OPTIMIZATION OF DELIVERY STRATEGIES FOR CARDIAC CELL THERAPY IN ISCHEMIC HEART DISEASE

Tycho I.G. van der Spoel

Optimization of delivery strategies for cardiac cell therapy in ischemic heart disease

ISBN: 978-90-820062-0-9

Design by:

DESIGN
ESIGN
SIGN
IGN
GN
N
ON PAPER

www.designonpaper.nl

OPTIMIZATION OF DELIVERY STRATEGIES FOR CARDIAC CELL THERAPY IN ISCHEMIC HEART DISEASE

OPTIMALISATIE VAN TRANSPLANTATIE STRATEGIEËN VOOR CARDIALE CEL THERAPIE IN ISCHEMISCHE HARTZIEKTEN

(met een samenvatting in het Nederlands)

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op dinsdag 18 december 2012 des middags te 4.15 uur

DOOR

Tycho Ids Gijsbert van der Spoel
geboren op 3 juni 1982 te Hilversum

Promotoren:
Prof. dr. P.A.F.M. Doevendans
Prof. dr. E. van Belle

Co-promotoren:
Dr. S.A.J. Chamuleau
Dr. J.P.G. Sluijter

Financial support by the Dutch Heart Foundation for the publication of this thesis is gratefully acknowledged. The research described in this thesis was supported by a grant of the Dutch Heart Foundation (DHF- 2010T025).
This research is part of the Project P1.04 SMARTCARE of the research program of the BioMedical Materials institute, co-funded by the Dutch Ministry of Economic Affairs, Agriculture and Innovation.

Additional support for publication of this thesis was granted by:
Samsung Medison, Volcano Corporation, Stichting Cardiovasculaire Biologie, Guerbet Nederland BV, Chipsoft, Boehringer Ingelheim BV, Biosense Webster Inc, Servier Nederland Farma B.V. en MSD.

VOOR MIJN OUDERS

Table of content


T.I.G van der Spoel

Introduction

INTRODUCTION

Acute myocardial infarction (MI) is one of the most important causes of heart failure and mortality in the western world.[1] MI is characterized by reduced myocardial perfusion and loss of cardiomyocytes. A typical human infarct involves the loss of approximately one billion cardiomyocytes.[2] Standard therapies are medication and percutaneous coronary intervention in the acute setting aiming at symptom relief and restoration of coronary flow. However, if blood flow is not immediately restored continuous loss of cardiomyocytes occurs. Neighbouring healthy cardiomyocytes cannot compensate for this ongoing process leading to chronic ischemic cardiomyopathy and clinical signs of heart failure. Unlike other organs (e.g. liver), the heart itself has unfortunately no to limited capacity to regenerate damaged myocardium. Patients with chronic ischemic heart failure can be treated via pharmacological or non-pharmacological modalities. Life style changes (e.g. weight loss, salt restriction) are recommended as a first step followed by treatment with various drugs to prevent left ventricular remodelling including angiotensin converting inhibitors, beta blockers, aldosterone antagonist and diuretics.[3] If clinical symptoms of heart failure are still present, revascularization procedures, resynchronization therapy, valvular and ventricular surgery should be considered.[3] Cardiac transplantation is an accepted treatment option for end-stage heart failure. However, due to donor shortage this is not a realistic treatment option; in 2011 only 40 heart transplantations were performed in the Netherlands. Despite optimal medical, reperfusion and device therapy many patients still remain symptomatic which leads to a clear restriction of their daily life. Cardiac cell therapy has been proposed as an alternative treatment option for chronic myocardial dysfunction. This transplantation therapy aims to replace dysfunctional cardiomyocytes or promote endogenous cardiac repair. This chapter provides an introduction from clinical perspective in the basic principles and first results of cardiac cell therapy.

Cardiac cell therapy

During the last decade several cell sources have been investigated for their potential effect on cardiac regeneration or neovascularisation. They can be divided into two groups: embryonic and adult stem cells. Embryonic stem cells have the advantage that they can divide indefinitely and are able to differentiate into all cell types and tissues of the body. This is also one of the disadvantages because this can lead to uncontrolled tumour formation (e.g. teratoma), next to relevant ethical issues.[4] In contrast to embryonic stem cells, adult stem cells are more predisposed for one type of tissue. Several adult cell types have been isolated and classically originate from bone marrow, peripheral blood or specific tissue (including cardiac tissue).[5] Figure 1 provides a schematic overview of adult cell sources for cardiac repair. In this thesis, we will focus on the most studied adult stem cells with in mind the clinical horizon and their proposed effects in pre-clinical animal models (Figure 2).

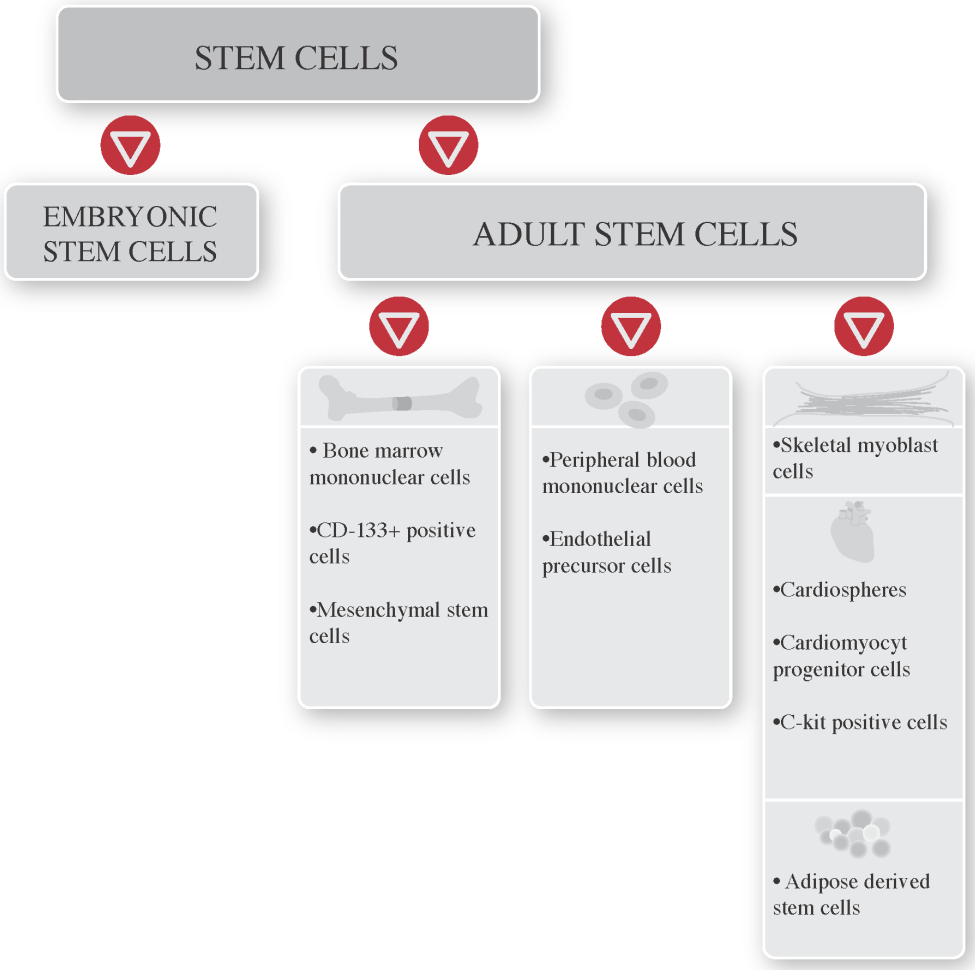


Figure 1. Different sources of stem cells for cardiac cell therapy.

In 2006, criteria were formulated by the International Society for Stem Cell Therapy to standardize and characterize human MSC isolation procedures.[13] The following criteria were formulated to characterize a MSC: adherence to plastic under culture conditions; expression of surface markers CD105, CD73, CD90 and a lack in expression of CD45, CD34, CD14 or CD11b, CD79 or CD19 and HLA-DR; *in vitro* differentiation into adipogenic, osteogenic or chondrogenic lineages. However, these criteria may not uniformly apply to other species due to the lack of comparison studies. A recent publication of our group demonstrated that pig MSC have comparable phenotyping, multi-lineage differentiation potential, immune-modulatory capacity, and functional improvement with human MSC indicating that data on porcine MSC therapy from experimental studies can be extrapolated

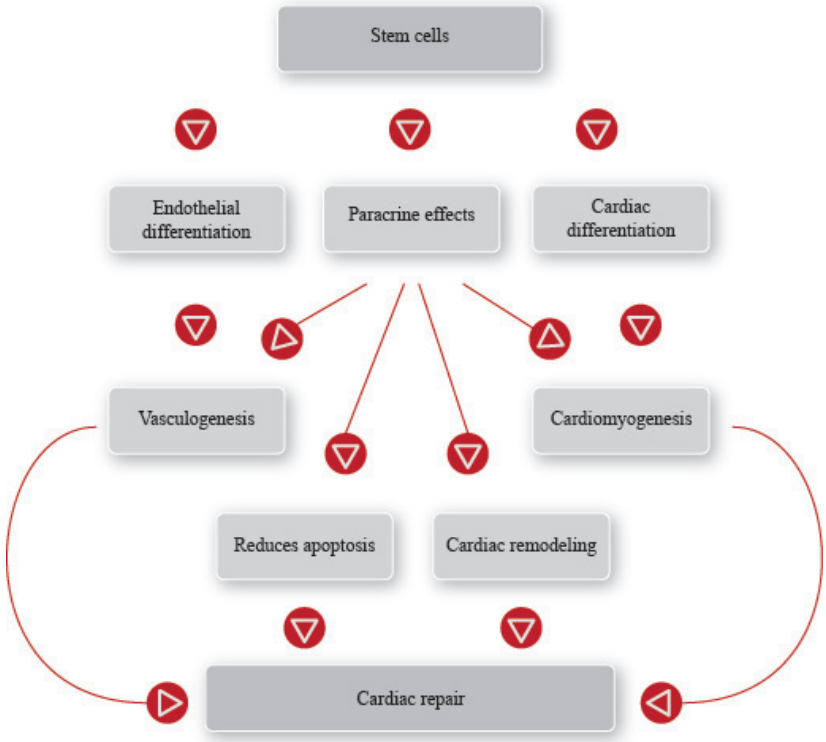


Figure 2. Working mechanisms of stem cells.

Bone marrow-derived stem cells

For patients, bone marrow is the main source to extract stem cells for cardiac cell therapy.[6] After density gradient centrifugation of the bone marrow mononucleair cell (BMMNC) fraction, hematopoetic, endothelial and mesenchymal stem cells (MSC) can be extracted based on their cell surface markers. Hematopoetic stem cells can be identified, among others, by a marker protein called CD-133 and were the first cells to be reported that were suggested to differentiate into cardiomyocytes when injected in infarcted mice[7] although this could not be reproduced by others.[8] A subgroup of hematopoetic stem cells are endothelial progenitor cells which can differentiate into endothelial cells *in vitro* and *in vivo* but not into cardiomyocytes *in vivo*.[9, 10] It has been postulated that endothelial progenitor cells stimulate neovascularisation, leading to a reduction in collagen formation and decreased apoptosis of cardiomyocytes in the infarcted borderzone.[11] Nevertheless, ischemic cardiomyopathy was associated with low number of endothelial progenitor cells and impaired migratory capacity[12] which could influence functional outcome after cell therapy.

to the clinical arena.[14] Early animal studies suggested that MSC can differentiate into functional cardiomyocytes[15] but current consensus is that this does not occur *in vivo*.[16] Their action can be explained by the secretion of paracrine factors that influence vessel formation, cardiac regeneration, prevent cardiomyocyte cell death and alter extracellular matrix.[17] Furthermore, MSC express moderate levels of human leukocyte antigens combined with their immuun-modulatory effect on innate and adapative immune cells makes them an attractive cell type for allogenic delivery after acute MI.[18] In 2009, Hare et al injected for the first time allogenic MSC in patients after acute MI and demonstrated that it was safe since no difference in adverse events rates between MSC treated and placebo treated patients were observed.[19]

Cardiac stem cells

Several types of adult cardiac stem cells (CSC) have been found in the heart.[20] Many of these cells have been identified based on different cell surface markers, including the expression of stem cell antigen (Sca-1), the tyrosine kinase receptor c-kit, the expression of the transcription factor Isl-1 or their ability to efflux Hoechst or Rhodamine dyes through ATP-binding transporters.[21-28]

Our group reported the isolation of a human progenitor cell based on Sca-1.[29] Transplantation of these cells into the MI mouse model prevented cardiac remodelling and maintained ejection fraction (EF) compared to vehicle treatment through generation of new cardiac tissue e.g. human cardiomyocytes and small vessel formation.[30] In 2007, Bearzi et al. isolated and expanded c-kit+ CSC from the human patient heart and injected into the infarcted myocardium of immunodeficient mice.[31] These cells generated a chimeric heart, which contains human myocardium composed of myocytes, coronary resistance arterioles, and capillaries. Isl-1 as well as side population cells could be expanded *in vitro*, but did not differentiate spontaneously towards the cardiac lineage unless co-cultured with cardiomyocytes.[32, 33] *In vivo* injection of human cardiosphere-derived cells into infarcted mouse hearts resulted in superior improvement of cardiac function, highest cell engraftment and myogenic differentiation rates, and the least-abnormal heart morphology three weeks after treatment compared with bone marrow derived cells.[34]

Several possible mechanisms may account for the observed beneficial effects of CSC after transplantation into injured myocardium. First, CSC can differentiate into the three cell lines that are necessary for cardiac regeneration: mature cardiomyocytes, smooth muscle cells and endothelial cells. Secondly, paracrine secretion of survival and angiogenic factors may also contribute to reported improvement of cardiac function and comparable with MSC.[30, 35, 36] Moreover, these factors could also act as survival factors working directly on constituting cells of the myocardium or perhaps mobilizing resident CSC involved in repair mechanisms. Till now, endomyocardial biopsies or atrial samples are necessary to obtain CSC which could lead to complications as bleeding, arrhythmias or tamponade in patients.

In summary, CSC can be identified through various surface markers. Some of the isolated CSC populations were shown to differentiate only *in vitro* but some did differentiate *in vivo* into mature cardiomyocytes as well. Whether the *in vivo* differentiation is efficient enough to be interpreted as true myocardial differentiation and if this can be used for complete or partial regeneration remains to be established.

Clinical trials with stem cells for ischemic heart disease

Since 2001 clinical trials were initiated to investigate the safety and efficacy of cardiac cell therapy in patients. Till now, about 29 randomized studies were conducted in patients with acute MI and 12 studies in patients with chronic ischemic cardiomyopathy, all with mixed results (Table 1). Most studies used unfractionated BMMNC and injected cells via intracoronary infusion. Small phase I-II clinical trials demonstrated a positive effect of 6-9% in EF due to a reduction in left ventricle (LV) volumes and improved myocardial perfusion at short-term follow-up.[37-39]

These results were confirmed by the REPAIR-AMI trial.[40] Later, Janssens et al. observed a positive but non-significant effect on EF[41]. Although promising, results from the ASTAMI (n=97), HEBE (n=200) and REGENT (n=200) trial showed no or even a negative effect on EF.[42 43 44] Nevertheless, three published meta-analysis showed that cell therapy overall was safe and led to an improvement of 3% on LV EF.[6, 45, 46] Interestingly, a recent published meta-analysis including 50 studies, enrolling a total of 2625 patients, reported that the effect on EF persists over long-term and led to a significant reduction in all cause mortality compared to placebo treated patients.[47] However, these trials were not designed to assess mortality as primary outcome. A new starting trial, supported by FP7 European grant, called the BMMNC on all cause mortality in acute MI study (BAMI trial), will address this issue.[48] This randomized and multicenter trial will enrol 3000 patients with reduced EF ≤45% after successful reperfusion for acute MI. Autologous BMMNC will be infused via intracoronary delivery and results will be compared to a placebo group receiving standard medical care.

In 2004, Chen et al. performed intracoronary infusions of MSC in 69 patients after acute MI and showed a significant improvement in LV function.[49] However, more randomized studies confirming these results are warrented.

Recently, two types of CSC have been used in patients namely cardiosphere-derived cells and c-kit+ CSC.[50, 51] Initial results were encouraging but safety issues are still present because of the small trial size and absence of long-term results.

Challenges in cardiac cell therapy

Since 1980 cardiac cell therapy started to evolve via *in vitro* and animal studies[52] which led to the initiation of clinical trials.[39, 47] It is debatable whether we had enough basic knowledge to treat patients on the start of these studies. Fact is that since a decade about 2600 patients were included in clinical stem cell trials to

Author	Year	Number of patients	Delivery method	Follow-up (months)	Cell type	Time to transplantation	Imaging modality	Change in LVEF stem cells vs. control
Acute MI								
Cao[60]	2009	86	IC	48	BMMNC	7d	Echo	3.6
Chen[49]	2004	69	IC	6	MSC	18d	LV angiography	12.0
Colombo[61]	2011	10	IC	12	CD-133$^+$ BMMNC	10-14d	Echo	3.8
Ge[62]	2006	20	IC	6	BMMNC	1d	Echo	6.7
Grajek[63]	2010	45	IC	12	BMMNC	5-6d	Echo	3.1
Herbots[64]	2009	67	IC	4	BMMNC	<1d	Echo	-1.5
Hirsch[42]	2011	200	IC	4	BMMNC/ PBMNC	3-8d	MRI	-0.2/0.2
Huikuri[65]	2008	80	IC	6	BMMNC	2-6d	Echo	5.4
Janssens[41]	2006	67	IC	4	BMMNC	1-2d	MRI	1.2
Kang[66]	2006	50	IC	6	PBCS	7d	MRI	5.2
Lipiec[67]	2009	36	IC	6	BMMNC	3-10d	SPECT	-0.8
Lunde[44]	2006	100	IC	6	BMMNC	6d	SPECT	1.1
Marban[51]	2012	31	IC	6	CDC	14-28d	MRI	0
Meluzin[68]	2006	66	IC	3	BMMNC	7d	SPECT	1.0
Meyer[38]	2006	60	IC	6	BMC	5d	MRI	2.0
Nogueira[69]	2009	20	IC	6	BMMNC	5.5d	Echo	2.8
Penicka[70]	2007	27	IC	4	BMMNC	4-11d	Echo	-5.1
Piepoli[71]	2010	38	IC	12	BMMNC	4-7d	SPECT	6.0
Plewka[72]	2010	56	IC	6	BMMNC	7d	Echo	4.0
Quyyumi[73]	2011	31	IC	6	CD34$^+$	8d	MRI	1.5
Ruan[74]	2005	20	IC	6	BMC	1d	Echo	9.2
Schachinger[40]	2006	187	IC	4	BMMNC	4d	LV	2.5

Author	Year	Number of patients	Delivery method	Follow-up (months)	Cell type	Time to transplantation	Imaging modality	Change in LVEF stem cells vs. control
Schachinger[40]	2006	187	IC	4	BMMNC	4d	LV angiography	2.5
Silva[75]	2009	30	IC	6	BMMNC	5.5d	RNV	5.0
Suarez de Lezo[76]	2007	20	IC	3	BMMNC	7d	LV angiography	15.0
Tendera[43]	2009	200	IC	6	BMC	7d	MRI	3.0
Traverse[77]	2010	40	IC	6	BMMNC	3-10d	MRI	-3.2
Traverse[78]	2011	87	IC	6	BMC	14-21d	MRI	-3.1
Turan[79]	2011	62	IC	12	BMC	7d	LV angiography	10.0
Wohrle[80]	2010	42	IC	6	BMMNC	6d	MRI	-3.9
Chronic MI								
Ang[81]	2008	25	IM or IC	6	BMMNC	>6 wk	MRI	1.5
Assmus[82]	2006	92	IC	3	BMMNC	2470d	LV angiography	4.1
Bolli[50]	2011	23	IC	4	CSC	113d	Echo/MRI	8.1
Erbs[83]	2005	26	IC	3	CPC	10d	MRI	7.2
Hendrikx[84]	2006	20	IM	4	BMMNC	217d	MRI	2.5
Kang[66]	2006	32	IC	6	PBCS	517d	MRI	-0.2
Perin[85]	2012	193	IM	6	BMMNC		SPECT	2.7
Pokushalov[86]	2010	109	IM	12	BMMNC	9-8y	Echo	6.1
Ramshorst[87]	2009	50	IM	3	BMMNC	6m	MRI	4.0
Tse[88]	2007	28	IM	6	BMMNC	NA	MRI	4.1
Yao[89]	2008	47	IC	6	BMMNC	repeated	MRI	0.8
Zhao[90]	2008	36	IM	6	BMMNC	NA	Echo	9.4

Legend: BMC bone marrow cells; BMMNC bone marrow mononuclear cells; CDC cardiosphere-derived cells; CPC circulating progenitor cells; CSC ckit positive cardiac stem cells; Echo echocardiography; PBCS peripheral blood mononuclear cells; IC intracoronary; IM intramyocardial; LVEF left ventricular ejection fraction (change in LVEF compared to control); MI myocardial infarction; MRI magnetic resonance imaging; NA not applicable; SPECT single photon emission computed tomography; RNV radionuclide ventriculography.

receive either stem cells or placebo treatment into the myocardium. These clinical studies have learned us a lot about the possibilities and impossibilities of cell therapy. However, they also pointed out that there are still some unresolved issues regarding cardiac cell therapy as also outlined by the ESC task force on stem cells and repair of the heart[53]:

- What is the best cell type for cardiac cell therapy?
- What is the optimal time-point to inject stem cells into the heart?
- What is the underlying mechanisms leading to the observed beneficial effect?
- What is the optimal delivery strategy?

Addressing these issues may further improve the clinical benefit of cell therapy, which is the major goal of this thesis.

Outline of this thesis

Irrespective of the chosen regenerative strategy, it is essential to deliver sufficient number of cells or amount of compounds to the infarcted myocardium to become effective which is important since low survival and retention of therapeutic cells after transplantation have been reported.[54, 55] This thesis focuses on optimization of cardiac cell delivery in chronic dysfunctional myocardium by thorough analysis of current literature, clinically available transplantation techniques and state-of-the art imaging modalities. **Chapter 2** provides an overview of the various cell tracking and non-surgical cell delivery techniques, which are highly important in view of experimental and clinical studies. Pre-clinical studies are essential to address safety and efficacy of new experimental therapies before advancing to the clinical arena, but a major unanswered question is if large animal models can accurately predict human clinical cell transplantation outcome? **In Chapter 3**, a meta-analysis of available pre-clinical studies for stem cell therapy in ischemic heart disease was performed to generate new insights for future human and pre-clinical trials. Based on the results of **Chapter 2 and 3**, we concluded that there was no consensus on the most optimal delivery strategy in chronic ischemic cardiomyopathy. To address this issue, a randomized study with blinded endpoint analysis was performed in a large animal model thereby comparing surgical, intracoronary, and transendocardial cell delivery. The results are presented in **Chapter 4**. Bone marrow cells have been well studied in both clinical and experimental studies as described earlier. However, no consensus exists regarding the optimal cell type. In addition, a decline of the positive effect of injected bone marrow cells over time has been reported[56], possibly due to low cell survival rates in the ischemic environment. To overcome this problem, we performed a direct comparison on functional endpoints between MSC and BMMNC

including long-term effects and a strategy of repeated cell injections (**Chapter 5**). Ejection fraction and parameters of regional function (e.g. strain) are closely related to prognosis in patients after acute MI.[57, 58] However, in cardiac cell transplantation studies, EF was mainly used as a parameter to assess the effect of cell therapy, thereby not able to detect changes in regional function.[59] These changes can be observed by deformation imaging using echocardiography to assess strain. However, this technique is hampered by confounding factors, low spatial and temporal resolution. In **Chapter 6**, a novel echocardiographic technique was tested in a large animal model of ischemia and reperfusion injury, using layer specific radiofrequency myocardial deformation measurements. This technique may be helpful to unravel the underlying mechanism of cell therapy and further optimize current delivery techniques. **Chapter 7** concludes this thesis providing a general discussion and summary together with their future implications and recommendations.

REFERENCES

1. Rosamond W, Flegal K, Furie K, Go A, Greenlund K, Haase N, Hailpern SM, Ho M, Howard V, Kissela B, Kittner S, Lloyd-Jones D, McDermott M, Meigs J, Moy C, Nichol G, O'Donnell C, Roger V, Sorlie P, Steinberger J, Thom T, Wilson M, Hong Y, for the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart Disease and Stroke Statistics--2008 Update: A Report From the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 2008;117:e25-146.

2. Reinecke H, Minami E, Zhu WZ, Laflamme MA. Cardiogenic Differentiation and Transdifferentiation of Progenitor Cells. Circ Res 2008;103:1058-1071.

3. Task FM, Dickstein K, Cohen-Solal A, Filippatos G, McMurray JJV, Ponikowski P, Poole-Wilson PA, Stromberg A, van Veldhuisen DJ, Atar D, Hoes AW, Keren A, Mebazaa A, Nieminen M, Priori SG, Swedberg K, ESC Committee for Practice Guidelines (CPG), Vahanian A, Camm J, De Caterina R, Dean V, Dickstein K, Filippatos G, Funck-Brentano C, Hellemans I, Kristensen SD, McGregor K, Sechtem U, Silber S, Tendera M, Widimsky P, Zamorano JL, Document Review, Tendera M, Auricchio A, Bax J, Bohm M, Corra U, la Bella P, Elliott PM, Follath F, Gheorghiade M, Hasin Y, Hernborg A, Jaarsma T, Komajda M, Kornowski R, Piepoli M, Prendergast B, Tavazzi L, Vachiery JL, Verheugt FWA, Zamorano JL, Zannad F. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2008. Eur Heart J 2008;29:2388-2442.

4. Nussbaum J, Minami E, Laflamme MA, Virag JAI, Ware CB, Masino A, Muskheli V, Pabon L, Reinecke H, Murry CE. Transplantation of undifferentiated murine embryonic stem cells in the heart: teratoma formation and immune response. FASEB J 2007;21:1345-1357.

5. Koudstaal S, Van der Spoel TIG, Van Slochteren FJ, Vrijsen K, Sluijter JPG, Cramer MJM, Doevendans PA, Chamuleau SAJ. Regeneratie van het beschadigde hart. Hartbulletin 2011;42:79-84.

6. Lipinski MJ, Biondi-Zoccai GG, Abbate A, Khianey R, Sheiban I, Bartunek J, Vanderheyden M, Kim HS, Kang HJ, Strauer BE, Vetrovec GW. Impact of intracoronary cell therapy on left ventricular function in the setting of acute myocardial infarction: a collaborative systematic review and meta-analysis of controlled clinical trials. J Am Coll Cardiol 2007;50:1761-1767.

7. Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine DM, Leri A, Anversa P. Bone marrow cells regenerate infarcted myocardium. Nature 2001;410:701-705.

8. Murry CE, Soonpaa MH, Reinecke H, Nakajima H, Nakajima HO, Rubart M, Pasumarthi KB, Virag JI, Bartelmez SH, Poppa V, Bradford G, Dowell JD, Williams DA, Field LJ. Hematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. Nature 2004;428:664-668.

9. Young PP, Vaughan DE, Hatzopoulos AK. Biologic Properties of Endothelial Progenitor Cells and Their Potential for Cell Therapy. Progress in Cardiovascular Diseases 2007;49:421-429.

10. Rubart M, Field LJ. CARDIAC REGENERATION: Repopulating the Heart. Annu Rev Physiol 2006;68:29-49.

11. Kocher AA, Schuster MD, Szabolcs MJ, Takuma S, Burkhoff D, Wang J, Homma S, Edwards NM, Itescu S. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. Nat Med 2001;7:430-436.

12. Kissel CK, Lehmann R, Assmus B, Aicher A, Honold J, Fischer-Rasokat U, Heeschen C, Spyridopoulos I, Dimmeler S, Zeiher AM. Selective Functional Exhaustion of Hematopoietic Progenitor Cells in the Bone Marrow of Patients With Postinfarction Heart Failure. J Am Coll Cardiol 2007;49:2341-2349.

13. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8:315-317.

14. Noort WA, Oerlemans MIFJ, Rozemuller H, Feyen D, Jaksani S, Stecher D, Naaijkens B, Martens AC, Buhring HJ, Doevendans PA, Sluijter JPG. Human versus porcine mesenchymal stromal cells: phenotype, differentiation potential, immunomodulation and cardiac improvement after transplantation. J Cell Mol Med 2011.

15. Shake JG, Gruber PJ, Baumgartner WA, Senechal G, Meyers J, Redmond JM, Pittenger MF, Martin BJ. Mesenchymal stem cell implantation in a swine myocardial infarct model: engraftment and functional effects. Ann Thorac Surg 2002;73:1919-1925.

16. Silva GV, Litovsky S, Assad JA, Sousa AL, Martin BJ, Vela D, Coulter SC, Lin J, Ober J, Vaughn WK, Branco RV, Oliveira EM, He R, Geng YJ, Willerson JT, Perin EC. Mesenchymal stem cells differentiate into an endothelial phenotype, enhance vascular density and improve heart function in a canine chronic ischemia model. Circulation 2005;111:150-156.

17. Gnecchi M, Zhang Z, Ni A, Dzau VJ. Paracrine Mechanisms in Adult Stem Cell Signaling and Therapy. Circ Res 2008;103:1204-1219.

18. Williams AR, Hare JM. Mesenchymal stem cells: biology, pathophysiology, translational findings, and therapeutic implications for cardiac disease. Circ Res 2011;109:923-940.

19. Hare JM, Traverse JH, Henry TD, Dib N, Strumpf RK, Schulman SP, Gerstenblith G, DeMaria AN, Denktas AE, Gammon RS, Hermiller JB, Jr., Reisman MA, Schaer GL, Sherman W. A Randomized, Double-Blind, Placebo-Controlled, Dose-Escalation Study of Intravenous Adult Human Mesenchymal Stem Cells (Prochymal) After Acute Myocardial Infarction. J Am Coll Cardiol 2009;54:2277-2286.

20. Van der Spoel TIG, Liu J, Sluijter JPG, Goumans MJ, Nathoe H, Van Belle E, Chamuleau SAJ, Doevendans PA. Human cardiac progenitor cells differentiate into cardiomyocytes. CML Cardiology 2009;24.

21. Laugwitz KL, Moretti A, Lam J, Gruber P, Chen Y, Woodard S, Lin LZ, Cai CL, Lu MM, Reth M, Platoshyn O, Yuan JXJ, Evans S, Chien KR. Postnatal isl1+ cardioblasts enter fully differentiated cardiomyocyte lineages. Nature 2005;433:647-653.

22. Pfister O, Mouquet F, Jain M, Summer R, Helmes M, Fine A, Colucci WS, Liao R. CD31- but Not

CD31+ Cardiac Side Population Cells Exhibit Functional Cardiomyogenic Differentiation. Circ Res 2005;97:52-61.

23. Martin CM, Meeson AP, Robertson SM, Hawke TJ, Richardson JA, Bates S, Goetsch SC, Gallardo TD, Garry DJ. Persistent expression of the ATP-binding cassette transporter, Abcg2, identifies cardiac SP cells in the developing and adult heart. Developmental Biology 2004;265:262-275.

24. Oh H, Bradfute SB, Gallardo TD, Nakamura T, Gaussin V, Mishina Y, Pocius J, Michael LH, Behringer RR, Garry DJ, Entman ML, Schneider MD. Cardiac progenitor cells from adult myocardium: Homing, differentiation, and fusion after infarction. Proceedings of the National Academy of Sciences 2003;100:12313-12318.

25. Messina E, De Angelis L, Frati G, Morrone S, Chimenti S, Fiordaliso F, Salio M, Battaglia M, Latronico MVG, Coletta M, Vivarelli E, Frati L, Cossu G, Giacomello A. Isolation and Expansion of Adult Cardiac Stem Cells From Human and Murine Heart. Circ Res 2004;95:911-921.

26. Matsuura K, Nagai T, Nishigaki N, Oyama T, Nishi J, Wada H, Sano M, Toko H, Akazawa H, Sato T, Nakaya H, Kasanuki H, Komuro I. Adult Cardiac Sca-1-positive Cells Differentiate into Beating Cardiomyocytes. Journal of Biological Chemistry 2004;279:11384-11391.

27. Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, Kasahara H, Rota M, Musso E, Urbanek K, Leri A, Kajstura J, Nadal-Ginard B, Anversa P. Adult Cardiac Stem Cells Are Multipotent and Support Myocardial Regeneration. 114 ed. 2003. p. 763-776.

28. Chamuleau SAJ, Vrijsen KR, Rokosh DG, Tang XL, Piek JJ, Bolli R. Cell therapy for ischaemic heart disease: focus on the role of resident cardiac stem cells. Neth Heart J 2009;17:199-207.

29. Van vliet P, Roccio M, Smits AM, Van Oorschot AA, Metz CH, Van Veen TA, Sluijter JPG, Doevendans PA, Goumans MJ. Progenitor cells isolated from the human heart: a potential cell source for regenerative therapy. Neth Heart J 2008;16:163-169.

