Intramyocardial Stem Cell Transplantation in Cardiac Surgery:
From Preclinical Backgrounds to the PERFECT trial

Peter Donndorf MD and Gustav Steinhoff MD, PhD,
Reference and Translation Center for Cardiac Stem Cell Therapy (RTC), Department of Cardiac Surgery, University Rostock, Germany

Received 19/12/2010, Reviewed 29/12/2010, Accepted 2/01/2011
Keywords: CABG, stem cell, intramyocardial, regenerative, surgery
DOI: 10.5083/ejcm.20424884.20

ABSTRACT
Cardiac cell therapy for regenerative purposes has been clinically applied in the fields of cardiac surgery and interventional cardiology for almost one decade. With preclinical studies showing promising regenerative concepts and results, the clinical efficacy of stem cell application reported until today in the setting of ischemic heart disease has been rather modest. However, clinical studies performed so far have been heterogeneous. Hence, for final evaluation of the possible clinical benefits completion of ongoing phase III trials are mandatory. The following article repeats preclinical and clinical prerequisites for cardiac stem cell application and introduces the German Phase III PERindopril Function of the Endothelium in Coronary artery disease Trial (PERFECT) for intramyocardial stem cell injection in combination with CABG surgery.

INTRODUCTION
Ten years ago, after promising preclinical results (1, 2), cardiac stem cell application for regenerative purposes was introduced in the fields of interventional cardiology and cardiac surgery for the treatment of acute myocardial infarction and chronic ischemic heart disease. Since intrinsic myocardial regeneration takes places but is reduced during a normal life span (3) it is conceivable that revascularisation procedures - especially in elderly patients who form the vast majority of cardiac and cardio-surgical patients - might profit from attempts aimed at stimulating these regenerative processes.

After initial reports of successful stem cell delivery to the injured heart, the clinical feasibility and safety of this new therapeutic strategy has been established, thereby focusing on the treatment of ischemic heart disease. Over 3000 patients with either recent myocardial infarction or chronic ischemic heart failure have been treated so far by means of interventional cardiology and cardiac surgery. Prospective clinical studies could prove the safety of both interventional intracoronary stem cell injection as well as surgical intramyocardial cell injection (4, 5).

Yet, the efficacy of cardiac stem cell application still remains an open question. In order to answer this question of surgical stem cell therapy in combination with CABG surgery, the German PEriNdropril Function of the Endothelium in Coronary artery disease Trial (PERFECT), a multicentre trial, was launched just over one year ago. The following article will repeat the preclinical background of cardiac stem cell application and review the rationale, as well as the clinical prerequisites for the PERFECT trial.

Background for intramyocardial stem cell transplantation in cardiac surgery:

Stem cells have the important properties of self-renewal and differentiation potential (6-9). Thus, they are ideal candidates for regeneration of damaged myocardial tissue (10), for example, in myocardial infarction or congestive heart failure. When acute myocardial infarction occurs, heart muscle tissue is regionally destroyed (11, 12). Myocardial regeneration by direct injection of c-kit+ bone marrow stem cells for treatment of acute heart failure following myocardial infarction in a mouse model was reported (1). This work initiated a controversial, but very inspiring discussion by reporting cardiac cell type regeneration by more than 50% in the infarcted area.

Although a straight reproduction of the reported results failed, and subsequent experimental studies showed that bone marrow-derived stem cells of haematopoetic or endothelial lineage (BMSC) are most likely not able to transdifferentiate into functional cardiomyocytes (13), plausible confirmation of the principle of cardiac regeneration caused by the transplantation of BMSC in different animal models followed until today (14). The isolation and the systemic delivery of bone marrow stem cells have been established before in the treatment of haematopoietic diseases (15).
Surface markers characterising BMSC in the adult human bone marrow include CD133, CD34 and CD117 (c-kit). Transplantation of bone marrow derived haematopoietic stem cells is related with myocardial regeneration most likely due to neovascularisation and reduction of apoptosis [16, 17]. Vessel formation is shown as a result of both stem cell and resident cell action in the infarction area. Thereby rescue of hibernating myocardium in the infarction border zone by improved oxygen supply leads to functional improvement due to improved ventricular wall function.

