences between MSCs-treated group and saline group regarding LV stroke volumes or LV ejection fraction was found. Moreover, along the period of this study the differentiation of MSCs into cardiomyocytes was incomplete, with only immature myofibrillar organization detected in the hearts of MSCs-treated rats. These results might indicate an early paracrine effect of the transplanted MSCs, as the improvement in LV function took place before the expression of muscle markers. Also the fact that no new muscle tissue showed to regenerate in the infarct zone, points towards a paracrine effect of the treatment. This beneficial effect however, did not last over a longer period of time, indicating the lack of long-term effects of allogenic MSCs transplantation in post-MI rats.

Another experimental trial was performed to investigate the effect of intravenous administration of MSCs on improving cardiac function in a pig model of myocardial infarction (92). This study assessed the hemodynamic evaluation of left ventricular function, as well as the histological evidence of cell engraftment in the ischemic myocardium. Either labeled MSCs or phosphate based saline were injected intravenously 48 hours after inducing MI in the study pigs through left anterior descending artery (LAD) blockage. One month after treatment, pigs in the MSCs group showed a higher increase in body weight, heavier hearts, as well as a smaller infarct size than the control pigs. Fluorescence microscopy showed that MSCs migrated and engrafted in the ischemic border area. This was shown to have resulted in improving systolic and diastolic left ventricular function compared to the control group. Left ventricular end diastolic pressures were significantly lower in the MSCs group, suggesting an enhancement of diastolic relaxation and a reduction of wall stress, which suggests a favorable ventricular remodeling. Systolic blood pressure was higher in the MSCs group, however insignificantly. Echocardiography also showed an improvement in left ventricular function of the MSCs-treated pigs. The increase in myocardial thickness of the MSCs-treated pigs with local infiltration of transplanted cells in the infarction zone also indicates the effect of

MSCs in reversing, or at least partially preventing ventricular remodeling. While this study showed the beneficial effects of MSCs transplantation, it unfortunately did not mention the safety of intravenous (IV) administration of MSCs, regarding their extra-cardiac homing and whether this route of administration caused any microembolisations in the myocardium or elsewhere.

More recently, an experimental study tackled the hypothesis that combining more than one type of stem cells could increase their therapeutic effects on post MI events. Based on the fact that MSCs transplantation alters CSCs proliferation (93), this study compared the therapeutic effect of transplanting MSCs or CSCs alone, to transplanting a combination between both MSCs and CSCs (95). Fourteen days after inducing MI in Yorkshire swine, intramyocardial combination of human CSCs (hCSCs) / human MSCs (hMSCs), hCSCs alone, hMSCs alone, or placebo was injected into the infarct zone. Ejection fraction was restored to base-line in stem cell-treated pig groups, while placebo treated pigs showed a persistence of left ventricular dysfunction. The results also showed a significant reduction of MI size in all cell therapy groups in comparison to placebo. However, the decrease in the infarct size was 2-fold greater in combination-therapy than either of the cell therapies alone. Moreover, ventricular chamber compliance and contractility where significantly increased in combination-therapy group. A 7-fold enhanced engraftment of stem cells was reported in the combination therapy group in comparison to other cell groups. Not only did this study prove the positive effects of both CSCs and MSCs in treating post-MI conditions, but also highlighted the importance of MSCs and CSCs interactions, that might lead to enhancing the cell-based therapy after MI.

The therapeutic effects of human umbilical cord mesenchymal cells (hUMCs) on post-MI conditions were also tested in an experimental trial (94). A total of 35 female rabbits were enrolled, and divided into five groups, with one group consisting of 7 intact rabbits. In the remaining four groups, MI was induced through LAD ligation. The rabbits in the four MI-induced groups received hUMCs, differentiated human umbilical cord mesenchymal cells (dhUMCs), phosphate buffered saline (PBS), or no therapy at all. After 30 days from inducing MI, hUMCs group and dhUMCs group showed a remarkable increase in left ventricular ejection fraction, as well as a significant improvement in the percentage of fractional shortening. Furthermore, these cell-receiving groups showed a significant reduction in the size of myocardial scar compared to the other groups. There were no

remarkable differences in these parameters between hUMCs and dhUMCs groups. The results reported in this study present human umbilical blood as a source of stem cells with therapeutic potential that might contribute in the regeneration of myocardial tissue damaged by ischemia. The ability of human umbilical mesenchymal cells to survive in rabbits shows their interesting immune properties. Indeed, hUMCs were reported to possess properties that make them suitable for allogenic transplantation (96), which is another reason why hUMCs constitutes an interesting source of stem cells.

The results of experimental trials showed the therapeutic effects of stem cell transplantation on post-MI conditions, and their potential role in myocardial regeneration, enhancing the ventricular function and decreasing the size of myocardial scar. Moreover, a partial reassurance was also offered through the animal studies, especially about the potential of tumor formation after stem cells transplantation. This led stem cell research to its next phase; human trials.

4.2 Human Trials

With the increased interest that stem cells attracted over the past decade, a large number of scientific research groups started testing the therapeutic effects of stem cells in clinical studies. As experimental studies showed a potential benefit of stem cells transplantation on post-MI conditions, the door was opened to testing such effects on human patients as well. Table 5 summarizes the most important human trials carried out over the past 10 years.

Strauer et al. (97) performed one of the early human trials at the beginning of this millennium. In this trial 10 patients were analyzed. After receiving the standard therapy after MI, they were treated by autologous, mononuclear bone marrow cells. In addition, 10 other patients took part in this trial, serving as a control group, and receiving only the standard therapy of MI. Each of the 20 patients had suffered a transmural myocardial infarction, with the mean duration of about 12 hours infarct pain before invasive diagnostics and therapy took place. All patients were under 70 years old, and did not have any severe co-morbidity, neither did they suffer a cardiac shock. Alcohol or drug dependant patients were excluded from this study.
All patients were treated with recanalization of the infarct-related artery by balloon angioplasty and (except for one patient) subsequent stent implantation.

