Cardiac bone marrow cell injection for chronic ischemic heart disease

Beeres, S.L.M.A.

Chapter 1

General Introduction

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Over the past decades, substantial advances in risk factor modification, pharmacological therapy, and revascularization therapy have significantly reduced the mortality of ischemic heart disease. Nevertheless, ischemic heart disease remains a leading cause of morbidity and mortality worldwide.

Cell therapy is currently being investigated as a potential treatment modality for patients with ischemic heart disease. Pre-clinical studies suggested that cell therapy may have a favorable effect on tissue perfusion and contractile performance by promoting vascularization and myocyte formation.[1] Following these encouraging pre-clinical results, cell therapy has rapidly been introduced in the clinical setting.

As an introduction to this thesis, an overview of the basic principles of cardiac cell therapy will be provided. At first, the different cell types that have been tested in pre-clinical studies and the specific mechanisms through which these cell types may contribute to functional improvement are discussed. Thereafter, the different routes of cell delivery are reviewed, along with the results of the initial clinical studies investigating the safety, feasibility and efficacy of cardiac cell therapy for patients with ischemic heart disease.

Cell Types for Cardiac Repair

The ideal cell population for cardiac repair would:

1) Improve the contractile properties of the myocardium through regeneration of substantial amounts of new cardiomyocytes which integrate structurally and functionally in the host myocardium.
2) Retain its proliferation potential for a determined period of time in order to enable colonization of large areas of damaged myocardium.
3) Generate new myocardial vasculature, either by direct incorporation into the newly formed vascular wall or by paracrine stimulation of resident cardiac cells.
4) Be highly resistant to myocardial ischemia and apoptosis.
5) Be of autologous origin, or retain minimum immunogenicity.
6) Be readily available in large quantities for cardiac transplantation.
7) Connect to the surrounding myocardium through functional connections without creating potentially proarrhythmogenic areas.

During the last decade, a variety of cell populations have been tested for cardiac repair, including bone marrow-derived cells, skeletal myoblasts, embryonic stem cells and more recently resident cardiac stem cells, adipose-tissue-derived stem cells and umbilical cord-derived stem cells (**Figure 1**).

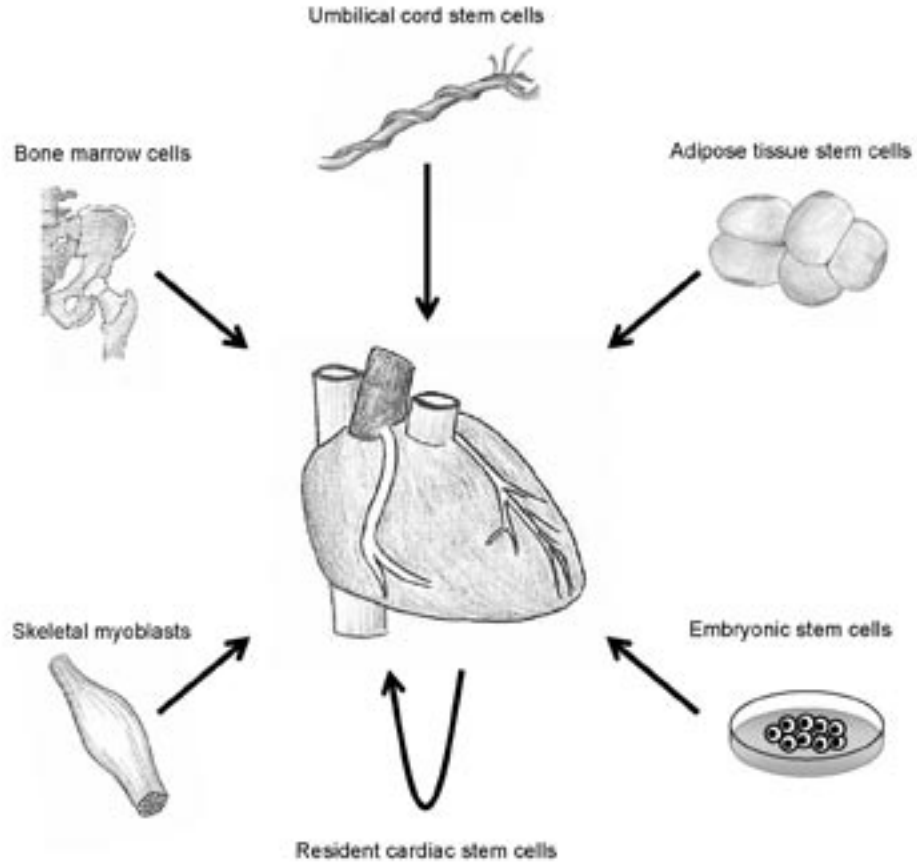

Figure 1

Different sources of cells for cardiac repair.

Bone Marrow-derived Cells

Bone marrow contains several cell types that have the capacity to proliferate, migrate and differentiate into various mature cell types. Among these are hematopoetic stem cells (HSC), endothelial progenitor cells (EPC), mesenchymal stem cells (MSC), and multipotent adult progenitor cells (MAPC).

Hematopoetic Stem Cells

HSC comprise <0.01% of the total bone marrow cell population and are identified by the expression of the human marker proteins CD34 or CD133. These cells have extensively been used for stem cell transplantation in hematological disorders since these cells can give rise to all types of blood cells.

In 2001, Orlic et al. suggested that HSC, when transplanted in the infarcted heart, may also transdifferentiate into cardiac cell lineages. In particular, HSC generated substantial amounts of de novo myocardium, comprising proliferating myocytes as well as vascular

structures.[2] Similarly, Jackson et al. described that a subset of HSC (the so-called side-population cells, which are characterized by a distinct Hoechst dye efflux pattern) were able to home to areas of damage in ischemic mouse hearts and transdifferentiate into vascular endothelial cells and cardiac myocytes.[3]

A number of recent studies, however, could not reproduce the promising in vivo transdifferentiation data.[4-6] For example, Balsam et al. reported that HSC acquired a leukocyte phenotype rather than differentiated into cardiomyocytes. Still, HSC transplantation prevented left ventricular (LV) dilatation and improved LV function. These data suggest that the functional improvement after HSC injection in infarcted myocardium is unlikely to be mediated by transdifferentiation into cardiomyocytes. Currently, it is presumed that the functional improvement is more likely to result from beneficial paracrine effects on LV remodeling or angiogenesis.

Endothelial Progenitor Cells

EPC reside in the bone marrow and have the capacity to migrate to the peripheral circulation, home to sites of ischemia and stimulate neovascularization. These cells can be isolated from the bone marrow or from peripheral blood following mobilization by cytokine treatment.

Traditionally, EPC were identified by the expression of the HSC markers CD34 or CD133, the co-expression of vascular endothelial growth factor receptor-2, and the ability to differentiate into endothelial cells.[7] However, recent evidence suggests that culture-expanded EPC also contain a CD14+/CD34- cell population, which promotes neovascularization by secreting pro-angiogenic growth factors.[8] This underscores the notion that additional characterization of the EPC population is warranted.

The ability of EPC to enhance neovascularization makes this cell type a promising candidate for cardiac cell therapy. For example, Kocher et al. reported that EPC transplantation after experimental infarction induced blood vessel formation and proliferation of pre-existing vasculature in the infarct bed.[9] Despite these encouraging experimental data, there is growing evidence that EPC numbers and their ability to promote neovascularization are impaired in patients with coronary artery disease, which limits the therapeutic usefulness in the clinical setting.[10;11]

Mesenchymal Stem Cells

MSC represent a rare population of self-renewing, multipotent cells present in the adult bone marrow stroma and in other mesenchymal tissues. These cells are characterized by the absence of hematopoetic surface markers, and their ability to differentiate in multiple phenotypes including chrondrocytes, osteoblasts, adipocytes and fibroblasts. Bone marrow provides an accessible and renewable source of adult MSC. Although MSC comprise only <0.01% of the bone marrow mononuclear cell fraction, these cells can easily be isolated (based on their adhesive properties) and expanded in vitro.