30. Smits AM, van Laake LW, den Ouden K, Schreurs C, Szuhai K, van Echteld CJ, Mummery CL, Doevendans PA, Goumans MJ. Human cardiomyocyte progenitor cell transplantation preserves long-term function of the infarcted mouse myocardium. Cardiovasc Res 2009;83:527-535.

31. Bearzi C, Rota M, Hosoda T, Tillmanns J, Nascimbene A, De AA, Yasuzawa-Amano S, Trofimova I, Siggins RW, Lecapitaine N, Cascapera S, Beltrami AP, D'Alessandro DA, Zias E, Quaini F, Urbanek K, Michler RE, Bolli R, Kajstura J, Leri A, Anversa P. Human cardiac stem cells. Proc Natl Acad Sci U S A 2007;104:14068-14073.

32. Hierlihy AM, Seale P, Lobe CG, Rudnicki MA, Megeney LA. The post-natal heart contains a myocardial stem cell population. FEBS Lett 2002;530:239-243.

33. Laugwitz KL, Moretti A, Lam J, Gruber P, Chen Y, Woodard S, Lin LZ, Cai CL, Lu MM, Reth M, Platoshyn O, Yuan JX, Evans S, Chien KR. Postnatal isl1+ cardioblasts enter fully differentiated cardiomyocyte lineages. Nature 2005;433:647-653.

34. Li TS, Cheng K, Malliaras K, Smith RR, Zhang Y, Sun B, Matsushita N, Blusztajn A, Terrovitis J, Kusuoka H, Marban L, Marban E. Direct Comparison of Different Stem Cell Types and Subpopulations Reveals Superior Paracrine Potency and Myocardial Repair Efficacy With

Cardiosphere-Derived Cells. J Am Coll Cardiol 2012;59:942-953.

35. Den Haan MC, Grauss RW, Smits AM, Winter EM, Van Tuyn J, Pijnappels DA, Steendijk P, Gittenberger-De Groot AC, Van der Laarse A, Fibbe WE, De Vries AA, Schalij MJ, Doevendans PA, Goumans MJ, Atsma DE. Cardiomyogenic differentiation-independent improvement of cardiac function by human cardiomyocyte progenitor cell injection in ischaemic mouse hearts. J Cell Mol Med 2012;16:1508-1521.

36. Gnecchi M, He H, Noiseux N, Liang OD, Zhang L, Morello F, Mu H, Melo LG, Pratt RE, Ingwall JS, Dzau VJ. Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement. FASEB J 2006;20:661-669.

37. Assmus B, Schachinger V, Teupe C, Britten M, Lehmann R, Dobert N, Grunwald F, Aicher A, Urbich C, Martin H, Hoelzer D, Dimmeler S, Zeiher AM. Tranplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). Circulation 2002;106:3009-3017.

38. Meyer GP, Wollert KC, Lotz J, Steffens J, Lippolt P, Fichtner S, Hecker H, Schaefer A, Arseniev L, Hertenstein B, Ganser A, Drexler H. Intracoronary Bone Marrow Cell Transfer After Myocardial Infarction: Eighteen Months' Follow-Up Data From the Randomized, Controlled BOOST (BOne marrOw transfer to enhance ST-elevation infarct regeneration) Trial. Circulation 2006;113:1287-1294.

39. Strauer BE, Brehm M, Zeus T, Kostering M, Hernandez A, Sorg RV, Kogler G, Wernet P. Repair of Infarcted Myocardium by Autologous Intracoronary Mononuclear Bone Marrow Cell Transplantation in Humans. Circulation 2002;106:1913-1918.

40. Schachinger V, Erbs S, Elsasser A, Haberbosch W, Hambrecht R, Holschermann H, Yu J, Corti R, Mathey DG, Hamm CW, Suselbeck T, Werner N, Haase J, Neuzner J, Germing A, Mark B, Assmus B, Tonn T, Dimmeler S, Zeiher AM, for the REPAIR-AMI Investigators. Improved clinical outcome after intracoronary administration of bone-marrow-derived progenitor cells in acute myocardial infarction: final 1-year results of the REPAIR-AMI trial. Eur Heart J 2006;27:2775-2783.

41. Janssens S, Dubois C, Bogaert J, Theunissen K, Deroose C, Desmet W, Kalantzi M, Herbots L, Sinnaeve P, Dens J, Maertens J, Rademakers F, Dymarkowski S, Gheysens O, Van Cleemput J, Bormans G, Nuyts J, Belmans A, Mortelmans L, Boogaerts M, Werf F. Autologus bone marrow-derived stem cell transfer in patients with ST-segment elevation myocardial infarction.: double blind, randomized controlled trial. Lancet 2006;367:112-121.

42. Hirsch A, Nijveldt R, van der Vleuten PA, Tijssen JGP, van der Giessen WJ, Tio RA, Waltenberger J, ten Berg JM, Doevendans PA, Aengevaeren WRM, Zwaginga JJ, Biemond BJ, van Rossum AC, Piek JJ, Zijlstra F, on behalf of the HEBE Investigators. Intracoronary infusion of mononuclear cells from bone marrow or peripheral blood compared with standard therapy in patients after acute myocardial infarction treated by primary percutaneous coronary intervention: results of the randomized controlled HEBE trial. Eur Heart J 2011;32:1736-1747.

43. Tendera M, Wojakowski W, Ruzyllo W, Chojnowska L, Kepka C, Tracz W, Musialek P, Piwowarska W, Nessler J, Buszman P, Grajek S, Breborowicz P, Majka M, Ratajczak MZ. Intracoronary infusion of bone marrow-derived selected CD34+CXCR4+ cells and non-selected mononuclear cells in patients with acute STEMI and reduced left ventricular ejection fraction: results of randomized, multicentre Myocardial Regeneration by Intracoronary Infusion of Selected Population of Stem

Cells in Acute Myocardial Infarction (REGENT) Trial. Eur Heart J 2009;30:1313-1321.

44. Lunde K, Solheim S, Aakhus S, Arnesen H, Abdelnoor M, Egeland T, Endresen K, Ilebekk A, Mangschau A, Fjeld JG, Smith HJ, Taraldsrud E, Grogaard HK, Bjornerheim R, Brekke M, Muller C, Hopp E, Ragnarsson A, Brinchmann JE, Forfang K. Intracoronary Injection of Mononuclear Bone Marrow Cells in Acute Myocardial Infarction. N Engl J Med 2006;355:1199-1209.

45. Abdel-Latif A, Bolli R, Tleyjeh IM, Montori VM, Perin EC, Hornung CA, Zuba-Surma EK, Al-Mallah M, Dawn B. Adult Bone Marrow-Derived Cells for Cardiac Repair: A Systematic Review and Meta-analysis. Arch Intern Med 2007;167:989-997.

46. Clifford DM, Fisher SA, Brunskill SJ, Doree C, Mathur A, Clarke MJ, Watt SM, Martin-Rendon E. Long-Term Effects of Autologous Bone Marrow Stem Cell Treatment in Acute Myocardial Infarction: Factors That May Influence Outcomes. PLoS ONE 2012;7:e37373.

47. Jeevanantham V, Butler M, Saad A, Abdel-Latif A, Zuba-Surma EK, Dawn B. Adult Bone Marrow Cell Therapy Improves Survival and Induces Long-Term Improvement in Cardiac Parameters / Clinical Perspective. Circulation 2012;126:551-568.

48. http://clinicaltrials.gov/ct2/show/NCT01569178. 2012.

49. Chen Sl, Fang Ww, Ye F, Liu YH, Qian J, Shan Sj, Zhang Jj, Chunhua RZ, Liao Lm, Lin S, Sun Jp. Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. The American Journal of Cardiology 2004;94:92-95.

50. Bolli R, Chugh AR, D'Amario D, Loughran JH, Stoddard MF, Ikram S, Beache GM, Wagner SG, Leri A, Hosoda T, Sanada F, Elmore JB, Goichberg P, Cappetta D, Solankhi NK, Fahsah I, Rokosh DG, Slaughter MS, Kajstura J, Anversa P. Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial. The Lancet 1926;378:1847-1857.

51. Makkar RR, Smith RR, Cheng K, Malliaras K, Thomson LE, Berman D, Czer LS, Marban L, Mendizabal A, Johnston PV, Russell SD, Schuleri KH, Lardo AC, Gerstenblith G, Marban E. Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial. The Lancet 2012;379:895-904.

52. Tomita S, Mickle DA, Weisel RD, Jia ZQ, Tumiati LC, Allidina Y, Liu P, Li RK. Improved heart function with myogenesis and angiogenesis after autologous porcine bone marrow stromal cell transplantation. J Thorac Cardiovasc Surg 2002;123:1132-1140.

53. Bartunek J, Dimmeler S, Drexler H, Fernández-Avilés F, Galinanes M, Janssens S, Martin J, Mathur A, Menasche P, Priori S, Strauer B, Tendera M, Wijns W, Zeiher A. The consensus of the task force of the European Society of Cardiology concerning the clinical investigation of the use of autologous adult stem cells for repair of the heart. Eur Heart J 2006;27:1338-1340.

54. Freyman T, Polin G, Osman H, Crary J, Lu M, Cheng L, Palasis M, Wilensky RL. A quantitative, randomized study evaluating three methods of mesenchymal stem cell delivery following myocardial infarction. Eur Heart J 2006;27:1114-1122.

55. Hou D, Youssef EA, Brinton TJ, Zhang P, Rogers P, Price ET, Yeung AC, Johnstone BH, Yock

PG, March KL. Radiolabeled cell distribution after intramyocardial, intracoronary, and interstitial retrograde coronary venous delivery: implications for current clinical trials. Circulation 2005;112:I150-I156.

56. Meyer GP, Wollert KC, Lotz J, Pirr J, Rager U, Lippolt P, Hahn A, Fichtner S, Schaefer A, Arseniev L, Ganser A, Drexler H. Intracoronary bone marrow cell transfer after myocardial infarction: 5-year follow-up from the randomized-controlled BOOST trial. Eur Heart J 2009;30:2978-2984.

57. Moller JE, Hillis GS, Oh JK, Reeder GS, Gersh BJ, Pellikka PA. Wall motion score index and ejection fraction for risk stratification after acute myocardial infarction. American Heart Journal 2006;151:419-425.

58. Antoni ML, Mollema SA, Delgado V, Atary JZ, Borleffs CJW, Boersma E, Holman ER, van der Wall EE, Schalij MJ, Bax JJ. Prognostic importance of strain and strain rate after acute myocardial infarction. Eur Heart J 2010;31:1640-1647.

59. Moller JE, Hillis GS, Oh JK, Reeder GS, Gersh BJ, Pellikka PA. Wall motion score index and ejection fraction for risk stratification after acute myocardial infarction. 151 ed. 2006. p. 419-425.

60. Cao F, Sun D, Li C, Narsinh K, Zhao L, Li X, Feng X, Zhang J, Duan Y, Wang J, Liu D, Wang H. Long-term myocardial functional improvement after autologous bone marrow mononuclear cells transplantation in patients with ST-segment elevation myocardial infarction: 4 years follow-up. Eur Heart J 2009;30:1986-1994.

61. Colombo A, Castellani M, Piccaluga E, Pusineri E, Palatresi S, Longari V, Canzi C, Sacchi E, Rossi E, Rech R, Gerundini P, Viecca M, Deliliers GL, Rebulla P, Soligo D, Giordano R. Myocardial blood flow and infarct size after CD133+ cell injection in large myocardial infarction with good recanalization and poor reperfusion: results from a randomized controlled trial. Journal of Cardiovascular Medicine 2011;12.

62. Ge J, Li Y, Qian J, Shi J, Wang Q, Niu Y, Fan B, Liu X, Zhang S, Sun A, Zou Y. Efficacy of emergent transcatheter transplantation of stem cells for treatment of acute myocardial infarction (TCT-STAMI). Heart 2006;92:1764-1767.

63. Grajek S, Popiel M, Gil L, Breborowicz P, Lesiak M, Czepczynski R, Sawinski K, Straburzynska-Migaj E, Araszkiewicz A, Czyz A, Kozlowska-Skrzypczak M, Komarnicki M. Influence of bone marrow stem cells on left ventricle perfusion and ejection fraction in patients with acute myocardial infarction of anterior wall: randomized clinical trial. Eur Heart J 2010;31:691-702.

64. Herbots L, D'hooge J, Eroglu E, Thijs D, Ganame J, Claus P, Dubois C, Theunissen K, Bogaert J, Dens J, Kalantzi M, Dymarkowski S, Bijnens B, Belmans A, Boogaerts M, Sutherland G, Van de Werf F, Rademakers F, Janssens S. Improved regional function after autologous bone marrow-derived stem cell transfer in patients with acute myocardial infarction: a randomized, double-blind strain rate imaging study. Eur Heart J 2009;30:662-670.

65. Huikuri HV, Kervinen K, Niemela M, Ylitalo K, Saily M, Koistinen P, Savolainen ER, Ukkonen H, Pietila M, Airaksinen JKE, Knuuti J, Makikallio TH. Effects of intracoronary injection of mononuclear bone marrow cells on left ventricular function, arrhythmia risk profile, and restenosis after thrombolytic therapy of acute myocardial infarction. Eur Heart J 2008;29:2723-2732.

66. Kang HJ, Lee HY, Na SH, Chang SA, Park KW, Kim HK, Kim SY, Chang HJ, Lee W, Kang WJ, Koo BK, Kim YJ, Lee DS, Sohn DW, Han KS, Oh BH, Park YB, Kim HS. Differential Effect of Intracoronary Infusion of Mobilized Peripheral Blood Stem Cells by Granulocyte Colony-Stimulating Factor on Left Ventricular Function and Remodeling in Patients With Acute Myocardial Infarction Versus Old Myocardial Infarction: The MAGIC Cell-3-DES Randomized, Controlled Trial. Circulation 2006;114:I-145.

67. Lipiec P, Krzeminska-Pakula M, Plewka M, Kusmierek J, Plachcinska A, Szuminski R, Robak T, Korycka A, Kasprzak J. Impact of intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction on left ventricular perfusion and function: a 6-month follow-up gated 99mTc-MIBI single-photon emission computed tomography study. European Journal of Nuclear Medicine and Molecular Imaging 2009;36:587-593.

68. Meluzin J, Mayer J, Groch L, Janousek S, Hornacek I, Hlinomaz O, Kala P, Panovsky R, Prasek J, Kaminek M, Stanicek J, Klabusay M, Koristek Z, Navratil M, Dusek L, Vinklarkova J. Autologous transplantation of mononuclear bone marrow cells in patients with acute myocardial infarction: The effect of the dose of transplanted cells on myocardial function. American Heart Journal 2006;152:975.

69. Nogueira FBdS, Silva SA, Haddad AF, Peixoto CM, Carvalho RMd, Tuche FA, Soares VcE, Sousa ALS, Rabischoffsky A, Mesquita CT, Borojevic R, Dohmann HFR. Systolic function of patients with myocardial infarction undergoing autologous bone marrow transplantation. Arquivos Brasileiros de Cardiologia 2009;93:374-379.

70. Penicka M, Horak J, Kobylka P, Pytlik R, Kozak T, Belohlavek O, Lang O, Skalicka H, Simek S, Palecek T, Linhart A, Aschermann M, Widimsky P. Intracoronary Injection of Autologous Bone Marrow-Derived Mononuclear Cells in Patients With Large Anterior Acute Myocardial Infarction: A Prematurely Terminated Randomized Study. J Am Coll Cardiol 2007;49:2373-2374.

71. Piepoli MF, Vallisa D, Arbasi M, Cavanna L, Cerri L, Mori M, Passerini F, Tommasi L, Rossi A, Capucci A. Bone marrow cell transplantation improves cardiac, autonomic, and functional indexes in acute anterior myocardial infarction patients (Cardiac Study). Eur J Heart Fail 2010;12:172-180.

72. Plewka M, Krzemiska-Pakula M, Lipiec P, Peruga JZ, Jezewski T, Kidawa M, Wierzbowska-Drabik K, Korycka A, Robak T, Kasprzak JD. Effect of Intracoronary Injection of Mononuclear Bone Marrow Stem Cells on Left Ventricular Function in Patients With Acute Myocardial Infarction. 104 ed. 2009. p. 1336-1342.

73. Quyyumi AA, Waller EK, Murrow J, Esteves F, Galt J, Oshinski J, Lerakis S, Sher S, Vaughan D, Perin E, Willerson J, Kereiakes D, Gersh BJ, Gregory D, Werner A, Moss T, Chan WS, Preti R, Pecora AL. CD34+ cell infusion after ST elevation myocardial infarction is associated with improved perfusion and is dose dependent. American Heart Journal 2011;161:98-105.

74. Ruan W, Pan CZ, Huang GQ, Li YL, GE JB, Shu XH. Assessment of left ventricular segmental function after autologous bone marrow stem cells transplantation in patients with acute myocardial infarction by tissue tracking and strain imaging. Chin Med J (Engl) 2005;118:1175-1181.

75. Silva SA, Sousa AL, Haddad AF, Azevedo JC, Soares VE, Peixoto CM, Soares AJ, Issa AF, Felipe LR, Branco RV, Addad JA, Moreira RC, Tuche FA, Mesquita CT, Drumond CC, Junior AO, Rochitte CE, Luz JH, rabischoffisky A, Nogueira FB, Vieira RB, Junior HS, Borojevic R, Dohmann HF.

Autologous bone-marrow mononuclear cell transplantation after acute myocardial infarction: comparison of two delivery techniques. Cell Transplant 2009;18:343-352.

76. Suarez de Lezo J, Herrera C, Romero MA, Pan M, Jiminez R, Carmona D, Segura JM, Nogueras S, Mesa D, Suarez de Lezo J, Pavlovic D, Ojeda S, Torres A. Functional Recovery Following Intracoronary Infusion of Autologous Mononuclear Bone Marrow Cells in Patients With Chronic Anterior Myocardial Infarction and Severely Depressed Ventricular Function. Revista Espanola de Cardiologia 2010;63:1127-1135.

77. Traverse JH, McKenna DH, Harvey K, Jorgenso BC, Olson RE, Bostrom N, Kadidlo D, Lesser JR, Jagadeesan V, Garberich R, Henry TD. Results of a phase 1, randomized, double-blind, placebo-controlled trial of bone marrow mononuclear stem cell administration in patients following ST-elevation myocardial infarction. American Heart Journal 2010;160:428-434.

78. Traverse JH, Henry TD, Ellis SG, Pepine CJ, Willerson JT, Zhao DX, Forder J, Byrne B, Hatzopoulos A, Penn M, Perin E, Cardiovascular Cell Therapy ResearchNetwork. Effect of intracoronary delivery of autologous bone marrow mononuclear cells 2 to 3 weeks following acute myocardial infarction on left ventricular function: the LateTIME randomized trial. JAMA 2011;306:2110-2119.

79. Turan RG, Bozdag T, Turan CH, Ortak J, Akin I, Kische S, Schneider H, Rauchhaus M, Rehders TC, Kleinfeldt T, Belu C, Amen S, Hermann T, Yokus S, Brehm M, Steiner S, Chatterjee T, Sahin K, Nienaber CA, Ince H. Enhanced mobilization of the bone marrow−derived circulating progenitor cells by intracoronary freshly isolated bone marrow cells transplantation in patients with acute myocardial infarction. Journal of Cellular and Molecular Medicine 2012;16:852-864.

80. Wohrle J, Merkle N, Mailander V, Nusser T, Schauwecker P, von Scheidt F, Schwarz K, Bommer M, Wiesneth M, Schrezenmeier H, Hombach V. Results of Intracoronary Stem Cell Therapy After Acute Myocardial Infarction. The American Journal of Cardiology 2010;105:804-812.

81. Ang KL, Chin D, Leyva F, Foley P, Kubal C, Chalil S, Srinivasan L, Bernhardt L, Stevens S, Shenje LT, Galinanes M. Randomized, controlled trial of intramuscular or intracoronary injection of autologous bone marrow cells into scarred myocardium during CABG versus CABG alone. Nat Clin Pract Cardiovasc Med 2008;5:663-670.

82. Assmus B, Honold J, Schachinger V, Britten MB, Fischer-Rasokat U, Lehmann R, Teupe C, Pistorius K, Martin H, Abolmaali ND, Tonn T, Dimmeler S, Zeiher AM. Transcoronary Transplantation of Progenitor Cells after Myocardial Infarction. N Engl J Med 2006;355:1222-1232.

83. Erbs S, Linke A, Adams V, Lenk K, Thiele H, Diederich KW, Emmrich F, Kluge R, Kendziorra K, Sabri O, Schuler G, Hambrecht R. Transplantation of Blood-Derived Progenitor Cells After Recanalization of Chronic Coronary Artery Occlusion: First Randomized and Placebo-Controlled Study. Circ Res 2005;97:756-762.

84. Hendrikx M, Hensen K, Clijsters C, Jongen H, Koninckx R, Bijnens E, Ingels M, Jacobs A, Geukens R, Dendale P, Vijgen J, Dilling D, Steels P, Mees U, Rummens JL. Recovery of Regional but Not Global Contractile Function by the Direct Intramyocardial Autologous Bone Marrow Transplantation: Results From a Randomized Controlled Clinical Trial. Circulation 2006;114:I-101.

85. Perin EC, Willerson JT, Pepine CJ, Henry TD, Ellis SG, Zhao DX, Silva GV, Lai D, Thomas JD, Kronenberg MW, Martin AD, Cardiovascular Cell Therapy Research Network (CCTRN). Effect

of transendocardial delivery of autologous bone marrow mononuclear cells on functional capacity, left ventricular function, and perfusion in chronic heart failure: the FOCUS-CCTRN trial. JAMA 2012;307:1717-1726.

86. Pokushalov E, Romanov A, Chernyavsky A, Larionov P, Terekhov I, Artyomenko S, Poveshenko O, Kliver E, Shirokova N, Karaskov A, Dib N. Efficiency of Intramyocardial Injections of Autologous Bone Marrow Mononuclear Cells in Patients with Ischemic Heart Failure: A Randomized Study. Journal of Cardiovascular Translational Research 2010;3:160-168.

87. van Ramshorst J, Bax JJ, Beeres SL, Dibbets-Schneider P, Roes SD, Stokkel MP, de Roos A, Fibbe WE, Zwaginga JJ, Boersma E, Schalij MJ, Atsma DE. Intramyocardial bone marrow cell injection for chronic myocardial ischemia: A randomized controlled trial. JAMA 2009;301:1997-2004.

88. Tse HF, Thambar S, Kwong YL, Rowlings P, Bellamy G, McCrohon J, Thomas P, Bastian B, Chan JKF, Lo G, Ho CL, Chan WS, Kwong RY, Parker A, Hauser TH, Chan J, Fong DYT, Lau CP. Prospective randomized trial of direct endomyocardial implantation of bone marrow cells for treatment of severe coronary artery diseases (PROTECT-CAD trial). Eur Heart J 2007;28:2998-3005.

89. Yao K, Huang R, Sun A, Qian J, Liu X, Ge L, Zhang Y, Zhang S, Niu Y, Wang Q, Zou Y, Ge J. Repeated autologous bone marrow mononuclear cell therapy in patients with large myocardial infarction. Eur J Heart Fail 2009;11:691-698.

90. Zhao Q, Sun Y, Xia L, Chen A, Wang Z. Randomized Study of Mononuclear Bone Marrow Cell Transplantation in Patients With Coronary Surgery. The Annals of Thoracic Surgery 2008;86:1833-1840.


T.I.G van der Spoel

NON-SURGICAL STEM CELL DELIVERY STRATEGIES AND IN VIVO CELL TRACKING TO INJURED MYOCARDIUM.

Tycho I.G. van der Spoel, MD[1]
Joe Chun-Tsu Lee[2]
Krijn R. Vrijsen, MSc[1]
Joost P.G. Sluijter, PhD[1,3]
Maarten Jan M. Cramer, MD, PhD.[1]
Pieter A. Doevendans, MD, PhD.[1,3]
Eric van Belle, MD, PhD.[1]
Steven A.J. Chamuleau, MD, PhD[1]

[1] Department of Cardiology, Division Heart and Lungs, University Medical Center Utrecht, the Netherlands.
[2] Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia.
[3] Interuniversity Cardiology Institute of the Netherlands (ICIN), Utrecht, the Netherlands.

ABSTRACT

Heart failure is a major economic and public health problem. Despite the recent advances in drug therapy and coronary revascularization, the lost cardiomyocytes due to necrosis and apoptosis are not replaced by new myocardial tissue. Cell therapy is an interesting therapeutic option as it potentially improves contractility and restores regional ventricular function. Early clinical data demonstrated that cell transplantation, mainly delivered through non-surgical methods, is safe and feasible. However, several important issues need to be elucidated. This includes, next to determining the best cell type, the optimal delivery strategy, the biodistribution and the survival of implanted stem cells after transplantation. In this view, pre-clinical animal experiments are indispensable. Reporter genes, magnetic or radioactive labeling of stem cells have been developed to observe the fate and the distribution of transplanted cells using non-invasive imaging techniques. Several studies have demonstrated that these direct and non-direct labeling techniques may become an important tool in cell therapy. Integration of cell delivery and cell tracking will probably be a key for the success of cell therapy in patients. This review will provide a comprehensive overview on the various cell tracking and non-surgical cell delivery techniques, which are highly important in view of experimental and clinical studies.

INTRODUCTION

Coronary heart disease is a major public and economic health problem leading to more than 7 million deaths world wide each year.[1, 2] Optimal pharmacologic treatment and coronary reperfusion therapy have led to improved survival of patients with coronary artery disease. Clearly, current therapies can not replace dysfunctional or lossed cardiomyocytes which finally lead to heart failure. A structural solution may be provided by cell therapy which has emerged as a potential new therapeutic strategy. Cell therapy is considered in the setting of acute myocardial infarction (MI) and chronic ischemic heart failure. The ultimate goals of cell therapy are myocardial regeneration and revascularization, thereby re-establishing synchronous contractility and bioelectrical conductivity to achieve overall clinical improvement of cardiac function without severe adverse effects. Transplantation strategies include percutaneous, surgical and systemic delivery of various types of stem cells.[3-7] To monitor the efficiency of implanted stem cells, most small animal studies use post mortem histology as a gold standard.[8, 9] For *in vivo* detection of cell retention, sophisticated imaging techniques are necessary. Additionally, non-invasive imaging is preferred to determine the effect of cell therapy on cardiac function (e.g. volume, mass and pressure). Nowadays it is possible to track and quantify transplanted stem cells by direct and non-direct labeling techniques using (1) nuclear imaging (positron emission tomography (PET) or single photon emission computer tomography (SPECT)) and (2) magnetic resonance imaging (MRI). Various clinically approved radiomarkers are suggested to be useful in cardiac cellular therapies like ^{18}F-fluorodeoxyglucose (^{18}F-FDG) for PET scan, indium ^{111} (^{111}In) for SPECT and superparamagnetic iron oxide (SPIO) for MRI.[10-12]

It is important to further optimize delivery strategies in view of ongoing (pre-) clinical studies for regenerative therapy. To this end, state-of-the-art cell tracking is highly necessary. This review will provide a robust update of available *in vivo* cell tracking strategies and non-surgical delivery techniques that will guide experimental set up of pre-clinical stem cell research.

Part 1: *in vivo* cell tracking strategies

In the following section the contrast agents and detectors that have been proposed for non-invasive cell tracking will be discussed. Thereafter, we will review the advantages and disadvantages of each imaging strategy and suggest future directions for research. Figure 1 and Table 1 will provide an overview of all available direct and non-direct labeling techniques.

MRI

For MRI, Gadolinium- and iron-based contrast agents can be used for direct labeling of stem cells. Gadolinium is bio-incompatible, cytotoxic in unchelated form and has a low relaxivity; therefore it is an unattractive agent for stem cell imaging. However,

novel Gadolinium-based particles are being investigated for this purpose, albeit not yet in the heart.[13]

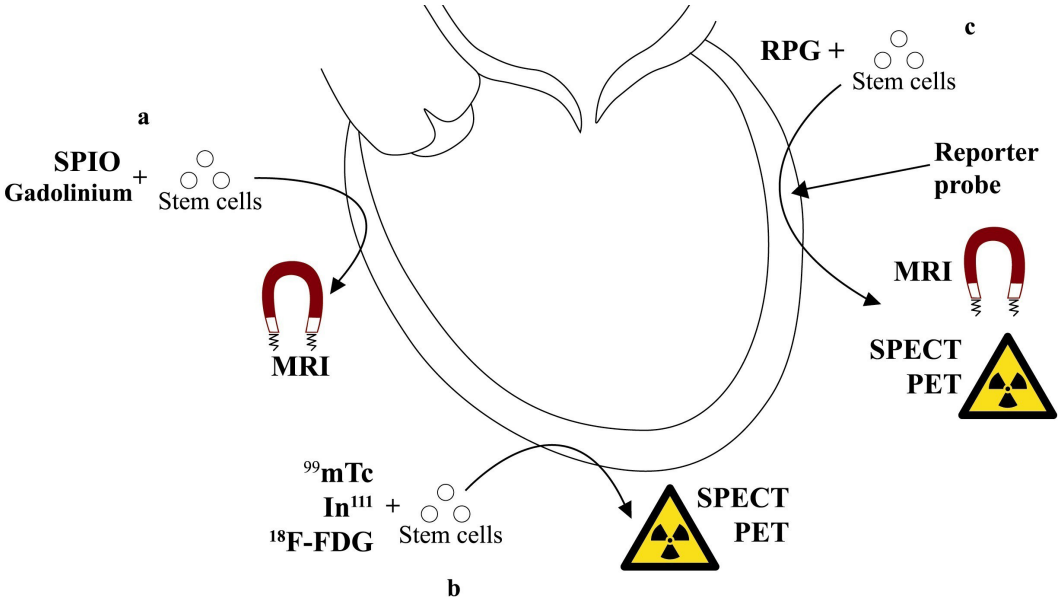


Figure 1. Different methods for non-invasive cell tracking.
(a) MRI= magnetic resonance imaging; SPIO= super paramagnetic iron oxide; (b) SPECT= single photon emission computer tomography; indium 111= In 111; 99Tc= 99Technetium; PET= positron emission tomography; 18F FDG = 18F-fluorodeoxyglucose; (c) RPG=reporter gene.

Method	Label	Advantages	Disadvantages
Direct labeling			
MRI	• Gadolinium	• Simple method	• Bio-incompatible, • Cytotoxic in unchelated form • Low relaxivity
	• SPIO	• Biocompatible • Cell friendly • High resolution • Stem cell imaging and anatomical function can be assessed simultaneously	• Long incubation time for labeling • Dilution of the contrast • Signal may not reflect living cells • Not suitable for patients with intracardiac defibrillator or pacemaker
SPECT	• In^{111} • ^{99}Tc	• High sensitivity • Stem cell imaging and perfusion can be assessed simultaneously	• Radiation exposure to patients and neighbouring cells • Low cellular retention • Possible effect of radioactivity on transplanted cells • Signal may not reflect living cells. • Signal loss due to radioactive decay
PET	• ^{18}F-FDG	• High spatial resolution • No cytotoxicity • Stem cell imaging and myocardial vitality can be assessed simultaneously	• Radiation exposure to patients • Signal may not reflect living cells. • Signal loss due to radioactive decay
Non-direct labeling			
RPG	• Reporter genes/probes	• Detection of viable cells • Observation of cell differentiation	• Cellular dysfunction or death • Immunogenicity of gene products • Potential risk of uncontrolled growth and malignancy • Costs

Table 1. Methods of direct and non-direct stem cell tracking.
MRI= magnetic resonance imaging; SPIO= super paramagnetic iron oxide; SPECT= single photon emission computer tomography; In111= indium111; 99Tc= 99technetium; PET= positron emission tomography; 18F-FDG=18F-fluorodeoxyglucose; RPG=reporter gene.

In 1996, SPIO's (30-200 nm) were approved as iron-based contrast agents for clinical use by the US Food and Drug Administration (Feridex, Guerbet, France). SPIO's are composed of an iron oxide core that is coated with a polymer shell to prevent aggregation. The polymer may contain dextran, polyethylene glycol or starch. The iron is biocompatible and can be recycled by cells using regular biochemical pathways. Labeling of targeted cells is accomplished by endocytosis. In addition, efficiency can be improved by using peptides/antibodies [14], magnetodendrimers [15] or transfection agents.[16] Labeled cells appeared to be hypo intense in T2*- and T2- weighted images. Numerous studies have shown that mesenchymal stem cells (MSC) can be labeled without affecting *in vitro* cell viability, proliferation and differentiation into adipogenic and osteogenic lineages by iron contrast agents.[10, 16, 17] Recently, pre-clinical studies were able to detect a minimum of about 10^5 pig MSC using different sized iron particles with a conventional cardiac MRI.[10, 12] Figure 3 shows an example of cell tracking by cardiac MRI using SPIO labeled MSC from our own laboratory. Detection of stem cells mainly depends on (1) magnetic field strength, (2) number of cells injected, (3) labeling efficiency and (4) cell size.