Study (Year) (Reference)	Delivery method	Number of patients treated/control	Study design	Cell type and dose	Time of cell delivery (days post MI)	Results
Strauer et al. (2002) (97)	IC	20/10	Case controlled	BMC $9–28 \times 10^6$	7	Decreased infarct size and improved contractility at 3 months
TOPCARE-AMI, Assmus et al. (2002)(98), Schachinger (2004)(99)	IC	30/29	Controlled Nonrandomized open-labeled	BMC 2.4×10^8 CPC 1.3×10^7	3-7	Reduced infract size and increased LVEF at 4 and 12 months Improved EF at 6 months, enhanced regional contractility, no effect at 18 months
BOOST, Wollert et al. (2004) (100) Meyer et al. (2006) (101)	IC	30/30	Controlled	BMC 24×10^9	6	AT 6 months: improved regional contractility increased EF. At 18 months: only insignificant difference
REPAIR-AMI, (102)	IC	102/102	Placebo controlled	BMC 2.4×10^8	4	Decreased infarct size and increased EF at 4 months.
Fernandez et al. (2004) (103)	IC	20/13	Controlled	BMC $11–90 \times 10^6$	10-15	Significantly improved contractile function and decreased infarct size
Janssens et al., (2004) (104)	IC	33/34	Placebo controlled	BMC 3.0×10^8	1	Reduced scar size, with no improvement in LVEF at 4 months

Chen et al. (2004)(105)	IC	34/35	Placebo controlled	MSC $48–68 \times 10^{10}$	18	At 3 and 6 months: Increase viability of infarct zone. Increased LVEF Increased regional contractility
ASTAMI, Lunde et al. (106)	IC	50/50	Randomised, controlled	BMC 8.7×10^{7}	5-8	No differences between both groups at 6 months Better improvement of exercise time at 3 years.
Hare et al. (2009) (71).	IV	39/21	Double-blind Placebo controlled	MSC 0.5, 1.6, 5 million cells/kg	N/A	Increased LVEF. Reduced ventricular arrhythmia Reverse remodeling
BALANCE study; Youssef et al. (2009) (46)	IC	62/62	Controlled	BMC 6.1×10^{7}	5-9	At 3 months: Significant increase in EF and SVI. Reduced infarct size. Significant improvement of contractility in the infarct zone. Significant increase in contraction velocities At 12 and 60 months: Sustained benefits, mortality was significantly reduced
HEBE study; Hirsch et al.(2011)(107)	IC	135/65	Randomized, controlled	BMC: $296 \pm 164 \times 10^{6}$ PBC: $287 \pm 137 \times 10^{6}$	3-8	No significant difference of therapeutic effects detected at 4 months.

Table 5: Mid- and long-term human trials examining the safety and therapeutic effects of stem cell transplantation after acute myocardial infarction.

In addition, all patients were subscribed with standard medication consisting of acetylsalicylic acid, an ACE-inhibitor, a beta-blocker, and a statin.
The cell transplantation was performed 5-9 days after onset of acute infarction. Cells were injected into the infarct zone through a balloon catheter, using multiple fractional high-pressure infusions of mononuclear BMCs.
All participating patients were followed up after 3 months by left heart catheterization, left ventriculography, and coronary angiography.
Comparison of both groups 3 months after cell transplantation or standard therapy alone showed several significant differences. The infarct region decreased significantly within the cell therapy group, and was also significantly smaller in comparison to the control group. On the other hand, only a statistically non-significant decrease in the infarct region was found within the standard therapy group. Wall movement velocity over the infarct area was significantly enhanced in the cell therapy group, but not in the standard therapy group. The difference between both groups was however non significant.
Ejection fraction increased in both groups, however insignificantly. Furthermore, other cardiac examinations were performed for the cell therapy group. Those examinations (dobutamine stress echocardiography, radionuclide ventriculography, and catheterization of the right heart) revealed significant improvement in SVI, LVESV and contractility, as well as myocardial perfusion of the infarct region.
Although this study is not as powerful as randomized double-blinded studies in proving the reached outcome, the results achieved through that trial did demonstratethat selective transplantation of autologous, mononuclear BMCs seemed to be safe and effective under clinical conditions.

The Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI) study (102) aimed to investigate the clinical outcome 2 years after intracoronary administration of BMCs in patients with acute myocardial infarction. 204 patients took part in this study. They have been randomized, 103 patients to receive placebo and 101 to be treated with BMCs. A total of 82% of the participants were men. During the 2 years of follow-up, a total of 11 deaths (5,4%) occurred, 8 in the placebo group and 3 in the BMCs group. Out of the 8 deaths in the placebo group, 5 deaths were due to cardiac reasons, 1 death due to cardiovascular reasons, and 2 deaths due to non-cardiovascular reasons. On the other hand all 3 deaths in the BMC group were due to cardiac reasons. This indicates that even though the number of deaths was less in the BMC

group, cardiac deaths were not significantly decreased compared to the placebo group (p=0.72).

Another end point of this study was the recurrence of myocardial infarction, which showed a significant difference between both groups (p=0.014), with 7 patients in the placebo group experiencing a total of 12 re-infarctions within the 2 years follow-up period, while none of the BMCs group experienced a myocardial re-infarction.

A subgroup of 59 patients (33 patients of the placebo group and 26 patients of the BMCs group) was subject to magnetic resonance imaging (MRI) analysis of LV function 2 years after therapy. This analysis demonstrated a beneficial effect on regional LV contractility over the infarct area in the BMCs group. However, the global LV function was not significantly different between both groups until the baseline left ventricular ejection fraction (LVEF) was adjusted. Similar results were achieved previously by Bone Marrow Transfer to Enhance ST-Elevation Infarct Regeneration (BOOST) trial (101), which was as well not able to detect a significant difference in EF between BMC group and the control group.

The BOOST trial (101) is one of the long-term randomized and controlled human trials. In this trial 60 patients were randomized and divided into a control group receiving only standard therapy, and a BMCs group that additionally received an intracoronary BMCs infusion around 5 days after PCI. In order to be eligible for this study, patients must have been admitted to hospital within 5 days from the symptom onset of their first STEMI, have been successfully treated via PCI and stent implantation in the infarct related artery. All patients were treated with aspirin, beta-blocker and ACE inhibitor, unless these medications were contraindicated.

24 hours after intracoronary BMCs transfer using „stop-flow“ balloon catheter technique, troponin-T serum levels were tested, and showed no increase. This indicated that the procedure did not cause any additional ischemic damage to the myocardium.