A number of experimental studies suggested that bone marrow-derived adult MSC have the potential to transdifferentiate in functional cardiomyocytes both in vivo and in vitro.[12-15] For instance, Shake et al. demonstrated that MSC expressed muscle-specific proteins after cardiac transplantation. In addition, MSC transplantation significantly improved LV function and beneficially affected LV remodeling.[13] Currently, however, it is under debate whether differentiation of MSC in cardiomyocytes can explain the functional improvement since recent studies questioned the cardiomyogenic differentiation potential of MSC.[16;17] In line with this discussion, experimental studies suggested that MSC transplantation may also stimulate neovascularization[16-18] and attenuate post-infarction remodeling.[19] Although the mechanism by which MSC may improve LV function is currently only partially understood, MSC are a promising candidate for cell-based cardiac repair because of the observed beneficial effects on LV function. In addition, MSC are reported to have low immunogenicity and therefore may be used in an allogenic setting in the future.

Multipotent Adult Progenitor Cells

Recently, a rare population of primitive cells with a remarkable differentiation potential has been identified within bone marrow-derived MSC cultures. These cells, the so-called MAPC, have extensive proliferation capacity and can give rise to adipocytes, osteoblasts, hepatocytes and endothelial cells in vitro. In addition, it has been shown that these cells can differentiate into myocytes after injection into the mouse tibialis anterior muscle[20] and into cardiomyocytes in chimeric mice.[21] Although the ability of MAPC to repair infarcted myocardium remains to be established, these cells may represent a promising population for cell-based cardiac repair.

In conclusion, there is evidence that the human adult bone marrow contains precursors of both myocytes and endothelial cells. The use of bone marrow cells in the cardiovascular setting has the advantage that bone marrow provides a relatively easily accessible (**Figure 2**), renewable and autologous source of therapeutic cells. This formed the basis for the use of

Figure 2
Bone marrow aspiration from the iliac crest under local anesthesia.

bone marrow in a number of clinical trials. Since head-to-head comparisons of the different bone marrow subpopulations are scarce, many investigators have chosen a pragmatic approach by using bone marrow mononuclear cell preparations. The mononuclear cell fraction (which can be easily isolated from a marrow aspirate with the use of density gradient separation) comprises a heterogeneous cell population including HSC, EPC, MSC and MAPC.

Skeletal Myoblasts

Skeletal myoblasts are precursors of skeletal muscle cells that normally lie in a quiescent state under the basal membrane of mature muscular fibers. Skeletal myoblasts can be isolated from skeletal muscle biopsies, amplified in large quantities in vitro, and then injected into myocardial scar tissue. Skeletal myoblasts exhibit many desirable qualities as of therapeutic donor cells for the treatment of ischemic heart disease. For example, they are highly resistant to ischemia and can be harvested as autologous cells. Moreover, when transplanted in infarcted myocardium, skeletal myoblasts have the potential to differentiate into functional myotubes and skeletal muscle fibers.[22]

A number of studies suggested that myoblast transplantation in infarcted myocardium may improve LV contractility.[22-24] However, skeletal myoblast transplantation has some disadvantages. A major caveat is the lack of electrical connections between skeletal muscle cells and the surrounding cardiomyocytes.[25] Injected myoblast may thereby form isolated, potentially proarrhythmogenic areas. In addition, there is conflicting evidence on whether these cells can survive over a longer time period in the host myocardium.[26]

Resident Cardiac Stem Cells

Traditionally, the adult mammalian heart was considered a terminally differentiated organ without regenerative capacity. However, recent data suggest that the adult myocardium itself contains undifferentiated cells that can be expanded in vitro to generate large numbers of cells capable of differentiating into cardiomyocytes and/or vascular cell lineages.[27-29] Moreover, in a pig infarction model, Nadal-Ginard et al. demonstrated that growth factor-induced stimulation of endogenous cardiac stem cells resulted in massive regeneration of the infarcted myocardium.[30]

Although resident cardiac stem cells have shown to be able to improve LV function and reconstitute well differentiated myocardium in a rat infarction model,[28] it is clear that in clinical practice the presence of these cells within the human adult heart does not translate into functionally significant cardiac differentiation following infarction. In addition, it remains to be investigated whether these cells are bone marrow-derived cells which have remained in local niches in the heart or represent a novel cell population. Nevertheless, the identification of resident cardiac stem cells opens new opportunities for cell-based cardiac repair in the future.

Adipose Tissue-derived Stem Cells

Adipose tissue is derived from the embryonic mesenchyme and contains adipocytes as well as many additional cell types, including MSC and EPC. Recently, Planat-Bénard et al. demonstrated that adipose tissue-derived cells could differentiate into beating cells with morphological, molecular and functional properties of cardiomyocytes.[31] In a mouse hind limb ischemia model, the same group reported that adipose tissue-derived cells have the potential to differentiate into an endothelial phenotype and promote tissue vascularization.[32]

The ease of access to fat and its abundance in humans make adipose tissue-derived cells an attractive source for cell-based cardiac repair. However, before widespread clinical application, additional characterization of the adipose tissue cell population is warranted. Moreover, the capacity of adipose tissue-derived cells to regenerate cardiac tissue in vivo still needs to be determined.

Umbilical Cord-derived Stem Cells

Human umbilical cord blood contains a number of stem cell populations, including HSC, mesenchymal precursor cells and unrestricted somatic stem cells (USSC). UCCS have a high proliferation potential, an extended life span and are capable of differentiating into various cell lineages, including cardiomyocytes.[33] Recently, Kim et al. demonstrated that human USSC transplantation in a porcine myocardial infarction model prevented LV dilatation and enhanced LV function.[34] The implanted cells also increased regional perfusion, suggesting that USSC may also participate in the formation of new blood vessels.

USSC have not yet been used in clinical studies. Nevertheless, umbilical cord-derived cells may represent an attractive cell source for cardiac repair in the future since these cells can easily be extracted and crypopreserved allowing individuals to store their own samples for potential future autologous use.

Embryonic Stem Cells

Embryonic stem cells are derived from the inner cell mass of the blastocyt-stage mammalian embryo. They are pluripotent, meaning that they are capable of giving rise to every somatic cell type of the adult organism as well as germ cells.[35] Embryonic stem cells have several features that make them an excellent candidate for cardiac cell therapy: they have unlimited proliferation potential[36] and can form cardiomyocytes[37;38] with a distinct electrophysiologic[39;40] and contractile phenotype.[41;42] Indeed, Min et al., demonstrated that transplanted mouse embryonic stem cells could differentiate into cardiomyocytes and improve the contractile function of previously infarcted myocardium.[43]

Although embryonic stem cell transplantation provides an exciting framework for cell-based cardiac repair, their use in clinical studies is hampered by unresolved ethical and legal issues, concern about induction of teratomas and the risk of rejection by the immune system. However, the generation of patient-specific immune-matched human embryonic

stem cells by nuclear transfer techniques has recently been demonstrated and may solve the latter issue in the long-term future.[44;45]

Comparison of Potential Donor Cells

Although experimental studies have tested various cell populations, it is clear that none of the aforementioned cell types meet all the criteria of the optimal cell population for cardiac repair. Nevertheless, skeletal myoblasts and bone marrow-derived cells have already been introduced in the clinical setting (**Figure 3**). Both cell types share advantages over the other cell types in that they are of autologous origin and readily available in large quantities for cardiac transplantation.