A practical drawback of iron-based contrast agents is that labeling is not permanent and self-replicable. Dilution of the contrast due to cellular fragmentation, fusion, division and migration also limits the use for follow-up after cell delivery. Also, variation in labeling efficiency among different cell types is present. For instance, SPIO-registered MR signals are still detectable in embryonic stem cells [18] 5 weeks after transplantation and 4-16 weeks for skeletal myoblasts [19] and MSC in murine models [20], respectively. Very little is known about the long-term survival after cell delivery in both pre-clinical models and humans. Furthermore, iron particles may still remain *in situ* and can be taken up by phagocytotic cells (e.g. cardiac macrophages) after cellular death [21]. Thus, MRI signal is still present leading to overestimation of the outcome of cellular survival ('false positive' results). Another potential drawback is negative image contrast artifacts due to air or hemorrhage after cell injection. Finally, patients with an intracardiac defibrillator or pacemaker are no candidates for MRI.

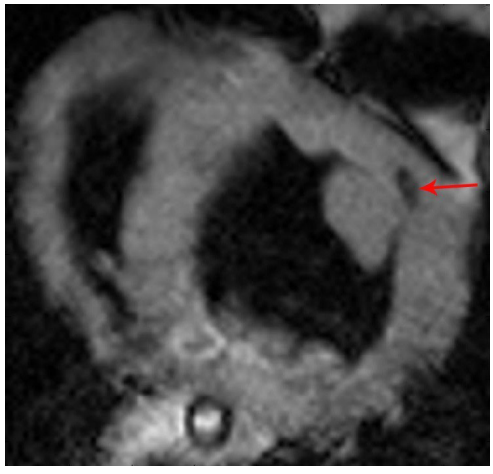


Figure 3. T2* image of SPIO labeled MSC (red arrow) after transepicardial injection in healthy myocardium.

MRI has become an appropriate imaging modality for stem cell tracking and therapeutic efficacy, without ionic radiation, high spatial resolution and detailed anatomical function. Nevertheless, at present this method is only useful for establishing initial retention of cells as it provides little evidence for long-term viability or functionality of transplanted cells. None of the MRI contrast agents have been used in the clinical field to monitor cellular survival. More information about long-term cell tracking and effects on cell behavior (e.g. differentiation and proliferation) in large animal studies is mandatory before applying this technique to clinical stem cell trials.

SPECT

Several radioisotopes are available for stem cell tracking in the heart, Technetium (^{99}Tc) (T½ 6 hours) and In^{111} (T½ 2.6 days). Labeling is based on established clinical protocols for white blood cells and performed by chelating agents that carry the radionuclides into the cell. Radioactivity is measured by a Gamma camera composing a 3D image.

In vitro studies have shown that cell integrity of both human and canine MSC, and endothelial progenitor cells (EPC) were unaffected after In^{111} labeling with 0,14-30 Bequerel per cell.[22-24]However, radiation induced cell damage was found after labeling hematopoietic progenitor cells (HPC) with In^{111}.[25, 26] In addition, low cellular retention after labeling was observed in all cell types. [26-28] Penicka et al. observed high retention of ^{99}Tc inside bone marrow mononuclear cells (BM-MNC) and no altered proliferation pattern after labeling [29]. Cell viability of MSC was also not influenced by ^{99}Tc [30]. The effect on cell differentiation was not determined in these studies. The use of SPECT is accompanied by a low detection threshold of about 10^4 cells [24] and therefore it is an attractive tool to determine *in vivo* biodistribution.

Both isotopes have been studied in various large animal models to determine cellular homing after surgical, intramyocardial (IM) and intravenous (IV) delivery.[12, 27, 31, 32] It was shown that a low number of cells accumulate in the heart after injection. However, when injecting cells into healthy myocardium 1/3 of the total radioactivity was still located in the heart.[33] Figure 4 shows a typical example of cellular retention of radioactive labeled stem cells after surgical injection in one of our experiments. Zhou and colleagues showed that it is possible to simultaneously asses stem cell imaging and perfusion in a rat model using dual isotope SPECT by combining both In^{111} (for cell imaging) and ^{99}Tc (for perfusion study).[34] This interesting finding should be confirmed in a pre-clinical model.

In humans, SPECT was employed to study the kinetics of ^{99}Tc or In^{111} labeled progenitor cells after intracoronary (IC) delivery in a small number of patients with ischemic heart disease. In general, low retention rates of progenitor cells (<10%) to the infarcted myocardium were found 1-2 hours after injection.[29, 35, 36] Signal loss due to reduction in activity also limits the use of radioisotopes for long-term follow-up.

SPECT is an attractive approach to determine delivery efficiency. In table 3, all studies on cell delivery efficiency are summarized per strategy. Animal studies have shown that SPECT imaging is a promising tool to visualize *in vivo* migration patterns and to assess functional effects of transplanted stem cells. However, the negative effect of radioisotopes on cell behavior (e.g. radiation induced cell damage, possible reduced differentiation rates) can not be neglected in view of clinical use.

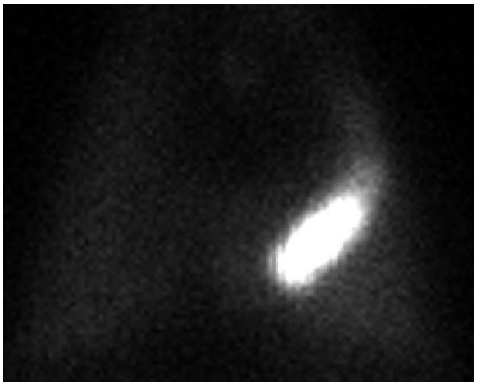


Figure 4. SPECT image of indium [111] labeled MSC in the heart after surgical injection in the left ventricle wall in a healthy porcine model.

PET

Positron emission tomography is a well known method to determine myocardial viability and perfusion by injecting ^{18}F-FDG. It is possible to label stem cells with ^{18}F-FDG to monitor homing and biodistribution (see table 3). No cytotoxicity, or impaired stem cell differentiation were documented after ^{18}F-FDG labeling. This could be due to the radioactive properties of ^{18}F-FDG, that emits a long range beta particle and thereby prevents radiation injury inside the cell. Although PET imaging offers high spatial resolution, the short half lifetime remains an obstacle for long-term cell tracking.

In a porcine MI model, dynamic cell tracking of percutaneous implemented ^{18}F-FDG labeled circulating progenitors cells was demonstrated: only 8-18 % of myocardial activity was retained one hour after IC delivery.[37] Similar results were obtained when autologous BM-MNC's were infused to the heart.[38] In addition, ^{18}F-FDG was used to label and determine myocardial homing and biodistribution of BM-MNC after IC and IV delivery in post-acute MI patients. Low amount of BM-MNC activity was detected in the infarcted myocardium after injection (less than 3%). [11] Both studies demonstrate the importance of metabolic myocardial imaging to determine cellular survival and a potential effect on scar tissue. However, larger (pre) clinical randomized studies on this topic are required to establish early and late biodistribution after cell delivery. Furthermore, a metabolic isotope with a longer half lifetime is necessary for chronic cell tracking.

Reporter Genes

To solve limitations in traditional cardiovascular imaging (i.e. false positive findings after cell death and cell toxicity), reporter genes (RPG) may be an attractive alternative. In short, a genetically engineered gene (the RPG) is incorporated into the genome of a cell prior to transplantation. The gene product should only be expressed by engrafted and still viable cells. Next, cells can be visualized after IV injection of an imaging tracer that targets the gene product. By its presence, the survival of the graft is certain because expression of the RPG and activity of the gene product depends on the viability of transplanted cells. Enzyme, transport and receptor based gene products are available for molecular imaging.

This strategy is particular well suited to overcome dilution effects which ensure long-term serial imaging of living transplanted stem cells. Also, repetitive imaging is possible and does not depend on decay of the radioisotope. Potential disadvantages include (1) costs, (2) cellular dysfunction or death, (3) immunogenicity of gene products, (4) potential risk of uncontrolled growth and malignancy; these aspects preclude clinical application in patients at this time. Several RPG's (transferrin receptor (TR), herpes simplex virus type 1 thymidine kinase (HSV1) and human sodium/iodide symporter) have been developed for non-invasive imaging in living animals.[39-41] The transferrin receptor has been proposed as a RPG for MRI.[42] High expression of TR on the cell membrane leads to increased iron uptake that is detectable by MRI and does not depend on intracellular iron concentration. Moreover, detection may be improved by covalent binding with iron nanoparticles [42]. However, accumulation of iron may lead to high levels of intracellular iron and diminished cellular function. Furthermore, not much is known about efficacy and safety of TR in large animal models and humans.

Herpes simplex virus type 1 thymidine kinase is being used for nuclear imaging [40]. Radioisotopes analogous to thymidine and guanosine are used as tracers. After metabolizing, the substrate is trapped intracellularly. Free radioactivity is detectable by PET or SPECT. In 2003, feasibility was tested to monitor survival of cardiomyoblasts after IM delivery using HSV1 thymidine kinase RPG. It was shown that optical imaging was more sensitive for detecting cardiomyoblasts (5 x 105) than PET ($3x10^6$).[40, 43] Furthermore, HSV1 thymidine kinase can be transduced in human MSC and visualized in a clinical relevant swine model with healthy myocardium.[44] In 2008, Gyöngyösi and colleagues demonstrated the feasibility of PET and optical imaging of the stable expressed of the trifusion gene protein (luciferase) for *in vivo* non-invasive tracking of IM injected MSC in a relevant animal model with survival up to 10 days after injection.[45] Data on HSV1 thymidine kinase and long-term follow-up are currently no available.

Human sodium/iodide symporter controls the membrane conductance of sodium and iodine. It is mainly expressed in the thyroid gland, and it is absent in cardiac cells.[46] Therefore, isotopes for both PET and gamma camera can be used to image

cells that express this gene. More detailed information about the effect of sodium influx on cardiomyocytes is required before entering the clinical field.
So far, the available data is limited to reveal the role of RPG in cellular tracking. Up till now, just one study attempted to initiate RPG imaging in an ischemic large animal model. Before human administration, a safe and stable RPG with no effect on cell behavior has to be developed. In parallel, optimal detection signal and more efficient delivery routes have to be established. Nevertheless, in our view RPG is a promising concept for reliable cell tracking with respect to pre-clinical studies that address optimal cell delivery strategies and chronic long-term follow-up.

Comparison of imaging techniques

At present, various direct and non-direct labeling strategies have been investigated for in vivo cell tracking. No technique has emerged as the most optimal tracking method. Fate and biodistribution after IV delivery by colabeling allogenic MSC with In^{111} and SPIO was observed. Migration of low amount of cells to the heart could be detected by SPECT, but not by MRI.[12] A combined approach using SPECT and cardiac MRI was used to determine function and precise visualization of In^{111} labeled stem cells in an ischemic rat model.[47] Simultaneous detection of stem cells and imaging of both perfusion deficit and myocardial function of the ischemic area was done by signal coregistration. Bioluminescence firefly luciferase RPG was more accurate compared to SPIO for long-term cell survival using optical and magnetic imaging.[48]

In patients, imaging is mainly performed to determine the effect of cell therapy on myocardial function and perfusion. To the best of our knowledge, no direct clinical comparison between imaging techniques has been performed to observe homing and distribution of transplanted human stem cells.

In summary, nuclear imaging is more sensitive than MRI for short-term cell tracking. For high spatial resolution and evaluation of cardiac function MRI is more appropriate. In case of long-term follow-up, iron particles and RPG can play an important role. In our view, a multimodality approach using both magnetic and nuclear radioagents in combination with RPG would provide a solution to current limitations in cell tracking in the near future.

Part 2: Non-surgical methods of cell delivery

The main objective of various cell delivery methods is to inject sufficient number of cells into the myocardium and to keep maximum retention of cells within the area of interest. A summary of the different cell delivery routes in clinical and pre-clinical setting will be provided (Figure 2 and Table 2) and also directions for future research are discussed.

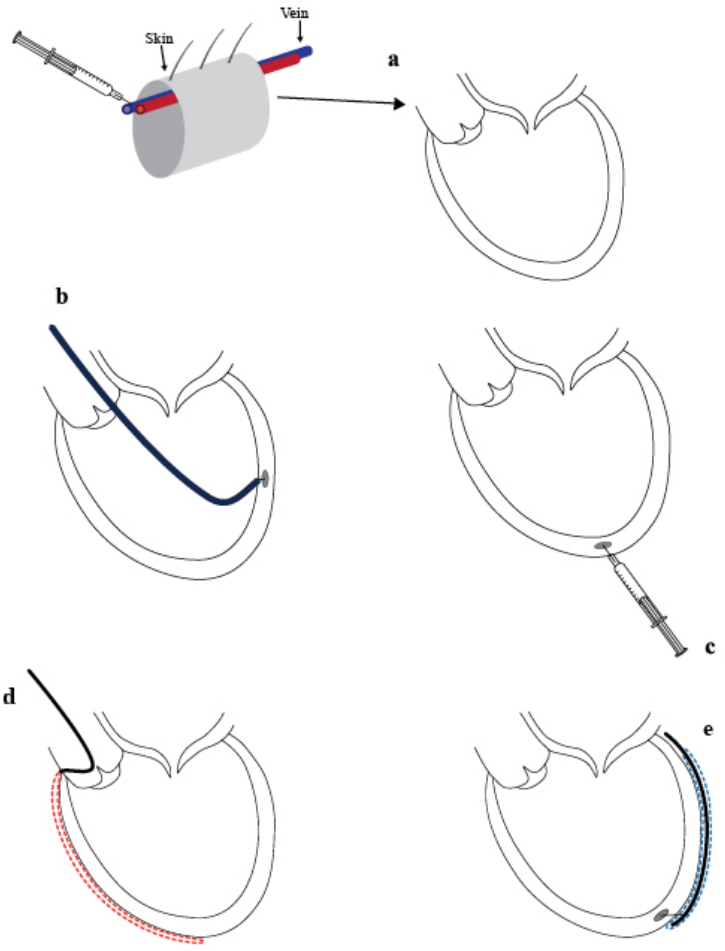


Figure 2. Schematic overview of the different delivery techniques to the injured myocardium. Legend: (a) Intravenous infusion (b) Trans-endocardial delivery (c) Surgical delivery (d) Intracoronary infusion (e) Retrograde coronary transvenous injection

Method	Advantages	Disadvantages
Intracoronary delivery	• Direct infusion infarct related or contralateral coronary artery • Well known technique by cardiologists	• In-stent restenosis • No access to occluded coronary artery • Embolisation of microvessels, leading to (micro) infarction • Intima dissection • Vascular access complications • Systemic delivery to non-cardiac tissues
Catheterized peripheral vein delivery	• Non-invasive and easy method • Allows intermittent cell infusion	• Microembolism • Low cellular migration and differentiation • Low delivery efficiency
Trans-endocardial injection	• Cell delivery in occluded areas • Implementation of high cell concentration in the ischemic region • Assess non-viable myocardium before transplantation	• Requires training; lengthen time of procedure • Expensive method • Risk of myocardial perforation • Arrhythmias • Vascular access complications
Retrograde coronary transvenous injection	• Low costs • May enter thinned myocardium due its co-axial injection technique	• May cause irreversible damage to venous wall • Perforation of the vein • Only access to the anterior wall along the vein • Technical difficult procedure • Vascular access complications

Table 2. Advantages and disadvantages of stem cell delivery methods.

Intracoronary delivery

During routine cardiac catheterization, IC delivery is performed through the central lumen of an over-wire balloon catheter that is advanced into the coronary artery of interest. By using transient balloon inflations, the duration of cell delivery is maximized, leading to migration of the delivered cells to the infarct related area. A major advantage of IC delivery is direct infusion into the target area using infarct related or a contralateral artery.

Based on animal and patient studies Strauer et al. looked for a non-surgical method for autologous cell therapy.[7, 49] In 2002, IC infusion of autologous BM-MNC appeared to be promising method for cell delivery in ten patients with acute MI.[50] Since then, a number of clinical trials have been conducted.[51-60] These studies showed that IC infusion was a safe delivery strategy and associated with a modest increase in myocardial function in patients with ischemic heart disease. Nevertheless, 5-year follow-up data of cell therapy demonstrated no significant improvement in left ventricle ejection fraction (EF) compared to placebo.[61, 62] In 31 clinical studies performed sofar, 22 used IC infusion as delivery strategy in approximately 1200 patients, despite unresolved issues regarding this transplantation technique.[63]

Important drawbacks of IC delivery are known, including the impossibility to access to the area of interest in patients with chronic occlusion. Other potential disadvantages of IC delivery of cells include intimal dissection [64, 65,] embolization of these cells from the site of injection to the microvasculature in the heart leading to micro infarctions [66] or abdominal region [67] and in-stent restenosis due to transient

balloon inflation.[68] Finally, imprecise localization and systemic delivery to non-cardiac tissues are limitations of IC therapy.[69] This can be explained by inadequate cellular migration into the myocardium during the first transit of coronary reperfusion causing a considerable loss of cells to the systemic circulation. A large portion of these cells are found in non-cardiac tissues, like lungs and liver. [69, 70] It has been shown that approximately 2% of the infused non-enriched BM-MNC home to the target area of cardiac injury in humans [11]. However, a higher retention (14-39%) in the infarcted myocardium was observed when using enriched BM-MNC.[11] This effect may be caused by differences in injected cell numbers. Notably, most clinical trials used non-enriched BM-MNC.

Many cell types have been used to treat MI using IC delivery in the (semi) acute setting. Although initial results were positive, low delivery efficiency remains an obstacle for clinical application. In general, this technique can not be used in chronic ischemic heart failure patients with occluded arteries. In addition, most studies related to IC infusion are small and lack of long-term follow-up data. In the future, research should focus on larger, blinded, randomized trials in MI patients with long-term follow-up to investigate the immediate and sustained effect of IC delivery.

Catheterized peripheral vein delivery

Cell delivery can be achieved by direct IV infusion of cells into a catheterized peripheral vein. Although it is an easy and safe method for cell delivery [3, 4,] non-cardiac uptake of stem cells after systemic delivery remains a major obstacle for clinical application. [27, 70, 71] Moreover, several studies have shown that no (0% of injected) cells retained in the heart (see Table 3). Additionally, the occurrence of microembolism in non-cardiac organs due to cellular entrapment of cell types with large diameter (e.g. skelet myoblasts or MSC) is an important drawback.

In our view, this technique is currently obsolete for clinical cardiac stem cell therapy. In case of future specific cardiac targeting of stem cells for optimal homing and engraftment, this technique can possibly re-enter the research arena.

Intramyocardial delivery

Nowadays, percutaneous injection of cells for cardiac repair directly into the injured myocardium is possible. Two delivery techniques are available for percutaneous IM injections: trans-endocardial injection (TE) and retrograde coronary transvenous (RCV) injection.

Trans-endocardial injection

Five different IM injection catheters are available for clinical use: Steerjet (MicroHeart)[72], Stiletto (Boston Scientific SciMed, Natick, MA) [10, 73 ,] Bioheart Myocath (Santa Rosa, CA), the Helix needle catheter (being developed) (BioCardia, CA) and Biosense Webster Myostar (Diamond Bar, CA).[5] All above stated devices

are developed for cell and gene based therapies. In general, IM injection of cells requires extensive fluoroscopic guidance to navigate within the ventricle, which is an important drawback for both patient and operator. To overcome this issue, the Myostar catheter is incorporated into a three dimensional electromechanical mapping system (NOGA). The target area can be determined by identifying viable, hibernating and infarcted myocardium, without the need of fluoroscopic guidance. Therapeutic cells can be injected in the region of interest, that is defined as a 'mismatch' area, i.e. presence of electrical activity in absence of mechanical movement. The use of the NOGA system was generally proven to be safe and feasible in animal studies and clinical trials for cellular [5, 74] and gene [75, 76] therapy. Perin et al. evaluated the safety and effect of TE delivered autologous BM-MNC in patients with severe heart failure. They observed an improved regional and global myocardial function compared to controls, without safety issues.[5] These encouraging results initiated a number of new trials.[77-80]

Other possible advantages of this technique include: cell delivery in occluded areas and implementation of high cell concentration in the myocardial region of interest. Potential drawbacks of IM delivery are the risk of myocardial perforation due to injection [81]. Furthermore, handling of the NOGA system requires technical training, is time consuming and expensive due to the use of a separate mapping and injection catheter. Another major drawback of TE injection is that direct cell injection may alter the gap junction orientation leading to ventricular arrhythmias.[82] Also, the ischemic environment and needle puncture may lead to a release of inflammatory stimuli which could be a trigger for arrhythmias.[83] Cellular retention ranges from 3-54% after TE injection. This wide variety is due to differences in animal model, TE catheter, cell type, imaging method and study design (see Table 3).

Over the past years, TE has rapidly evolved from an experimental technique towards a promising IM delivery technique. In the coming years research should focus on determining the most efficient TE catheter and long-term effects of this strategy.

	Setting / Study design	n	Cell type	Label	Labeling efficiency (%)	Cell viability(%)	Imaging method	Cell injection to detection (time)	Delivery efficiency to the heart (%)
Intracoronary delivery									
Hofmann et al.[11]	AMI / Observational	3	BM-MNC	^{18}F-FDG	>99	92-96	PET	55-75 min	1.3-2.6
Hou et al.[31]	AMI / Randomized	5	PBMNC	In^{111}	66	N/A	PET	60 min	1.6
Freyman et al.[70]	AMI / Randomized	6	MSC	Iridium particles	N/A	>70	Histology	14 days	6
Doyle et al.[37]	AMI / Observational	3	CPC	^{18}F-FDG	>90	>98	PET	60 min	8.7
Blocket et al.[99]	AMI / Observational	6	HPC	^{18}F-FDG	6	N/A	PET	60 min	5.5
Kang et al.[100]	AMI / Observational	17	PBCS	^{18}F-FDG	72	N/A	PET	120 min	1.5
Schachinger et al.[36]	AMI, OMI / Observational	17	CPC	In^{111}	10	90	Gamma camera	60 min	6.9
Caveliers et al.[35]	OMI / Observational	2	PBCS	In^{111}	51	88	SPECT	60 min	6.9-8
Qian et al.[38]	AMI / Observational	7	BM-MNC	^{18}F-FDG	91	97	PET	60 min	6.8
Penicka et al.[29]	AMI, OMI / Observational	10	BM-MNC	^{99}Tc	90	94-99	SPECT	120 min	1-5
Intravenous delivery									
Hofmann et al.[11]	AMI / Observational	3	BM-MNC	^{18}F-FDG	>99	92-96	PET	50-60 min	0
Kang et al.[100]	AMI / Observational	3	PBCS	^{18}F-FDG	72	N/A	PET	120 min	0
Freyman et al.[70]	AMI / Randomized	6	MSC	Iridium particles	N/A	>70	Histology	14 days	0
Chin et al.[27]	AMI / Observational	2	MSC	In^{111}	86	>95	SPECT	<24 hours	0
Kupatt et al.[32]	AMI / Observational	3	EPC	^{99}Tc	45-80	>80	SPECT	60 min	0.5
Retrograde coronary transvenous delivery									
Hou et al.[31]	AMI / Randomized	5	PBMNC	In^{111}	66	N/A	PET	60 min	3.2
Kupatt et al.[32]	AMI / Observational	3	EPC	^{99}Tc	45-80	>80	SPECT	60 min	2.7
Surgical delivery									
Mitchell et al.[101]	AMI / Observational	6	EPC	In^{111}	N/A	N/A	SPECT	40 min	57
Hou et al.[31]	AMI / Randomized	6	PBMNC	In^{111}	66	N/A	PET	60 min	11
Trans-endocardial delivery									
Dib et al.[102]	AMI / Observational	1	Skelet myoblasts	Iridium particles	N/A	N/A	Histology	120 min	4
Lyngbaek et al.[33]	Healthy/ Observational	6	MSC	In^{111}	N/A	96	Gamma camera	30 min	35
Mitchell et al.[103]	AMI / Observational	7	EPC	In^{111}	N/A	N/A	SPECT	40 min	54
Freyman et al.[70]	AMI / Randomized	6	MSC	Iridium particles	N/A	>70	Histology	14 days	3

Table 3. Comparison of delivery efficiency of unselected stem cells to the heart observed in patient and large animal studies. AMI= acute myocardial infarction; OMI=old myocardial infarction; N= number of animals or patients;SPECT= single positron emission computer tomography; In^{111}= indium^{111}; ^{99}Tc= ^{99}technetium; PET= positron emission tomography; ^{18}F-FDG=^{18}F-fluorodeoxyglucose; PBSC= peripheral blood stem cells; MSC= mesenchymal stem cell; BM-MNC= bone marrow mononuclear cell; HPC= hematopoietic stem cell; PBMNC= peripheral blood mononuclear cell; CPC= circulating progenitor cell; EPC= endothelial progenitor cell; N/A= not available.

Retrograde coronary transvenous injection

During a routine transvenous catheterization procedure a roadmap coronary venogram will be performed to gain access to all areas of the heart. Of note: no left-sided catheterization procedure is necessary for this technique. A composite catheter (TransAccess, Menlo Park, California) with a nitinol needle will be inserted into the venous wall under intravascular ultrasound, followed by microinfusion of stem cells by an IntraLume (Trans Vascular Inc.) catheter that will penetrate the myocardium under fluoroscopic guidance.[84] Thompson and colleagues were the first to demonstrate the safety and feasibility of RCV delivery in a non-infarcted swine model.[84] In addition, retrograde infusion of bone marrow cells induced angiogenesis and improved cardiac function in ischemic pigs compared to controls.[85] It was shown that RCV is a safe and feasible method for myoblast transplantation in patients 3 months after MI.[86] The authors also suggested that the RCV catheter rotates better which may improve target accuracy compared to TE injection. Furthermore, RCV is advantageous in cost, time performance thereby preventing cell loss and may enter thinned myocardium (<5mm) due its co-axial injection technique.[84, 85] However, possible irreversible damage to the venous wall may occur during the injection procedure [87] and it is technical difficult to implement cells in the coronary venous system. With this technique only access to the anterior wall can be achieved, and only along the veins anatomy. Incorrect position of the needle may cause perforation of the venous wall leading to a pericardial hemorrhage. A small number of studies [84-87] have been conducted, but it is still early to draw a conclusion regarding the efficacy of RCV.

Other delivery methods

Cell transplantation into the coronary venous system and the pericardial space has been tested in pre-clinical models and may have promising clinical applications in the future. Local intrapericardial delivery can be achieved by transatrial or subxyphoid access.[88, 89] Both techniques were well tolerated without apparent complications. However, to our knowledge no studies have investigated cell injections to the injured heart. Moreover, clinical experience with this technique is limited. Only one study has been conducted so far.[90]

Coronary sinus venous infusion is performed by advancing a single or double balloon catheter via the coronary sinus into the area of interest.[31, 91] Before cell infusion, a detailed anatomical map will be obtained by a coronary sinus venogram. During the procedure infusion pressure should be monitored closely to prevent disruption of the venocappillary system.[92] Studies shave shown that it is feasible to access most myocardial segments through the cardiac venous system.[93] Therefore, this technique may be an alternative for patients with a coronary arterial occlusion. Compared to IC delivery brief periods of venous balloon occlusion are unlikely to cause clinical complaints or myocardial ischemia due to the existence of venous

anastomoses.[93] The limitations of this approach are similar to RCV injections.

It was demonstrated that coronary venous infusion does not produce hemodynamical changes in a porcine model of myocardial injury. The authors concluded that this strategy was effective because autologous unfractioned bone marrow cells were observed in the myocardium and enhanced angiogenesis.[94] Later, the same research group conducted a prospective study in 14 patients with chronic stable angina. Autologous cell infusion was safe and tolerable. Significant improvement in myocardial perfusion and EF were observed during follow-up. Coronary angiography showed more collateral vessels in 9/14 patients.[95] However, these results do not prove efficacy assessed by a randomized trial.

Comparison of delivery techniques

Hou and colleagues assessed cell distribution of human mononuclear cells after surgical, IC and coronary venous delivery in an ischemic swine model. Only 11%, 2.6% and 3.2% were retained in the heart after surgical, IC and venous delivery, respectively.[31] Although surgical delivery appeared to be the most efficient technique, there was a huge variation in efficiency. The group of Freyman compared allogenic MSC engraftment after IV, IC and IM (Stiletto) delivery in a porcine MI model.[70] They found that IC delivery was associated with significant higher engraftment rates after 14 days compared to IM and IV. However, decreased coronary bloodflow and greater myocardial injury were observed after IC delivery. This could be due to high cell numbers injected. Perin and colleagues demonstrated that IM injection (using NOGA technology) of autologous MSC significantly improved left ventricle EF and reduced myocardial ischemia in a canine model. Conversely, no change in the IC group was observed.[96] Another study compared IM and RCV delivery of microspheres and found no significant difference in myocardial retention between these techniques. The authors also suggested that IM injection is superior to RCV in the infarct region, but that RCV is preferred for treatment of the peri-infarct region were to be treated based on differences in target areas of the devices.[97] Recently, it was demonstrated that RCV injection of BM-MNC is better than IC delivery in view of cell retention and tissue penetration in an acute MI model. However, the study is limited by a very small sample size (n=2 per group).[98]

In summary, several large animal studies showed conflicting results in the efficacy of different transplantation strategies. Notably, the optimal transplantation technique also depends on type of model (acute MI vs chronic heart failure). To provide a definite answer to the most optimal delivery strategy, we believe that a randomized trial in a clinically relevant animal model (porcine) is necessary, using state-of-the-art cell tracking techniques, including determination of biodistribution after the various delivery strategies.

CONCLUSION

Cell based cardiac repair showed beneficial effect on myocardial function in animal experiments. A number of clinical trials have already been conducted, although important unresolved issues concerning cell therapy are present. Interestingly, the most optimal delivery strategy still needs to be determined. Non-invasive imaging plays an essential role in determining biodistribution, survival and functional effects to the heart, that is of importance for several aspects of cell therapy (e.g. delivery strategy, cell type). Imaging parameters like contractility, perfusion, and viability of myocardium do not grant direct visualization of transplanted cells. New advancements in MRI and nuclear imaging have shown to provide reliable and highly sensitive visualization of transplanted cells, although mainly performed in animal models. The introduction of molecular cell tracking will contribute immensely to future studies of cellular mechanisms attributable to functional improvement. Until now, a small number of studies compared biodistribution between different delivery techniques in acute MI models. Unfortunately, results are still inconclusive due to differences in cell type, animal model, labeling method and delivery techniques.

In view of clinical trials it is important to determine the most optimal delivery strategy in a pre-clinical MI model using state-of-the-art cell tracking for both biodistribution and long-term survival. Adequate cell tracking is essential to guide molecular approaches to enhance homing, engraftment and survival of transplanted stem cells. Therefore, additional and more focused pre-clinical studies are mandatory before designing new clinical trials.

Acknowledgments

This work was supported by the Netherlands Heart Foundation '[2003B07304 and 2010T025]', BSIK program "Dutch Program for Tissue Engineering", '[grant 6746]', and a Bekalis price (PD). This research also forms part of the Project P1.04 SMARTCARE of the research program of the BioMedical Materials institute, co-funded by the Dutch Ministry of Economic Affairs.

REFERENCES

1. Rosamond W, Flegal K, Furie K, Go A, Greenlund K, Haase N, Hailpern SM, Ho M, Howard V, Kissela B, Kittner S, Lloyd-Jones D, McDermott M, Meigs J, Moy C, Nichol G, O'Donnell C, Roger V, Sorlie P, Steinberger J, Thom T, Wilson M, Hong Y, for the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart Disease and Stroke Statistics--2008 Update: A Report From the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 2008;117:e25-146.

2. World Health Organization. The atlas of heart disease and stroke. 2007. p. 48-49.

3. Halkos M, Zhao ZQ, Kerendi F, Wang NP, Jiang R, Schmarkey L, Martin B, Quyyumi A, Few W, Kin H, Guyton R, Vinten-Johansen J. Intravenous infusion of mesenchymal stem cells enhances regional perfusion and improves ventricular function in a porcine model of myocardial infarction. Basic Research in Cardiology 2008;103:525-536.

4. Hare JM, Traverse JH, Henry TD, Dib N, Strumpf RK, Schulman SP, Gerstenblith G, DeMaria AN, Denktas AE, Gammon RS, Hermiller JB, Jr., Reisman MA, Schaer GL, Sherman W. A Randomized, Double-Blind, Placebo-Controlled, Dose-Escalation Study of Intravenous Adult Human Mesenchymal Stem Cells (Prochymal) After Acute Myocardial Infarction. J Am Coll Cardiol 2009;54:2277-2286.

5. Perin EC, Dohmann HFR, Borojevic R, Silva SA, Sousa ALS, Mesquita CT, Rossi MID, Carvalho AC, Dutra HS, Dohmann HJF, Silva GV, Belem L, Vivacqua R, Rangel FOD, Esporcatte R, Geng YJ, Vaughn WK, Assad JAR, Mesquita ET, Willerson JT. Transendocardial, Autologous Bone Marrow Cell Transplantation for Severe, Chronic Ischemic Heart Failure. Circulation 2003;107:2294-2302.