Follow up examinations showed that LVEF increased in the cell group by 6.7 and 5.9 percentage points after 6 and 18 months respectively. In the control group, LVEF raised by 0.7 and 3.1 percentage points, after 6 and 18 months respectively. LVEF enhancement was significantly higher in the BMCs group after 6 months (P=0.0026) but not after 18 months (P=0.27). Furthermore, LVEF recovery along the period of 18 months was significantly more rapid in the cell receiving patients. Thus, in comparison to the six months' follow-up (100), the eighteen months' follow-up data from the BOOST trial showed that a single treatment with intracoronary BMCs did not achieve sustained long-term benefits on left

ventricular systolic functions after acute myocardial infarction. This study suggested, however, that BMCs transplantation after myocardial infarction does accelerate the LVEF recovery. It also showed that cell transplantation did not increase the risk of adverse clinical events, in-stent restenosis, or arrhythmic side effects, suggesting the safety of this treatment.

The five years' follow-up data of the same study (108) confirmed that the benefits of the BMCs treatment on LVEF in STEMI patients were not sustained over a longer period of time. It did however show, that even after 5 years of BMCs application, there were no significant differences in mortality and morbidity, providing reassurance concerning the safety of such treatments. Moreover, at 5 years follow-up, patients with bigger transmural infarcts tended to sustain the benefits from BMCs therapy. This should be however studied separately in randomized trials in the future in order to understand such a trend.

One of the long term human trials that was first published in 2002 by Assmus et al. (98), the TOPCARE-AMI trial, also shows the beneficial effects of progenitor cells therapy. Twenty patients that suffered their first STEMI and were acutely treated by coronary stenting were enrolled in this study. Patients were randomized to receive intracoronary infusion of either bone marrow-derived or circulating blood-derived progenitor cells (CPC) directly into the infarct artery. Another group of 11 patients who suffered as well a STEMI and matched the study group in primary treatment, infarct size and localization, but did not receive any progenitor cells, acted as a control group for this study.

The progenitor cells treated group showed a significant increase in the global LVEF, significantly improved regional wall motion over the infarct zone, as well as reduced end-systolic left ventricular volumes after 4 months of the cell injection. On the other hand, there was only a slight increase in the LVEF in the reference group, while the end-systolic volumes remained unchanged. Moreover, a profound enhancement of the regional contractile function, as well as a significant increase of the coronary blood flow reserve in the infarct artery of the progenitor cells groups were reported. Interestingly, there were no significant differences for any of the measured parameters between blood-derived and bone marrow-derived progenitor cell groups. Over the period of 4 months, no signs of inflammatory reactions were observed. Malignancy was as well not observed over the period of this study. This study however was continued further after the initial phase of the first 4 months. More patients were included according the same criteria, and were again randomized to either BMCs or CPCs.

The next follow-up took place 12 months after the initial treatment. The data analysis of this follow-up (99) revealed a significant increase in EF ($P<0.001$) as well as reduction of the infarct size ($P<0.001$). Absence of reactive hypertrophy was also reported, suggesting functional regeneration of the infarcted ventricles.
A five-years' follow-up report of the same trial was published in 2011(109), were follow-up data of 55 patients was completed. This report revealed that 16 patients underwent target vessel revascularization, whereas only two of those revascularizations occurred after 1 year of cells administration. MRI subgroup analysis in 31 patients showed a sustained improvement in LVEF. It was as well reported that the reduction of infarct size was still sustained over the 5 years' follow-up period. However, even though the LV end-systolic volume was stable, LV end-diastolic volume did increase significantly. In total, over the 5 years of follow-up, this trial did provide reassurance regarding the long-term safety of intracoronary cell therapy, and suggested long term benefits of this treatment on LV function.

In 2004, Fernandez et al. (103) published an interesting study concerning the regenerative capability of BMCs and the possible role BMCs transplantation therapy might play in treating myocardial infarction. This study was designed to test BMCs on two dimensions; a clinical study testing the safety and the beneficial effect of intracoronary transplantation of autologous BMCs in patients that suffered a myocardial infarction, and an *in vitro* study, assessing their myocardial regenerative capability and their effect on post-infarction left ventricular remodeling. *In vitro*, human BMCs were able to graft into damaged mouse cardiac tissue, acquiring a cardiomyocyte phenotype and expressing cardiac proteins after 1 week of culturing. As for the clinical trial, BMCs transplantation did not cause any adverse effects on microvascular function or myocardial injury. Using magnetic resonance 6 months after the onset of treatment, it was shown that the end-diastolic and the end-systolic thickness of the infarcted wall did increase significantly, whereas the end-systolic volume was decreased. Moreover, the regional and global LV function improved within those 6 months.
The outcome of this study showed that BMCs can nest into damaged myocardium, differentiating into cardiac cell phenotype *in vitro*, and that treatments using BMCs are safe and might have positive effects on ventricular remodeling.

The effect of mesenchymal stem cells transplantation to treat MI was examined by Chen et al. (105) and published in 2004. Sixty-nine patients that suffered AMI, and were treated primarily through angiography or angioplasty, were randomized to receive either bone marrow-derived mesenchymal stem cells (BMSCs) (n=34) or saline (n=35). Three months after BMSCs transplantation the wall movement velocity over the infarct area was significantly increased in the BMSCs group, while it only increased minimally in the control group. Over the same period of time, LVEF showed a significant increase in the BMSCs group in comparison to before cell transplantation. LVEF was also significantly higher in the BMSCs group than the control group. LVEF showed however no further change at 6 months after transplantation compared to the 3 months results.
In another double-blind, randomized, placebo-controlled human trial, Hare et al. (71) examined the safety and efficacy of intravenous injection of adult human mesenchymal stem cells (hMSCs) in patients that suffered an acute myocardial infarction, and underwent a successful reperfusion (71). A total of 53 patients that met the enrollment criteria were randomized to receive a single intravenous infusion of high-dose hMSCs, low-dose hMSCs, or placebo. The primary end point of this study was the incidence of treatment-emergent adverse events in the first 6 months after hMSCs transplantation. This primary end point of safety was met, with similar adverse event rates between hMSCs and placebo groups. Moreover, the other exploratory efficacy end points demonstrated reduced ventricular arrhythmia episodes, as well as a better LV function in the hMSCs groups relative to the placebo group. In a cardiac MRI substudy, LVEF was increased in the hMSCs groups, while it did not increase in the placebo group. This study suggested the safety of hMSC when administrated intravenously, as well as a possible beneficial effects leading to reversing the remodeling process after MI.