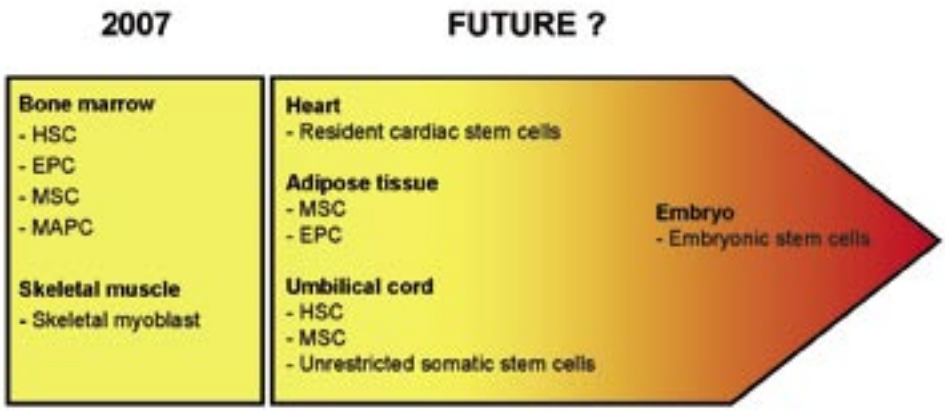

Figure 3

Cell types for cardiac repair. Cells in current human trials include bone marrow-derived cells and skeletal myoblasts. Cells in pre-clinical studies include resident cardiac stem cells, adipose tissue-derived cells, umbilical cord-derived stem cells and embryonic stem cells. Although embryonic stem cells exhibit many desirable qualities as of the ideal cell population for cardiac repair, their use in clinical studies in the near future is hampered by a number of safety, ethical and legal issues.

Studies comparing the ability of skeletal myoblasts and bone marrow-derived cells to functionally repair ischemically-damaged myocardium have not yet been performed in humans. Animal studies, however, suggested that bone marrow-derived cells and skeletal myoblasts may improve regional systolic function to a similar degree.[35;46]

While deciding on which of these 2 cell types to use in patients with ischemic heart disease, several factors should be taken into account. Skeletal myoblasts are more resistant to ischemia, and therefore may be more suitable for the transplantation into scarred myocardium. Conversely, a major advantage of bone marrow cells over skeletal myoblasts is the plastic nature of the bone marrow cells. An additional advantage of bone marrow cells is that these cells may not only give rise to contractile elements, but also promote neovascularization, which may be of particular benefit for patients with ischemic heart disease. The latter argument, combined

with the more favorable safety profile of bone marrow cell transplantation, has provided the rationale for the use of bone marrow cells in the majority of clinical studies performed to date.

Cell Delivery Routes

The goal of any cell delivery strategy is to transplant sufficient numbers of cells into the myocardial region of interest and to achieve maximum retention of therapeutic cells within that area. At present, the following cell delivery strategies are available for cell transplantation in patients with ischemic heart disease: intravenous infusion, intracoronary infusion, and intramyocardial injection (**Table 1, Figure 4**).

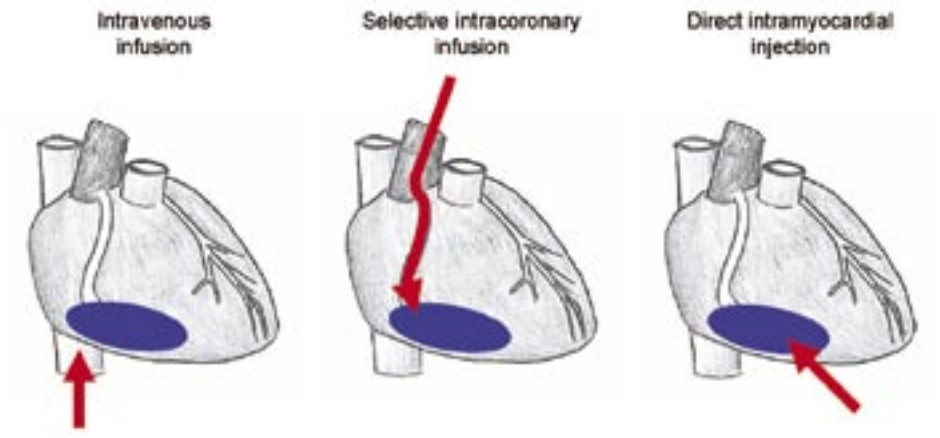

Figure 4
Different routes for cell delivery to the heart.

Intravenous Cell Infusion

The intravenous technique involves infusion of cells through a central venous catheter. Although intravenous infusion is the simplest method of cell delivery, a high percentage of the injected cells may become trapped in the lungs, liver and spleen. Therefore, only a small number of cells enters the coronary circulation and is available for trans-endothelial migration into the myocardium. Indeed, Barbash et al. reported that intravenous infusion of MSC in a rat infarction model resulted in cardiac engraftment rates of less than 1%.[47] Another potential disadvantage of intravenous cell delivery is that clusters of larger cells (such as MSC) may create micro-emboli in the vasculature of multiple organ systems.

Intracoronary Cell Infusion

Selective intracoronary cell delivery involves cell infusion through the central lumen of an over-the-wire balloon catheter which is positioned into a coronary artery. In order to enhance myocardial cell retention, the cells are delivered during transient balloon inflations.

TABLE 1. Delivery routes for cardiac cell transplantation

Method	Advantages	Disadvantages
Intravenous infusion	- Simple, non-invasive - Allows repetitive cell delivery	- High systematic exposure - Low percentage of cells enters coronary circulation - Transmigration dependent on homing signals - Requires administration of large numbers of cells - Risk of micro-emboli
Intracoronary infusion	- Relatively simple technique - Low systematic exposure - Cell delivery in a specific coronary territory - Cells delivery in myocardial regions with preserved oxygen supply	- Transmigration dependent on homing signals - Inability to deliver cells to regions with occluded coronary artery - Risk of micro-infarctions - Risk of endothelial damage due to balloon inflations
Intramyocardial injection	- Low systematic exposure - High cell concentration in myocardial region of interest - Allows cell delivery in territories with occluded coronary artery	- Risk for unwanted differentiation when injecting cells in scar tissue - Risk of increased tissue heterogeneity after focal cell injection
Trans-epicardial	- Relatively simple technique - Direct visualization of cell injections	- Need for thoracotomy
Trans-endocardial	- Assessment of myocardial viability before cell injection	- More complex technique - Risk of cardiac perforation
Trans-venous	- Visualization of cell injections with echocardiography - Cell delivery deep in myocardium	- More complex technique - Inability to deliver cells in all myocardial territories - Risk of damage of coronary veins

This maneuver halts coronary blood flow and prevents rapid outwash of the infused cells.
The intracoronary infusion technique is particularly well-suited for the delivery of cells to a specific coronary territory and has the advantage that cells can travel directly into myocardial regions with preserved oxygen supply, which ensures a favorable environment for cell survival. Nevertheless, retention of cells in the target myocardium remains a critical issue since intracoronary infused cells still have to transmigrate the vascular endothelium. Accordingly, this technique will be predominantly suited for the treatment of recently infarcted and reperfused myocardium when chemoattractants and cell adhesion molecules are expressed at high levels.[48;49]
Potential disadvantages of intracoronary cell infusion are the inability to deliver cells in myocardial territories with an occluded coronary artery, the risk of cardiac micro-infarctions when infusing large cells,[50] and the risk of coronary endothelial damage due to repetitive balloon inflations at the time of cell transfer.[51]