6. Perin EC, Silva GV, Assad JAR, Vela D, Buja LM, Sousa ALS, Litovsky S, Lin J, Vaughn WK, Coulter S, Fernandes MR, Willerson JT. Comparison of intracoronary and transendocardial delivery of allogeneic mesenchymal cells in a canine model of acute myocardial infarction. Journal of Molecular and Cellular Cardiology 2008;44:486-495.

7. Tomita S, Mickle DAG, Weisel RD, Jia ZQ, Tumiati LC, Allidina Y, Liu P, Li RK. Improved heart function with myogenesis and angiogenesis after autologous porcine bone marrow stromal cell transplantation. Journal of Thoracic and Cardiovascular Surgery 2002;123:1132-1140.

8. Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine DM, Leri A, Anversa P. Bone marrow cells regenerate infarcted myocardium. Nature 2001;410:701-705.

9. Wang JS, Shum-Tim D, Galipeau J, Chedrawy E, Eliopoulos N, Chiu RC. Marrow stromal cells for cellular cardiomyoplasty: Feasibility and potential clinical advantages. Journal of Thoracic and Cardiovascular Surgery 2000;120:999-1006.

10. Hill JM, Dick AJ, Raman VK, Thompson RB, Yu ZX, Hinds KA, Pessanha BSS, Guttman MA, Varney TR, Martin BJ, Dunbar CE, McVeigh ER, Lederman RJ. Serial Cardiac Magnetic Resonance Imaging of Injected Mesenchymal Stem Cells. Circulation 2003;108:1009-1014.

11. Hofmann M, Wollert KC, Meyer GP, Menke A, Arseniev L, Hertenstein B, Ganser A, Knapp WH, Drexler H. Monitoring of Bone Marrow Cell Homing Into the Infarcted Human Myocardium.

Circulation 2005;111:2198-2202.

12. Kraitchman DL, Tatsumi M, Gilson WD, Ishimori T, Kedziorek D, Walczak P, Segars WP, Chen H, Fritzges D, Izbudak I, Young RG, Marcelino M, Pittenger MF, Solaiyappan M, Boston RC, Tsui BMW, Wahl RL, Bulte JWM. Dynamic Imaging of Allogeneic Mesenchymal Stem Cells Trafficking to Myocardial Infarction. Circulation 2005;112:1451-1461.

13. Nolte IS, Gungor S, Erber R, Plaxina E, Scharf J, Misselwitz B, Gerigk L, Przybilla H, Groden C, Brockmann MA. In vitro labeling of glioma cells with gadofluorine M enhances T1 visibility without affecting glioma cell growth or motility. Magn Reson Med 2008;59:1014-1020.

14. Lewin M, Carlesso N, Tung CH, Tang XW, Cory D, Scadden DT, Weissleder R. Tat peptide-derivatized magnetic nanoparticles allow in vivo tracking and recovery of progenitor cells. Nat Biotechnol 2000;18:410-414.

15. Bulte JW, Douglas T, Witwer B, Strable E, Lewis BK, Zywicke H, Miller B, van Gelderen P, Moskowitz BM, Duncan ID, Frank JA. Magnetodendrimers allow endosomal magnetic labeling and in vivo tracking of stem cells. Nat Biotechnol 2001;19:1141-1147.

16. Frank JA, Miller BR, Arbab AS, Zywicke HA, Jordan EK, Lewis BK, Bryant LH, Jr., Bulte JWM. Clinically Applicable Labeling of Mammalian and Stem Cells by Combining Superparamagnetic Iron Oxides and Transfection Agents. Radiology 2003;228:480-487.

17. Kostura L, Kraitchman DL, Mackay AM, Pittenger MF, Bulte JW. Feridex labeling of mesenchymal stem cells inhibits chondrogenesis but not adipogenesis or osteogenesis. NMR in Biomedicine 2004;17:513-517.

18. Himes N, Min JY, Lee R, Brown C, Shea J, Huang X, Xiao YF, Morgan JP, Burstein D, Oettgen P. In vivo MRI of embryonic stem cells in a mouse model of myocardial infarction. Magn Reson Med 2004;52:1214-1219.

19. Cahill KS, Germain S, Byrne BJ, Walter GA. Non-invasive analysis of myoblast transplants in rodent cardiac muscle. Int J Cardiovasc Imaging 2004;20:593-598.

20. Stuckey DJ, Carr CA, Martin-Rendon E, Tyler DJ, Willmott C, Cassidy PJ, Hale SJM, Schneider JE, Tatton L, Harding SE, Radda GK, Watt S, Clarke K. Iron Particles for Noninvasive Monitoring of Bone Marrow Stromal Cell Engraftment into, and Isolation of Viable Engrafted Donor Cells from, the Heart. Stem Cells 2006;24:1968-1975.

21. Amsalem Y, Mardor Y, Feinberg MS, Landa N, Miller L, Daniels D, Ocherashvilli A, Holbova R, Yosef O, Barbash IM, Leor J. Iron-Oxide Labeling and Outcome of Transplanted Mesenchymal Stem Cells in the Infarcted Myocardium. Circulation 2007;116:I-38.

22. Aicher A, Brenner W, Zuhayra M, Badorff C, Massoudi S, Assmus B, Eckey T, Henze E, Zeiher AM, Dimmeler S. Assessment of the Tissue Distribution of Transplanted Human Endothelial Progenitor Cells by Radioactive Labeling. Circulation 2003;107:2134-2139.

23. Bindslev L, Haack-Sorensen M, Bisgaard K, Kragh L, Mortensen S, Hesse B, Kjaer A, Kastrup J. Labelling of human mesenchymal stem cells with indium-111 for SPECT imaging: effect on cell proliferation and differentiation. European Journal of Nuclear Medicine and Molecular Imaging

2006;33:1171-1177.

24. Jin Y, Kong H, Stodilka RZ, Wells RG, Zabel P, Merrifield PA, Sykes J, Prato FS. Determining the minimum number of detectable cardiac-transplanted 111In-tropolone-labelled bone-marrow-derived mesenchymal stem cells by SPECT. Phys Med Biol 2005;19:4445-4455.

25. Brenner W, Aicher A, Eckey T, Massoudi S, Zuhayra M, Koehl U, Heeschen C, Kampen WU, Zeiher AM, Dimmeler S, Henze E. 111In-Labeled CD34+ Hematopoietic Progenitor Cells in a Rat Myocardial Infarction Model. J Nucl Med 2004;45:512-518.

26. Nowak B, Weber C, Schober A, Zeiffer U, Liehn E, von Hundelshausen P, Reinartz P, Schaefer W, Buell U. Indium-111 oxine labelling affects the cellular integrity of haematopoietic progenitor cells. European Journal of Nuclear Medicine and Molecular Imaging 2007;34:715-721.

27. Chin B.B, Nakamoto Y, Bulte J.W, Pittenger M.F, Whal R, Kraitchman D.L. 111In oxine labelled mesenchymal stem cell SPECT after intravenous administration in myocardial infarction. Nucl Med Commun 2003;24:1149-1154.

28. Patterson RB, Mayfield G, Silberstein EB, Kempczinski RF. The potential unreliability of indium 111 oxine labeling in studies of endothelial cell kinetics. J Vasc Surg 1989;10:650-655.

29. Penicka M, Lang O, Widimsky P, Kobylka P, Kozak T, Vanek T, Dvorak J, Tintera J, Bartunek J. One-day kinetics of myocardial engraftment after intracoronary injection of bone marrow mononuclear cells in patients with acute and chronic myocardial infarction. Heart 2007;93:837-841.

30. Barbash IM, Chouraqui P, Baron J, Feinberg MS, Etzion S, Tessone A, Miller L, Guetta E, Zipori D, Kedes LH, Kloner RA, Leor J. Systemic Delivery of Bone Marrow-Derived Mesenchymal Stem Cells to the Infarcted Myocardium: Feasibility, Cell Migration, and Body Distribution. Circulation 2003;108:863-868.

31. Hou D, Youssef EA-S, Brinton TJ, Zhang P, Rogers P, Price ET, Yeung AC, Johnstone BH, Yock PG, March KL. Radiolabeled Cell Distribution After Intramyocardial, Intracoronary, and Interstitial Retrograde Coronary Venous Delivery: Implications for Current Clinical Trials. Circulation 2005;112:I-150.

32. Kupatt C, Hinkel R, Lamparter M, von Bruhl ML, Pohl T, Horstkotte J, Beck H, Muller S, Delker S, Gildehaus FJ, Buning H, Hatzopoulos AK, Boekstegers P. Retroinfusion of Embryonic Endothelial Progenitor Cells Attenuates Ischemia-Reperfusion Injury in Pigs: Role of Phosphatidylinositol 3-Kinase/AKT Kinase. Circulation 2005;112:I-117.

33. Lyngbaek S, Ripa R, Haack-Sorensen M, Cortsen A, Kragh L, Andersen C, Jorgensen E, Kjaer A, Kastrup J, Hesse B. Serial in vivo imaging of the porcine heart after percutaneous, intramyocardially injected 111In-labeled human mesenchymal stromal cells. The International Journal of Cardiovascular Imaging 2010;26:273-284.

34. Zhou R, Thomas DH, Qiao H, Bal HS, Choi SR, Alavi A, Ferrari VA, Kung HF, Acton PD. In Vivo Detection of Stem Cells Grafted in Infarcted Rat Myocardium. J Nucl Med 2005;46:816-822.

35. Caveliers A, De Keulenaer G, Everaert H, Everaert H, Van Camp G, Verheye S, Roland J, Schoors D, Franken PR, Schots R. In vivo visualization of 111In labeled CD133+ peripheral blood stem cells

after intracoronary administration in patients with chronic ischemic heart disease. 51 ed. 2007. p. 61-66.

36. Schachinger V, Aicher A, Dobert N, Rover R, Diener J, Fichtlscherer S, Assmus B, Seeger FH, Menzel C, Brenner W, Dimmeler S, Zeiher AM. Pilot Trial on Determinants of Progenitor Cell Recruitment to the Infarcted Human Myocardium. Circulation 2008;118:1425-1432.

37. Doyle B, Kemp BJ, Chareonthaitawee P, Reed C, Schmeckpeper J, Sorajja P, Russell S, Araoz P, Riederer SJ, Caplice NM. Dynamic Tracking During Intracoronary Injection of 18F-FDG-Labeled Progenitor Cell Therapy for Acute Myocardial Infarction. J Nucl Med 2007;48:1708-1714.

38. Qian H, Yang Y, Huang J, Gao R, Dou K, Yang G, Li J, Shen R, He Z, Lu M, Zhao S. Intracoronary delivery of autologous bone marrow mononuclear cells radiolabeled by 18F-fluoro-deoxy-glucose: Tissue distribution and impact on post-infarct swine hearts. Journal of Cellular Biochemistry 2010;102:64-74.

39. Louie AY, Hüber MM, Ahrens ET, Rothbächer U, Moats R, Jacobs RE, Fraser SE, Meade TJ. In vivo visualization of gene expression using magnetic resonance imaging. Nat Biotechnol 2000;18:321-325.

40. Tjuvajev JG, Finn R, Watanabe K, Joshi R, Oku T, Kennedy J, Beattie B, Koutcher J, Larson S, Blasberg RG. Noninvasive Imaging of Herpes Virus Thymidine Kinase Gene Transfer and Expression: A Potential Method for Monitoring Clinical Gene Therapy. Cancer Res 1996;56:4087-4095.

41. Weissleder R, Moore A, Mahmood U, Bhorade R, Benveniste H, Basilion JP. In vivo magnetic resonance imaging of transgene expression. Nat Med 2000;6:351-355.

42. Moore A, Josephson L, Bhorade RM, Basilion JP, Weissleder R. Human Transferrin Receptor Gene as a Marker Gene for MR Imaging. Radiology 2001;221:244-250.

43. Wu JC, Chen IY, Sundaresan G, Min JJ, De A, Qiao JH, Fishbein MC, Gambhir SS. Molecular imaging of cardiac cell transplantation in living animals using optical bioluminescence and positron emission tomography. Circulation 2003;108:1302-1305.

44. Willmann JK, Paulmurugan R, Rodriguez-Porcel M, Stein W, Brinton TJ, Connolly AJ, Nielsen CH, Lutz AM, Lyons J, Ikeno F, Suzuki Y, Rosenberg J, Chen IY, Wu JC, Yeung AC, Yock P, Robbins RC, Gambhir SS. Imaging Gene Expression in Human Mesenchymal Stem Cells: From Small to Large Animals. Radiology 2009;252:117-127.

45. Gyongyosi M, Blanco J, Marian T, Tron L, Petnehazy O, Petrasi Z, Hemetsberger R, Rodriguez J, Font G, Pavo IJ, Kertesz I, Balkay L, Pavo N, Posa A, Emri M, Galuska L, Kraitchman DL, Wojta J, Huber K, Glogar D. Serial Noninvasive In Vivo Positron Emission Tomographic Tracking of Percutaneously Intramyocardially Injected Autologous Porcine Mesenchymal Stem Cells Modified for Transgene Reporter Gene Expression. Circ Cardiovasc Imaging 2008;1:94-103.

46. Miyagawa M, Beyer M, Wagner B, Anton M, Spitzweg C, Gansbacher B, Schwaiger M, Bengel FM. Cardiac reporter gene imaging using the human sodium/iodide symporter gene. Cardiovasc Res 2005;65:195-202.

47. Shen D, Liu D, Cao Z, Acton P, Zhou R. Coregistration of Magnetic Resonance and Single Photon Emission Computed Tomography Images for Noninvasive Localization of Stem Cells Grafted in the Infarcted Rat Myocardium. Molecular Imaging and Biology 2007;9:24-31.

48. Chen I, Greve J, Gheysens O, Willmann J, Rodriguez-Porcel M, Chu P, Sheikh A, Faranesh A, Paulmurugan R, Yang P, Wu J, Gambhir S. Comparison of Optical Bioluminescence Reporter Gene and Superparamagnetic Iron Oxide MR Contrast Agent as Cell Markers for Noninvasive Imaging of Cardiac Cell Transplantation. Molecular Imaging and Biology 2009;11:178-187.

49. Hamano K, Nishida M, Hirata K, Mikamo A, Li TS, Harada M, Miura T, Matsuzaki M, Esato K. Local Implantation of Autologous Bone Marrow Cells for Therapeutic Angiogenesis in Patients With Ischemic Heart Disease Clinical Trial and Preliminary Results. Japanese circulation journal 2001;65:845-847.

50. Strauer BE, Brehm M, Zeus T, Kostering M, Hernandez A, Sorg RV, Kogler G, Wernet P. Repair of Infarcted Myocardium by Autologous Intracoronary Mononuclear Bone Marrow Cell Transplantation in Humans. Circulation 2002;106:1913-1918.

51. Chen Sl, Fang Ww, Ye F, Liu YH, Qian J, Shan Sj, Zhang Jj, Chunhua RZ, Liao Lm, Lin S, Sun Jp. Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. The American Journal of Cardiology 2004;94:92-95.

52. Erbs S, Linke A, Schachinger V, Assmus B, Thiele H, Diederich KW, Hoffmann C, Dimmeler S, Tonn T, Hambrecht R, Zeiher AM, Schuler G. Restoration of Microvascular Function in the Infarct-Related Artery by Intracoronary Transplantation of Bone Marrow Progenitor Cells in Patients With Acute Myocardial Infarction: The Doppler Substudy of the Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI) Trial. Circulation 2007;116:366-374.

53. Hirsch A, Nijveldt R, van der Vleuten PA, Biemond BJ, Doevendans PA, van Rossum AC, Tijssen JG, Zijlstra F, Piek JJ. Intracoronary infusion of autologous mononuclear bone marrow cells or peripheral mononuclear blood cells after primary percutaneous coronary intervention: Rationale and design of the HEBE trial--A prospective, multicenter, randomized trial. American Heart Journal 2006;152:434-441.

54. Janssens S, Dubois C, Bogaert J, Theunissen K, Deroose C, Desmet W, Kalantzi M, Herbots L, Sinnaeve P, Dens J, Maertens J, Rademakers F, Dymarkowski S, Gheysens O, Van Cleemput J, Bormans G, Nuyts J, Belmans A, Mortelmans L, Boogaerts M, Van de Werf F. Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: double-blind, randomised controlled trial. The Lancet367:113-121.

55. Kang HJ, Kim HS, Zhang SY, Park KW, Cho HJ, Koo BK, Kim YJ, Lee DS, Sohn DW, Han KS, Oh BH, Lee MM, Park YB. Effects of intracoronary infusion of peripheral blood stem-cells mobilised with granulocyte-colony stimulating factor on left ventricular systolic function and restenosis after coronary stenting in myocardial infarction: the MAGIC cell randomised clinical trial. The Lancet 2004;363:751-756.

56. Lunde K, Solheim S, Aakhus S, Arnesen H, Abdelnoor M, Egeland T, Endresen K, Ilebekk A, Mangschau A, Fjeld JG, Smith HJ, Taraldsrud E, Grogaard HK, Bjornerheim R, Brekke M, Muller

C, Hopp E, Ragnarsson A, Brinchmann JE, Forfang K. Intracoronary Injection of Mononuclear Bone Marrow Cells in Acute Myocardial Infarction. N Engl J Med 2006;355:1199-1209.

57. Schachinger V, Assmus B, Britten MB, Honold J, Lehmann R, Teupe C, Abolmaali ND, Vogl TJ, Hofmann WK, Martin H, Dimmeler S, Zeiher AM. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction: final one-year results of the TOPCARE-AMI Trial. J Am Coll Cardiol 2004;44:1690-1699.

58. Strauer BE, Brehm M, Zeus T, Bartsch T, Schannwell C, Antke C, Sorg RV, Kogler G, Wernet P, Mnller HW, Kostering M. Regeneration of Human Infarcted Heart Muscle by Intracoronary Autologous Bone Marrow Cell Transplantation in Chronic Coronary Artery Disease: The IACT Study. J Am Coll Cardiol 2005;46:1651-1658.

59. Wollert KC, Meyer GP, Lotz J, Ringes Lichtenberg S, Lippolt P, Breidenbach C, Fichtner S, Korte T, Hornig B, Messinger D, Arseniev L, Hertenstein B, Ganser A, Drexler H. Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. The Lancet364:141-148.

60. Ge J, Li Y, Qian J, Shi J, Wang Q, Niu Y, Fan B, Liu X, Zhang S, Sun A, Zou Y. Efficacy of emergent transcatheter transplantation of stem cells for treatment of acute myocardial infarction (TCT-STAMI). Heart 2006;92:1764-1767.

61. Schaefer A, Zwadlo C, Fuchs M, Meyer GP, Lippolt P, Wollert KC, Drexler H. Long-term effects of intracoronary bone marrow cell transfer on diastolic function in patients after acute myocardial infarction: 5-year results from the randomized-controlled BOOST trial−an echocardiographic study. Eur J Echocardiogr 2010;11:165-171.

62. Yousef M, Schannwell CM, Kostering M, Zeus T, Brehm M, Strauer BE. The BALANCE Study: Clinical Benefit and Long-Term Outcome After Intracoronary Autologous Bone Marrow Cell Transplantation in Patients With Acute Myocardial Infarction. J Am Coll Cardiol 2009;53:2262-2269.

63. Segers VFM, Lee RT. Stem-cell therapy for cardiac disease. Nature 2008;451:937-942.

64. Hirsch A, Nijveldt R, van der Vleuten PA, Tio RA, van der Giessen WJ, Marques KMJ, Doevendans PA, Waltenberger J, ten Berg JM, Aengevaeren WRM, Biemond BJ, Tijssen JG, van Rossum AC, Piek JJ, Zijlstra F. Intracoronary infusion of autologous mononuclear bone marrow cells in patients with acute myocardial infarction treated with primary PCI: Pilot study of the multicenter HEBE trial. Catheterization and Cardiovascular Interventions 2008;71:273-281.

65. Meluzin J, Mayer J, Groch L, Janousek S, Hornacek I, Hlinomaz O, Kala P, Panovsky R, Prasek J, Kaminek M, Stanicek J, Klabusay M, Koristek Z, Navratil M, Dusek L, Vinklarkova J. Autologous transplantation of mononuclear bone marrow cells in patients with acute myocardial infarction: The effect of the dose of transplanted cells on myocardial function. American Heart Journal 2006;152:975.

66. Vulliet PR, Greeley M, Halloran SM, MacDonald KA, Kittleson MD. Intra-coronary arterial injection of mesenchymal stromal cells and microinfarction in dogs. The Lancet 2004;363:783-784.

67. Perin EC, Silva GV, Assad JAR, Vela D, Buja LM, Sousa ALS, Litovsky S, Lin J, Vaughn WK, Coulter S, Fernandes MR, Willerson JT. Comparison of intracoronary and transendocardial delivery of allogeneic mesenchymal cells in a canine model of acute myocardial infarction. Journal of Molecular and Cellular Cardiology 2008;44:486-495.

68. Bartunek J, Vanderheyden M, Vandekerckhove B, Mansour S, De Bruyne B, De Bondt P, Van Haute I, Lootens N, Heyndrickx G, Wijns W. Intracoronary Injection of CD133-Positive Enriched Bone Marrow Progenitor Cells Promotes Cardiac Recovery After Recent Myocardial Infarction: Feasibility and Safety. Circulation 2005;112:I-178.

69. Tossios P, Krausgrill B, Schmidt M, Fischer T, Halbach M, Fries JWU, Fahnenstich S, Frommolt P, Heppelmann I, Schmidt A, Schomacker K, Fischer JH, Bloch W, Mehlhorn U, Schwinger RHG, Muller-Ehmsen J. Role of balloon occlusion for mononuclear bone marrow cell deposition after intracoronary injection in pigs with reperfused myocardial infarction. Eur Heart J 2008;29:1911-1921.

70. Freyman T, Polin G, Osman H, Crary J, Lu M, Cheng L, Palasis M, Wilensky RL. A quantitative, randomized study evaluating three methods of mesenchymal stem cell delivery following myocardial infarction. Eur Heart J 2006;27:1114-1122.

71. Price MJ, Chou CC, Frantzen M, Miyamoto T, Kar S, Lee S, Shah PK, Martin BJ, Lill M, Forrester JS, Chen PS, Makkar RR. Intravenous mesenchymal stem cell therapy early after reperfused acute myocardial infarction improves left ventricular function and alters electrophysiologic properties. International Journal of Cardiology 2006;111:231-239.

72. Boekstegers P, Giehrl W, Degenfeld Gv, Steinbeck G. Selective Suction and Pressure-Regulated Retroinfusion: An Effective and Safe Approach to Retrograde Protection Against Myocardial Ischemia in Patients Undergoing Normal and High Risk Percutaneous Transluminal Coronary Angioplasty. J Am Coll Cardiol 1998;31:1525-1533.

73. Schuleri KH, Amado LC, Boyle AJ, Centola M, Saliaris AP, Gutman MR, Hatzistergos KE, Oskouei BN, Zimmet JM, Young RG, Heldman AW, Lardo AC, Hare JM. Early improvement in cardiac tissue perfusion due to mesenchymal stem cells. Am J Physiol Heart Circ Physiol 2008;294:H2002-H2011.

74. Tse HF, Kwong YL, Chan JK, Lo G, Ho CL, Lau CP. Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation. The Lancet 2003;361:47-49.

75. Kornowski R, Leon MB, Fuchs S, Vodovotz Y, Flynn MA, Gordon DA, Pierre A, Kovesdi I, Keiser JA, Epstein SE. Electromagnetic guidance for catheter-based transendocardial injection: a platform for intramyocardial angiogenesis therapy: Results in normal and ischemic porcine models. J Am Coll Cardiol 2000;35:1031-1039.

76. Losordo DW, Vale PR, Hendel RC, Milliken CE, Fortuin FD, Cummings N, Schatz RA, Asahara T, Isner JM, Kuntz RE. Phase 1/2 Placebo-Controlled, Double-Blind, Dose-Escalating Trial of Myocardial Vascular Endothelial Growth Factor 2 Gene Transfer by Catheter Delivery in Patients With Chronic Myocardial Ischemia. Circulation 2002;105:2012-2018.

77. Briguori C, Reimers B, Sarais C, Napodano M, Pascotto P, Azzarello G, Bregni M, Porcellini A, Vinante O, Zanco P, Peschle C, Condorelli G, Colombo A. Direct intramyocardial percutaneous

delivery of autologous bone marrow in patients with refractory myocardial angina. American Heart Journal 2006;151:674-680.

78. Krause K, Jaquet K, Schneider C, Haupt S, Lioznov MV, Otte KM, Kuck KH. Percutaneous intramyocardial stem cell injection in patients with acute myocardial infarction: first-in-man study. Heart 2009;95:1145-1152.

79. Nyolczas N, Gyongyosi M, Beran G, Dettke M, Graf S, Sochor H, Christ G, Edes I, Balogh L, Krause KT, Jaquet K, Kuck KH, Benedek I, Hintea T, Kiss R, Preda I, Kotevski V, Pejkov H, Dudek D, Heba G, Sylven C, Charwat S, Jacob R, Maurer G, Lang I, Glogar D. Design and rationale for the Myocardial Stem Cell Administration After Acute Myocardial Infarction (MYSTAR) Study: A multicenter, prospective, randomized, single-blind trial comparing early and late intracoronary or combined (percutaneous intramyocardial and intracoronary) administration of nonselected autologous bone marrow cells to patients after acute myocardial infarction. American Heart Journal 2007;153:212.

80. van Ramshorst J, Bax JJ, Beeres SLMA, bbets-Schneider P, Roes SD, Stokkel MPM, de Roos A, Fibbe WE, Zwaginga JJ, Boersma E, Schalij MJ, Atsma DE. Intramyocardial Bone Marrow Cell Injection for Chronic Myocardial Ischemia: A Randomized Controlled Trial. JAMA 2009;301:1997-2004.

81. Gyongyosi M, Lang I, Dettke M, Beran G, Graf S, Sochor H, Nyolczas N, Charwat S, Hemetsberger R, Christ G, Edes I, Balogh L, Krause KT, Jaquet K, Kuck KH, Benedek I, Hintea T, Kiss R, Preda I, Kotevski V, Pejkov H, Zamini S, Khorsand A, Sodeck G, Kaider A, Maurer G, Glogar D. Combined delivery approach of bone marrow mononuclear stem cells early and late after myocardial infarction: the MYSTAR prospective, randomized study. Nat Clin Pract Cardiovasc Med 2009;6:70-81.

82. Gutstein DE, Morley GE, Tamaddon H, Vaidya D, Schneider MD, Chen J, Chien KR, Stuhlmann H, Fishman GI. Conduction Slowing and Sudden Arrhythmic Death in Mice With Cardiac-Restricted Inactivation of Connexin43. Circ Res 2001;88:333-339.

83. Klein RM, Vester EG, Brehm MU, Dees H, Picard F, Niederacher D, Beckmann MW, Strauer BE. Inflammation of the myocardium as an arrhythmia trigger. Z Kardiol 2000;89:24-35.

84. Thompson CA, Nasseri BA, Makower J, Houser S, McGarry M, Lamson T, Pomerantseva I, Chang JY, Gold HK, Vacanti JP, Oesterle SN. Percutaneous transvenous cellular cardiomyoplasty: A novel nonsurgical approach for myocardial cell transplantation. J Am Coll Cardiol 2003;41:1964-1971.

85. Yokoyama SI, Fukuda N, Li Y, Hagikura K, Takayama T, Kunimoto S, Honye J, Saito S, Wada M, Satomi A, Kato M, Mugishima H, Kusumi Y, Mitsumata M, Murohara T. A strategy of retrograde injection of bone marrow mononuclear cells into the myocardium for the treatment of ischemic heart disease. Journal of Molecular and Cellular Cardiology 2006;40:24-34.

86. Siminiak T, Fiszer D, Jerzykowska O, Grygielska B, Rozwadowska N, Kalmucki P, Kurpisz M. Percutaneous trans-coronary-venous transplantation of autologous skeletal myoblasts in the treatment of post-infarction myocardial contractility impairment: the POZNAN trial. Eur Heart J 2005;26:1188-1195.

87. Brasselet C, Morichetti MC, Messas E, Carrion C, Bissery A, Bruneval P, Vilquin JT, Lafont A, Hagege AA, Menasche P, Desnos M. Skeletal myoblast transplantation through a catheter-based

coronary sinus approach: an effective means of improving function of infarcted myocardium. Eur Heart J 2005;26:1551-1556.

88. Hou DM, March KL. A novel percutaneous technique for accessing the normal pericardium: a single-center successful experience of 53 porcine procedures. J invasive cardiol 2003;15:13-17.

89. Waxman S, Pulerwitz TC, Rowe KA, Quist WC, Verrier RL. Preclinical safety testing of percutaneous transatrial access to the normal pericardial space for local cardiac drug delivery and diagnostic sampling. Catheterization and Cardiovascular Interventions 2000;49:472-477.

90. Macris MP, Igo SR. Minimally invasive access of the normal pericardium: initial clinical experience with a novel device. Clin Cardiol 1999;22:36-39.

91. Hagikura K, Fukuda N, Yokoyama SI, Yuxin L, Kusumi Y, Matsumoto T, Ikeda Y, Kunimoto S, Takayama T, Jumabay M, Mitsumata M, Saito S, Hirayama A, Mugishima H. Low invasive angiogenic therapy for myocardial infarction by retrograde transplantation of mononuclear cells expressing the VEGF gene. International Journal of Cardiology 2009;In Press.

92. Corday E, Meerbaum S, Drury J.K. The coronary sinus: an alternate channel for administration of arterial blood and pharmacologic agents for protection and treatment of acute cardiac ischemia. J Am Coll Cardiol 1986;7:711-714.

93. Herity NA, Lo ST, Oei F, Lee DP, Ward MR, Filardo SD, Hassan A, Suzuki T, Rezaee M, Carter AJ, Yock PG, Fitzgerald PJ. Selective regional myocardial infiltration by the percutaneous coronary venous route: A novel technique for local drug delivery. Catheterization and Cardiovascular Interventions 2000;51:358-363.

94. Vicario J, Piva J, Pierini A, Ortega HH, Canal A, Gerardo L, Pfeiffer H, Campos C, Fendrich I, Novero R, Monti A. Transcoronary sinus delivery of autologous bone marrow and angiogenesis in pig models with myocardial injury. Cardiovascular Radiation Medicine 2004;3:91-94.

95. Vicario J, Campos C, Piva J, Faccio F, Gerardo L, Becker C, Ortega HH, Pierini A, Lofeudo C, Novero R, Licheri A, Milesi R, Perez Balino N, Monti A, Amin A, Pfeiffer H, De Giovanni E, Fendrich I. Transcoronary sinus administration of autologous bone marrow in patients with chronic refractory stable angina: Phase 1. Cardiovascular Radiation Medicine 2004;5:71-76.

96. Perin EC, Silva GV, Assad JAR, Vela D, Buja LM, Sousa ALS, Litovsky S, Lin J, Vaughn WK, Coulter S, Fernandes MR, Willerson JT. Comparison of intracoronary and transendocardial delivery of allogeneic mesenchymal cells in a canine model of acute myocardial infarction. Journal of Molecular and Cellular Cardiology 2008;44:486-495.

97. Baklanov DV, Moodie KM, McCarthy FE, Mandrusov E, Chiu J, Aswonge G, Cheng J, Chow M, Simons M, de Muinck ED. Comparison of transendocardial and retrograde coronary venous intramyocardial catheter delivery systems in healthy and infarcted pigs. Catheter Cardiovasc Interv 2006;68:416-423.

98. George JC, Goldberg J, Joseph M, Abdulhameed N, Crist J, Das H, Pompili VJ. Transvenous Intramyocardial Cellular Delivery Increases Retention in Comparison to Intracoronary Delivery in a Porcine Model of Acute Myocardial Infarction. Journal of Interventional Cardiology 2009;21:424-431.

99. Blocklet D, Toungouz M, Berkenboom G, Lambermont M, Unger P, Preumont N, Stoupel E, Egrise D, Degaute JP, Goldman M, Goldman S. Myocardial Homing of Nonmobilized Peripheral-Blood CD34+ Cells After Intracoronary Injection. Stem Cells 2006;24:333-336.

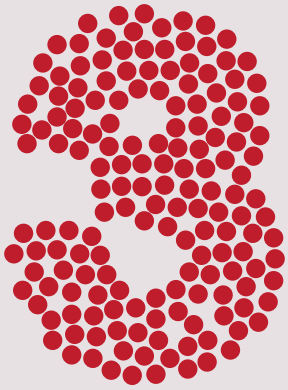
100. Kang WJ, Kang HJ, Kim HS, Chung JK, Lee MC, Lee DS. Tissue Distribution of 18F-FDG-Labeled Peripheral Hematopoietic Stem Cells After Intracoronary Administration in Patients with Myocardial Infarction. J Nucl Med 2006;47:1295-1301.

101. Mitchell AJ, Sabondjian E, Sykes J, Deans L, Zhu W, Lu X, Feng Q, Prato FS, Wisenberg G. Comparison of Initial Cell Retention and Clearance Kinetics After Subendocardial or Subepicardial Injections of Endothelial Progenitor Cells in a Canine Myocardial Infarction Model. J Nucl Med 2010.