On the other hand, a study performed by Jassens et al. in 2004 showed relatively different results concerning the effect of BMCs transfer on LVEF (104). The primary endpoint of this study was the increase in LV ejection fraction. Secondary endpoints included decrease of the infarct size as well as changes in the regional LV function at 4 months' follow-up. In this double-blind study, 67 patients were randomized in the ratio of 1:1 to receive either BMCs or placebo. All patients participating in this study had suffered an acute myocardial infarction with cumulative ST-segment elevation of at least 6 mm, and a significant LV dysfunction after undergoing a successful PCI with stent implantation. After bone marrow harvesting from each patient under local anesthesia and isolation of BMCs, the final cell or

placebo preparation were injected in three fractions over 2-3 minutes, using a perfusion catheter. After the cell transfer was completed, injection of contrast medium into the infarct-related artery was carried on to assure vessel patency. Out of the 67 participating patients, one patient from the BMCs group died of hemorrhagic shock, while all the other 66 patients continued the 4 months' follow-up. Over the time of the study, global LV ejection fraction increased in both groups. The changes in left ventricular end diastolic volume (LVEDV) and left ventricular end systolic volume (LVESV) did not differ between BMCs group and controls. Myocardial perfusion and metabolism increased in both groups, with no significant benefit of BMCs transplantation. However, in comparison to placebo infusion, BMCs transplantation had a significant effect on reducing the size of the myocardial infarct, with a BMCs treatment effect of 28 %, as well as better recovery of regional systolic function.

The findings of this study indicated that intracoronary BMCs transplantation does not have an extra beneficial effect on global LV ejection functional recovery, but might however play a role in reducing the infarct size, suggesting a potentially biological positive effect of bone marrow derived cells on infarct remodeling. Furthermore, this study did not record any adverse effects related to intracoronary BMCs transfer, indicating the safety of this procedure.

In 2006 Lunde et al. published the results of the ASTAMI study (106), a randomized human trial, examining the therapeutic effects of intracoronary injection of bone marrow derived mononuclear cells after myocardial infarction. In this study, 100 patients were enrolled, and were randomly assigned to receive either autologous mononuclear BMC or to act as controls. The end points of this study were changes in LVEF, end-diastolic volume and the infarct size. Six months after treatment medical examination was carried out, using electrocardiogram-gated single-photon-emission computed tomography (SPECT) and MRI. The results showed no significant differences between patients that received BMCs and controls, suggesting that intracoronary injection of autologous mononuclear BMC has no effect on global left ventricular function.

The results of the 3 years' follow up of this study (110) showed that the rates of adverse events were low and equal in both groups, suggesting the long term safety of this treatment. It also showed that the patients receiving BMCs had a larger improvement in exercise time, compared to the controls. However, other than this improvement in exercise time, no other effects of BMCs treatment were identified 3 years after cell injection.

The lack of beneficial therapeutic effects of stem cell injection after AMI was also demonstrated in the results of the HEBE trial (107). This multicenter randomized controlled study enrolled 200 patients that suffered their first, large size AMI. After successful reperfusion via PCI, patients were randomized into three groups, receiving BMCs, mononuclear peripheral blood cells (PBC), or standard therapy. The primary endpoint was the percentage of dysfunctional left ventricular segments improvement at 4-months' follow-up. This did not differ significantly between the cell receiving groups and the control group. Improvement of left ventricular ejection fraction showed as well no significant difference between the three groups. Changes in left ventricular volumes, infarct size, and rates of clinical events were comparable between all groups.
The authors of that study suggested that the lack of demonstration of benefits of the BMCs therapy in the results might be due to a number of factors, such as the enrollment of patients with relatively short total ischemic time. Another factor is the relatively short follow-up period, lacking the demonstration of any long-term effects on LV function and remodeling. Also this study powered to detect a 6% improvement in the LVEF, while other studies reporting beneficial effects of such therapies suggest an average improvement of LVEF by around 3%. Nevertheless, the results of this study, as well as other studies showing no therapeutic benefits of stem cell therapies on post-MI conditions should be taken into consideration before such therapies make their way in clinical application.

One of the largest long-term studies up to date is in fact the Clinical Benefit and Long-Term Outcome After Intracoronary Autologous Bone Marrow Cell Transplantation in Patients With Acute Myocardial Infarction (BALANCE) study (46), published by Youssef et al. A total of 124 patients that suffered an acute myocardial infarction were recruited into this clinical study. Recanalization of the infarct-related artery was performed on all patients, using balloon angioplasty, stent implantation, or both. Further medication included aspirin, clopidogrel, beta-blocker, ACE inhibitor as well as a diuretic agent.
The therapeutic follow-up took place at three points of time; 3, 12 and 60 months after treatment. Patients were divided into two groups with 64 patients in each group. The first group received bone marrow derived cells in addition to the standard treatment, while the second group served as a control group, receiving only the standard treatment. BMCs were infused directly into the infarct-related artery. This was carried out using an angioplasty balloon catheter placed within the stented coronary segment.
The results recorded by this study were indeed in favor of BMCs treatment. The frequency

of the events of repeated coronary intervention and rehospitilisation did not differ between both groups along the full term of this study. At three months follow-up, improvements in the LV performance were observed in the BMCs group. A significant improvement of the ejection fraction by 7.9% was reported, while the stroke volume index (SVI) at rest increased by 16.5%. Moreover, the infarct size decreased by 8%. A significant decrease in end systolic volume and end diastolic volume was also reported. In addition, velocity of circumferential fiber shortening (VCF) increased significantly by 31% and the end-systolic wall stress was reduced by 8.6% over the infarction region in the BMCs group.
At 12 and 60 months' follow-up, a worsening of LV performance was noticed in the control group, in the form of an increase in LVEDV and LVESV, as well as a reduction of LVEF and SVI. On the other hand, patients in the BMCs group showed to have sustained the improvements in LV performance even for 60 months after treatment.
Another significant difference was shown between both groups concerning mortality of participants. While only 1 patient died from the BMCs group, 7 patients died from the control group, along the follow-up term of this study. The authors of this study interpreted these numbers as average mortality rates of 0.35% per year in the BMCs group and of 2.35% per year in the control group, indicating a significant difference in mortality rates between both groups. Furthermore, no adverse effects related to BMCs transplantation were detected throughout the course of this study, indicating a long-term safety of BMCs transplantation when intracoronary application is used.
The results of this long-term study could as well be summed up characterizing the time course of beneficial events that takes place after BMCs treatment, as an initially maximum improvement reached within the first few months after treatment, and an anti-remodeling effect on the longer run, preserving the ventricular performance.