By intramyocardial injection these cells are accumulated within the infarct zone and the border zone where they contribute to myocardial regeneration and scar size reduction, although contractile neo tissue is not generated. The first intramyocardial injection of bone marrow-derived haematopoietic stem cells for treatment of chronic ischemic heart disease due to myocardial infarction applying this rationale was performed in 2001 by the Rostock group [18]. To date, several clinical trials investigating safety and efficacy of surgical intramyocardial BMSC injection have followed.

Another cell type of special interest for cardiac regeneration is represented by skeletal myoblasts, which in fact were among the first cell types considered for cardiac repair. Skeletal myoblasts are capable to differentiate into non-muscle cell types and are resistant to ischemia [19], which is an obvious obstacle to the function of other stem cells in injured myocardium. Animal studies in cardiac disease models have been performed with encouraging results. However, skeletal myoblasts do not fully differentiate into cardiomyocytes in vivo after transplantation. The contracting myotubules do not operate in synchrony with the surrounding myocardium, which is most likely due to a lack of the gap-junction protein connexin 43 activity and lack of electrical coupling with the surrounding cardiac cells (20, 21). Formation of functional myocardium and preclinical evidence of successful cardiomyocyte differentiation from human BMSC in vitro.

A principle problem however, is formed by the fact that autologous stem cells are subject to age- and disease-related impairment of their proliferative capacity, paracrine activity and plasticity [22]. In order to overcome these intrinsic limitations of autologous stem cell therapy, strategies have been developed in the recent past which include the use of allogeneic donors, neonatal cells (i.e. embryonic stem cells) and pre-treated or genetically manipulated cells (i.e. induced pluripotent stem cells, IPS). Since this article primarily reviews the current clinical status of surgical intramyocardial stem cell therapy, these complex strategies aimed to optimise the regenerative approaches in cardiovascular medicine - although of high interest - will not be further discussed here.

**Stem cell isolation and surgical cell delivery**

Important conditions for clinical stem cell therapy are the precise and careful techniques of bone marrow cell preparation, availability of large cell concentrations within the area of interest (border zone of infarction), migration of stem cells into the apoptotic or necrotic myocardial area and prevention of homing of transplanted cells to other extracardiac organs.

For stem cell transplantation in cardiac diseases, adult bone marrow (80–200 ml) is aspirated under local anaesthesia from the iliac crest. Although coronary artery bypass grafting (CABG), when performed “on-pump” using the heart-lung machine, requires systemic anticoagulation, up to now in our clinic, we have had no bleeding complications related to bone marrow aspiration [23]. Respective bone marrow stem cell populations then need to be isolated under good manufacturing practice (GMP) conditions. During cell preparation, viability needs to be determined several times and finally must reach around 95%. All microbiological tests of the clinically used cell preparations must prove negative for endogenous (HIV, HBV, HCV) or exogenous contamination. Also any haematologic disease, compromising bone marrow cell quality has to be excluded prior to cell transplantation.

Surgical stem cell implantation is performed into well exposed ischemic areas, allowing for multiple injections within and principally around the infarct area with a thin needle. The first clinical studies performed stem cell injection in combination with CABG. Once the graft-coronary artery anastomoses are completed the ischemic area is visualised and the cells are injected into the border zone of the infarcted area [5]. To date this method of direct injection represents the standard method of surgical stem cell therapy and has been applied successfully also during off-pump CABG as well as during “stand-alone” minimally invasive procedures where cell injection is performed without cardiac arrest via lateral minithoracotomy [25, 26].