102. Dib N, Campbell A, Jacoby DB, Zawadzka A, Ratliff J, Miedzybrocki BM, Gahremanpour A, Diethrich EB, Opie SR. Safety and feasibility of percutaneous autologous skeletal myoblast transplantation in the coil-infarcted swine myocardium. Journal of Pharmacological and Toxicological Methods 2007;54:71-77.

103. Mitchell AJ, Sabondjian E, Sykes J, Deans L, Zhu W, Lu X, Feng Q, Prato FS, Wisenberg G. Comparison of Initial Cell Retention and Clearance Kinetics After Subendocardial or Subepicardial Injections of Endothelial Progenitor Cells in a Canine Myocardial Infarction Model. J Nucl Med 2010;


T.I.G van der Spoel



HUMAN RELEVANCE OF PRE-CLINICAL STUDIES IN STEM CELL THERAPY; SYSTEMATIC REVIEW AND META-ANALYSIS OF LARGE ANIMAL MODELS OF ISCHEMIC HEART DISEASE.

Tycho I.G. van der Spoel, M.D.[1]
Sanne J. Jansen of Lorkeers, MSc.[1]
Pierfrancesco Agostoni, M.D., Ph.D.[1]
Eric van Belle, M.D., Ph.D. [1]
Mariann Gyöngyösi M.D., Ph.D. [2]
Joost P.G. Sluijter, Ph.D. [1,3]
Maarten Jan M. Cramer, M.D., Ph.D. [1]
Pieter A. Doevendans, M.D., Ph.D. [1,3]
Steven A.J. Chamuleau, M.D., Ph.D. [1]

[1] Department of Cardiology, Division Heart and Lungs, University Medical Center Utrecht, the Netherlands.
[2] Department of Cardiology, Medical University of Vienna, Austria.
[3] Interuniversity Cardiology Institute of the Netherlands (ICIN), Utrecht, the Netherlands.

ABSTRACT

Background: Stem cell therapy is a treatment strategy for ischemic heart disease in patients. Meta-analysis of randomized human trials showed <5% improvement in left ventricular ejection fraction (LVEF). Meta-analysis of available pre-clinical data of ischemic heart disease could provide important clues to design human clinical trials.

Methods: Random-effects meta-analysis was performed on pig, dog or sheep studies investigating the effect of cardiac stem cell therapy in ischemic cardiomyopathy (52 studies; N=888 animals). Endpoints were LVEF and death.

Results: Ischemia/reperfusion infarction was performed in 23 studies and chronic occlusion in 29 studies. Pooled analysis showed a LVEF difference of 7.5% at follow-up after cell therapy vs. control (95% confidence interval (CI), 6.2% to 8.9%; P<0.001). By exploratory multivariable meta-regression significant predictors of LVEF improvement were: cell type (bone marrow mononuclear cells (BM-MNC) showed less effect than other cell types, e.g. mesenchymal stem cells; P=0.040) and type of infarction (left anterior descending artery 8.0% vs. left circumflex artery 5.8%; P=0.045). Cell therapy was not associated with increased mortality (P=0.68). Sensitivity analysis showed trends towards more improvement with higher cell number (≥107), chronic occlusion models and late injections (>1 week). After follow-up of 8 weeks the effect of cell therapy decreased to 6%.

Conclusions: This meta-analysis showed that large animal models are valid to predict outcome of clinical trials. Our results showed that cell therapy is safe and led to a preserved LVEF. Future trials should focus on cell types other than BM-MNC, large infarction and strategies to obtain sustained effects overtime.

INTRODUCTION

Coronary heart disease is a major public and economic health problem leading to more than 7 million deaths world wide each year [1, 2]. Myocardial infarction (MI) is characterized by loss of cardiomyocytes, scar formation, ventricular remodeling and it can develop into end-stage heart failure. Optimal pharmacologic treatment and coronary reperfusion therapy have led to improved survival of patients with coronary artery disease, even if current medical therapies cannot replace dysfunctional cardiomyocytes. Cell therapy has emerged as a potential therapeutic strategy. The ultimate goals of cell therapy are myocardial regeneration and revascularization, thus, re-establishing synchronous contractility and bioelectrical conductivity to achieve overall clinical improvement of cardiac function without severe adverse effects.

Many large animal studies in acute MI and ischemic cardiomyopathy have been performed, mostly with heterogeneous design and conflicting outcomes. These pre-clinical results led to the initiation of clinical trials and showed at best marginal results [3]. Nevertheless, pre-clinical studies are mandatory to assess risk of a new therapy and predict safety, feasibility and efficacy. Moreover, they address unresolved issues regarding clinical cell therapy (i.e. choice of cell type, cell number, method of delivery, time of delivery and follow-up after cell transplantation), which questions have been outlined by the task force of the European Society of Cardiology on stem cell repair of the heart [4]. Therefore, large animal models are valid and relevant for clinical practice, and have important clues regarding these yet unanswered questions. Similar to the human cardiac stem cell therapies, large number of animal studies have been performed including relative small number of animals. We hypothesize, that meta-analysis of these pre-clinical data might be helpful to design future clinical studies similarly to the meta-analysis of human cardiac stem cell trials.

We performed a systematic overview of the pertinent literature including a quantitative meta-analytical pooling of the data to assess the effects of stem cell transplantation in large animals with acute or chronic ischemic cardiomyopathy. A pre-specified sub-analysis is performed to focus on aforementioned unresolved issues.

METHODS

Eligibility criteria

Acute MI or chronic ischemic cardiomyopathy models in large animals were screened. Randomized controlled (RCT) and cohort trials investigating the effect of stem cell therapy on cardiac function as determined by left ventricular ejection fraction (LVEF) were analyzed. In addition, a placebo or sham operated control group had to be included in the study. Trials that only investigated transfected or genetically engineered stem cells altering cell behavior, or studies using conditioned medium were excluded, but studies using reporter genes (solely for stem cell imaging

purposes) were included. Reviews, editorials, comments, reports from scientific sessions and discussions were excluded.

Search Strategy

A Pubmed search was performed (January 1980 to March 2010) using the following search terms: *"(pig OR porcine OR swine OR canine OR dog OR sheep OR ovine) AND (stem cells OR progenitor cells OR bone marrow) AND (myocardial infarction OR heart failure OR coronary artery disease OR cardiac repair OR myocardial regeneration)"*. Only English and published reports were included. The retrieved studies were carefully examined to exclude potentially duplicate or overlapping data. The complete search strategy is available on request.

Data abstraction

Two reviewers (TS and SJ) independently screened abstracts and the resulting manuscripts were approved by a third reviewer (SC). The following information was extracted from the complete manuscripts of the qualified studies: basal characteristics of the study, LVEF, end-diastolic volume (EDV), end-systolic volume (ESV) and mortality. If necessary, data were estimated from graphics or recalculated by available data: LVEF was recalculated as follows: (EDV-ESV)/EDV *100%. Accordingly, standard deviations were determined or recalculated from standard errors. Volume data were recalculated for body-weight. For final analysis we preferably used MRI data. Alternatively, data derived by echocardiography, nuclear imaging, left ventricle angiography or pressure-volume (PV) loops respectively, were used in absence of MRI data. In case of missing data, corresponding authors were contacted. Thirty-six emails were sent and 18 authors responded. Standard guidelines[5] for quality assessment of clinical trials could not be completely applied in these pre-clinical experiments. Therefore, we used modified criteria to assess selection, performance and detection bias: randomization (yes/no), adequate allocation (y/n), adequate method of randomization (y/n), blinding of the operator (y/n) and blinding of the functional analysis (y/n).

Data analysis

Our primary outcome was difference in mean LVEF (reported in %) at follow-up between control and treated animals. Secondary endpoints were difference in EDV and ESV (reported as volume in mL) at follow-up and mortality after treatment. In case of multiple measurements over time, data measured at the longest duration of follow-up were used for analysis. A random-effect model was applied. Continuous variables were reported as weighted mean differences with 95% confidence intervals (CI) between the cell-treated animals and control groups. In case of dichotomous data, the pooled estimate of effect was presented as odds ratio (OR) with 95% CI. In case of multiple experimental groups next to one control group within one study,

the number of animals in the control group was divided equally by the number of experimental groups. Details of enrolled subgroups are provided in data supplement table 1. Unadjusted P values are reported throughout, with hypothesis testing set at the 2-tailed 0.05 level. Heterogeneity was considered significant at P<0.106. Inconsistency was estimated by using the I^2 statistic; values of 25%, 50% and 75% were considered low, moderate and high inconsistency, respectively [7].

Based on clinical scenario a multivariate analysis was performed for: MI model (ischemia/reperfusion or chronic occlusion); location of infarct-related artery (left anterior descending artery (LAD) or left circumflex artery (LCX)); type of animal (pig, dog, sheep); cell type; number of cells injected; method of cell delivery (retrograde coronary transvenous injection, surgical, intracoronary (IC), and trans-endocardial (TE) delivery); timing of cell therapy after acute MI and follow-up after cell therapy. Furthermore, from a clinical point of view the following subgroup analyses were performed: MI model (ischemia/reperfusion or chronic occlusion); type of infarction (LAD or LCX); cell type (bone marrow mononuclear stem cells (BM-MNC) or mesenchymal stem cells (MSC)); number of cells injected ($<10^7$, 10^7-10^8, 10^8-10^9, or $\geq 10^9$), timing of cell therapy after acute MI ($\leq$ 1 day, 1-7 days, $\geq$ 7 days) and follow-up after cell therapy (1-4 weeks, 5-8 weeks, 9-12 weeks, >12 weeks). A Funnel plot was drawn for LVEF to explore publication bias. A power-analysis for future studies in ischemic heart disease was performed. All analyses were performed with Review Manager version 5 (The Nordic Cochrane Center, Købehvn Denmark) and SPSS 17.0; SPSS, Chicago, IL.

RESULTS

Included study characteristics

The electronic database search identified 304 articles, among which 52 articles were eligible for review (34 RCT and 18 cohort studies; see Figure 1). In total 1251 animals were described in the included articles but 888 animals met our inclusion criteria and were analyzed. Characteristics of the enrolled studies are depicted in Table 1. Most studies used a porcine model (41 studies). In 23 studies ischemia/reperfusion was used as a MI model. Myocardial infarction was mainly induced in the left anterior descending coronary artery (38 studies), but site of ligation/constriction of the vessel (proximal, mid or distal) varied. Ten different cell types have been studied. In most cases surgical or IC delivery was performed. Timing of cell therapy after induction of MI was <1 day (15 studies), 1-7 days (11 studies) or >7 days (26 studies). Median and interquartile range of time to follow-up imaging was 6 weeks (4-8 weeks). Functional endpoints were assessed by MRI (18 studies), echocardiography (23 studies), nuclear imaging (5 studies), left ventricle angiography (4 studies) or PV-loop (2 studies). Volume data were reported in 25 studies and mortality in 32 studies.

Author	N	Type of animal	Study design	Type of infarction	MI model	Cell type	Number of cells	Route of delivery	Timing of cell therapy after MI*	Follow up (weeks)
Bel et al.[24] 2003	18	Sheep	RCT	LCX	No I/R	BM-MNC	$4.2*10^8$	Surgical	21d	8
Brasselet et al.[25] 2005	14	Sheep	RCT	LAD	No I/R	Skeletal myoblasts	$2.4*10^8$	RCV	14d	8
Chacques et al.[26] 2004	11	Sheep	RCT	LAD	No I/R	Skeletal myoblasts	$7.0*10^7$	Surgical	21d	12
Chen et al.[27] 2009	13	Pig	RCT	LCX	No I/R	MSC	$4.0*10^7$	Surgical	42d	4
De Silva et al.[28] 2008	11	Pig	Cohort	LAD	I/R	BM-MNC	$1.0*10^9$	IC	4d	6
Dixon et al.[29] 2009	46	Sheep	RCT	LAD	No I/R	MSC	$2.5*10^7$-$4.5*10^8$	Surgical	1h	8
Doyle et al.[30]	18	Pig	Cohort	LCX	I/R	EPC	$3.0*10^7$	IC	2d	8
Gavira et al.[31] 2006	16	Pig	Cohort	LAD	No I/R	Skeletal myoblasts	$4.0*10^8$	TE/Surgical	56d	12
Ghodsizad et al.[32] 2007	10	Pig	RCT	LCX	No I/R	USSC	$1.3*10^7$	Surgical	1h	8
Ghostine et al.[33] 2002	16	Sheep	RCT	LCX	No I/R	Skeletal myoblasts	$4.2*10^8$	Surgical	14d	17
Gyöngyösi et al.[34] 2008	11	Pig	RCT	LAD	I/R	MSC	$7.1*10^6$	TE	16d	1.5
Hagikura et al.[35] 2009	10	Pig	Cohort	LAD	No I/R	MNC	$5.0*10^6$	Coronary sinus venous infusion	4h	4
Haider et al.[36] 2004	11	Pig	RCT	LCX	No I/R	Skeletal myoblasts	$3.0*10^8$	Surgical	21d	6
Halkos et al.[37] 2008	33	Pig	RCT	LAD	I/R	MSC	$3.9*10^7$-$3.7*10^8$	IV	1h	12
Hamamoto et al.[38] 2009	35	Sheep	Cohort	LAD	No I/R	MSC	$2.3*10^7$-$4.4*10^8$	Surgical	1h	4-8
Hashemi et al.[39] 2008	31	Pig	RCT	LAD	I/R	MSC	$2.4*10^7$-$4.4*10^8$	TE	3d	8-12
He et al.[40] 2005	19	Dog	Cohort	LAD	No I/R	Skeletal myoblasts	$3.6*10^8$-$5.4*10^8$	Surgical/TE	7d	10
Jiang et al.[41] 2010	11	Pig	RCT	LAD	I/R	MSC	$1.0*10^7$	IC	1h	13
Johnston et al.[42]	14	Pig	RCT	LAD	I/R	CDC	$1.0*10^7$	IC	28d	8

2009									
Kawamoto et al.[43]	24	Pig	Cohort	LCX	No I/R	MNC/EPC	$1.0*10^7$	TE	28d	4
2003									
Kim et al.[44]	16	Pig	RCT	LAD	No I/R	USSC	$1.0*10^8$	Surgical	28d	4
2005									
Li et al.[45]	16	Pig	RCT	LAD	I/R	BM-MNC	$3.5*10^8$	IC	1h	4
2008									
Li et al.[46]	25	Pig	Cohort	LAD	No I/R	MSC/BM-MNC	$6.2*10^5$-$4.7*10^7$	IC	2h	4
2008									
Lim et al.[47]	24	Pig	Cohort	LAD	I/R	MSC	$1.0*10^7$	IC	3d	4
2006									
Mäkelä et al.[48]	14	Pig	RCT	LCX	I/R	BM-MNC	$1.0*10^8$	Surgical	1h	3
2007									
Makkar et al.[49]	16	Pig	RCT	LAD	No I/R	MSC	$2.0*10^8$	Surgical	28d	8
2005									
Memon et al.[50]	12	Dog	Cohort	LAD	No I/R	Skeletal myoblasts/BM-MNC	$3.0*10^6$-$1.0*10^8$	Surgical	14d	4
2005									
Menard et al.[51]	18	Sheep	RCT	LCX	No I/R	ESC	$3.0*10^7$	Surgical	14d	4
2005									
Messas et al.[52]	13	Sheep	RCT	LCX	No I/R	Skeletal myoblasts	$2.5*10^8$	Surgical	56d	8
2006									
Moelker et al.[53]	30	Pig	RCT	LCX	I/R	BM-MNC	$5.0*10^8$	IC	7d	4
2007									
Patila et al.[55]	15	Pig	RCT	LCX	No I/R	Skeletal myoblasts	$2.0*10^6$	Surgical	24d	4
2009									
Perin et al.[56]	17	Dog	RCT	LAD	I/R	MSC	$1.0*10^8$	TE/IC	7d	2
2008									
Price et al.[57]	15	Pig	RCT	LAD	I/R	MSC	$3.2*10^8$	IV	1h	13
2006									
Qi et al.[58]	20	Pig	RCT	LAD	I/R	MSC	$1.0*10^8$	IC	5d	4-8
2008									
Qian et al.[59]	12	Pig	Cohort	LAD	I/R	MSC	$1.0*10^9$	IC	7	6
2007									
Quevedo et al.[60]	10	Pig	Cohort	LAD	I/R	MSC	$2.0*10^8$	IC	84d	12
2009									
Schneider et al.[61]	23	Pig	RCT	LCX	No I/R	MSC/BM-MNC	NA/$1.7*10^7$	TE	14d	4
2009									

Schuleri et al.[62] 2008	15	Pig	RCT	LAD	I/R	MSC	$2.0*10^8$	TE	2d	8
Schuleri et al.[63] 2009	12	Pig	RCT	LAD	I/R	MSC	$2.0*10^8$	Surgical	111d	12
Sheu et al.[64] 2009	18	Pig	Cohort	LAD	No I/R	BM-MNC	$3.0*10^7$	Surgical	1h	13
Thompson et al.[65] 2005	8	Pig	Cohort	LAD	No I/R	BM-MNC	$3.0*10^8$	RCV	35d	8
Tomita et al.[66] 2002	11	Pig	RCT	LAD	No I/R	MSC†	$1.0*10^8$	Surgical	28d	4
Valina et al.[67] 2007	21	Pig	RCT	LAD	I/R	MSC/ADSC	$2.0*10^6$	IC	1h	4
Wang et al.[68] 2009	12	Pig	RCT	LAD	I/R	MSC	$5.0*10^7$	Trans coronary injection	1h	4
Yang et al.[69] 2009	12	Pig	RCT	LAD	I/R	MSC	$3.0*10^7$	Surgical	1h	6
Yang et al.[70] 2006	12	Pig	RCT	LAD	No I/R	MSC	$5.0*10^6$	IC	28d	4
Yang et al. [71] 2007	18	Pig	RCT	LAD	I/R	MSC	$5.0*10^6$	IC	28d	4
Yi et al.[72] 2006	14	Pig	RCT	LAD	No I/R	MSC	$8.0*10^7$	TE	28d	4
Yokoyama et al.[73] 2006	21	Pig	RCT	LAD	No I/R	BM-MNC	$3.2*10^9$	Coronary sinus venous infusion	6h or 14d	4
Zeng et al.[74] 2007	14	Pig	RCT	LAD	No I/R	MSC	$5.0*10^7$	Surgical	1h	4
Zhang et al.[75] 2007	20	Pig	Cohort	LAD	No I/R	HPC	$5.0*10^7$	IC	14d	4

Table 1. Study Characteristics

ADSC= Adipose tissue derived stem cells; BM-MNC= Bone marrow mononuclear cells; CDC= Cardiosphere derived cells; EPC=Endothelial progenitor cells; ESC= Embryonic stem cells; HPC=Hematopoietic progenitor cells; IC= Intracoronary infusion; I/R= Ischemia/reperfusion; IV= Peripheral intravenous; LAD= Left anterior descending artery; LCX= Left circumflex artery; MI=Myocardial infarction; MNC= Peripheral mononuclear cells; MSC= Mesenchymal stem cells; N= number of animals (treated group and control group); NA= Not applicable; RCT=Randomized controlled trail; RCV= Retrograde coronary transvenous injection; TE= Trans-endocardial injection; USSC= Unrestricted somatic stem cells. *timing in days (d) or hours (h); †MSC cultured with 5-azacytidine

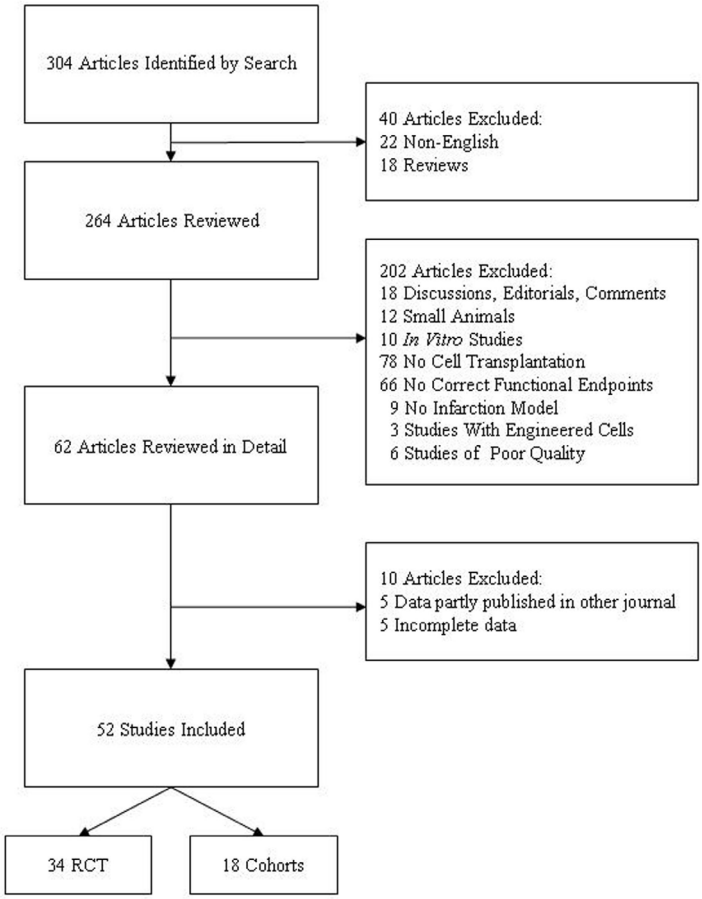


Figure 1. Flowchart of enrolled studies on cell therapy in large animals with acute myocardial infarction and chronic ischemic cardiomyopathy. RCT= Randomized controlled trial.

Quality of included studies

Data supplement table 2 shows the methodological quality of the enrolled studies. Blinded analysis of LVEF was performed in 12 RCT and 10 cohort studies. The operator was blinded in 5 studies. One article reported the method of randomization. Thirty-six studies (69%) were published in journals with an impact factor ≥3.0.

Meta-analyses

Pooled analysis showed a LVEF difference of 7.5% at follow-up after cell therapy vs. control (95% CI, 6.2% to 8.9%; P<0.001) with significant heterogeneity (p<0.01) and inconsistency (I^2: 77%; Figure 2). At follow-up, mean LVEF after cell transplantation and control was 56% and 48%, respectively. Consistently an ESV difference of -7.4 mL (95% CI, -12.9 mL to -1.8 mL; P=0.01) and EDV difference of -5.3 mL (95% CI, -12.7 mL to 2.1 mL; P=0.16) was found with significant

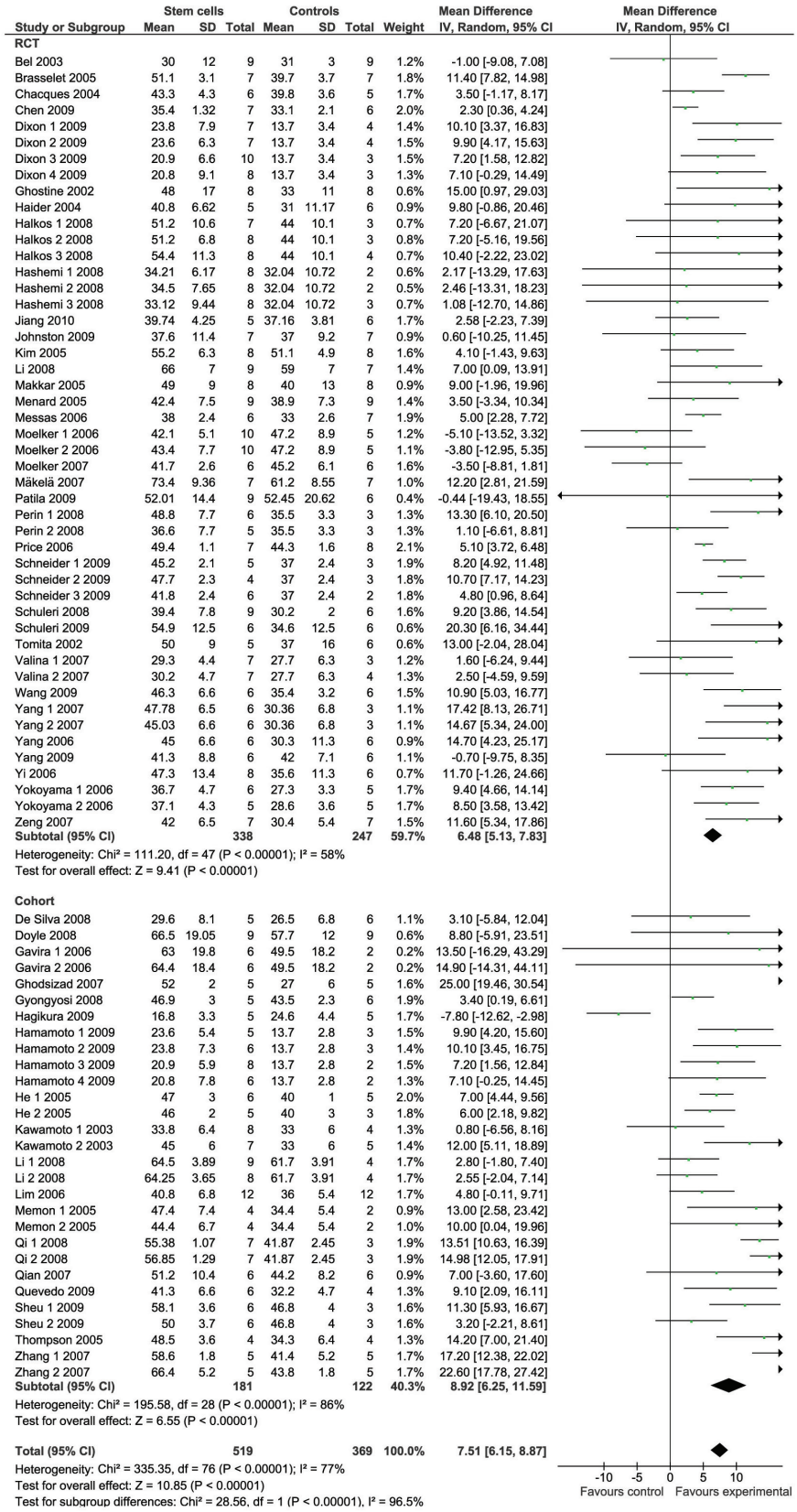
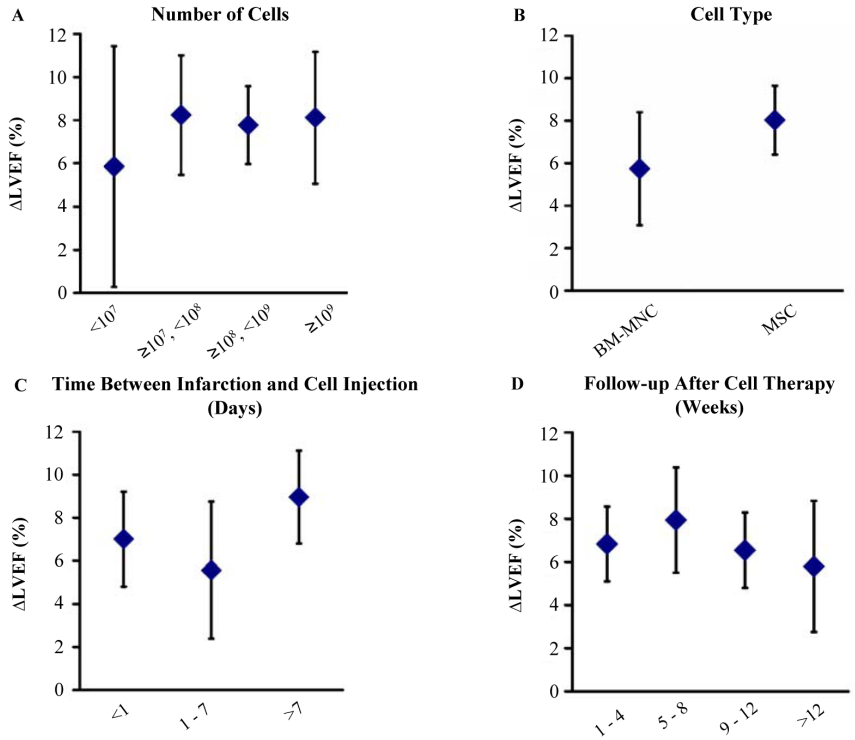


Figure 2. Forest plot showing the impact of stem cell therapy on LVEF improvement compared to controls. RCT= Randomized controlled trial; 95% CI= 95% Confidence interval; WMD= Weighted mean difference.

heterogeneity ($p<0.001$ for both) and inconsistency (I^2 >90% for both). Overall, no significant difference in LVEF at baseline between the control group and cell treated group was found (P=0.31); however only 69 % of the studies reported these baseline data. No significant differences were found in mortality after cell transplantation: 9.5% (36/380) in cell treated group vs. 8.4 % (21/251) in the control group (OR 1.13 [0.63 to 2.02], I^2=0%, P=0.68). The majority of deaths were due to arrhythmias (data not shown).

Sensitivity-analyses

A multivariable meta-regression analysis showed that cell type (P=0.040) and type of infarction (P=0.045) are the only independent significant predictors of LVEF improvement. A trend was observed (Figure 3) towards more improvement of cell therapy regarding: anterior infarction with LAD as infarct-related artery, high cell number ($\geq 10^7$) and late injections (>1 week after MI). BM-MNC showed less effect than MSC. In addition, less benefit was observed in ischemia/reperfusion MI models compared to chronic MI models. During follow-up the effect of cell therapy appeared to decline over time. No trend in LVEF improvement was observed regarding animal model (P=0.49) and route of cell delivery (P=0.90). The funnel plot for LVEF suggests a lack of publication bias as values were evenly distributed around the overall estimate (Figure 4).



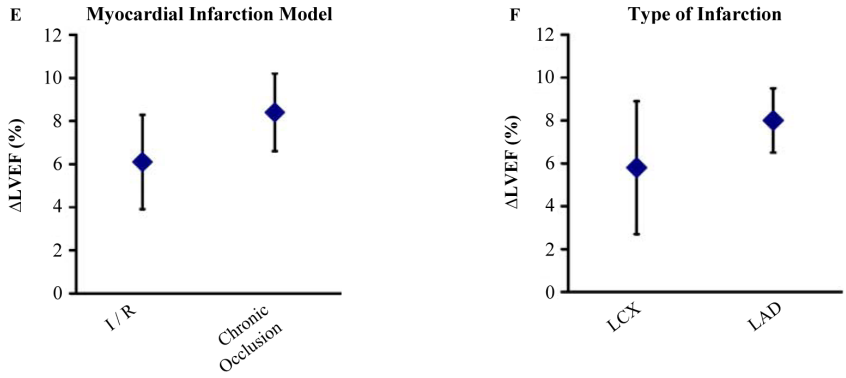


Figure 3. Sensitivity analysis by visual inspection showed a trend towards more improvement of cell therapy compared to control regarding: (A) high cell number (≥107) (P=0.52), (B) other cell types than bone marrow (P=0.040), (C) late injections (>1wk) (P=0.68), (E) chronic occlusion model (P=0.70) and (F) LAD infarction (P=0.045). After 8 weeks follow-up (D) the effect of cell therapy fades away (P=0.11). LVEF= Left ventricular ejection fraction; LAD= Left anterior descending artery; LCX= Left circumflex artery; I/R= ischemia/reperfusion; BM-MNC= Bone marrow mononuclear stem cells; MSC=Mesenchymal stem cells; P values are derived from the multivariate analysis.

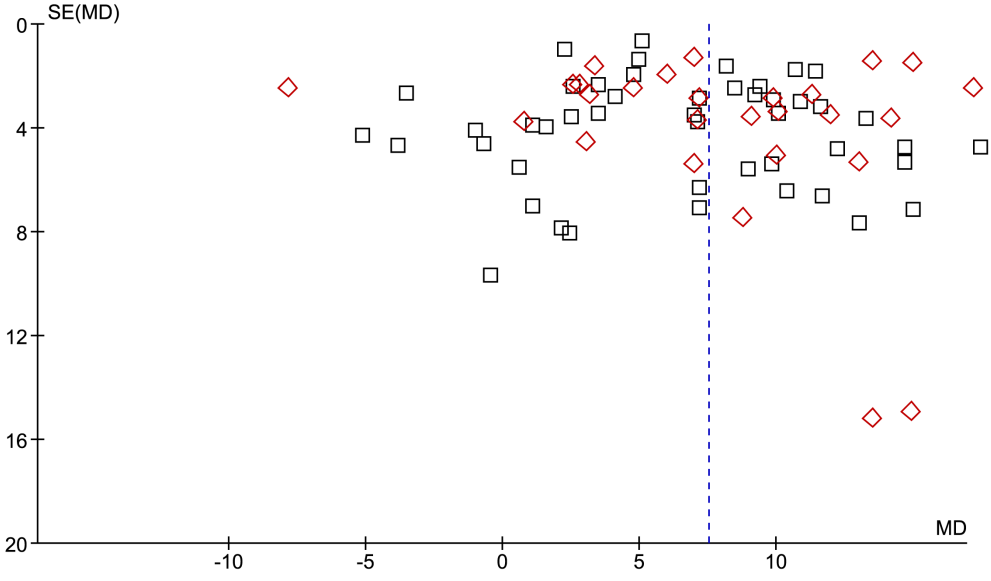


Figure 4. Funnel plot for LVEF improvement. Blue dotted line shows the overall estimated mean difference. The red diamond shaped color displays cohort studies and black squares displays RCT. No evidence for publication bias was found. SE=Standard Error; MD= Mean difference.