4.3 Safety of stem cells transplantation to treat patients with MI

Despite the fact that most of the reviewed pre-clinical and clinical trials suggested the therapeutic benefits of stem cell transplantation on patients that suffered a myocardial infarction, a lot of concerns have been raised about the safety of such therapies. While most human studies, especially the long-term ones (46,108), did not report an increased incidence of stem cell-related side effects in the patients treated, other studies mentioned the occurrence of some adverse events. A meta-analysis published earlier this year summarized the adverse events that took place when stem cell transplantation therapy was used for treating patient with myocardial infarction (111). A total of 1,796 patients were

enrolled in those trials. Out of 26 trials, 7 reported side effects related to cell transplantation therapy. Intracoronary (IC) cell transplantation, being the most frequently used method of cell delivery in those trials, showed a 7.5% incidence of adverse events related to this method of transplantation. A total of 36 ischemic ST-segment changes related to balloon inflation during cell transplantation using „stop-flow“ technique were reported. One trial reported ST-segment elevation in most of the patients during the same procedure. Endovascular complications due to stem cell transplantation were also reported by a number of trials. This included coronary artery dissection, stent thrombosis, as well as a case of no-reflow phenomenon. Moreover, five patients suffered an arrhythmia, while four patients developed transient hypotension after cell therapy.

Some points should be put into consideration regarding this meta-analysis. First, this meta-analysis did not mention whether those adverse events, which are thought to be related to cell therapy, also appeared in the control groups, and whether there was any statistically significant differences in their incidence between cell receiving patients and controls or not. Second, most of the adverse events occurred during balloon inflation, within the framework of „stop-flow“ technique cell delivery. The efficacy of such techniques depends majorly on the experience of the performer. This could explain the appearance of such adverse events in only a few studies, with most of the patients in one study suffering from balloon inflation-related adverse events. Moreover, the „stop-flow“ technique involves multiple balloon inflations, increasing the possibility of such events. As for arrhythmic side effects, their incidence was remarkably increased in patients receiving skeletal myoblasts, which suggests a relation between the type of stem cells transplanted, and the frequency of developing arrhythmia. In addition, there was no cases of stem cell related tumor formation reported, offering a reassurance to one of the major concerns about stem cell therapy.

While it has been suggested by the authors of this meta-analysis that using alternative delivery methods, such as transepicardial or transendocardial injections, would decrease the rate of adverse events, those techniques have to be intensively examined, as there is not enough data to confirm a less frequent occurrence of adverse effects when such methods are used instead of IC infusion to deliver stem cells to the myocardium.

Another meta-analysis comparing the outcomes of human trials using bone marrow stem cells to treat patients after STEMI reported that there was no significant difference in the rate of adverse effects between cell receiving groups and control groups. In fact, the rates of revascularization were found to be lower in the cell receiving groups (112).

5 Discussion

Stem cell transplantation to treat patients that suffered an acute myocardial infarction has attracted a lot of attention in the past 15 years. This is because of the interesting beneficial effects such therapy offers, reversing ventricular remodeling and assisting myocardial regeneration, with the consequence of improving the cardiac functions.

The publications reviewed in this paper showed that most of the human trials were conducted to examine either the beneficial effects of stem cells transplantation, or the safety of this therapy, or both.

While most of the short and long term human trials showed a beneficial effect of stem cell transplantation therapy on post MI conditions, some trials failed to find any benefits of such therapies on cardiac functions. This is why some groups conducted meta-analyses, comparing the results of different human trials.

A meta-analysis examining the efficacy and safety of bone marrow stem cell therapy was published recently (112). This meta-analysis compared 29 randomized controlled human trials that used BMSC therapy for treating patients that suffered a myocardial infarction with ST-segment elevation. An improvement of LVEF was reported by most of the reviewed trials, with an average increase of about 3% in LVEF. A dose-response relationship between the amount of cells transplanted and the beneficial effect on LVEF was suggested. No significant differences were found between cell receiving groups and controls regarding the frequency of adverse events. BMSC treated groups showed less rates of revascularization than control groups.

Another meta-analysis comparing 10 studies using as well BMSC therapies to treat AMI reported similar results concerning LVEF improvement in BMSC patients in comparison to placebo receiving patients (113). Moreover, a beneficial effect on other cardiac performance parameters, such as LVESV and LVEDV, was also reported. Those parameters were all found to be significantly improved in the BMSC treated groups. Significantly lower rates of repeated MI in the BMSC groups were also reported.

Although the reviewed preclinical and clinical data suggest a beneficial effect of stem cell transplantation in increasing the cardiac functions after myocardial infarction, this novel therapy needs to be further developed before it finds its way to becoming a standard therapy. At the moment, the used timing, dosing and mode of cell delivery is not according

to clinical evidence, but are rather based mainly on practical feasibility in the clinical setting and analysis from small animal trials (55). Before this therapy could make it into guidelines, more long-term, double-blind, randomized clinical trials have to be performed, examining other aspects than just the safety and therapeutic effects of stem cell transplantation. Those aspects that need to be determined through clinical research include the optimal cell type for stem cell therapies, the most effective method of cell application, the best timing for cell transplantation and the ideal dosage of cells that should be transplanted. The mechanism of action of stem cells in regenerating the myocardial mass needs to be more clarified as well.