Interventional therapy protocols utilising stem cell transplantation for the treatment of acute myocardial infarction and chronic ischemic heart disease include intra coronary as well as endocardial stem cell injection. Since homing of injected BMSC towards injured tissue is very complex and depends on the interplay of many factors, including stromal cell-derived factor-1 (SDF-1) and the presence of an adequate inflammatory stimulus, adhesion molecules and a sufficient endothelial nitric oxide production, it may be hard to design adequately timed intracoronary treatment strategies. In contrast to intracoronary application, endocardial stem cell injection using the NOGA® injection catheter facilitates the delivery of stem cells directly into the target area of the myocardium without depending on sufficient cell migration across the endothelial barrier. The first clinical studies were able to prove safety and feasibility of the transendocardial route in the setting of chronic ischemic heart disease [27] as well as for intreatable angina [28].

**Indications for intramyocardial stem cell transplantation**

To date, there is robust preclinical as well as growing clinical evidence that stem cells might offer beneficial effects in the surgical treatment of subacute and chronic ischemic heart failure and in the interventional therapy treatment of acute myocardial infarction. There is only limited experience for the effects of bone marrow stem cell application in non-ischemic heart disease. However, via paracrine effects, gain in cardiac function in the absence of myocardial ischemia is reported [29].

Stem cell therapy for ischemic heart disease is indicated in patients presenting with impaired left ventricular ejection fraction between 20 to 40 per cent due to myocardial infarction leading to symptoms of heart failure with or without angina. The underlying myocardial infarction should be of mild extension—approximately between 9 and 14 cm² with the presence of a distinct area of “hibernating” myocardium in the infarction border zone as verified by pre-procedural viability tests. Left ventricular wall thickness in echocardiographic evaluation should be greater than 4mm in order to avoid extramyocardial injection and the risk of iatrogenic ventricular wall injury [30].
Early injection after infarction could be beneficial to prevent a large fibrotic scar. On the other hand, since myocardial infarction leads to severe impairment of heart function associated with rhythmic instability and poorer tolerance of additional treatment, including further ischemia during cardiac surgery, it might be reasonable to wait for the acute phase to pass until the infarction zone is consolidated. For surgical reasons myocardial consolidation is also preferable for any elective operative revascularisation procedure. Furthermore cell transplantation should be more effective after the post-ischemic inflammatory reaction has subsided i.e. after day 8-12 following an acute attack.  

Stem cell transplantation within the ‘hot’ phase post-myocardial infarction inflammation might lead them to take part in the inflammation cascade rather than in the formation of functional myocardium and vessels.

In our clinic we assign patients with impaired heart function after myocardial infarction and the presence of hibernating myocardium to stem cell treatment in combination with CABG surgery when indicated. The goal of intramyocardial stem cell transplantation in these patients is the improvement of myocardial function by augmentation of myocardial perfusion by cell supported angiogenesis and arteriogenesis in the infarction border zone or regions of low contractility due to poor perfusion.

In the selected patients the treatment indication is verified by cardiac stress MRI or myocardial scintigraphy for evaluation of myocardial viability as well as global ventricular function, coronary angiogram, echocardiography and Holter-ECG.

Clinical studies

Several clinical studies have revealed beneficial stem cell effects in subacute and chronic ischemic heart failure. Most of the surgical trials designed for this setting (Table 1) performed intramyocardial stem cell transplantation in combination with "on-pump" coronary artery bypass surgery. Surgical studies completed so far have been randomised as well as non randomised. Nearly all studies were able demonstrate clinical feasibility and a high level of patient safety.

However, all of these studies included a rather small number of patients and have been heterogenous regarding the type and number of cells applied, the time point of cell application and the inclusion criteria for the respective studies. The improvement of cardiac function by intramyocardial cell transplantation has been described as an moderate increase in left ventricular ejection fraction from 5 to 10 per cent compared with the respective control groups, as well as an improvement of wall motion caused by enhanced myocardial perfusion. Yet, the study heterogeneity mentioned above make it difficult to interpret conclusively the efficacy of surgical stem cell application so far.