Power calculation

Based on our results we performed a sample size calculation for future studies in ischemic heart disease. To obtain a power of at least 80% in a two-sided two-sample t-test with an alfa of 0.05, 11 animals needed to be included in each group to detect a significant difference of 8% in LVEF.

DISCUSSION

The current analysis comprises data of 52 published pre-clinical studies involving large animals treated with cardiac stem cells in order to investigate the effects of cell therapy for ischemic heart disease. The main findings are: (1) Cell therapy improves LVEF by 7.5% due to a significant decrease in ESV; (2) There is no increased mortality after cell treatment; (3) Cell type and type of infarction are important predictors of functional outcome; (4) Sensitivity analysis suggests that MSC, LAD infarction, chronic occlusion MI models, a higher number of cells ($\geq 10^7$) and cell injection at least one week after MI have a beneficial effect on LVEF; (5) No effect on animal type and route of delivery was found.

Safety and efficacy of cardiac stem cell therapy in pre-clinical trials

Safety of cell therapy is still an important issue [8], regarding the no reflow after intracoronary cell injections and myocardial perforation by intramyocardial application. In spite of cell delivery- associated adverse events, human trials did not report increased mortality. Similarly, the present meta-analysis of pre-clinical trials showed no significant difference in death in animals receiving cell transplantation compared to controls, although only 32 studies (61%) addressed this issue. Although global LV function improves after cell therapy, no significant difference in EDV (-1.92 mL) was documented, indicating that cell therapy led to increase in contractility, but did not prevent ventricular remodelling. Similar result was observed in two clinical meta-analyses[3, 9]. This could be due to the relative short-term follow-up (less <4 months) of the enrolled studies in our analysis. However, it is possible that structural myocardial changes and effects on diastolic filling occur after 4 months.

Transplantation of higher number of cells ($\geq 10^7$) appears to have a more pronounced impact on improvement in LVEF. Our results are in agreement with clinical meta-analysis in that significant effect on LVEF may only be achieved when infusing doses are higher than 10^8 cells [9]. Moreover, meta-regression analysis showed that choice of cell type is an important predictor for LVEF. Subanalysis revealed a trend towards larger benefit in case of transplantation of MSC, as compared to BM-MNC. Scarce evidence is available that these cell types can regenerate new cardiomyocytes *in vivo*. This suggests the stimulation of an endogenous regenerative capacity of the heart upon cell transplantation, by release of growth factors, cytokines and other paracrine molecules by the transplanted and host cells, enhancing angiogenesis and reducing apoptosis[10-12].

Unfortunately, no complete data on infarct size were reported. However, our meta-regression analysis showed that type of infarction is an important significant independent predictor for clinical outcome. In detail, LAD-related anterior wall infarction showed more benefit after stem cell therapy compared to LCX infarction (LAD 8.0% vs. LCX 5.8%). Interestingly, there was no important difference in ratio of MI model for LAD and LCX infarction. Therefore, the observed effect may be caused by a greater degree of expansion after LAD infarction [13] leading to lower LVEF and a higher risk for mortality therefore more benefit from cell therapy is expected in this patient group. Indeed, post hoc analyses of the REPAIR-AMI trial database and a clinical meta-analyses suggested that the effects of bone marrow cells were significantly higher in the subgroup with a baseline LVEF<49% who may have a tendency to develop heart failure[14, 15]. Ischemia/reperfusion MI models were associated with less improvement in LVEF compared to chronic occlusion models (LVEF; 6.3 vs. 8.3) although there was a higher incidence of permanent ligation animal studies. No reliable insight in baseline LVEF was available between these groups. In theory, percutaneous ischemia/reperfusion models are considered most reliable for translational research as it mimics more closely the clinical practice of primary percutaneous coronary intervention. Timing of injection is important with more pronounced benefit if applied 7 days after MI. Our findings are comparable to clinical studies [3, 16]. In the acute setting (<24 hours) cellular retention and survival is likely influenced by the local hostile microenvironment.

In large animals, the effect of cell therapy fades away 8 weeks after cell injection. This phenomenon is in accordance with initial observations in patient studies [17]. This finding should trigger researchers towards novel applications and strategies of stem cell therapy (e.g. slow release agents, genetic engineering of stem cells, or repetitive injections overtime).

Effect of study design on study outcome

An overall beneficial effect of cardiac stem cell therapy has been observed in this analysis. However, this effect appears to be more pronounced in cohort studies as compared to RCT (LVEF; RCT 6.5% vs. Cohorts 8.9%). It is conceivable that cohort studies are designed for practical reasons and might systematically overestimate the effect of cell therapy.

The capability of animal studies to predict human clinical outcome have been questioned by some authors [18, 19]. However, the results from the animal RCT studies are comparable to clinical meta-analyses [3, 20] (RCT; LVEF 6,5% vs. 4%) indicating that ischemic large animal models are relevant for translational purposes.

Recommendations for future translational stem cell research

In view of clinical practice it is mandatory that pre-clinical studies are performed according to high standards. In our opinion, the following items should be reported

in pre-clinical studies for establishing standards for translational stem cell research with in mind the clinical horizon: randomized study design; blinded functional analysis; number of animals used in the study protocol must be clear and include the measured data before treatment and at the follow-up, mortality after treatment and during follow-up.

Over the next few years, adequately powered large animal studies and clinical trials should focus on transplantation of $\geq 10^7$ stem cells, other cell types than BM-MNC and later time point of injection (>1 week after MI). To maintain the beneficial effect on LVEF over time, repeated cell injections or the use of biomaterials to enhance survival of transplanted cells should be evaluated. No difference between species was observed and we therefore recommend the use of pigs to evaluate the effect of cell therapy since many studies are available for comparison. We suggest the use of IC in the setting of acute MI and TE for chronic MI since no difference was found in our meta-analysis between these transplantation techniques.

Meta-analyses of animal studies are not common, yet they are recommended in several settings[21] and can often guide research and clinical endeavours[22]. Performing pre-clinical meta-analysis may also be attractive to evaluate the effect of other therapies to design future (pre-)clinical trials.

Limitations

Limitations of meta-analysis are well known[23]. In particular, in our study the diversity in animal type, incidence of permanent occlusion, delivery method, time of injection after MI, follow-up after cell therapy and number of cells may play a role in the observed outcomes in the present study. However, multivariate analysis (used as an exploratory tool) showed no differences, but should be used with caution to generate new hypothesis. Heterogeneity may be present due to the extremely sensitive end-points chosen (all continuous: LVEF, EDV and ESV). By using random-effect analysis the risk of finding erroneous estimates is minimized. Although various imaging modalities have been used to measure our endpoints. Univariate analysis showed no significant difference between these techniques (P=0.44). Our analysis was based on study outcomes and we did not have access to individual data. Accordingly we provided mean values. As some studies did not report all data necessary for the analysis, effort was made to contact corresponding authors to complete the database: only 5 studies were finally excluded due to incomplete data.

To date, numerous human clinical trials have already been conducted in order to assess the efficacy and safety aspects of cardiac stem cell therapy[3]. Obviously, differences exist between large animal models and clinical practice. Healthy young large animals differ from older patients with long standing coronary artery disease, and frequently comorbidities (e.g. diabetes, hypertension, renal failure) are present. Consequently, many patients are routinely treated with other drugs (e.g. ACE-inhibitors, beta-blockers, anti-diabetic medication), in contrast to research animals.

Furthermore, autologous stem cells extracted from young large animals are 'fresh' whereas cells from patients are 'aged'. Finally, duration of follow-up is relatively short in animal studies. Despite these differences we have shown that pre-clinical data are highly relevant to predict outcome for clinical trials.

CONCLUSIONS

To the best of our knowledge, this is the first systematic review and meta-analysis in large animal models to evaluate the effect of cell therapy in ischemic heart disease. This analysis showed that large animal models are valid to predict outcome of clinical trials. Moreover, the results showed that cardiac cell therapy is safe, led to an improved LVEF and revealed important clues for designing (pre-) clinical trials.

Funding

This work was supported by the Netherlands Heart Foundation '[2003B07304 and 2010T025]', BSIK program "Dutch Program for Tissue Engineering", '[grant 6746]', and a Bekalis price (PD). This research also forms part of the Project P1.04 SMARTCARE of the research program of the BioMedical Materials institute, co-funded by the Dutch Ministry of Economic Affairs.

Conflict of Interest: none declared.

REFERENCES

1. Rosamond W, Flegal K, Furie K, Go A, Greenlund K, Haase N et al. for the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart Disease and Stroke Statistics--2008 Update: A Report From the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 2008;117:e25-146.

2. World Health Organization. The atlas of heart disease and stroke. 2007. p. 48-49.

3 Abdel-Latif A, Bolli R, Tleyjeh IM, Montori VM, Perin EC, Hornung CA et al. Adult Bone Marrow-Derived Cells for Cardiac Repair: A Systematic Review and Meta-analysis. Arch Intern Med 2007;167:989-997.

4. Bartunek J, Dimmeler S, Drexler H, Fernández-Avilés F, Galinanes M, Janssens S et al. The consensus of the task force of the European Society of Cardiology concerning the clinical investigation of the use of autologous adult stem cells for repair of the heart. Eur Heart J 2006; 27:1338-1340.

5. Jüni P, Altman DG, Egger M. Assessing the quality of controlled clinical trials. BMJ 2001;323:42-46.

6. Green S, Higgins JP, Alderson P, Clarke M, Mulrow CD, Oxman AD. Cochrane Handbook for Systematic Reviews of Interventions: Cochrane Book Series. John Wiley & Sons, Ltd; 2008.

7. Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003;327:557-560.

8. Mansour S, Vanderheyden M, De Bruyne B, Vandekerckhove B, Delrue L, Van Haute I et al. Intracoronary Delivery of Hematopoietic Bone Marrow Stem Cells and Luminal Loss of the Infarct-Related Artery in Patients With Recent Myocardial Infarction. J Am Coll Cardiol 2006; 47:1727-1730.

9. Martin-Rendon E, Brunskill SJ, Hyde CJ, Stanworth SJ, Mathur A, Watt SM. Autologous bone marrow stem cells to treat acute myocardial infarction: a systematic review. Eur Heart J 2008;29:1807-1818.

10. Vrijsen KR, Sluijter JP, Schuchardt MW, van Balkom BW, Noort WA, Chamuleau SA et al. Cardiomyocyte progenitor cell-derived exosomes stimulate migration of endothelial cells. J Cell Mol Med 2010.

11. Lai RC, Arslan F, Lee MM, Sze NS, Choo A, Chen TS et al. Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. Stem Cell Res 2010;4:214-222.

12. Gnecchi M, Zhang Z, Ni A, Dzau VJ. Paracrine Mechanisms in Adult Stem Cell Signaling and Therapy. Circ Res 2008;103:1204-1219.

13. Pirolo JS, Hutchins GM, Moore GW. Infarct expansion: pathologic analysis of 204 patients with a single myocardial infarct. J Am Coll Cardiol 1986;7:349-354.

14. Schachinger V, Erbs S, Elsasser A, Haberbosch W, Hambrecht R, Holschermann H et al. Intracoronary Bone Marrow-Derived Progenitor Cells in Acute Myocardial Infarction. N Engl J Med 2006;355:1210-1221.

15. Brunskill SJ, Hyde CJ, Doree CJ, Watt SM, Martin-Rendon E. Route of delivery and baseline left ventricular ejection fraction, key factors of bone-marrow-derived cell therapy for ischaemic heart disease. Eur J Heart Fail 2009;11:887-896.

16. Zhang S, Sun A, Xu D, Yao K, Huang Z, Jin H et al. Impact of timing on efficacy and safetyof intracoronary autologous bone marrow stem cells transplantation in acute myocardial infarction: a pooled subgroup analysis of randomized controlled trials. Clin Cardiol 2009;32:458-466.

17. Schaefer A, Zwadlo C, Fuchs M, Meyer GP, Lippolt P, Wollert KC et al. Long-term effects of intracoronary bone marrow cell transfer on diastolic function in patients after acute myocardial infarction: 5-year results from the randomized-controlled BOOST trial−an echocardiographic study. Eur J Echocardiogr 2010;11:165-171.

18. Lafont A, Faxon D. Why do animal models of post-angioplasty restenosis sometimes poorly predict the outcome of clinical trials? Cardiovas Res 1998;39:50-59.

19. Rosenzweig A. Cardiac Cell Therapy -- Mixed Results from Mixed Cells. N Engl J Med 2006;355:1274-1277.

20. Jiang M, He B, Zhang Q, Ge H, Zang MH, Han ZH et al. Randomized controlled trials on the therapeutic effects of adult progenitor cells for myocardial infarction: meta-analysis. Expert Opin Biol Ther 2010;10:667-680.

21. Sandercock P, Roberts I. Systematic reviews of animal experiments. The Lancet 2002;360:586.

22. Biondi-Zoccai GGL, Abbate A, Parisi Q, Agostoni P, Burzotta F, Sandroni C et al. Is vasopressin superior to adrenaline or placebo in the management of cardiac arrest? A meta-analysis. Resuscitation 2003;59:221-224.

23. Petitti DB. Meta-analysis, decision analysis, and cost-effectiveness analysis: methods for quantitative synthesis in medicine. New York, NY: Oxford University Press; 2000.

24. Bel A, Messas E, Agbulut O, Richard P, Samuel JL, Bruneval P et al. Transplantation of autologous fresh bone marrow into infarcted myocardium: a word of caution. Circulation 2003;108 Suppl 1:II247-II252.

25. Brasselet C, Morichetti MC, Messas E, Carrion C, Bissery A, Bruneval P et al. Skeletal myoblast transplantation through a catheter-based coronary sinus approach: an effective means of improving function of infarcted myocardium. Eur Heart J 2005;26:1551-1556.

26. Chachques JC, Duarte F, Cattadori B, Shafy A, Lila N, Chatellier G et al. Angiogenic growth factors and/or cellular therapy for myocardial regeneration: a comparative study. J Thorac Cardiovasc Surg 2004;128:245-253.

27. Chen SL, Zhu CC, Liu YQ, Tang LJ, Yi L, Yu BJ et al. Mesenchymal stem cells genetically modified with the angiopoietin-1 gene enhanced arteriogenesis in a porcine model of chronic myocardial ischaemia. J Int Med Res 2009;37:68-78.

28. deSilva R, Raval AN, Hadi M, Gildea KM, Bonifacino AC, Yu ZX et al. Intracoronary infusion of autologous mononuclear cells from bone marrow or granulocyte colony-stimulating factor-

mobilized apheresis product may not improve remodelling, contractile function, perfusion, or infarct size in a swine model of large myocardial infarction. Eur Heart J 2008;29:1772-1782.

29. Dixon JA, Gorman RC, Stroud RE, Bouges S, Hirotsugu H, Gorman JH, III et al. Mesenchymal cell transplantation and myocardial remodeling after myocardial infarction. Circulation 2009;120:S220-S229.

30. Doyle B, Sorajja P, Hynes B, Kumar AH, Araoz PA, Stalboerger PG et al. Progenitor cell therapy in a porcine acute myocardial infarction model induces cardiac hypertrophy, mediated by paracrine secretion of cardiotrophic factors including TGFbeta1. Stem Cells Dev 2008;17:941-951.

31. Gavira JJ, Perez-Ilzarbe M, Abizanda G, Garcia-Rodriguez A, Orbe J, Paramo JA et al. A comparison between percutaneous and surgical transplantation of autologous skeletal myoblasts in a swine model of chronic myocardial infarction. Cardiovasc Res 2006;71:744-753.

32. Ghodsizad A, Niehaus M, Kogler G, Martin U, Wernet P, Bara C et al. Transplanted human cord blood-derived unrestricted somatic stem cells improve left-ventricular function and prevent left-ventricular dilation and scar formation after acute myocardial infarction. Heart 2009;95:27-35.

33. Ghostine S, Carrion C, Souza LC, Richard P, Bruneval P, Vilquin JT et al. Long-term efficacy of myoblast transplantation on regional structure and function after myocardial infarction. Circulation 2002;106:I131-I136.

34. Gyongyosi M, Blanco J, Marian T, Tron L, Petnehazy O, Petrasi Z et al. Serial noninvasive in vivo positron emission tomographic tracking of percutaneously intramyocardially injected autologous porcine mesenchymal stem cells modified for transgene reporter gene expression. Circ Cardiovasc Imaging 2008;1:94-103.

35. Hagikura K, Fukuda N, Yokoyama SI, Yuxin L, Kusumi Y, Matsumoto T et al. Low invasive angiogenic therapy for myocardial infarction by retrograde transplantation of mononuclear cells expressing the VEGF gene. Int J Cardiol 2009;142:56-64.

36. Haider HK, Ye L, Jiang S, Ge R, Law PK, Chua T et al. Angiomyogenesis for cardiac repair using human myoblasts as carriers of human vascular endothelial growth factor. J Mol Med 2004;82:539-549.

37. Halkos ME, Zhao ZQ, Kerendi F, Wang NP, Jiang R, Schmarkey LS et al. Intravenous infusion of mesenchymal stem cells enhances regional perfusion and improves ventricular function in a porcine model of myocardial infarction. Basic Res Cardiol 2008;103:525-536.

38. Hamamoto H, Gorman JH, III, Ryan LP, Hinmon R, Martens TP, Schuster MD et al. Allogeneic mesenchymal precursor cell therapy to limit remodeling after myocardial infarction: the effect of cell dosage. Ann Thorac Surg 2009;87:794-801.

39. Hashemi SM, Ghods S, Kolodgie FD, Parcham-Azad K, Keane M, Hamamdzic D et al. A placebo controlled, dose-ranging, safety study of allogenic mesenchymal stem cells injected by endomyocardial delivery after an acute myocardial infarction. Eur Heart J 2008;29:251-259.

40. He KL, Yi GH, Sherman W, Zhou H, Zhang GP, Gu A et al. Autologous skeletal myoblast transplantation improved hemodynamics and left ventricular function in chronic heart failure dogs.

J Heart Lung Transplant 2005;24:1940-1949.

41. Jiang Y, Chen L, Tang Y, Ma G, Shen C, Qi C et al. HO-1 gene overexpression enhances the beneficial effects of superparamagnetic iron oxide labeled bone marrow stromal cells transplantation in swine hearts underwent ischemia/reperfusion: an MRI study. Basic Res Cardiol 2010;105:431-442.

42. Johnston PV, Sasano T, Mills K, Evers R, Lee ST, Smith RR et al. Engraftment, Differentiation, and Functional Benefits of Autologous Cardiosphere-Derived Cells in Porcine Ischemic Cardiomyopathy. Circulation 2009;120:1075-1083.

43. Kawamoto A, Tkebuchava T, Yamaguchi J, Nishimura H, Yoon YS, Milliken C et al. Intramyocardial transplantation of autologous endothelial progenitor cells for therapeutic neovascularization of myocardial ischemia. Circulation 2003;107:461-468.

44. Kim BO, Tian H, Prasongsukarn K, Wu J, Angoulvant D, Wnendt S et al. Cell transplantation improves ventricular function after a myocardial infarction: a preclinical study of human unrestricted somatic stem cells in a porcine model. Circulation 2005;112:I96-104.

45. Li CJ, Gao RL, Yang YJ, Hu FH, Yang WX, You SJ et al. Implantation of autologous bone marrow mononuclear cells into ischemic myocardium enhances coronary capillaries and systolic function in miniswine. Chin Med Sci J 2008;23:234-238.

46. Li SR, Qi XY, Hu FL, Zhang JQ, Wang TH, Dang Y et al. Mechanisms of improvement of left ventricle remodeling by trans-planting two kinds of autologous bone marrow stem cells in pigs. Chin Med J (Engl) 2008;121:2403-2409.

47. Lim SY, Kim YS, Ahn Y, Jeong MH, Hong MH, Joo SY et al. The effects of mesenchymal stem cells transduced with Akt in a porcine myocardial infarction model. Cardiovasc Res 2006;70:530-542.

48. Makela J, Ylitalo K, Lehtonen S, Dahlbacka S, Niemela E, Kiviluoma K et al. Bone marrow-derived mononuclear cell transplantation improves myocardial recovery by enhancing cellular recruitment and differentiation at the infarction site. J Thorac Cardiovasc Surg 2007;134:565-573.

49. Makkar RR, Price MJ, Lill M, Frantzen M, Takizawa K, Kleisli T et al. Intramyocardial injection of allogenic bone marrow-derived mesenchymal stem cells without immunosuppression preserves cardiac function in a porcine model of myocardial infarction. J Cardiovasc Pharmacol Ther 2005;10:225-233.

50. Memon IA, Sawa Y, Miyagawa S, Taketani S, Matsuda H. Combined autologous cellular cardiomyoplasty with skeletal myoblasts and bone marrow cells in canine hearts for ischemic cardiomyopathy. J Thorac Cardiovasc Surg 2005;130:646-653.

51. Menard C, Hagege AA, Agbulut O, Barro M, Morichetti MC, Brasselet C et al. Transplantation of cardiac-committed mouse embryonic stem cells to infarcted sheep myocardium: a preclinical study. Lancet 2005;366:1005-1012.

52. Messas E, Bel A, Morichetti MC, Carrion C, Handschumacher MD, Peyrard S et al. Autologous myoblast transplantation for chronic ischemic mitral regurgitation. J Am Coll Cardiol 2006;47:2086-2093.

53. Moelker AD, Baks T, van den Bos EJ, van Geuns RJ, de Feyter PJ, Duncker DJ et al. Reduction in infarct size, but no functional improvement after bone marrow cell administration in a porcine model of reperfused myocardial infarction. Eur Heart J 2006;27:3057-3064.

54. Moelker AD, Baks T, Wever KM, Spitskovsky D, Wielopolski PA, van Beusekom HM et al. Intracoronary delivery of umbilical cord blood derived unrestricted somatic stem cells is not suitable to improve LV function after myocardial infarction in swine. J Mol Cell Cardiol 2007;42:735-745.

55. Patila T, Ikonen T, Kankuri E, Uutela A, Lommi J, Krogerus L et al. Improved diastolic function after myoblast transplantation in a model of ischemia-infarction. Scand Cardiovasc J 2009;43:100-109.

56. Perin EC, Silva GV, Assad JA, Vela D, Buja LM, Sousa AL et al. Comparison of intracoronary and transendocardial delivery of allogeneic mesenchymal cells in a canine model of acute myocardial infarction. J Mol Cell Cardiol 2008;44:486-495.

57. Price MJ, Chou CC, Frantzen M, Miyamoto T, Kar S, Lee S et al. Intravenous mesenchymal stem cell therapy early after reperfused acute myocardial infarction improves left ventricular function and alters electrophysiologic properties. Int J Cardiol 2006;111:231-239.

58. Qi CM, Ma GS, Liu NF, Shen CX, Chen Z, Liu XJ et al. Transplantation of magnetically labeled mesenchymal stem cells improves cardiac function in a swine myocardial infarction model. Chin Med J (Engl) 2008;20;121:544-550.

59. Qian H, Yang Y, Huang J, Gao R, Dou K, Yang G et al. Intracoronary delivery of autologous bone marrow mononuclear cells radiolabeled by 18F-fluoro-deoxy-glucose: tissue distribution and impact on post-infarct swine hearts. J Cell Biochem 2007;102:64-74.

60. Quevedo HC, Hatzistergos KE, Oskouei BN, Feigenbaum GS, Rodriguez JE, Valdes D et al. Allogeneic mesenchymal stem cells restore cardiac function in chronic ischemic cardiomyopathy via trilineage differentiating capacity. Proc Natl Acad Sci U S A 2009;106:14022-14027.

61. Schneider C, Jaquet K, Geidel S, Rau T, Malisius R, Boczor S et al. Transplantation of Bone Marrow-Derived Stem Cells Improves Myocardial Diastolic Function: Strain Rate Imaging in a Model of Hibernating Myocardium. J Am Soc Echocardiogr 2009;22:1180-1189.

62. Schuleri KH, Amado LC, Boyle AJ, Centola M, Saliaris AP, Gutman MR et al. Early improvement in cardiac tissue perfusion due to mesenchymal stem cells. Am J Physiol Heart Circ Physiol 2008;294:H2002-H2011.

63. Schuleri KH, Feigenbaum GS, Centola M, Weiss ES, Zimmet JM, Turney J et al. Autologous mesenchymal stem cells produce reverse remodelling in chronic ischaemic cardiomyopathy. Eur Heart J 2009;30:2722-2732.

64. Sheu JJ, Yuen CM, Sun CK, Chang LT, Yen CH, Chiang CH et al. Six-month angiographic study of immediate autologous bone marrow mononuclear cell implantation on acute anterior wall myocardial infarction using a mini-pig model. Int Heart J 2009;50:221-234.

65. Thompson CA, Reddy VK, Srinivasan A, Houser S, Hayase M, Davila A et al. Left ventricular functional recovery with percutaneous, transvascular direct myocardial delivery of bone marrow-derived cells. J Heart Lung Transplant 2005;24:1385-1392.

66. Tomita S, Mickle DA, Weisel RD, Jia ZQ, Tumiati LC, Allidina Y et al. Improved heart function with myogenesis and angiogenesis after autologous porcine bone marrow stromal cell transplantation. J Thorac Cardiovasc Surg 2002;123:1132-1140.

67. Valina C, Pinkernell K, Song YH, Bai X, Sadat S, Campeau RJ et al. Intracoronary administration of autologous adipose tissue-derived stem cells improves left ventricular function, perfusion, and remodelling after acute myocardial infarction. Eur Heart J 2007;28:2667-2677.

68. Wang X, Jameel MN, Li Q, Mansoor A, Qiang X, Swingen C et al. Stem cells for myocardial repair with use of a transarterial catheter. Circulation 2009;120:S238-S246.

69. Yang YJ, Qian HY, Huang J, Li JJ, Gao RL, Dou KF et al. Combined Therapy With Simvastatin and Bone Marrow-Derived Mesenchymal Stem Cells Increases Benefits in Infarcted Swine Hearts. Arterioscler Thromb Vasc Biol 2009;29:2076-2082.

70. Yang ZJ, Ma DC, Wang W, Xu SL, Zhang YQ, Chen B et al. Experimental study of bone marrow-derived mesenchymal stem cells combined with hepatocyte growth factor transplantation via noninfarct-relative artery in acute myocardial infarction. Gene Ther 2006;13:1564-1568.

71. Yang ZJ, Ma DC, Wang W, Xu SL, Zhang YQ, Chen B et al. Neovascularization and cardiomyocytes regeneration in acute myocardial infarction after bone marrow stromal cell transplantation: comparison of infarct-relative and noninfarct-relative arterial approaches in swine. Clin Chim Acta 2007;381:114-118.

72. Yi F, Guo WY, Lu AL, Wang HC, Li H, Li WJ et al. Vascular endothelial growth factor expressing mesenchymal stem cells improves cardiac function in chronic myocardial infarction in pigs. Chin Med J (Engl) 2006;119:1664-1668.

73. Yokoyama S, Fukuda N, Li Y, Hagikura K, Takayama T, Kunimoto S et al. A strategy of retrograde injection of bone marrow mononuclear cells into the myocardium for the treatment of ischemic heart disease. J Mol Cell Cardiol 2006;40:24-34.

74. Zeng L, Hu Q, Wang X, Mansoor A, Lee J, Feygin J et al. Bioenergetic and functional consequences of bone marrow-derived multipotent progenitor cell transplantation in hearts with postinfarction left ventricular remodeling. Circulation 2007;115:1866-1875.

75. Zhang S, Ge J, Zhao L, Qian J, Huang Z, Shen L et al. Host vascular niche contributes to myocardial repair induced by intracoronary transplantation of bone marrow CD34+ progenitor cells in infarcted swine heart. Stem Cells 2007;25:1195-1203.

Author	Number of Groups	Subgroups
Dixon et al.(1) 2009	4	• $2.5*10^7$ • $7.5*10^7$ • $2.2*10^8$ • $4.5*10^8$
Halkos et al.(2) 2008	3	• $3.9*10^7$ • $1.1*10^8$ • $3.7*10^8$
Hamamoto et al.(3) 2009	4	• $2.3*10^7$ • $7.2*10^7$ • $2.1*10^8$ • $4.4*10^8$
Hashemi et al.(4) 2008	3	• $2.4*10^7$ • $2.4*10^7$ • $4.4*10^8$
He et al.(5) 2005	2	• Surgical • TE
Kawamoto et al.(6) 2003	2	• MNC • EPC
Li et al.(7) 2008	2	• MSC • BM-MNC
Memon et al.(8) 2005	2	• Skeletal myoblasts • BM-MNC
Moelker et al.(9) 2006	2	• Bone marrow

		• BM-MNC
Perin et al.(10) 2008	2	• IC
		• TE
Qi et al. (11)2008	2	• Labeled MSC
		• Unlabeled MSC
Schneider et al.(12)	3	• Autologous MSC
2009		• Allogeneic MSC
		• BM-MNC
Sheu et al.(13) 2009	2	• Infarct- relative arterial approach
		• Noninfarct-relative arterial approach
Valina et al.(14) 2007	2	• MSC
		• ADSC
Yang et al.(15) 2007	2	• Infarct- relative arterial approach
		• Non-infarct relative arterial approach
Yokoyama et al.(16)	2	• AMI group
2006		• OMI group
Zhang et al.(17) 2007	2	• Rentrop score=0
		• Rentrop score =1

Supplemental table 1. Details of the subgroups of the enrolled studies.
ADSC= Adipose tissue derived stem cells; AMI= Acute myocardial infarction; BM-MNC= Bone marrow mononuclear cells; EPC=Endothelial progenitor cells; IC= Intracoronary infusion; MNC= Peripheral mononuclear cells; MSC= Mesenchymal stem cells; TE= Trans-endocardial injection; OMI= Old myocardial infarction.

Author	RCT	Adequate allocation	Method of randomization described	Operator blinded	Analyst blinded
Bel et al.(18) 2003	Y	N	N	N	N
Brasselet et al.(19) 2005	Y	N	N	N	Y
Chacques et al.(20) 2004	Y	N	N	N	N
Chen et al.(21) 2009	Y	N	N	N	Y
De Silva et al.(22) 2008	N	N	N	Y	Y
Dixon et al.(1) 2009	Y	N	N	N	N
Doyle et al.(23) 2008	N	N	N	N	Y
Gavira et al.(24) 2006	N	N	N	N	Y
Ghodsizad et al.(25) 2007	Y	N	N	N	Y
Ghostine et al.(26) 2002	Y	N	N	N	N
Gyöngyösi et al.(27) 2008	Y	N	N	N	Y
Hagikura et al.(28) 2009	N	N	N	N	N
Haider et al.(29) 2004	Y	N	N	N	Y
Halkos et al.(2) 2008	Y	N	N	Y	Y
Hamamoto et al.(3) 2009	N	N	N	N	Y
Hashemi et al.(4) 2008	Y	N	N	N	N
He et al.(5) 2005	N	N	N	N	Y
Jiang et al.(30) 2010	Y	N	N	N	N
Johnston et al.(31) 2009	Y	N	N	N	N

Kawamoto et al.(6) 2003	N	N	N	N	Y
Kim et al.(32)2005	Y	N	N	N	N
Li et al.(33) 2008	Y	N	N	N	N
Li et al.(7) 2008	N	N	N	N	N
Lim et al.(34) 2006	N	N	N	N	N
Mäkelä et al.(35) 2007	Y	N	N	Y	Y
Makkar et al.(36) 2005	Y	N	N	N	Y
Memon et al.(8) 2005	N	N	N	N	N
Menard et al.(37) 2005	Y	N	N	N	N
Messas et al.(38) 2006	Y	N	N	N	Y
Moelker et al.(9) 2006	Y	N	N	N	N
Moelker et al.(39) 2007	Y	N	N	N	N
Patila et al.(40) 2009	Y	N	N	N	Y
Perin et al.(10) 2008	Y	N	N	N	Y
Price et al.(41) 2006	Y	N	N	N	N
Qi et al. (11)2008	Y	N	N	N	N
Qian et al.(42) 2007	Y	N	N	N	N
Quevedo et al.(43) 2009	N	N	N	N	N
Schneider et al.(12) 2009	Y	N	N	N	Y
Schuleri et al.(44) 2008	Y	N	N	N	Y
Schuleri et al.(45) 2009	Y	N	N	Y	N
Sheu et al.(13) 2009	N	N	N	N	Y
Thompson et al.(46) 2005	N	N	N	N	Y

Tomita et al. (47)2002	Y	N	N	N	N
Valina et al.(14) 2007	Y	Y	Y	Y	Y
Wang et al.(48) 2009	Y	N	N	N	N
Yang et al.(49) 2009	Y	N	N	N	N
Yang et al.(50) 2006	Y	N	N	N	N
Yang et al.(15) 2007	Y	N	N	N	N
Yi et al.(51) 2006	Y	N	N	N	N
Yokoyama et al.(16) 2006	Y	N	N	N	N
Zeng et al.(52) 2007	Y	N	N	N	N
Zhang et al.(17) 2007	N	N	N	N	N

Supplemental table 2. Methodological quality of the included studies according to the Jüni guidelines. RCT= Randomized trial; Yes=Y; No=N.