While it is still unclear, which cell type would be the optimal cell for stem cell therapies, bone marrow-derived stem cell is the most frequently used cell type for clinical studies. This might be due to their advantages in comparison to other cell types, especially the availability of a range of populations of cells with the ability to differentiate into various types of cells, with no need of in vitro expansion. In addition to BMSCs, MSCs were as well used in many human trials, showing good results due to their ability to transdifferentiate into cardiomyocytes and being tolerated by the immune system (56)

One of the other questions that need to be settled is the method of stem cells delivery to the myocardium. There are different ways to deliver stem cells to the required cardiac area (Figure 7). This could be performed intravascular, either through intravenous (IV) injection or through intracoronary (IC) application. Direct cell injection into the ventricular wall represents another method to deliver stem cells. Either transepicardial, or trandendocardial transplantation could be used to deliver stem cells directly into the ventricular wall.
While IV application seems to be the simplest way, the stem cells tend to home in non-cardiac organs as well (114), limiting the clinical applications of this method. A clinical study was performed comparing IV infusion and IC delivery through transplantation of labeled BMCs in patients that suffered a MI. It showed cardiac homing of these cells only after IC delivery, but not after IV infusion (115). However, Hare et al. examined the safety and efficacy of IV application of human MSCs after MI in a randomized double-blind clinical trial, and reported both the safety and positive effects of such therapy on cardiac function (71).
On the other hand, the overwhelming majority of clinical trials evolving MI patients were performed through intracoronary application. Those studies used the „stop-flow“

technique. With the use of a balloon catheter, and by performing percutaneous transluminal coronary angioplasty (PTCA), the cell transplantation is performed over multiple fractional high-pressure infusions (97). This technique is used to maximize the contact time of the transplanted cells with the microvascular circulation. Depending on the experience of the performer, this would need about one hour to be performed. Although it was reported that IC artery injection of MSCs caused microinfarctions in dogs (116), the efficacy and safety of IC stem cell delivery in humans were also reported by multiple mid-term and long-term clinical trials.

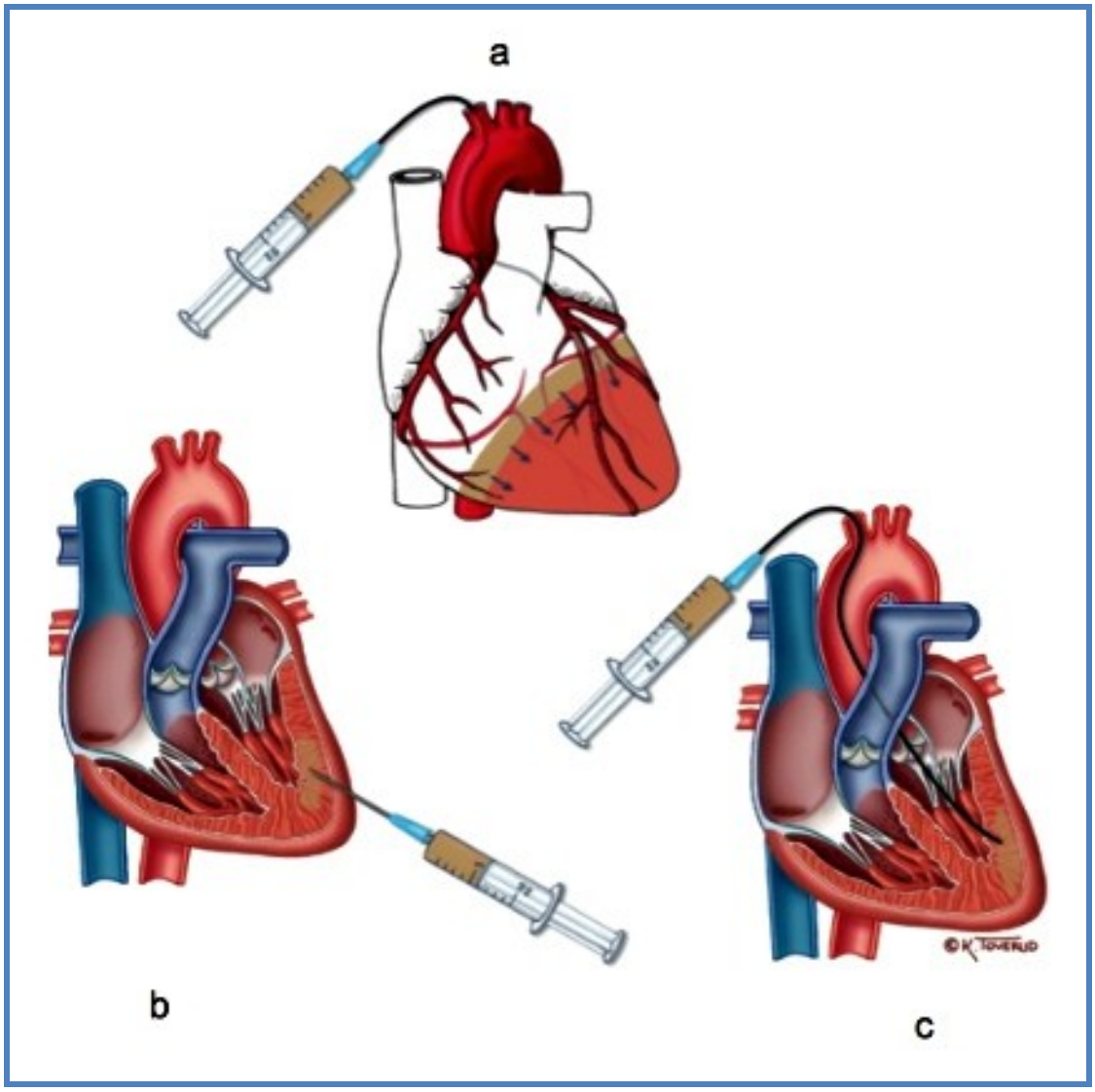

Figure 6 Methods of stem cell delivery to the myocardium; a) intracoronary. b) transepicardial. c) transendocardial [modified after (62)]

Transepicardial cell injection is another way to deliver stem cells, and could be performed during open heart surgery, as it is the case with coronary artery bypass grafting. The

myocardium is under direct vision of the surgeon and direct application of the cells to the required area could be done. However, the efficacy of cell transplantation is hard to assess when performed parallel to open heart surgery (117).
Transendocardial (TE) cell injection could be achieved using an electromechanical mapping catheter, which is then replaced with a needle catheter positioned against the endocardial surface, injecting cells directly into the ventricular wall (118). In a canine study comparing TE and IC transplantation of MSCs, the TE group showed higher rates of cell retention, with cell clusters forming in the infarct zone, while such clusters failed to form in the IC group. The TE group showed as well an increase in vascularity and a better functional improvement than the IC group (75).
It is still not very clear, which method of cell delivery achieves the best results. However, depending on the condition of the patient, one method could be more beneficial than the other. Indeed, trans vascular application is thought to benefit patient that suffered a recent myocardial infarction that was successfully reperfused the most (117), as chemoattractants and adhesion molecules are present in high concentrations at this point of time (119). On the other hand, direct injection seems to be better in case of late disease process, such as chronic myocardial ischemia, when transvascular cell delivery is not possible due to coronary artery occlusion. Moreover, direct injection technique could be used to deliver cells that are relatively large in size, such as MSCs, overcoming the problems of microembolisation when such cells are delivered through IC injection (117).