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Sample Size/ Design</th>
<th>Primary intervention</th>
<th>Co-Intervention; Mean stem cell Dose (SD)</th>
<th>Route of injection</th>
<th>Follow-up duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hendrikx (2006)</td>
<td>20/RCT</td>
<td>CABG</td>
<td>CD 133 BMSC aspiration; 60.25 (31)x10^6</td>
<td>IM</td>
<td>4 month</td>
</tr>
<tr>
<td>Mocini (2006)</td>
<td>36/cohort</td>
<td>CABG</td>
<td>BMC-MN aspiration; 292 (232)x10^6</td>
<td>IM</td>
<td>3 months</td>
</tr>
<tr>
<td>Klein (2007)</td>
<td>10/cohort</td>
<td>&quot;stand alone&quot; cell injection</td>
<td>CD 133 BMSC aspiration; 1.5-9.7 X 10^6 cells</td>
<td>IM</td>
<td>9 months</td>
</tr>
<tr>
<td>Ahmadi (2007)</td>
<td>27/cohort</td>
<td>CABG</td>
<td>CD 133 BMSC aspiration; 1.89 (0.03)x10^6</td>
<td>IM</td>
<td>6 months</td>
</tr>
<tr>
<td>Pompilio (2008)</td>
<td>5/cohort</td>
<td>&quot;stand alone&quot; cell injection</td>
<td>CD 133 BMSC aspiration (3), BMSC mobilisation; (2); 7.6x10^6</td>
<td>IM</td>
<td>12 months</td>
</tr>
<tr>
<td>Stamm (2007)</td>
<td>40/RCT</td>
<td>CABG</td>
<td>CD 133 BMSC aspiration; 5.80x10^6</td>
<td>IM</td>
<td>6 months</td>
</tr>
<tr>
<td>Zhao (2008)</td>
<td>36/RCT</td>
<td>CABG</td>
<td>BMC-MN aspiration; 6.59(5.12)10^6</td>
<td>IM</td>
<td>6 months</td>
</tr>
<tr>
<td>Patel (2005)</td>
<td>20/RCT</td>
<td>CABG (off-pump)</td>
<td>CD 34 BMSC aspiration; 22x10^6</td>
<td>IM</td>
<td>6 months</td>
</tr>
</tbody>
</table>

CABG, coronary artery bypass grafting; CD, cluster of differentiation; BMSC, bone marrow stem cell; BMC-MN, bone marrow mononuclear cell; IM, intramyocardial; RCT, randomised controlled trial; cohort, cohort study; SD, standard deviation; SC, stem cells.
The PERFECT clinical trial

The PERFECT trial (ClinicalTrials.gov Identifier:NCT00950274) is a Phase III trial, aimed to provide evidence for the effects of cardiac stem cell therapy in combination with CABG surgery on ventricular function as well as patients’ clinical outcomes and quality of life. It has been designed as a double blinded, placebo controlled, multi-centre trial, and is one of the first surgical trials stringently applying GMP standards for all therapeutic steps.

As the primary endpoint, left ventricular ejection fraction after six months will be analysed; secondary endpoints are left ventricular dimensional parameters, physical performance measured by six minutes walking test, change in NYHA score and patients’ quality of life.

Establishing consequent pre-procedural assessment of regional myocardial viability and function by cardiac MRI leading to exact targeting of revascularisation procedures and possible additional cell applications is another aim addressed by this trial. In addition, basic research protocols carried out concomitantly with the trial will support the ongoing design of an optimal “cell product” for clinical application in the future.

Patient inclusion criteria:

- Coronary artery disease with indication for CABG and previous myocardial infarction
- Time frame infarction to cell transplantation > 2 weeks
- LV EF between 25 and 40 per cent measured by cardiac MRI
- Akinetic/hypokinetic/hibernating left ventricular areas localised by cardiac stress MRI
- Absence of any valve pathologies requiring replacement or reconstructive surgery

Exclusion criteria:

- Emergency operation
- Resuscitation in combination with ventricular arrhythmias within the last 14 days before treatment
- Active cancer, organ transplantation, end-stage renal disorder
- Infection (CRP ≥ 20 mg/l, Temp ≥ 38.5 °C)
- Contraindication for MRI

Future perspectives

Since the first description of bone marrow stem cell transplantation during coronary artery bypass surgery in 2001, several clinical studies have suggested intramyocardial stem cell injection to be beneficial for patients with chronic ischemic heart failure. Still, the underlying molecular mechanisms of stem cell related gain of cardiac function need to be further clarified. Besides myocardial regeneration due to enhanced neovascularisation, alternative modes of stem cell action like paracrine activity and immunomodulation have to be considered. Especially MSC possess potent immunomodulatory properties which might be useful for therapy strategies aiming for cardiovascular regeneration.