REFERENCES SUPPLEMENTS

1. Dixon JA, Gorman RC, Stroud RE, Bouges S, Hirotsugu H, Gorman JH, III et al. Mesenchymal cell transplantation and myocardial remodeling after myocardial infarction. Circulation 2009; 120:S220-S229.

2. Halkos ME, Zhao ZQ, Kerendi F, Wang NP, Jiang R, Schmarkey LS et al. Intravenous infusion of mesenchymal stem cells enhances regional perfusion and improves ventricular function in a porcine model of myocardial infarction. Basic Res Cardiol 2008;103:525-536.

3 Hamamoto H, Gorman JH, III, Ryan LP, Hinmon R, Martens TP, Schuster MD et al. Allogeneic mesenchymal precursor cell therapy to limit remodeling after myocardial infarction: the effect of cell dosage. Ann Thorac Surg 2009;87:794-801.

4. Hashemi SM, Ghods S, Kolodgie FD, Parcham-Azad K, Keane M, Hamamdzic D et al. A placebo controlled, dose-ranging, safety study of allogenic mesenchymal stem cells injected by endomyocardial delivery after an acute myocardial infarction. Eur Heart J 2008;29:251-259.

5. He KL, Yi GH, Sherman W, Zhou H, Zhang GP, Gu A et al. Autologous skeletal myoblast

transplantation improved hemodynamics and left ventricular function in chronic heart failure dogs. J Heart Lung Transplant 2005;24:1940-1949.

6. Kawamoto A, Tkebuchava T, Yamaguchi J, Nishimura H, Yoon YS, Milliken C et al. Intramyocardial transplantation of autologous endothelial progenitor cells for therapeutic neovascularization of myocardial ischemia. Circulation 2003;107:461-468.

7. Li SR, Qi XY, Hu FL, Zhang JQ, Wang TH, Dang Y et al. Mechanisms of improvement of left ventricle remodeling by trans-planting two kinds of autologous bone marrow stem cells in pigs. Chin Med J (Engl) 2008;121:2403-2409.

8. Memon IA, Sawa Y, Miyagawa S, Taketani S, Matsuda H. Combined autologous cellular cardiomyoplasty with skeletal myoblasts and bone marrow cells in canine hearts for ischemic cardiomyopathy. J Thorac Cardiovasc Surg 2005;130:646-653.

9. Moelker AD, Baks T, van den Bos EJ, van Geuns RJ, de Feyter PJ, Duncker DJ et al. Reduction in infarct size, but no functional improvement after bone marrow cell administration in a porcine model of reperfused myocardial infarction. Eur Heart J 2006;27:3057-3064.

10. Perin EC, Silva GV, Assad JA, Vela D, Buja LM, Sousa AL et al. Comparison of intracoronary and transendocardial delivery of allogeneic mesenchymal cells in a canine model of acute myocardial infarction. J Mol Cell Cardiol 2008;44:486-495.

11. Qi CM, Ma GS, Liu NF, Shen CX, Chen Z, Liu XJ et al. Transplantation of magnetically labeled mesenchymal stem cells improves cardiac function in a swine myocardial infarction model. Chin Med J (Engl) 2008;121:544-550.

12. Schneider C, Jaquet K, Geidel S, Rau T, Malisius R, Boczor S et al. Transplantation of Bone Marrow-Derived Stem Cells Improves Myocardial Diastolic Function: Strain Rate Imaging in a Model of Hibernating Myocardium. J Am Soc Echocardiogr 2009 ;22:1180-1189.

13. Sheu JJ, Yuen CM, Sun CK, Chang LT, Yen CH, Chiang CH et al. Six-month angiographic study of immediate autologous bone marrow mononuclear cell implantation on acute anterior wall myocardial infarction using a mini-pig model. Int Heart J 2009;50:221-234.

14. Valina C, Pinkernell K, Song YH, Bai X, Sadat S, Campeau RJ et al. Intracoronary administration of autologous adipose tissue-derived stem cells improves left ventricular function, perfusion, and remodelling after acute myocardial infarction. Eur Heart J 2007; 28:2667-2677.

15. Yang ZJ, Ma DC, Wang W, Xu SL, Zhang YQ, Chen B et al. Neovascularization and cardiomyocytes regeneration in acute myocardial infarction after bone marrow stromal cell transplantation: comparison of infarct-relative and noninfarct-relative arterial approaches in swine. Clin Chim Acta 2007;381:114-118.

16. Yokoyama S, Fukuda N, Li Y, Hagikura K, Takayama T, Kunimoto S et al. A strategy of retrograde injection of bone marrow mononuclear cells into the myocardium for the treatment of ischemic heart disease. J Mol Cell Cardiol 2006;40:24-34.

17. Zhang S, Ge J, Zhao L, Qian J, Huang Z, Shen L et al. Host vascular niche contributes to myocardial repair induced by intracoronary transplantation of bone marrow CD34+ progenitor cells in infarcted

swine heart. Stem Cells 2007;25:1195-1203.

18. Bel A, Messas E, Agbulut O, Richard P, Samuel J, Bruneval P et al. Transplantation of Autologous Fresh Bone Marrow Into Infarcted Myocardium: A Word of Caution. Circulation 2003;108:II-247.

19. Brasselet C, Morichetti MC, Messas E, Carrion C, Bissery A, Bruneval P et al. Skeletal myoblast transplantation through a catheter-based coronary sinus approach: an effective means of improving function of infarcted myocardium. Eur Heart J 2005;26:1551-1556.

20. Chachques JC, Duarte F, Cattadori B, Shafy A, Lila N, Chatellier G et al. Angiogenic growth factors and/or cellular therapy for myocardial regeneration: a comparative study. J Thorac Cardiovasc Surg 2004;128:245-253.

21. Chen SL, Zhu CC, Liu YQ, Tang LJ, Yi L, Yu BJ et al. Mesenchymal stem cells genetically modified with the angiopoietin-1 gene enhanced arteriogenesis in a porcine model of chronic myocardial ischaemia. J Int Med Res 2009;37:68-78.

22. deSilva R, Raval AN, Hadi M, Gildea KM, Bonifacino AC, Yu ZX et al. Intracoronary infusion of autologous mononuclear cells from bone marrow or granulocyte colony-stimulating factor-mobilized apheresis product may not improve remodelling, contractile function, perfusion, or infarct size in a swine model of large myocardial infarction. Eur Heart J 2008;29:1772-1782.

23. Doyle B, Sorajja P, Hynes B, Kumar AH, Araoz PA, Stalboerger PG et al. Progenitor cell therapy in a porcine acute myocardial infarction model induces cardiac hypertrophy, mediated by paracrine secretion of cardiotrophic factors including TGFbeta1. Stem Cells Dev 2008;17 :941-951.

24. Gavira JJ, Perez-Ilzarbe M, Abizanda G, Garcia-Rodriguez A, Orbe J, Paramo JA et al. A comparison between percutaneous and surgical transplantation of autologous skeletal myoblasts in a swine model of chronic myocardial infarction. Cardiovasc Res 2006;71:744-753.

25. Ghodsizad A, Niehaus M, Kogler G, Martin U, Wernet P, Bara C et al. Transplanted human cord blood-derived unrestricted somatic stem cells improve left-ventricular function and prevent left-ventricular dilation and scar formation after acute myocardial infarction. Heart 2009;95:27-35.

26. Ghostine S, Carrion C, Souza LC, Richard P, Bruneval P, Vilquin JT et al. Long-term efficacy of myoblast transplantation on regional structure and function after myocardial infarction. Circulation 2002;106:I131-I136.

27. Gyongyosi M, Blanco J, Marian T, Tron L, Petnehazy O, Petrasi Z et al. Serial noninvasive in vivo positron emission tomographic tracking of percutaneously intramyocardially injected autologous porcine mesenchymal stem cells modified for transgene reporter gene expression. Circ Cardiovasc Imaging 2008;1:94-103.

28. Hagikura K, Fukuda N, Yokoyama SI, Yuxin L, Kusumi Y, Matsumoto T et al. Low invasive angiogenic therapy for myocardial infarction by retrograde transplantation of mononuclear cells expressing the VEGF gene. Int J Cardiol 2009;142:56-64.

29. Haider HK, Ye L, Jiang S, Ge R, Law PK, Chua T et al. Angiomyogenesis for cardiac repair using human myoblasts as carriers of human vascular endothelial growth factor. J Mol Med 2004;82:539-549.

30. Jiang Y, Chen L, Tang Y, Ma G, Shen C, Qi C et al. HO-1 gene overexpression enhances the beneficial effects of superparamagnetic iron oxide labeled bone marrow stromal cells transplantation in swine hearts underwent ischemia/reperfusion: an MRI study. Basic Res Cardiol 2010;105:431-442.

31. Johnston PV, Sasano T, Mills K, Evers R, Lee ST, Smith RR et al. Engraftment, Differentiation, and Functional Benefits of Autologous Cardiosphere-Derived Cells in Porcine Ischemic Cardiomyopathy. Circulation 2009;120:1075-1083.

32. Kim BO, Tian H, Prasongsukarn K, Wu J, Angoulvant D, Wnendt S et al. Cell transplantation improves ventricular function after a myocardial infarction: a preclinical study of human unrestricted somatic stem cells in a porcine model. Circulation 2005;112:I96-104.

33. Li CJ, Gao RL, Yang YJ, Hu FH, Yang WX, You SJ et al. Implantation of autologous bone marrow mononuclear cells into ischemic myocardium enhances coronary capillaries and systolic function in miniswine. Chin Med Sci J 2008;23:234-238.

34. Lim SY, Kim YS, Ahn Y, Jeong MH, Hong MH, Joo SY et al. The effects of mesenchymal stem cells transduced with Akt in a porcine myocardial infarction model. Cardiovasc Res 2006; 70:530-542.

35. Makela J, Ylitalo K, Lehtonen S, Dahlbacka S, Niemela E, Kiviluoma K et al. Bone marrow-derived mononuclear cell transplantation improves myocardial recovery by enhancing cellular recruitment and differentiation at the infarction site. J Thorac Cardiovasc Surg 2007;134:565-573.

36. Makkar RR, Price MJ, Lill M, Frantzen M, Takizawa K, Kleisli T et al. Intramyocardial injection of allogenic bone marrow-derived mesenchymal stem cells without immunosuppression preserves cardiac function in a porcine model of myocardial infarction. J Cardiovasc Pharmacol Ther 2005;10:225-233.

37. Menard C, Hagege AA, Agbulut O, Barro M, Morichetti MC, Brasselet C et al. Transplantation of cardiac-committed mouse embryonic stem cells to infarcted sheep myocardium: a preclinical study. Lancet 2005;366:1005-1012.

38. Messas E, Bel A, Morichetti MC, Carrion C, Handschumacher MD, Peyrard S et al. Autologous myoblast transplantation for chronic ischemic mitral regurgitation. J Am Coll Cardiol 2006;47: 2086-2093.

39. Moelker AD, Baks T, Wever KM, Spitskovsky D, Wielopolski PA, van Beusekom HM et al. Intracoronary delivery of umbilical cord blood derived unrestricted somatic stem cells is not suitable to improve LV function after myocardial infarction in swine. J Mol Cell Cardiol 2007; 42:735-745.

40. Patila T, Ikonen T, Kankuri E, Uutela A, Lommi J, Krogerus L et al. Improved diastolic function after myoblast transplantation in a model of ischemia-infarction. Scand Cardiovasc J 2009;43:100-109.

41. Price MJ, Chou CC, Frantzen M, Miyamoto T, Kar S, Lee S et al. Intravenous mesenchymal stem cell therapy early after reperfused acute myocardial infarction improves left ventricular function and alters electrophysiologic properties. Int J Cardiol 2006;111:231-239.

42. Qian H, Yang Y, Huang J, Gao R, Dou K, Yang G et al. Intracoronary delivery of autologous bone marrow mononuclear cells radiolabeled by 18F-fluoro-deoxy-glucose: tissue distribution and impact on post-infarct swine hearts. J Cell Biochem 2007;102:64-74.

43. Quevedo HC, Hatzistergos KE, Oskouei BN, Feigenbaum GS, Rodriguez JE, Valdes D et al. Allogeneic mesenchymal stem cells restore cardiac function in chronic ischemic cardiomyopathy via trilineage differentiating capacity. Proc Natl Acad Sci U S A 2009;106: 14022-14027.

44. Schuleri KH, Amado LC, Boyle AJ, Centola M, Saliaris AP, Gutman MR et al. Early improvement in cardiac tissue perfusion due to mesenchymal stem cells. Am J Physiol Heart Circ Physiol 2008;294:H2002-H2011.

45. Schuleri KH, Feigenbaum GS, Centola M, Weiss ES, Zimmet JM, Turney J et al. Autologous mesenchymal stem cells produce reverse remodelling in chronic ischaemic cardiomyopathy. Eur Heart J 2009;30:2722-2732.

46. Thompson CA, Reddy VK, Srinivasan A, Houser S, Hayase M, Davila A et al. Left ventricular functional recovery with percutaneous, transvascular direct myocardial delivery of bone marrow-derived cells. J Heart Lung Transplant 2005;24:1385-1392.

47. Tomita S, Mickle DA, Weisel RD, Jia ZQ, Tumiati LC, Allidina Y et al. Improved heart function with myogenesis and angiogenesis after autologous porcine bone marrow stromal cell transplantation. J Thorac Cardiovasc Surg 2002;123:1132-1140.

48. Wang X, Jameel MN, Li Q, Mansoor A, Qiang X, Swingen C et al. Stem cells for myocardial repair with use of a transarterial catheter. Circulation 2009;120:S238-S246.

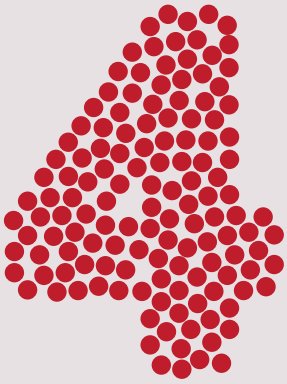
49. Yang YJ, Qian HY, Huang J, Li JJ, Gao RL, Dou KF et al. Combined Therapy With Simvastatin and Bone Marrow-Derived Mesenchymal Stem Cells Increases Benefits in Infarcted Swine Hearts. Arterioscler Thromb Vasc Biol 2009;29:2076-2082.

50. Yang ZJ, Ma DC, Wang W, Xu SL, Zhang YQ, Chen B et al. Experimental study of bone marrow-derived mesenchymal stem cells combined with hepatocyte growth factor transplantation via noninfarct-relative artery in acute myocardial infarction. Gene Ther 2006; 13:1564-1568.

51. Yi F, Guo WY, Lu AL, Wang HC, Li H, Li WJ et al. Vascular endothelial growth factor expressing mesenchymal stem cells improves cardiac function in chronic myocardial infarction in pigs. Chin Med J (Engl) 2006;119:1664-1668.

52. Zeng L, Hu Q, Wang X, Mansoor A, Lee J, Feygin J et al. Bioenergetic and functional consequences of bone marrow-derived multipotent progenitor cell transplantation in hearts with postinfarction left ventricular remodeling. Circulation 2007;115:1866-1875.


T.I.G van der Spoel



TRANSENDOCARDIAL CELL INJECTION IS NOT SUPERIOR TO INTRACORONARY INFUSION IN A PORCINE MODEL OF ISCHEMIC CARDIOMYOPATHY: A STUDY ON DELIVERY EFFICIENCY.

Tycho I.G. van der Spoel, MD[1]
Krijn R. Vrijsen, MSc[1]
Stefan Koudstaal, MD[1]
Joost P.G. Sluijter, PhD[1,2]
Johannes F.W. Nijsen, PhD[3]
Hugo W. de Jong, PhD[3]
Imo E. Hoefer, MD PhD[4]
Maarten Jan M. Cramer, MD PhD[1]
Pieter A. Doevendans, MD PhD[1,2]
Eric van Belle, MD PhD[1]
Steven A.J. Chamuleau, MD PhD[1]

[1] Department of Cardiology, Division Heart and Lungs, University Medical Center Utrecht, Utrecht, the Netherlands.
[2] Interuniversity Cardiology Institute of the Netherlands (ICIN), Utrecht, the Netherlands.
[3] Department of Radiology and Nuclear Medicine, University Medical Center Utrecht, Utrecht, the Netherlands.
[4] Department of Experimental Cardiology, Division Heart and Lungs, University Medical Center Utrecht, Utrecht, the Netherlands.

ABSTRACT

Background: Stem cell therapy is a new strategy for chronic ischemic heart disease in patients. However, no consensus exists on the most optimal delivery strategy. This randomized study was designed to assess cell delivery efficiency of 3 clinical relevant strategies: intracoronary (IC) and transendocardial (TE) using electromechanical mapping guidance (NOGA) compared to surgical delivery in a chronic pig model of ischemia-reperfusion injury.

Methods: Twenty-four animals underwent delivery of 10^7 autologous Indium-oxine labeled bone marrow-derived mesenchymal stem cells (MSC) 4 weeks after infarction and were randomized to 1 of 3 groups (n=8 each group): IC, TE or surgical delivery (reference group). Primary endpoint was defined as percentage (%) of injected dose per organ and assessed by *in vivo* gamma-emission counting. In addition, troponin and coronary flow were assessed before and after MSC injection.

Results: Blinded endpoint analysis showed no significant difference in efficiency after surgical (16±4%), IC (11±1%) and TE (11±3%) (P=0.52) injections. IC showed less variability in efficiency compared with TE and surgical injection. Overall, TE injection showed less distribution of MSC to visceral organs compared with other modalities. Troponin rise and intracoronary flow did not differ between the percutaneous groups.

Conclusion: This randomized study showed no significant difference in cell delivery efficiency to the myocardium in a clinically relevant ischemic large animal model between IC and TE delivery. In addition, no differences in safety profile were observed. These results are important in view of the choice of percutaneous cell delivery modality in future clinical stem cell trials.

INTRODUCTION

After myocardial infarction (MI), chronically ischemic myocardium may result in variable degrees of scar tissue. Native endogenous repair mechanisms are insufficient to prevent cardiac remodelling to occur, consequently infarct-related heart failure remains a major cause of morbidity and mortality[1]. Cell therapy emerged as an innovative and attractive therapeutic approach for patients with chronic myocardial ischemia. The ultimate goal of this treatment is to support and enhance the endogenous repair mechanisms by replacing dysfunctional cardiomyocytes and inducing angiogenesis.

A modest beneficial effect was observed in clinical and pre-clinical studies [2, 3]. One of the critical issues for the limited success of stem cell based therapy for myocardial repair is an efficient method for cell delivery[4]. Currently, two percutaneous approaches (e.g. intracoronary (IC) and transendocardial (TE) delivery) have been applied for treatment with different cell populations in patients with chronic ischemia[5-8].

It is suggested that TE injection is superior to IC in terms of efficiency and safety. This is based on personal experience and non-blinded studies [9, 10]. However, a direct randomized comparison between IC and TE using the NOGA system with blinded endpoint analysis in a chronic MI model has not been performed. Surgical injection is considered as a reference strategy, because direct visualization of the area of interest, and direct monitoring of the injection is possible. A (pre-) clinically applicable method for accurate quantification of cell retention is direct cell radiolabeling by Indium-111 oxine (^{111}In). This allows determination of cell transplantation efficiency, and thereby enables optimization of cell delivery. Therefore, our primary objective was to determine the most efficacious cell delivery technique in a randomized comparison between these clinically available transplantation modalities in a chronic ischemic large animal model.

MATERIALS AND METHODS

Animals

Twenty-four female Dutch Landrace pigs (±70 kg) received humane care in compliance with the *"Guide for the Care and Use of Laboratory Animals,"* published by the National Institutes of Health (National Institutes of Health publication 85-23, revised 1985). The study protocol was approved by the Animal Experimentation Committee of the University of Utrecht.

Anesthesia and euthanasia

Animals were anesthetized in the supine position and intubated with an endotracheal tube. The animals were mechanically ventilated with the use of a positive-pressure ventilator with a mix of oxygen and air (FiO2 0.5). General anesthesia/analgesia was maintained with midazolam (0.7 mg kg^{-1} h^{-1}), sufentanyl citrate (2 μg kg^{-1} h^{-1}) and

pancuronium bromid (0.1 mg kg^{-1} h^{-1}). Metoprolol was administered intravenously (5 mg) to reduce the mechanical stress of the heart. The day before operation 160 mg acetylic salicylic acid and a bolus of 75 mg clopidogrel was administered. During surgery, animals were anticoagulated with heparin (ACT>250s). At the end of the experiment the animals were euthanized by pentobarbital overdose.

Myocardial infarction procedure

During the entire procedure, electrocardiogram, arterial pressure and capnogram were continuously monitored. MI was created by temporary proximal ligation of the left circumflex artery (LCX) for 75 minutes as previously described[11]. To prevent ventricular arrhythmias, 300 mg amiodarone intravenously was given.

Randomized comparison on delivery efficiency

In 24 healthy animals, MI was surgically induced after median sternotomy. Four weeks later the animals received autologous cell transplantation and were sacrificed after nuclear imaging (Figure 1). After surgery, animals were randomly assigned to 1 of 3 groups (n=8 per group): IC delivery, TE delivery or surgical delivery (reference group). The randomization scheme was stored in a sealed envelope and retrieved after induction of MI by a person not involved in the study. After recovery, the animals received daily an oral dose of 50 mg metoprolol, 400 mg amiodarone, 75 mg clopidogrel and 160 mg acetylic salicylic acid until termination to prevent thrombosis and arrhythmias. Primary blinded endpoint was defined as percentage (%) of injected dose per organ derived from whole-body images observed at 4 hours after injection. To evaluate myocardial damage of percutaneous interventions, blood samples (2.5 mL) were collected after MI, before and 6 hours after the intervention for the measurement of plasma concentration of cTnI.

Cell culture and labeling

A total of 20-25 mL bone marrow was extracted from the sternum by a heparinized syringe before creating MI. Bone marrow-derived mesenchymal stem cells (MSC) were isolated by Ficoll density gradient centrifugation. Autologous MSC were isolated and cultured in M-199 (Lonza, Verviers, Belgium) supplemented with 10% FBS, heparin and 1% penicillin/ streptomyocin. The cells were incubated at 37°C and medium was changed every 3 days. Cells were cultured in 75 cm^2 flask and passaged when they reached confluence till passage 5-7. MSC were frozen in 10% DMSO and 90% culture medium. MSC were characterized as previously described[12]. Seven days prior to transplantation cells were thawed, plated in flasks and grown to confluency. At the day of cell delivery, before trypanization, cells were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE, Invitrogen, Carlsbad, CA, USA) according to manufactures protocol.

Subsequently, MSC (10^7) were labeled with 30 MBq ^{111}In at 37°C for 20 minutes.

After incubation, cells were washed three times with HANKS buffer (Invitrogen, Carlsbad, CA, USA) to remove unbound label. Radiolabel uptake efficiency was measured with a dose calibrator (Veenstra, Joure, the Netherlands). After labeling, cell viability was assessed via trypan-blue (Sigma-Aldrich, St. Louis, MO, USA) counting. Before injection, MSC were resuspended in 2 or 10 mL PBS, depending on the delivery technique.

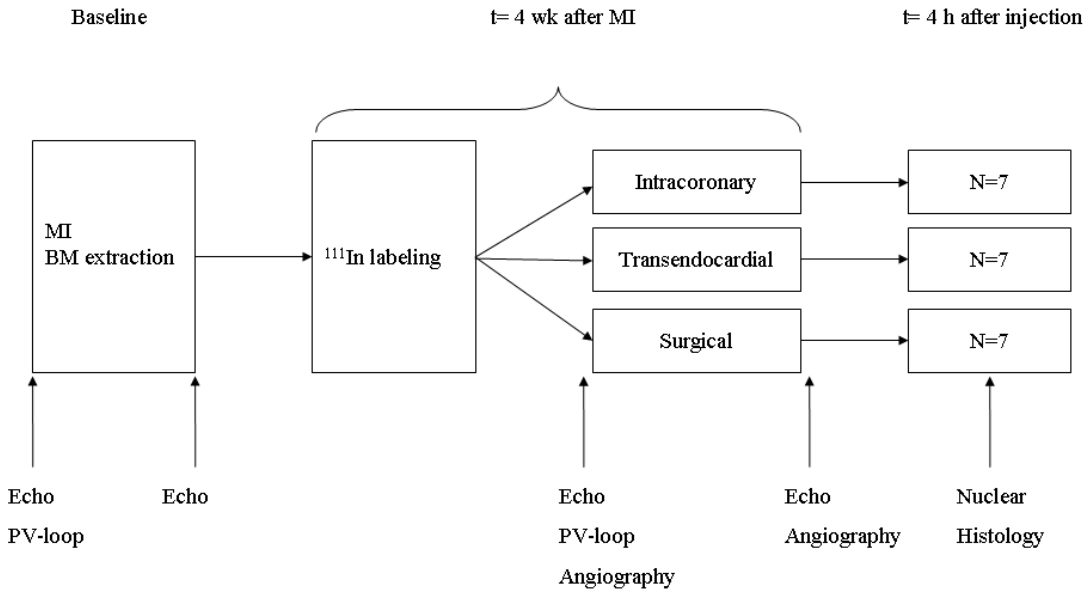


Figure 1. Study design
Intracoronary infusion was performed by stop-flow technique and transendocardial injection by using electromechanical mapping guidance (NOGA). Surgical injection was used as a reference group in this randomized study. BM= Bone marrow; MI= myocardial infarction; ^{111}In= Indium; PV loop=pressure-volume loop.

Intracoronary delivery

Four weeks after MI an over-the-wire balloon (Boston Scientific Corp, Natick, MA, USA) of equivalent size to the proximal LCX artery was placed. The balloon was inflated at low pressure (2 atm) at the same location and 3.3 mL of cell suspension was infused over 30 to 45 seconds. The angioplasty balloon was deflated after 3 minutes. This procedure was repeated for 3 times and a total of 10 mL (10^7 cells) was infused. After the procedure, coronary angiography was performed to confirm vessel patency using the Thrombolysis in Myocardial Infarction (TIMI) score[13]. Blinded image analysis was performed by an independent observer not involved in the study protocol.

Transendocardial delivery

After placement of an 8-F sheath into the femoral artery, a 3-dimensional electromechanical map of the left ventricle (LV) was obtained using the NOGA system (Biosense Webster, Cordis, Johnson & Johnson, USA) as described previously[14, 15]. First, an electromechanical map was obtained by retrograde passage of the catheter through the aortic valve into the cavity of the LV. Next, 10 TE injections of approximately 0.2 mL were slowly performed (30-40 seconds) using the MYOSTAR® injection catheter (Biosense Webster, Cordis, Johnson & Johnson, Diamond Bar, USA). Two injections were placed in the infarct zone and 8 in the border zone. Injections were only given in areas with a unipolar voltage >6mV[14, 15].

Surgical delivery

Lateral thoracotomy was performed and the pericardium was opened to expose the lateral surface. A 1 mL syringe with a 27 gauge needle was used to inject 10^7 labeled cells. In total, 10 injections of 0.2 mL were performed across the lateral wall in the border and infarcted zone delimitated by superficial stitches.

Imaging and analysis

The anaesthetized pigs were positioned in supine position and scanned with a dual-head gamma camera (Forte, Philips, Best, the Netherlands) within 4 hours after injection to quantify *in vivo* MSC distribution. A whole-body scan was acquired using the following imaging parameters: medium-energy general-purpose collimator and 512x1024 projection matrix. After termination, whole organs (heart; lungs; liver; spleen; kidneys; bladder including urinary catheter) and catheter systems were scanned ex vivo as static anterior and posterior images for 5 minutes with the following parameters: medium-energy general-purpose collimator and 256x256 projection matrix. Two energy windows were acquired at 174 keV and 247 keV. The retained activity in syringes was measured by the dose calibrator (Veenstra, Joure, the Netherlands). After correction for half-life, background and attenuation reconstruction, regions of interest were placed over the major visceral organs and whole-body, using manufacturer's software (Pegasys, Philips, Best, the Netherlands). Post-mortem segmental analysis of the LV was performed in a subset of animals by cutting the LV into 5 slices from base to apex.

Echocardiography

Chamber dimensions were obtained from transthoracic ultrasound images (5-MHz probe, IE-33, Philips, Best, the Netherlands) in short-axis view at the mid-papillary level. All echocardiographic data were analyzed using the same protocol. The LV internal diameter (LVID) was measured in longitudinal length and the internal area (LVIA) was obtained without including the papillary muscles in end-systole and end-diastole. The FAS was calculated as $((LVIA_{ED}-LVIA_{ES})/ LVIA_{ED})\times100$.

Echocardiographic data were collected after stabilization of the hemodynamics at baseline, MI and before MSC injection. A short echocardiogram was performed after cell injection to exclude a tamponade. Analysis was performed in a blinded fashion.

Pressure-Volume loop protocol

A 7-F conductance catheter was inserted via the left carotid artery into the LV and connected with a signal processor (Leycom CFL, CD-Leycom, Zoetermeer, the Netherlands). The catheter was placed retrogradely along the long axis of the LV. The correct position of the conductance catheter was verified by echocardiography or angiography and by inspection of the segmental conductance signals. The conductance signals were calibrated by thermodilution and hypertonic saline dilution[16, 17]. For thermodilution cardiac output measurements and hypertonic saline infusion, a 7-F Swan-Ganz catheter was placed via the right jugular vein into the right pulmonary artery. Data were collected during steady-state conditions with the respirator systems turned off. From these signals, hemodynamic indices were derived. Data analysis and calculations were performed using custom-made software (CD Leycom, Zoetermeer, the Netherlands), as previously described[18]. Parameters of global systolic and diastolic function were calculated during steady-state conditions. Data were collected after stabilization of the hemodynamics at baseline and before MSC injection.

Post-mortem examination

After euthanasia transverse slices of the heart were obtained. All major visceral organs were weighed. Heart samples were snap-frozen by liquid nitrogen. Before cutting sections of 7 micrometer samples were mounted in Tissue Tek OCT. To detect autologous MSC in histology sections, cells were prelabeled with CFSE and nuclei were stained with Hoechst dye. Samples were analyzed by fluorescence microscopy.

Statistical analysis

Values derived from echocardiography, nuclear imaging and cTnI were analyzed in a blinded fashion. Statistical comparison of data between three delivery groups was done using one-way ANOVA with Bonferroni post-hoc correction or in case of two groups with an independent T-test. Hemodynamics, cardiac dimensions and cTnI were compared to baseline using a paired sample T-test. Accuracy of in-vivo imaging was determined by a Pearson correlation and intraclass correlation coefficient (ICC). Data are presented as mean±SE. *P*-values <0.05 were considered statistically significant.

RESULTS

Procedural and safety data

In total 24 animals were included in the efficiency study. Three animals were

excluded from the study due to cardiac tamponade evidenced by obduction (Surgical group; day 1 after MI), sudden death probably due to a fatal arrhythmia since signs of heart failure where absent (IC group; day 28 after MI) and mechanical dysfunction of the MYOSTAR® injection catheter (TE group). After TE injections, no cardiac tamponade was observed by echocardiography. After TE and IC infusion TIMI 3 flow of the LCX was established in all cases. In one animal (surgical group), pressure-volume loop measurements could not be performed due to instable catheter position. Four hours after MI induction, Troponin levels increased to 27±7 μg/L. Four weeks later, a slight increase in cTnI after cell injection was observed in both TE (n=6) and IC (n=5) groups from 0.12±0.1 μg/L to 2.70±1.0 μg/L (P=0.052) and from 0.14±0.04 μg/L to 1.47±0.8μg/L (P=0.389), respectively. However, no statistical difference between groups before and after injection was observed (P=0.789 and P=0.377), respectively. No significant differences in hemodynamic and echocardiographic parameters between the delivery groups at baseline and 4 weeks after MI were observed indicating a correct randomization during the study (Table 1). Overall labeling uptake was 61±2% and cell viability was 69±3% before injection. No significant differences were found in cell characteristics (e.g. label uptake, viability, number of cells, labeling time and time from injection till scan) between the 3 delivery modalities.