The timing of cell transplantation plays as well an important role in affecting the outcome of cell therapy (56). During the first 4 days after MI, cell adhesion molecule concentration is high enough to promote the transplanted cells into the inflammatory process, rather than transdifferentiation into myocardial cells. About a week after MI the concentration of those cell adhesion molecules start to decline, allowing for an optimal differentiation of the transplanted cells (120,121). By the end of the second week after scar formation, the beneficial effects of the transplanted cells are reduced. This is why it is thought that the best time point for cell transplantation is between 7 and 14 days after MI (122). This has been shown in the results reported by the REPAIR AMI trial, as patients treated within 4 days after MI showed no beneficial effects of cell transplantation, while patients treated between 4 and 8 days after MI were found to have better EF in follow-up (123). This should be examined more closely in upcoming randomized clinical experiments, in order to define the optimal time point for cell transplantation.

While the beneficial effects of stem cells on cardiac repair are being reported by multiple animal and human studies, the mechanism underlying their therapeutic effect remains questionable. Originally, it was suggested that stem cells induce myocardial repair through their transdifferentiation into cardiomyocytes, reversing the loss of cardiacmyocytes caused by ischemia (84,124). Transplantation of MSCs pretreated with 5-azacytidine into injured rat hearts showed their homing and differentiation into cardiac-like cells (125). Direct injection of bone marrow derived cells into the hearts of mice after AMI regenerated about 68% of the infarct zone with newly formed cardiomyocytes (126). It was also suggested that cell fusion of donor cells with recipient cardiomyocytes might play a role in explaining the mechanism of action of stem cell transplantation (127,128). However, with deeper investigation into those theories, the number of newly formed cardiomyocytes was not found to be big enough to explain the significant functional enhancement (56). On the other hand, it was reported that fibroblasts, which have no contractile function like cardiomyocytes, did as well improve the diastolic function when transplanted into injured rabbit hearts (129).This lead to a new hypothesis, proposing that stem cell transplantation benefits the cardiac function in a paracrine fashion (Figure 6), through releasing soluble factors that enhance cardiac protection, neuvascularization as well as cardiac repair (130). MSCs were found to be involved in the production and secretion of a number of cytokines, chemokines and growth factors that are thought to be involved in cardiac repair (131). Interestingly, the production of many of those factors is normally increased under hypoxic conditions (132). Gnecchi et al. reported that they were able to reproduce most of the beneficial effects of MSCs transplantation on cardiac repair by injecting conditioned medium (CM), a cell-free medium harvested from MSC cultures and containing metabolites and growth factors secreted by those cells in culture, into the injured hearts (133). Takahashi et al. performed a similar study, injecting supernatants of bone marrow mononuclear cells into infracted rat hearts. They reported a protective effect of those cytokines, which showed to have preserved the contractile capacity of the myocardium and inhibited apoptosis of cardiomyocytes. They also reported a decrease in infarct size, an increase in capillary density, as well as an enhanced cardiac function in comparison to controls (134). A recent study compared the therapeutic effects of adipose-derived stem cells (ADSC) to these of a conditioned medium collected from the same amount of ADSC (ADSC-CM) on MI in mice (135). Compared to control group, both ADSC and ADSC-CM showed a significant decrease in the infarct size, as well as an improvement in cardiac

function. Even though the improvement of angiogenesis was stronger in the ADSC-treated mice, both ADSC and ADSC-CM significantly reduced the rate of cardiomyocyte apoptosis at the border zone. While ADSCs differentiated into vascular cells, amounting to about 9% of the enhanced angiogenesis, they did not differentiate into cardiomyocytes.

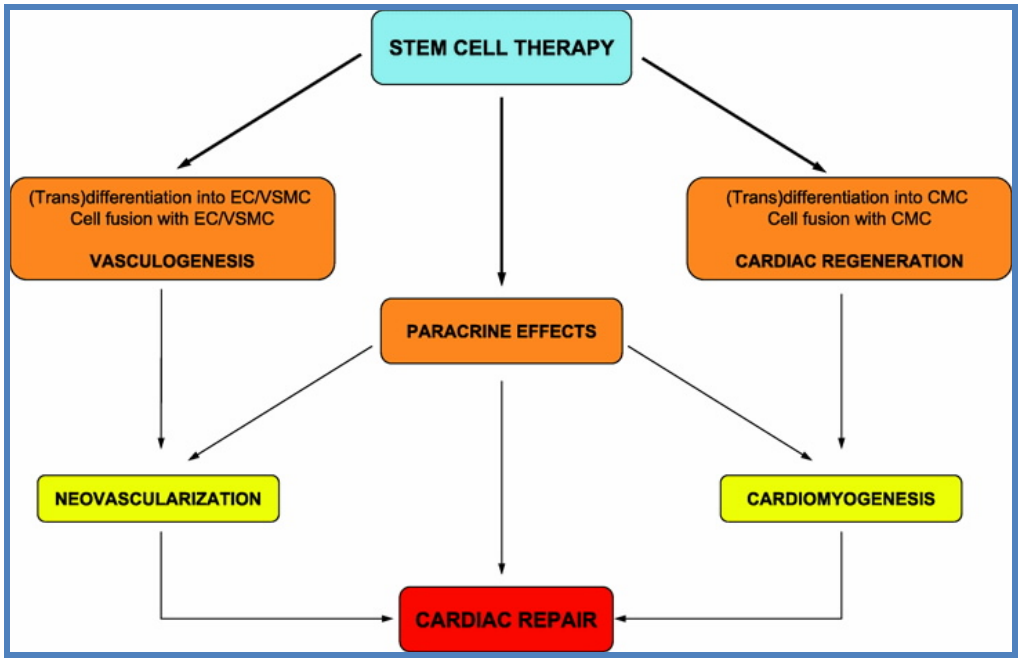

Figure 7: Proposed mechanisms of stem cell action in cardiac repair. Paracrine effects could possibly influence many processes, such as cardiomyogenesis and neovascularization, leading to cardiac repair (133)**.**

The authors of this study concluded that the therapeutic effects of ADSC transplantation is majorly through their paracrine functions, enhancing cardiac protection and angiogenesis, while ADSC differentiation only plays a minor role in angiogenesis.