On the other hand, especially the use of MSC might also hold some pitfalls, like secondary infections due to uncontrolled immunosuppression and tumour growth. For establishing BMSC transplantation in the treatment of ischemic heart failure randomised, double-blinded, multi-centre Phase III trials need to be accomplished as soon as possible. These trials should also be aimed to evaluate whether a relevant gain in cardiac function is reached and to which this possible gain affects clinical outcome (NYHA score etc.) of stem-cell treated patients in the long term. Attempts to optimise the cells applied for cardiac regeneration purposes, for example by genetic reprogramming, hold great promises.

Furthermore the aim to create dynamic “multi-lineage” regenerative strategies by combining adequately optimised “cell products” with tissue engineered scaffolds, heart valves or cardiac resynchronisation therapy (38, 37-39) should be further supported since they offer a realistic perspective to come to an integrated regenerative approach. To reach these perspectives, co-operative studies between clinical and preclinical research are mandatory.

REFERENCES


ABSTRACT

Determining operability in patients with aortic valve disease is dependent on two major factors: physical characteristics of the patient and the extent of damage induced by strain on the myocardium from stenotic and regurgitant lesions, and co-morbidities, and when myocardial contractile reserve is poor.

On the other hand, the surgeon may be reluctant to perform the intervention in the presence of co-morbidities, and when myocardial contractile reserve is poor. This procedure is not appropriate for patients with severe chronic heart failure and absolute contraindications to surgery, such as advanced age and co-morbidities, and when myocardial contractile reserve is poor. At the same time, the patient's age and physical condition are key factors in determining operability. In patients with severe chronic heart failure and severe co-morbidities, the decision to perform intervention before establishment of severe myocardial damage may be considered. The extent of damage induced by strain on the myocardium from stenotic and regurgitant lesions and the patient's degree of myocardial contractile reserve are key factors in determining operability.

Keywords: valve disease, percutaneous valve therapy, surgery-valve treatment.

Received 31/12/2010, Reviewed 10/1/2011, Accepted 18/01/2011

1. Introduction

Aortic stenosis (AS) is the most common type of valvular heart disease and is characterized by the narrowing of the aortic valve orifice. The incidence of AS is on the rise since this pathology is more frequent in older adults.1 The natural history of AS has shown that in the absence of surgical management, patients become symptomatic.2

2. Indication of surgery

Indications for aortic valve surgery (AVR) are based on the presence of severe AS, which is defined as a peak aortic valve gradient of over 40 mmHg or an effective orifice area of less than 0.6 cm²/m² body surface area) or the patient be symptomatic.3

3. Technical considerations

The extent of damage induced by strain on the myocardium from stenotic and regurgitant lesions and the extent of myocardial contractile reserve are key factors in determining operability. In patients with severe chronic heart failure and severe co-morbidities, the decision to perform intervention before establishment of severe myocardial damage may be considered. The extent of damage induced by strain on the myocardium from stenotic and regurgitant lesions and the patient's degree of myocardial contractile reserve are key factors in determining operability.

4. Conclusion

The extent of damage induced by strain on the myocardium from stenotic and regurgitant lesions and the patient's degree of myocardial contractile reserve are key factors in determining operability. In patients with severe chronic heart failure and severe co-morbidities, the decision to perform intervention before establishment of severe myocardial damage may be considered. The extent of damage induced by strain on the myocardium from stenotic and regurgitant lesions and the patient's degree of myocardial contractile reserve are key factors in determining operability.