Intramyocardial Cell Injection

Therapeutic cells can also be injected directly into the myocardium. At present, three delivery routes have been described for intramyocardial injection of therapeutic cells in patients with ischemic heart disease: trans-epicardial injection, trans-endocardial injection and trans-venous injection.
Trans-epicardial injection, which has been performed as an adjunct to coronary artery bypass grafting, has the advantage that it allows direct visualization of the infarcted myocardium and target cell injections within this area. However, cells injected directly in necrotic tissue will most probably not receive the necessary cues and environment to engraft and differentiate along the cardiomyocyte pathway. Moreover, the invasiveness of this approach hampers its use as a stand-alone procedure.
On the contrary, catheter based trans-endocardial injection can be performed as a stand-alone procedure during cardiac catheterization. This technique involves direct injection of therapeutic cells through a needle inserted into the myocardium guided by a 3-D electro-mechanical mapping system (NOGA system (Biosense-Webster, Waterloo, Belgium (**Figure 5**)).[52;53] This 3-D mapping system can be used to distinguish viable, hibernating or infarcted myocardium. Accordingly, the viability of an endocardial injection site can be determined prior to injection. Potential disadvantages of trans-endocardial injection are the risk of endocardial damage and the risk of ventricular perforation, which may limit its use in recently infarcted myocardium.[54]
Recently, the trans-venous injection technique has been proposed as an alternative route for direct intramyocardial cell injection.[23] This technique involves injection of therapeutic cells trough the coronary veins into the myocardium with the use of a catheter system incorporating an ultrasound tip for guidance and an extendable needle. In contrast with the trans-endocardial approach, the trans-venous injection technique delivers cells parallel to the ventricular wall thereby delivering cells deep into the injured myocardium.

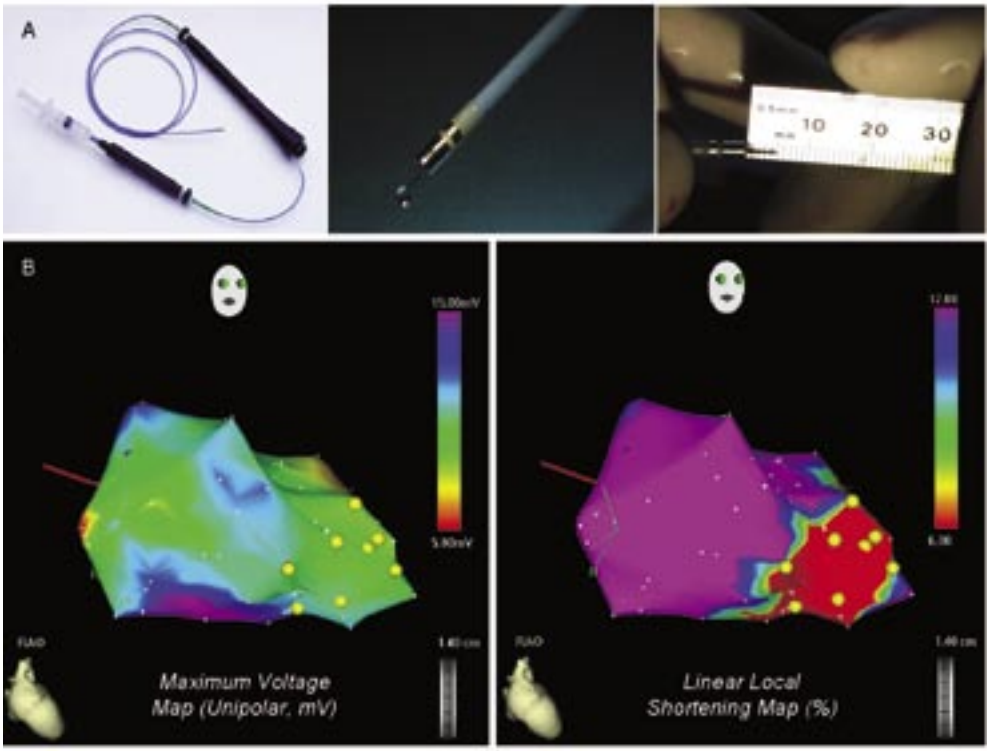

Figure 5
(A) NOGA compatible MyoStar injection catheter from which a hollow needle can be advanced by for direct intramyocardial fluid delivery. (B) Color coded NOGA maps (right anterior oblique view). Unipolar voltage map showing normal voltages in the apical region. Linear local shortening map showing reduced linear local shortening in the same myocardial region. The yellow dots indicate the 8 injection sites.

However, not all myocardial territories can be reached with this somewhat bulky catheter system, and positioning of the injection catheter in a specific coronary vein is technically challenging and not possible in all patients.

Comparison of Different Delivery Routes

At present, no delivery strategy has emerged as the most optimal administration route for cardiac cell transplantation. Recently, Freyman et al. compared 3 methods of MSC delivery in a porcine infarction model.[55] Fourteen days after cell transplantation, the percentage of MSC retained in the infarct zone was 6% in the intracoronary infusion group, 3% in the trans-endocardial injection group and 0% in the intravenous infusion group. Analysis of representative samples of the lungs and liver revealed that trans-endocardial injection was associated with less remote organ engraftment as compared with the intravenous and intracoronary infusion techniques. Whereas no complications were observed with intravenous infusion and trans-endocardial injection, intracoronary cell infusion was associated with myocardial damage.

Until now, a head-to-head comparison of the different delivery routes has not been performed in humans. While deciding on which technique to use in patients with ischemic heart disease, several factors should be taken into consideration. First, certain large cell types (such as MSC) are best administered by means of intramyocardial injection because of the potential risk of embolization when large numbers of these cells are infused intracoronary or intravenously. Second, the percentage of transplanted cells retained in the myocardium is strongly dependent on the local milieu. In particular, the strength of homing signals may vary in different clinical scenarios. Homing signals include chemoattractants and cell adhesion molecules that stimulate the therapeutic cells to adhere to the endothelium, transmigrate through the endothelium and invade the myocardium. In general, the intracoronary infusion technique seems to be most suited for the treatment of recently infarcted and reperfused myocardium when high levels of homing signals are expressed. On the contrary, direct intramyocardial injection techniques seem to be more appropriate for the treatment of patients with chronic disease, when an occluded epicardial coronary artery precludes intracoronary cell infusion or when homing signals are expressed at low levels in the heart.

Clinical Applications: Bone Marrow Cells for Myocardial Infarction

Early reperfusion strategies and advances in pharmacological management have considerably improved post-infarction survival rates. However, in a substantial number of the survivors, the loss of a significant number of viable cardiomyocytes and the development of myocardial fibrosis eventually leads to LV dysfunction with remodeling, resulting in heart failure. Despite recent therapeutic advances, the long-term prognosis of patients who develop heart failure after infarction remains poor. Accordingly, it is warranted to develop novel treatment strategies that reduce infarct size, prevent remodeling and improve LV function.

Experimental Background of Bone Marrow Cell Therapy for Myocardial Infarction

In 2001, a landmark study by Orlic et al. generated excitement as it provided evidence that locally delivered bone marrow cells could generate de novo myocardium thereby improving the outcome after infarction.[56] Since then, the potential of bone marrow cells to functionally repair infarcted myocardium has been investigated in numerous pre-clinical studies.[4-6;57] While the initial studies mainly focused on repopulating the infarcted myocardial area with a pool of new functional cardiomyocytes, recent studies demonstrated that cell therapy may improve myocardial performance through a variety of mechanisms (**Figure 6**). Regardless of the mechanism, there appears to be agreement that bone marrow cell transplantation has the potential to improve cardiac function in animal models of myocardial infarction.