One of the instant paracrine effects of stem cells in a hypoxic environment is the release of cytoprotective molecules that decrease cardiomyocytes apoptosis (130). As for cardiac repair and cardiomyocytes regeneration, it was suggested that stem cells transplantation might activate resident CSCs and alter circulating stem cells to migrate to the infarct area, stimulating cardiomyocytes replication through a paracrine action, and activating cardiac repair (130).

While the scientific debate between the different hypothesizes continues, new information pointing to the importance of the paracrine effects of stem cells are of great value. It is clear that those paracrine actions do play a role in cardiac repair. Treatments based on this hypothesis should be further enhanced and examined, as such treatments might support the benefits of stem cell therapy, and even overcome some of the obstacles that faces stem cell transplantation, such as oncogenesis and immune rejection.

The different aspects concerning future research of stem cell transplantation after MI are already attracting the attention of researches. A big project is currently conducted, aiming to standardize the methods of bone marrow collection, preparation procedures and delivery post AMI. The BAMI project (136) will conduct the first large all course mortality clinical trial, enrolling around 3000 patients, with the intention of demonstrating that transcoronary infusion of bone marrow-derived progenitor cells is safe and would reduce the mortality rate by 25% and reduce the rehospitalisation rate by 15%.

This and similar projects should be further conducted, in order to achieve the optimal results using stem cell transplantation, and settle all the unclarified issues. With such projects taking place, stem cells could be used in the future as a standard therapy for treating patients after MI, enhancing their cardiac functions, and saving millions of lives around the world.

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7 Curriculum Vitae

Profile

First Name: Karim
Last Name: Morsi
Gender: Male
Date of birth: 15/05/1985
Place of birth: Cairo – Egypt
Nationality: Egyptian

Education

Primary Education:
Misr International Language Schools; Cairo, Egypt. 1992-1997
Preparatory Education:
Misr International Language Schools; Cairo, Egypt. 1997-2000
Secondary Education:
Misr International Language Schools; Cairo, Egypt. 2000-2003
Higher Education:
Cairo University, Faculty of medicine; Cairo, Egypt. 2003-2008
Medizinische Universität Graz 2010-2013

Degrees

Diploma of achievement of Egyptian secondary education, Cairo, Egypt: 2003

- **Languages:** Advanced Arabic language, high-level English language, intermediate French as a second language.
- **Majors:** Biology, Chemistry, Physics, Economics, Statistics
- **Minors:** Math 1, Geography
- **Grade:** 96.4%
- **Bonuses:** 1.5% Sports Achievement bonus in parachuting.

Diploma of achievement of the Ford Foundation Leadership and Self-discovery program: 2007

- **Awarded by:** The Institute of International Education.
- **Prospects:** Leadership and Self-discovery peer trainer

Deustchsprache für Hochschule (C1) 2010
- Awarded by: Georg-Simon-Ohm Hochschule, Nürenberg, Germany

Medical practical Training

2003-2005: Clinical training courses of Human Anatomy, Cairo University)
2006-2007: General Ophthalmology, Otorhinolaryngology,(Kasr Al Ainy Hospitals)
2007-2008: General Pediatric medicine, General Obstetrics and Gynecology, Community Medicine and Public Health. (Kasr Al Ainy Hospitals)
July 2010: General surgery internship, Krankenhaus Rotthalmünster, Germany
October 2010: Orthopedic Surgery, LKH Graz, Austria
July 2011: Vascular Surgery, Cairo University hospitals, Egypt
July-August 2012: Neurology, Krankenhaus Passau, Germany
September 2012: Gynecology and Obstetrics, Krankenhaus Passau, Germany
October 2012: Cardio surgery, Krankenhaus Passau, Germany
December 2012: Training at a general practitioner's clinic, Voitsberg, Austria.

Volunteer work experiences

- Organizing Committee member in the Best Buddy and Special Olympics Projects, 1998-2000.
- Active membership of the Student Scientific Society, Cairo University, 2006
- Community Development and general public awareness activist, rural Egypt, 2007.
- Organizing Committee Member in the European Underwater and Baro-medical Society annual congress and general assembly, Sharm El-Sheikh September 2007.
- Co-Founder of the Egyptian Medical Students' Association (EMSA), 2007

Responsibilities and leadership positions

- Head of the Egyptian delegation in the Euro-Med Youth In Action Exchange Program in Partnership with UNESCO, Tunisia, 2005.
- Head of the Organizing Committee of the Egyptian Medical Students Association general assembly meeting, 2006

- Appointed Executive board member and Local Incoming Exchange Officer in the Students' Scientific Society, Cairo University, 2007
- Project coordinator of the annual Kasr El Ainy international Winter School in tropical medicine, Cairo, 2007
- Project coordinator of the first international summer school in hyperbaric medicine, Red Sea, 2007
- Team leader of the Cairo university exchange season and international summer school, Cairo, 2007
- Founding member of the Egyptian Medical Students' Association NGO, 2007
- Project coordinator of the Smoking Free Life initiative organized by the Egyptian Medical Students' Association in partnership with the Egyptian Ministry of Health and the World Health Organization, 2008
- Vice President of the student NGO EMSA, 2007-2011
-

Languages Spoken

- **Arabic** Native
- **English** Very Good
- **German** Very Good
- **French** Basic

Hobbies

- Parachuting: Over 5 jumps record, 1st position in National Championship in 2001, and 3rd position in 2002.
- Horse back riding, Arabian Horses breading and training on oriental dressage
- Desert Safari expeditions
- Traveling
- Skiing
- Kite Surfing
- Soccer
- Squash
- Fishing