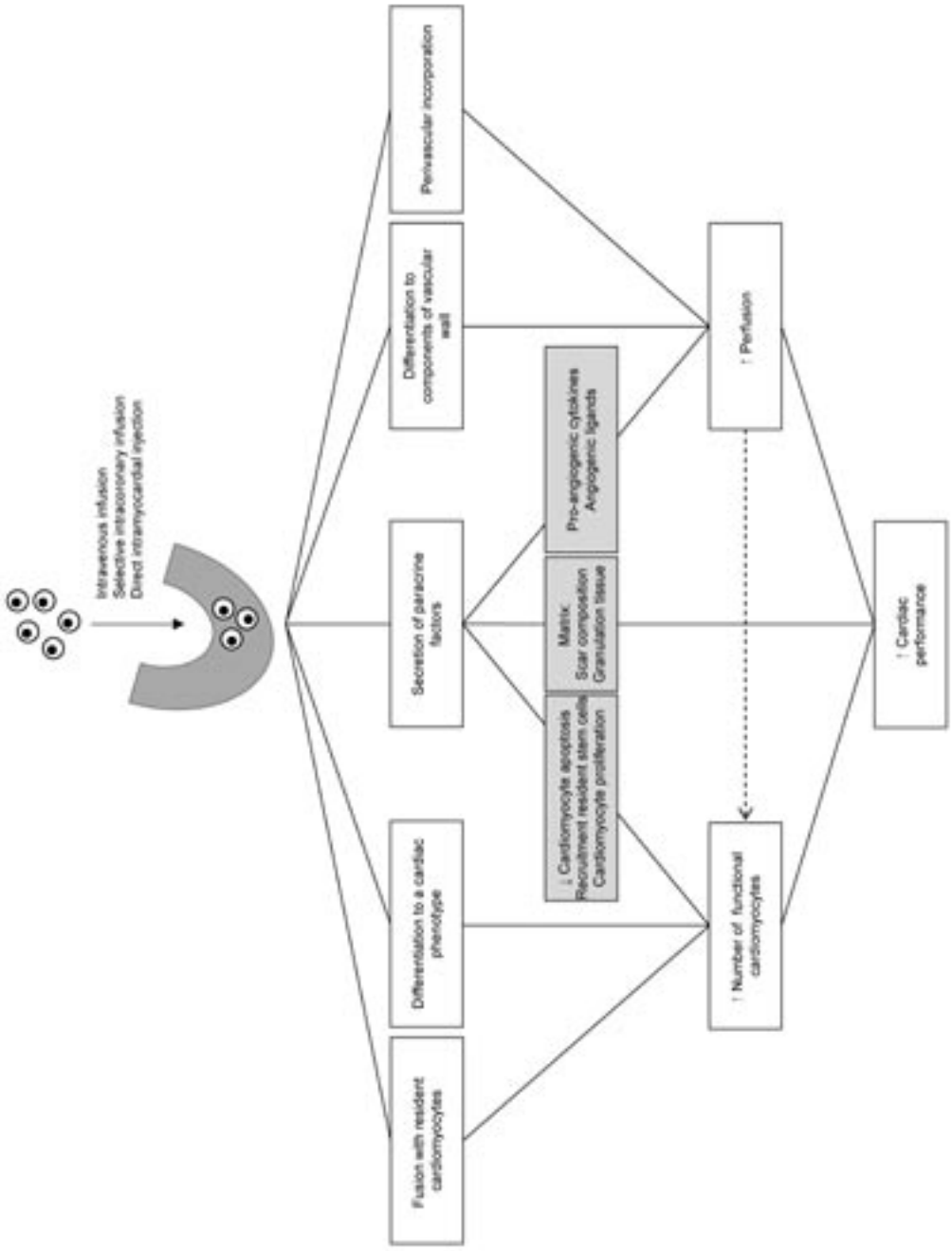

Figure 6
Potential mechanisms by which bone marrow cells may improve cardiac performance.

Clinical Studies in Patients with Acute Myocardial Infarction

To date, the results of 10 clinical studies,[58-71] in which bone marrow cells were transplanted as a sole procedure in ≥10 acute myocardial infarction patients, have been published in peer-reviewed journals (**Table 2**). In these studies, cells were infused in the infarct-related coronary artery 1-18 days after primary percutaneous coronary intervention. The follow-up period ranged from 3 to 18 months.

Safety and Feasibility

Most studies concluded that intracoronary bone marrow cell infusion in the immediate post-infarction period is safe in the short- and mid-term follow-up. In particular, the clinical trials have not reported an increased risk of ventricular arrhythmia. In addition, intracoronary bone marrow cell infusion did not appear to inflict myocardial micro-infarctions, even when large cells were infused.[66] Two studies, however, suggested that intracoronary cell infusion after acute infarction may be associated with aggravation of coronary atherosclerosis. The MAGIC study reported that intracoronary infusion of G-CSF mobilized cells was associated with an unexpected high rate of in-stent restenosis.[65] Similarly, Bartunek et al. reported that patients treated with intracoronary infusion of CD133+ enriched bone marrow cells showed a higher incidence of coronary events as compared to control patients.[51;67] Possible explanations for bone marrow cell-induced aggravation of atherosclerosis include promotion of angiogenesis within atherosclerotic lesions, aggregation of inflammatory cells within the plaque, differentiation of transplanted cells into neointimal smooth muscle cells, and impaired re-endothelialization of the stented coronary segment due to repetitive balloon inflations at the time of cell infusion. In contrast to these results, repeat coronary angiography in 3 other studies did not show aggravation of coronary atherosclerosis after intracoronary bone marrow cell infusion for acute myocardial infarction.[62;63;69]

Efficacy

Intracoronary bone marrow cell infusion following acute myocardial infarction aims to functionally repair the infarcted myocardium by promoting vascularization of the infarcted area and repopulate the infarcted region with viable cardiomyocytes.[1] The results of several observational studies suggested a favorable effect of bone marrow cell infusion on LV function with a reduced infarct size.[59-62] However, a number of randomized controlled studies yielded mixed results in terms of therapeutical benefit. For example, magnetic resonance imaging in the BOOST and the ASTAMI study revealed that bone marrow cell transfer did not improve LV ejection fraction (LVEF) nor decrease infarct size.[63;64;69] On the contrary, Chen et al. reported that intracoronary bone marrow cell infusion was associated with a significant improvement in LVEF and a reduced infarct size.[66] In the largest study to date (the REPAIR-AMI study), the absolute improvement in LVEF at 4 months was 5.5±7.3% in the bone marrow cell group, as compared to 3.0±6.5% in the placebo group ($P<0.01$).[70] Subgroup analysis revealed that patients with an impaired baseline LVEF (≤48.9%)

TABLE 2. Clinical studies of bone marrow cell transplantation for acute myocardial infarction

	Year	Nr pts	Study design	Delivery route	Cell type	Follow-up (months)	Safety	LVEF	Infarct size
Strauer et al. [58]	2002	10 vs. 10 controls	Observational, Control +	Intracoronary	2.8x10^7 BMC	3	+	=	↓ (% dysfunctional segments)
TOPCARE-AMI[59-61]	2002	29 BMC 30 CPC	Observational, Control -	Intracoronary	2.1x10^8 BMC 1.6x10^7 CPC	12	+	↑ (9.3%)	↓ (contrast-enhanced volume)
Fernández-Avilés et al.[62]	2004	20 vs. 13 controls	Observational, Control +	Intracoronary	7.8x10^7 BMC	6	+	↑ (5.8%)	↓ (% dysfunctional segments)
BOOST [63,64]	2004	30 vs. 30 controls	Randomized, Sham -	Intracoronary	2.4x10^9 BMC	18	+	=	= (contrast-enhanced volume)
MAGIC [65]	2004	10 vs. 17 controls	Randomized, Sham -	Intracoronary	1.5x10^9 G-CSF-mobilized cells	6	- ↑ restenosis	↑ (6.4%)	NA
Chen et al.[66]	2004	34 vs. 35 controls	Randomized, Sham +	Intracoronary	4.8-6.0x10^{10} BMC	6	+	↑ (18.0%)	↓ (% dysfunctional segments)
Bartunek et al.[67]	2005	19 vs. 16 controls	Observational, Control +	Intracoronary	1.3x10^7 BMC	4	- ↑atherosclerosis	↑ (7.0%)	NA
Janssens et al.[68]	2006	33 vs. 34 controls	Randomized, Sham +	Intracoronary	4.8x10^8 BMC	4	+	=	↓ (contrast-enhanced volume)
ASTAMI[69]	2006	50 vs. 50 controls	Randomized, Sham -	Intracoronary	6.8x10^7 BMC	6	+	=	= (contrast-enhanced volume)
REPAIR-AMI[70,71]	2006	101 vs. 103 controls	Randomized, Sham +	Intracoronary	2.4x10^8 BMC	12	+	↑ (5.5%)	NA

BMC = bone marrow cells; CPC = circulating progenitor cells; G-CSF = granulocyte-colony stimulating factor; LVEF = left ventricular ejection fraction; NA = not available

and patients in whom cells were transplanted >4 days after infarction derived most benefit (**Figure 7**). Intriguingly, patients receiving bone marrow cell infusion exhibited a significantly lower rate of pre-specified major cardiovascular events, although the study was not adequately powered to evaluate clinical outcome.[71]

Conclusions

The current evidence for the clinical safety of intracoronary cell transfer for acute myocardial infarction is overall optimistic, but there are conflicting data regarding the efficacy of intracoronary cell infusion in the immediate post-infarction period. The lack of consistent results on the efficacy of bone marrow cell transfer for acute infarction is probably related to differences in enrollment criteria, bone marrow cell processing, the moment of cell delivery after infarction, and the imaging method used to assess changes in LV function and infarct size. Accordingly, studies using state-of-the-art imaging techniques are needed to identify the most favorable cell type, the optimal cell number to be administered and the optimal point of time for cell delivery after acute infarction. Simultaneously, large randomized, double-blind, placebo-controlled multi-centre studies are warranted to assess the clinical efficacy of bone marrow cell transfer for acute myocardial infarction.

Clinical Studies in Patients with Chronic Myocardial Infarction

Apart from the study described in this thesis, 4 studies assessed the safety, feasibility and potential efficacy of bone marrow cell transfer for chronic myocardial infarction (**Table 3**).[72-75] The mean time that had elapsed since myocardial infarction ranged from 8±3 months in the study by Erbs et al. to 81±72 in the TOPCARE-CHD study. Nevertheless, LVEF was remarkably preserved in the 4 study populations. In all studies, the therapeutic cells were infused in the previously-opened infarct related artery.

Safety and Feasibility

The combined experience from the 4 studies suggests that intracoronary bone marrow cell transfer for chronic myocardial infarction is safe during the short and mid-term follow-up. However, in the TOPCARE-CHD study, the incidence of in-stent restenosis appears to be relatively high. In-stent restenosis occurred in 4/35 patients in the bone marrow cell group, in 1/34 patients in the circulating progenitor cell group and in 0/23 patients in the control group.[75] Future studies are warranted to investigate whether the incidence of in-stent restenosis is higher in cell-treated patients as compared to control patients.

Efficacy

Conceptually, transplantation of bone marrow cells into akinetic scar tissue may improve cardiac function and impede LV remodeling. Indeed, 3 studies noted a significant improvement in LV function after cardiac cell transplantation (**Table 3**). For example, the TOPCARE-CHD investigators reported that bone marrow cell transplantation in

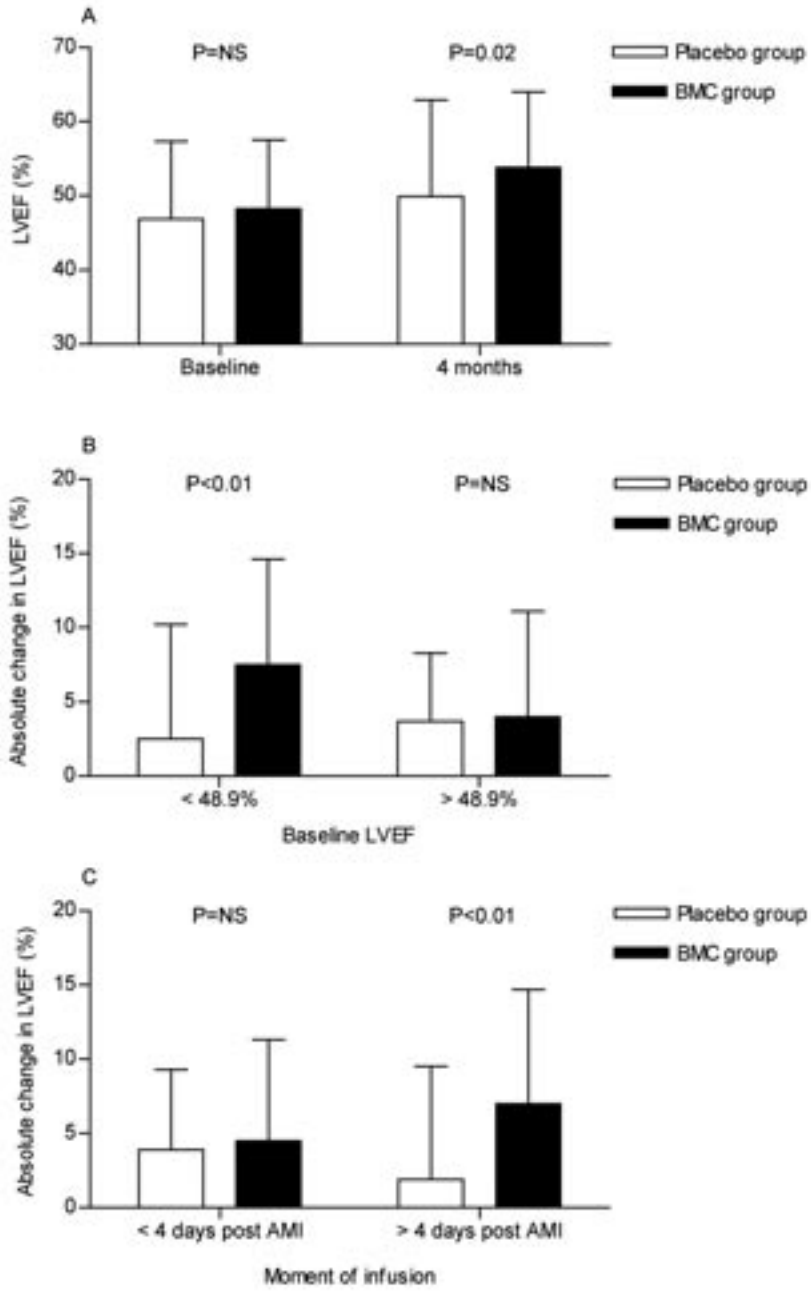

Figure 7
(A) In the REPAIR-AMI study, LVEF at baseline was similar in the placebo and the bone marrow cell (BMC) group (P=NS). At 4 months, LVEF was significantly higher in the BMC group than in the placebo group (P=0.02). The absolute increase in LVEF was 5.5±7.3% in the BMC group and 3.0±6.5% in the placebo group (P=0.01). (B) Subgroup analysis revealed that patients with a baseline LVEF at or below the median value of 48.9% and (C) patients in whom BMC were transplanted >4 days after acute myocardial infarction (AMI) derived the most benefit. Adapted from Schachinger et al.[70]

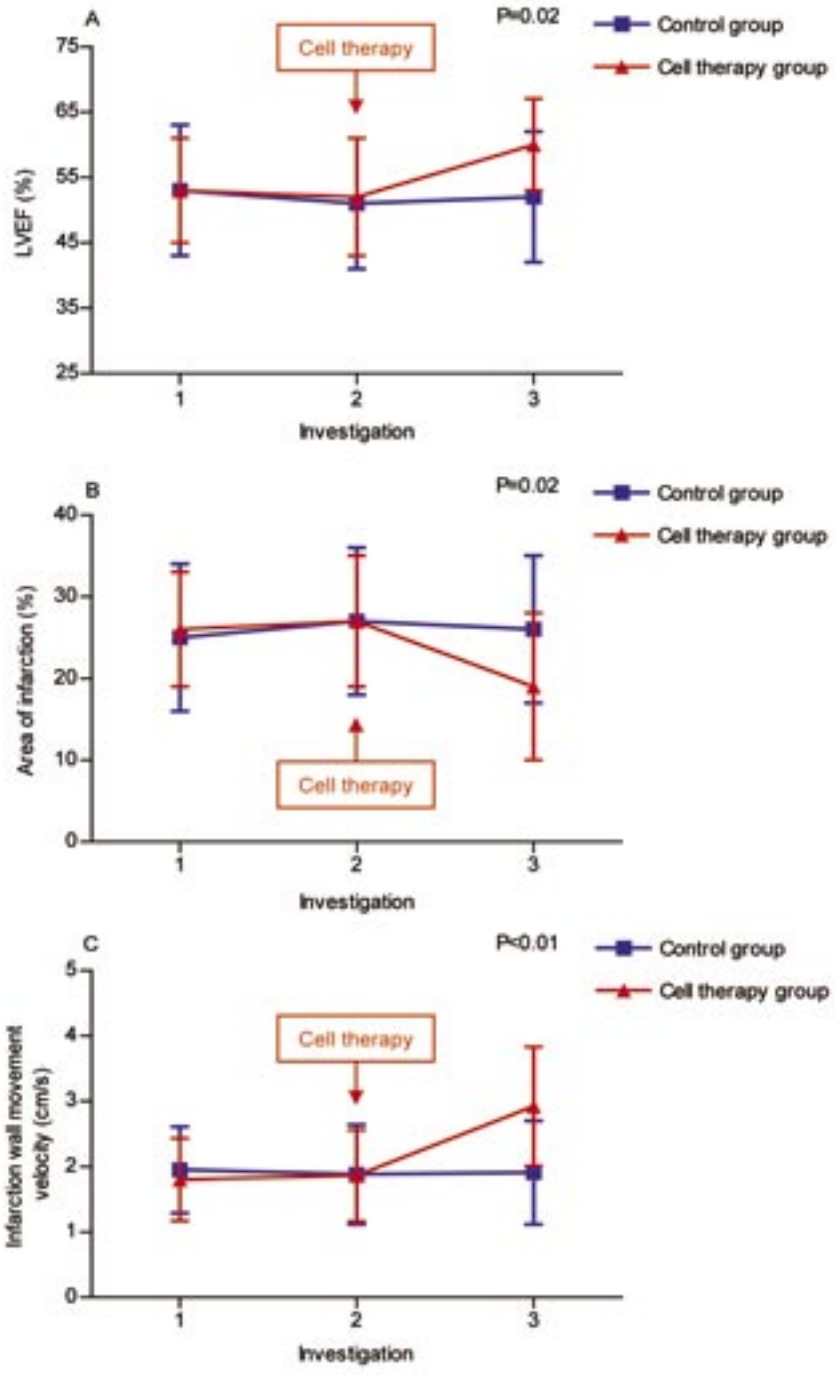

Figure 8
In the IACT study, bone marrow cell transfer was associated with (A) a significant increase in LVEF, (B) a significant decrease in the area of infarction, and (C) a significant improvement in infarct wall movement velocity. Investigation 1 was 9±6 months before cell transplantation, investigation 2 was at the time of cell therapy, and investigation 3 was at 3 months follow-up. Adapted from Strauer et al.[72]

TABLE 3. Clinical studies of bone marrow cell transplantation for chronic myocardial infarction

	Year	Nr pts	Setting	Study design	Delivery route	Cell type	Follow-up (months)	Safety	LVEF	Infarct size
IACT[72]	2005	18 vs. 18 controls	27±31 months post infarction	Observational, Control +	Intracoronary	9x10^7 BMC	3	+	↑ (8.0%)	↓ (% dysfunctional segments)
Katritsis et al.[73]	2005	11 vs. 11 controls	8±15 months post infarction	Observational, Control +	Intracoronary	2-4x10^6 BMC (MSC and EPC)	4	+	=	↓ (no. of scar segments on SPECT)
Erbs et al.[74]	2005	13 vs. 13 controls	8±3 months post infarction	Randomized, Sham +	Intracoronary	7x10^7 G-CSF-mobilized cells	3	+	↑ (7.2%)	= (contrast-enhanced volume)
TOPCARE-CHD[75]	2006	28 BMC vs. 24 CPC vs. 23 controls	81±72 months post infarction	Randomized, Sham -	Intracoronary	2x10^8 BMC 2x10^7 CPC	3	- (↑ restenosis)	↑ (2.9%)	= (contrast-enhanced volume)

BMC = bone marrow cells; CPC = circulating progenitor cells; EPC = endothelial progenitor cells; G-CSF = granulocyte-colony stimulating factor; LVEF = left ventricular ejection fraction; MSC = mesenchymal stem cells; SPECT = single photon emission computed tomography

TABLE 4. Clinical studies of bone marrow cell transplantation for chronic myocardial ischemia

	Year	Nr pts	Setting	Study design	Delivery route	Cell type	Follow-up (months)	Safety	LVEF	Myocardial perfusion
Perin et al.[78]	2003	14 vs. 7 controls	Heart failure	Observational, Control +	Intramyocardial	3x10^7 BMC	12	+	↑ (9.0%) at 3 mo = at 12 mo	= Perfusion defect at rest ↓ Extent of ischemia
Fuchs et al.[79]	2006	27	Angina	Observational, Control -	Intramyocardial	8x10^7 BMC	12	+	=	↑ Stress perfusion in injected territories = Stress perfusion in remote territories
Briguori et al.[80]	2006	10	Angina	Observational, Control -	Intramyocardial	2 x10^8 BMC	12	+	=	↓ Extent of ischemia
Tse et al.[81]	2006	12	Angina	Observational, Control -	Intramyocardial	12-16x10^7 BMC	44	+	=	NA

BMC = bone marrow cells; LVEF = left ventricular ejection fraction; NA = not available

patients with chronic myocardial infarction was associated with a small but significant 2.9% increase in LVEF at 3 months. No improvement was observed in patients receiving circulating progenitor cells or in patients who received no cell infusion.[75] Similarly, the IACT investigators reported that bone marrow cell injection was associated with an 8% increase in LVEF. In this study, the increase in LVEF was associated with a reduced infarct size and an improved infarct wall motion velocity (**Figure 8**).[72]

Conclusions

The currently available clinical experience suggests that cardiac cell therapy for chronic myocardial infarction is techniqually feasible and can be performed safely, although the aggravation of in-stent restenosis after intracoronary cell infusion has been posed as a potential danger. The preliminary efficacy results seem to support the hypothesis that cell therapy may improve LVEF. However, observations are limited by the small sample examined, the short follow-up period and the non-randomized trial design of the majority of studies. Therefore, randomized, double-blind, placebo controlled studies are needed to rigorously assess the safety and efficacy of cell therapy in patients with chronic myocardial infarction. In addition, it remains to be investigated whether bone marrow cell transplantation may also improve LVEF in patients with severe post-infarction heart failure and a severely depressed LV function. Future studies may also assess the safety and potential efficacy of intramyocardial cell delivery in patients with chronic myocardial infarction since it has been proposed that the intramyocardial delivery route may be more appropriate for the treatment of patients with chronic infarction in whom homing signals are expressed at low levels in the heart.

Clinical Applications: Bone Marrow Cells for Chronic Ischemia

Despite significant advances in catheter interventional and surgical techniques, there remain a substantial number of patients with coronary artery disease who are ineligible for a conventional revascularization procedure. As these patients can have stress-inducible myocardial ischemia despite optimal medical therapy and myocardial ischemia may be associated with anginal symptoms and an impaired LV function, new therapeutic strategies aimed at improving myocardial blood flow should be developed.

Experimental Background of Bone Marrow Cell Therapy for Chronic Ischemia

Enthusiasm about the potential of bone marrow cell transplantation to improve myocardial blood flow arose in 2001 when Fuchs et al. reported that trans-endocardial bone marrow cell injection enhanced collateral flow and improved myocardial contractility in pigs with chronic ischemia.[76] Since then, various animal model studies confirmed these results.[16;77]

For example, Kawamoto et al. reported that trans-endocardial bone marrow cell injection in swine resulted in histological, angiographic and functional evidence of enhanced neovascularization of ischemic myocardium.[77] The transplanted cells incorporated into foci of myocardial neovascularization, differentiated into mature endothelial cells, enhanced vascularity in the ischemic myocardium and preserved LV function. These studies provided the rationale for the initiation of clinical studies investigating bone marrow cell transplantation as a novel treatment modality for patients with chronic ischemia.

Clinical Studies in Patients with Chronic Ischemia

To date, 4 pilot studies (apart from the studies described in this thesis) addressed the safety, and feasibility of autologous bone marrow cell injection in patients with stress-induced ischemia (**Table 4**).[78-81] Patients were not amenable for conventional coronary revascularization and all received trans-endocardial bone marrow cell injections in the ischemic region under 3-D electromechanical guidance. Three studies included patients with angina; 1 study included patients with ischemic heart failure.

Safety and Feasibility

From the initial studies it appears as if intramyocardial bone marrow cell injection in patients with chronic ischemia is safe. In particular, procedural-related complications (such as myocaridal infarction and pericardial effusion) were not reported and sustained ventricular arrhythmia were not observed after bone marrow cell injection. However, Perin et al. reported that 1 patient with severe heart failure died suddenly 14 weeks after cell transplantation.[82] Although sudden cardiac death is relatively common in patients with severe ischemic heart failure, it cannot be ruled out that this death was related to the cell injections. Therefore, additional studies that aim to systematically evaluate the electrophysiological effects of intramyocardial bone marrow cell transplantation are warranted.

From the initial clinical studies, limited data are available regarding the progression of coronary atherosclerosis after bone marrow cell injection for chronic ischemia. In the study by Fuchs et al. 7/27 patients required a coronary intervention within 12 months after cell transfer because of in-stent restenosis or aggravation of coronary atherosclerosis.[79] Although the majority of revascularization procedures were performed in coronary artery segments supplying non-injected territories, it cannot be excluded that cell injection contributed to accelerated progression of coronary atherosclerosis since a control group was not included. Since routine repeat coronary angiography has not yet been performed after intramyocardial bone marrow cell injection, final conclusions regarding the safety of trans-endocardial bone marrow cell injection cannot be drawn.

Efficacy

From the initial non-randomized studies only preliminary conclusions can be drawn regarding the ability of cell therapy to improve myocardial perfusion in patients with chronic ischemia. Nevertheless, the results from the observational studies are encouraging. For example, Briguori et al. reported a reduced frequency of anginal symptoms which was paralleled by a reduction in the severity and the extent of the ischemic area in 4 of 8 patients.[80] Similarly, Fuchs et al, using dual-isotope SPECT imaging, showed an improved myocardial stress perfusion in the injected territories, whereas no improvement was noted in non-injected territories.[79] In the only study in patients with ischemic heart failure, SPECT at 2 months revealed a significant reduction in myocardial ischemia in the treatment group as compared with the control group.[82] Simultaneously, LVEF increased in the treatment group, but not in the control group. At 12 months, the beneficial effect on myocardial perfusion sustained, but there was no more a statistically significant difference between the treatment and the control group in terms of LVEF.[78]

Conclusions

In summary, the current pilot studies suggest that intramyocardial bone marrow cell injection is feasible and safe. Moreover, it appears as if bone marrow cell injection can reduce anginal symptoms and improve myocardial perfusion. However, the enthusiasm must be tempered by the small size of the investigated study populations and the relatively short follow-up period. It is warranted that additional studies investigate the electrophysiological effects of intramyocardial bone marrow cell injection, and assess whether bone marrow cell injection in patients with chronic ischemia is associated with aggravation of coronary atherosclerosis. Furthermore, the long-term safety remains to be demonstrated. At the same time, the capacity of intramyocardial bone marrow cell injection to enhance myocardial contractility and improve myocardial perfusion should be evaluated with the use of state-of-the-art non-invasive imaging techniques.

Aim and Outline of the Thesis

The aim of this thesis is to evaluate the safety, feasibility and potential efficacy of autologous bone marrow mononuclear cell injection in patients with chronic ischemic heart disease.

In **Chapter 2**, the electrophysiological characteristics of bone marrow-derived mesenchymal stem cells (which are a component of the mononuclear cell fraction) are evaluated with the use of an in vitro model of conduction block.

Chapter 3 describes the safety and feasibility results of the first 15 intramyocardial bone marrow cell injection procedures performed at the Leiden University Medical Center. In the subsequent 2 chapters, the safety profile of this novel treatment strategy is further explored.

In particular, **Chapter 4** describes the electrophysiological effects of intramyocardial bone marrow cell injection and **Chapter 5** evaluates whether intramyocardial bone marrow cell transplantation is associated with aggravation of coronary atherosclerosis.

Subsequently, the potential efficacy of bone marrow cell injection for drug-refractory angina and stress-inducible ischemia is investigated. In **Chapter 6,** magnetic resonance imaging and Tc-99m tetrofosmin SPECT imaging are used to assess changes in LV systolic function and myocardial perfusion at 3 months follow-up. In **Chapter 7**, the effect of cell therapy on LV diastolic function is evaluated with the use of magnetic resonance imaging and tissue Doppler imaging. In **Chapter 8** nuclear imaging techniques are used to provide more insight in the mechanism of benefit from bone marrow cell injection in patients with chronic myocardial ischemia. **Chapter 9** addresses the question whether the beneficial effects on myocardial perfusion and LV function that were observed at 3 and 6 months follow-up, are sustained over a longer period of time.

In contrast to the previous chapters, in which cells were injected in patients with drug-refractory angina and stress-inducible ischemia, **Chapter 10** describes the safety, feasibility and potential efficacy of bone marrow cell injection in patients with chronic myocardial infarction and a severely impaired LVEF.

Finally, in **Chapter 11** an overview of the most promising non-invasive imaging techniques for in vivo tracking of transplanted cells is provided, followed by a comprehensive summary of the currently available clinical studies investigating a cell therapy-related effect on LV function, myocardial perfusion, scar tissue, and myocardial viability.

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