	Baseline			4 Weeks after MI			*P* value between groups	
Parameter	IC	TE	Surgical	IC	TE	Surgical	Baseline	Termination
	n=7	n=7	n=7	n=7	n=7	n=6		
HR (beats/min)	53±4	55±3	52±2	62±3	63±2	57±4	0.864	0.456
MAP (mmHg)	100±3	107±4	100±4	89±7	99±5	91±6	0.308	0.455
$LVID_{ED}$(cm)	4.8±0.2	4.5±0.2	4.6±0.2	4.8±0.2	4.8±0.2	4.8±0.2	0.400	0.943
$LVID_{ES}$(cm)	3.3±0.1	3.1±0.2	3.5±0.1	3.6±0.1	3.7±0.2	3.9±0.3	0.144	0.541
FAS (%)	49±1	50±2	47±1	41±2	36±3	32±3	0.333	0.107
PV-loop derived								
ESP (mmHg)	108±6	103±8	100±6	95±10	87±8	76±3*	0.721	0.270
EDP (mmHg)	9.0±0.5	8.0±0.2	8.1±0.3	13±0.7*	15±0.7*	15±1.0*	0.293	0.231
dP/dt_{MAX} (mmHg/s)	1242±34	1329±86	1293±54	1056±128	1004±87*	843±53*	0.610	0.323
dP/dt_{MIN} (mmHg/s)	-1074±120	-933±116	-948±84	-960±102	-949±79	-833±27	0.711	0.493
EDV (mL)	106±3	99±6	104±5	131±7*	138±13	147±5*	0.528	0.530
ESV (mL)	41±3	45±7	39±3	62±4*	66±7*	69±4*	0.602	0.655
EF (%)	59±3	61±4	64±3	52±4	49±6	52±3*	0.486	0.868

Table 1. Hemodynamics, cardiac geometry and function at baseline and 4 weeks after myocardial infarction

No difference in delivery efficiency to the heart

Whole-body γ-scan revealed a trend towards higher retention of MSC after surgical delivery (16±4%) compared with IC (11±1%) and TE (11±3%) but this difference was not statistical significant (P=0.52). Variation in delivery efficiency was less in the IC group (Figure 2). Qualitative analysis after TE delivery showed a higher local retention of cells at the mid-papillary level in the targeted area compared with widespread distribution of cells in the infarcted area after IC infusion (Figure 3).

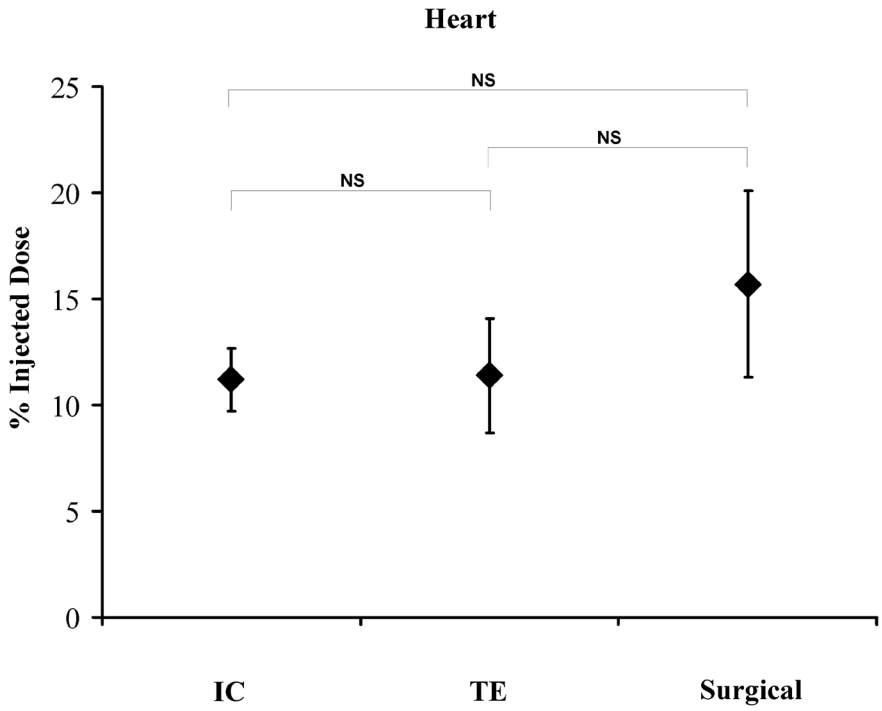


Figure 2. Myocardial retention of autologous MSC at 4 hours after IC, TE and surgical delivery.
No difference in myocardial retention between delivery techniques was detected (n=7/group; *P*=0.52). Variation in efficiency was higher after TE and surgical transplantation (reference group) compared with IC infusion. IC=intracoronary; NS=not significant; TE=transendocardial.

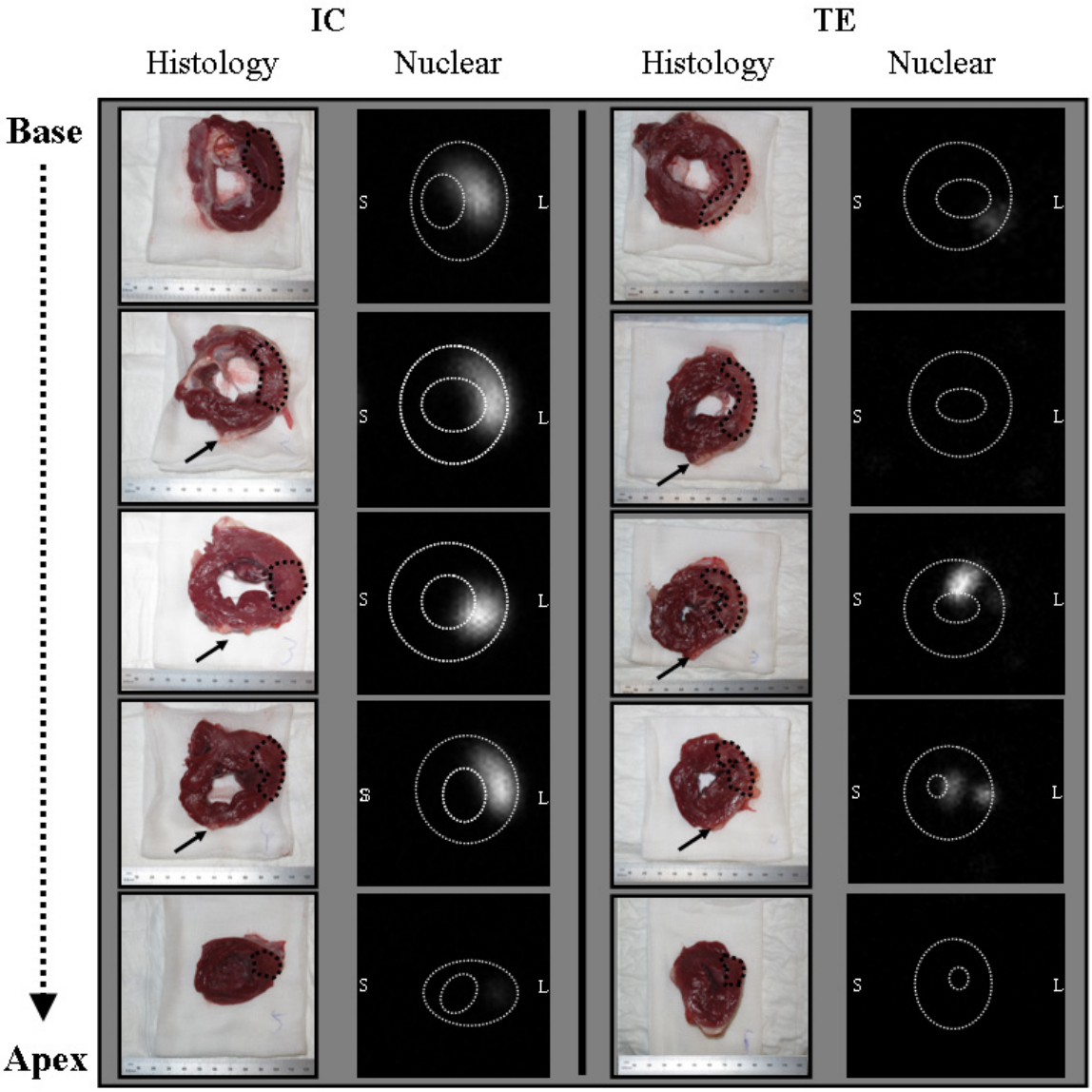


Figure 3. Segmental analysis of the LV after percutaneous delivery.
To visualize cell retention in the LV, the heart was cut into 5 slices from apex to base (histological images). Static anterior images were obtained from all slices within 4 hours after cell delivery. Note that after TE delivery cells were mainly retained in the midlateral wall whereas IC infusion showed a more scattered distribution of MSC in the posterolateral wall. Representative histological and nuclear images were derived from the same animals for both groups.
Black arrow=left anterior descending artery; IC=intracoronary; L=lateral wall; TE=transendocardial; White dotted lines=endocardial and epicardial border; S=septal wall; Black dotted line= area of interest;

Variation in biodistribution of injected MSC between delivery modalities

High accumulation of ^{111}In labeled cells in the lungs occurred in all injection groups (Figure 4). However, TE administration led to significant less retention of cells in the pulmonary tract compared with surgical delivery (P<0.05). No trend in the lungs was seen for IC (P=0.52). Low numbers of MSC were detected in the kidneys, liver and spleen. Significantly more labeled cells were distributed to the kidneys after TE injection compared with the other techniques (TE vs. IC P<0.05; TE vs. surgical P<0.001). A minimal amount of labeled MSC was located in the musculoskeletal system and none in the brain. For each delivery modality about 45% of radioactive cells accumulated in non-target organs.

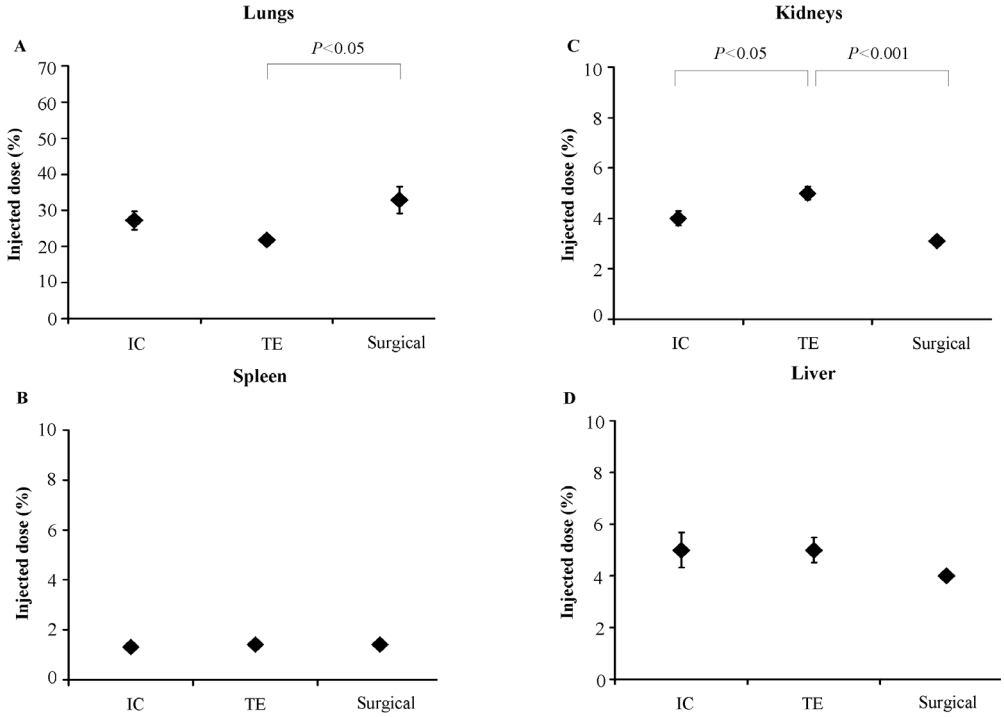


Figure 4. Biodistribution of ^{111}In labeled MSC to non-target organs.
Distribution of ^{111}In labeled MSC in various organs after IC, TE and surgical (reference) injection (n=7/group). IC= intracoronary; TE=transendocardial.

Accuracy of in-vivo imaging

Figure 5 displays a high correlation between whole-body radiation data and *ex vivo* measurements (R^2=0.827). These data support the translational potential of whole-body γ-scan to guide cell therapy approaches from pre-clinical to clinical applications. To describe how strongly measurements resemble each other within the same group a reliability analysis was performed. The intraclass correlation coefficient (ICC) was above 0.79 in all cases, underlying a strong reliability within groups.

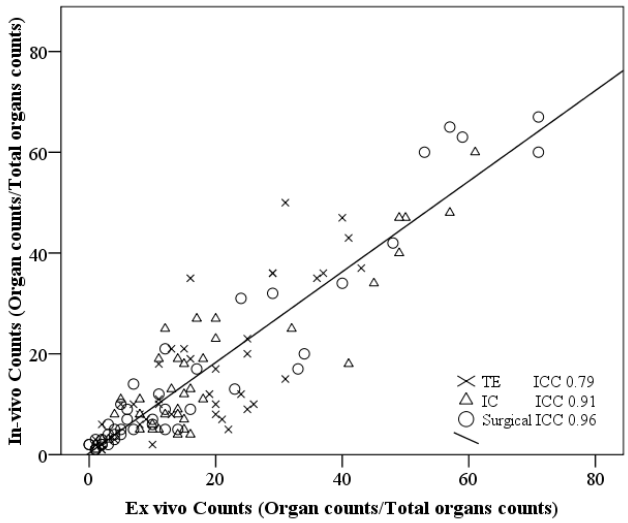


Figure 5. Correlation between *in vivo* and *ex vivo* gamma emission counts from major organs.
Correlation between in vivo and ex vivo gamma emission counts from heart, lungs, liver, spleen and bladder including urine catheter. The R^2 and the intraclass correlation coefficient (ICC) demonstrate a high agreement between quantitative whole-body imaging and post-mortem images.

Histological analysis

Fluorescent microscopy confirmed the presence of CFSE labeled MSC in the heart, direct after IC, TE and surgical injection (Figure 6). Clusters of cells were present within the infarcted area and border zone in the histological samples of TE and surgical injected animals, whereas after IC infusion MSC were observed scattered throughout the targeted myocardium. Control samples taken from the remote area showed no CFSE labeled MSC (data not shown).

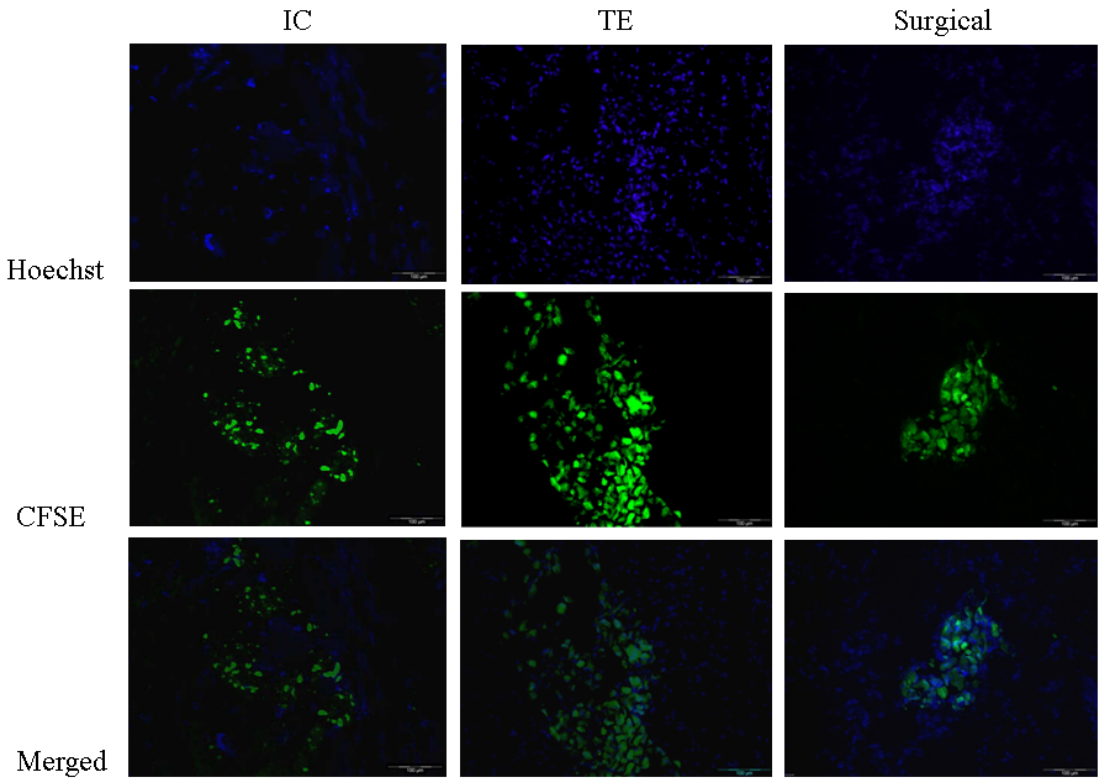


Figure 6. Fluoroscopic images of labeled MSC in the heart.
Representative histological sections were stained with Hoechst for nuclei (blue). The green color indicates presence of CFSE labeled MSC after IC, TE or surgical delivery. Sections were derived from the same animals as shown in figure 3. Samples taken from remote area showed no CFSE labeled MSC (data not shown). Scale bar 100 μmeter, 200x magnification.

DISCUSSION

In this study, for the first time a randomized comparison with blinded end-point analysis between IC and TE using the NOGA system was performed and related to surgical injection (reference group). We used a stable chronic ischemic porcine model and used state-of-the-art nuclear cell tracking to determine the most optimal stem cell delivery strategy in terms of delivery efficiency. The main novel findings are: (1) TE injection was not superior to IC in delivery efficiency; (2) Both techniques were safe, with no significant difference in myocardial damage and no mortality after cell injection.

We observed a slightly higher efficiency (11%) in chronic ischemic myocardium at 4 hours after percutaneous cell delivery which is in contrast to previous studies[10, 19-21], that reported lower retention of transplanted cells (<10%) in acute porcine MI

models. The discrepancy between our observations and previous observations can be explained by the differences in study design, MI model, cell type, and delivery method. We performed an adequately powered randomized study for one time-point (4 hours after injection) with blinded end-point analysis and standard medical care comparable with the clinical treatment of patients with chronic ischemic cardiomyopathy. MSC retention was assessed in a chronic ischemia model (i.e. 4 weeks after MI) whereas others performed cell delivery within 7 days after acute MI. In addition, we used autologous MSC in contrast to allogeneic or human MSC thereby preventing an immunological response or ectopic tissue formation[22-24]. Until now, no study determined MSC retention via nuclear imaging in a chronic MI model using the NOGA electronic mapping system which allows accurate injection by identifying the area of MI and border-zone without fluoroscopic guidance[25]. Previous studies on this topic used the Stiletto catheter (Boston Scientific Corporation, Natick, MA, USA) or performed surgical injection[10, 19].

It was anticipated by others that TE would yield better results than IC. Surprisingly, we could not observe a significant difference in efficiency between percutaneous delivery groups. Moreover, IC infusion clearly demonstrated less variation in efficiency compared to TE. A possible explanation for this finding could be that TE is relatively hampered by (1) the occurrence of premature ventricular arrhythmias as a result of the myocardial injection, or (2) the presence of the posterolateral papillary muscle leading to less stable catheter and needle position during TE delivery. IC is an easy method and relatively operator independent. Our segmental analysis by *ex vivo* γ-scan revealed site specific retention of MSC after TE in the ischemic area compared with uniform distribution of cells after IC. These observations were also found in large acute MI models[21]but until now not confirmed in a chronic model. Our findings are also in line with a recent published rodent study showing superiority of IC in terms of uniformity of cell distribution but additionally also more myocyte regeneration and amount of viable tissue in the area at risk[26].

Common mentioned safety issues of cell delivery include (1) arrhythmias[27] and death, (2) no reflow[19] and myocardial damage due to cell plugging[28], (3) cardiac tamponade[29] and (4) cell distribution to non-target organs. We did not observe decreased coronary flow after IC or TE delivery during coronary angiogram. This may be due to the anti-coagulation protocol and lower injected cell number compared to other studies[19, 30]. This study evaluated the effect of 10^7 MSC and no dose-finding was performed. Regarding myocardial damage, we observed an increase in cTnI (<10%) after percutaneous delivery. Echocardiographic images obtained after TE showed no signs of myocardial perforation. In agreement with other groups[10, 19] we noted a substantial redistribution of delivered MSC for all three techniques to non-targeted organs (about 45%). Cells were mainly retained in the lungs and to a lesser extent in the left-sided circulation indicating that most cells left the target area via the myocardial venous or lymphatic system. The possibility that less homing signals are

present in chronic damaged myocardium and could lead to the higher redistribution remains to be elucidated. Although no acute adverse effects (e.g. respiratory failure) were observed due to extra-cardiac distribution, long-term side-effects cannot be ruled out yet, since the follow-up was 4 hours.

Recommendations for future stem cell studies

Based on our results (e.g. delivery efficiency and safety data) and others [3] the choice of delivery method could depend on medical indication and practical aspects, since TE and IC yield similar results.

Since MSC were injected through an open coronary artery, we suggest the use of IC for patients with a patent coronary artery and TE for occluded arteries when using similar amount of cells. This is important in view of (large scaled) clinical trials. Since the majority of the delivered cells were retained in non-target organs, organ toxicity should be evaluated in future (pre-) clinical studies.

In our study, low efficiency (11%) to the heart was found using percutaneous delivery techniques. This finding should trigger researchers to develop new catheters or strategies (e.g. microtissues, image fusion or molecular approaches) to improve targeted cell retention.

Limitations

The design of our study did not include a functional evaluation of the different cell distribution, as the goal was to determine the most optimal delivery strategy by evaluating short term MSC retention. In a pre-clinical meta-analysis (52 studies; 900 animals) we have previously shown that improvements in ejection fraction (EF) after cell therapy were observed[3]. Interestingly, no differences in EF between IC, TE and surgical injection could be noticed. In addition, multivariate analysis showed that the method of delivery was a non-significant predictor of EF improvement[3].

To date, numerous human and pre-clinical trials have already been conducted in order to assess the efficacy and safety aspects of cardiac stem cell therapy. Obviously, differences exist between large animal models and clinical practice. Healthy young large animals differ from older patients with long standing coronary artery disease, and frequently co-morbidities (e.g. diabetes, hypertension, renal failure) are frequently present. Despite these differences, a recent published pre-clinical meta-analysis showed that large animal models are valid to predict the outcome of clinical trials[3].

Relatively low cell viability after ^{111}In labeling was observed, however no difference between groups was found. Moreover, the overall viability was comparable to other groups (69% vs.74%)[31].

CONCLUSIONS

This randomized study showed no significant difference in delivery efficiency to the myocardium in a clinically relevant ischemic large animal model. Moreover, no differences in safety profile were observed. These results suggest that, the choice of delivery modality could depend on medical indication and practical aspects (costs, side effects on non-target organs and operator experience), since TE and IC yield similar results.

Acknowledgements

This work was supported by the Netherlands Heart Foundation '[2003B07304 and 2010T025]', BSIK program "Dutch Program for Tissue Engineering", '[grant 6746]', and a Bekalis price (PD). This research is part of the Project P1.04 SMARTCARE of the research program of the BioMedical Materials institute, co-funded by the Dutch Ministry of Economic Affairs, Agriculture and Innovation. The final contribution of the Netherlands Heart Foundation is gratefully acknowledged.

We gratefully acknowledge Cees Verlaan, Merel Schurink, Marlijn Jansen, Maringa Emons, Ben van Middelaar and Joyce Visser for excellent technical assistance and animal care. In addition, the authors acknowledge the technical assistance provided by Fred van het Schip and Sridevi Jaksani. The authors would also like to thank John Bemelmans and Sieneke Heeres for their skilled technical assistance with the MSC labeling with ^{111}In and with the pig *in vivo* and *ex vivo* scintigraphy studies. We thank P. Agostoni MD, PhD (interventional cardiologist) for grading the coronary angiographies.

Conflict of interest statement

None

REFERENCES

1. Rosamond W, Flegal K, Furie K, Go A, Greenlund K, Haase N, Hailpern SM, Ho M, Howard V, Kissela B, Kittner S, Lloyd-Jones D, McDermott M, Meigs J, Moy C, Nichol G, O'Donnell C, Roger V, Sorlie P, Steinberger J, Thom T, Wilson M, Hong Y, for the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart Disease and Stroke Statistics--2008 Update: A Report From the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 2008;117:e25-146.

2. Abdel-Latif A, Bolli R, Tleyjeh IM, Montori VM, Perin EC, Hornung CA, Zuba-Surma EK, Al-Mallah M, Dawn B. Adult Bone Marrow-Derived Cells for Cardiac Repair: A Systematic Review and Meta-analysis. Arch Intern Med 2007;167:989-997.

3. van der Spoel TIG, Jansen of Lorkeers S, Agostoni P, van Belle E, Gyongyosi M, Sluijter JPG, Cramer MJ, Doevendans PA, Chamuleau SAJ. Human relevance of pre-clinical studies in stem cell therapy; systematic review and meta-analysis of large animal models of ischemic heart disease. Cardiovasc Res 2011;91:649-658.

4. Bartunek J, Dimmeler S, Drexler H, Fernández-Avilés F, Galinanes M, Janssens S, Martin J, Mathur A, Menasche P, Priori S, Strauer B, Tendera M, Wijns W, Zeiher A. The consensus of the task force of the European Society of Cardiology concerning the clinical investigation of the use of autologous adult stem cells for repair of the heart. Eur Heart J 2006;27:1338-1340.

5. Assmus B, Honold J, Schachinger V, Britten MB, Fischer-Rasokat U, Lehmann R, Teupe C, Pistorius K, Martin H, Abolmaali ND, Tonn T, Dimmeler S, Zeiher AM. Transcoronary Transplantation of Progenitor Cells after Myocardial Infarction. N Engl J Med 2006;355:1222-1232.

6. Katritsis DG, Sotiropoulou PA, Karvouni E, Karabinos E, Korovesis S, Perez SA, Voridis EM, Papamichail M. Transcoronary transplantation of autologous mesenchymal stem cells and endothelial progenitors into infarcted human myocardium. Catheterization and Cardiovascular Interventions 2005;65:321-329.

7. Tse HF, Thambar S, Kwong YL, Rowlings P, Bellamy G, McCrohon J, Thomas P, Bastian B, Chan JKF, Lo G, Ho CL, Chan WS, Kwong RY, Parker A, Hauser TH, Chan J, Fong DYT, Lau CP. Prospective randomized trial of direct endomyocardial implantation of bone marrow cells for treatment of severe coronary artery diseases (PROTECT-CAD trial). Eur Heart J 2007;28:2998-3005.

8. van Ramshorst J, Bax JJ, Beeres SLMA, Dibbets-Schneider P, Roes SD, Stokkel MPM, de Roos A, Fibbe WE, Zwaginga JJ, Boersma E, Schalij MJ, Atsma DE. Intramyocardial Bone Marrow Cell Injection for Chronic Myocardial Ischemia: A Randomized Controlled Trial. JAMA 2009;301:1997-2004.

9. Dib N, Menasche P, Bartunek JJ, Zeiher AM, Terzic A, Chronos NA, Henry TD, Peters NS, Fernandez-Aviles F, Yacoub M, Sanborn TA, DeMaria A, Schatz RA, Taylor DA, Fuchs S, Itescu S, Miller LW, Dinsmore JH, Dangas GD, Popma JJ, Hall JL, Holmes Jr DR. Recommendations for Successful Training on Methods of Delivery of Biologics for Cardiac Regeneration: A Report of the International Society for Cardiovascular Translational Research. JACC: Cardiovascular Interventions 2010;3:265-275.

10. Hou D, Youssef EA-S, Brinton TJ, Zhang P, Rogers P, Price ET, Yeung AC, Johnstone BH, Yock PG, March KL. Radiolabeled Cell Distribution After Intramyocardial, Intracoronary, and Interstitial Retrograde Coronary Venous Delivery: Implications for Current Clinical Trials. Circulation 2005;112:I-150.

11. Timmers L, Henriques JPS, de Kleijn DPV, DeVries JH, Kemperman H, Steendijk P, Verlaan CWJ, Kerver M, Piek JJ, Doevendans PA, Pasterkamp G, Hoefer IE. Exenatide Reduces Infarct Size and Improves Cardiac Function in a Porcine Model of Ischemia and Reperfusion Injury. J Am Coll Cardiol 2009;53:501-510.

12. Noort WA, Oerlemans MIFJ, Rozemuller H, Feyen D, Jaksani S, Stecher D, Naaijkens B, Martens AC, Buhring HJ, Doevendans PA, Sluijter JPG. Human versus porcine mesenchymal stromal cells: phenotype, differentiation potential, immunomodulation and cardiac improvement after transplantation. Journal of Cellular and Molecular Medicine 2011;no.

13. Gibson CM, Cannon CP, Daley WL, Dodge JT, Jr., Alexander B, Marble SJ, McCabe CH, Raymond L, Fortin T, Poole WK, Braunwald E. TIMI Frame Count : A Quantitative Method of Assessing Coronary Artery Flow. Circulation 1996;93:879-888.

14. Ben-Haim SA, Osadchy D, Schuster I, Gepstein L, Hayam G, Josephson ME. Nonfluoroscopic, in vivo navigation and mapping technology. Nat Med 1996;2:1393-1395.

15. Gepstein L, Hayam G, Ben-Haim SA. A Novel Method for Nonfluoroscopic Catheter-Based Electroanatomical Mapping of the Heart : In Vitro and In Vivo Accuracy Results. Circulation 1997;95:1611-1622.

16. Baan J, van der Velde ET, de Bruin HG, Smeenk GJ, Koops J, van Dijk AD, Temmerman D, Senden J, Buis B. Continuous measurement of left ventricular volume in animals and humans by conductance catheter. Circulation 1984;70:812-823.

17. Steendijk P, Baan J. Comparison of intravenous and pulmonary artery injections of hypertonic saline for the assessment of conductance catheter parallel conductance. Cardiovasc Res 2000;46:82-89.

18. Steendijk P, BaanJr. J, Van Der Velde ET, Baan J. Effects of critical coronary stenosis on global systolic left ventricular function quantified by pressure-volume relations during dobutamine stress in the canine heart. J Am Coll Cardiol 1998;32:816-826.

19. Freyman T, Polin G, Osman H, Crary J, Lu M, Cheng L, Palasis M, Wilensky RL. A quantitative, randomized study evaluating three methods of mesenchymal stem cell delivery following myocardial infarction. Eur Heart J 2006;27:1114-1122.

20. Gyongyosi M, Hemetsberger R, Wolbank S, Kaun C, Posa A, Marian T, Balkay L, Emri M, Galuska L, Mikecz P, Petrasi Z, Charwat S, Hemetsberger H, Blanco J, Maurer G. Imaging the Migration of Therapeutically Delivered Cardiac Stem Cells. JACC: Cardiovascular Imaging 2010;3:772-775.

21. Perin EC, Silva GV, Assad JA, Vela D, Buja LM, Sousa AL, Litovsky S, Lin J, Vaughn WK, Coulter S, Fernandes MR, Willerson JT. Comparison of intracoronary and transendocardial delivery of allogeneic mesenchymal cells in a canine model of acute myocardial infarction. J Mol Cell Cardiol 2008;44:486-495.

22. Breitbach M, Bostani T, Roell W, Xia Y, Dewald O, Nygren JM, Fries JWU, Tiemann K, Bohlen H, Hescheler J, Welz A, Bloch W, Jacobsen SE, Fleischmann BK. Potential risks of bone marrow cell transplantation into infarcted hearts. Blood 2007;110:1362-1369.

23. Lyngbaek S, Ripa R, Haack-Sorensen M, Cortsen A, Kragh L, Andersen C, Jorgensen E, Kjaer A, Kastrup J, Hesse B. Serial in vivo imaging of the porcine heart after percutaneous, intramyocardially injected 111In-labeled human mesenchymal stromal cells. The International Journal of Cardiovascular Imaging 2010;26:273-284.

24. Huang XP, Sun Z, Miyagi Y, Donald Kinkaid H, Zhang L, Weisel RD, Li RK. Differentiation of Allogeneic Mesenchymal Stem Cells Induces Immunogenicity and Limits Their Long-Term Benefits for Myocardial Repair. Circulation 2010;122:2419-2429.

25. Kornowski R, Hong MK, Gepstein L, Goldstein S, Ellahham S, Ben-Haim SA, Leon MB. Preliminary Animal and Clinical Experiences Using an Electromechanical Endocardial Mapping Procedure to Distinguish Infarcted From Healthy Myocardium. Circulation 1998;98:1116-1124.

26. Li Q, Guo Y, Ou Q, Chen N, Wu WJ, Yuan F, OBrien E, Wang T, Luo L, Hunt G, Zhu X, Bolli R. Intracoronary administration of cardiac stem cells in mice: a new, improved technique for cell therapy in murine models. Basic Research in Cardiology 2011;1-16.

27. Dib N, Dinsmore J, Lababidi Z, White B, Moravec S, Campbell A, Rosenbaum A, Seyedmadani K, Jaber WA, Rizenhour CS, Diethrich E. One-Year Follow-Up of Feasibility and Safety of the First U.S., Randomized, Controlled Study Using 3-Dimensional Guided Catheter-Based Delivery of Autologous Skeletal Myoblasts for Ischemic Cardiomyopathy (CAuSMIC Study). JACC: Cardiovascular Interventions 2009;2:9-16.

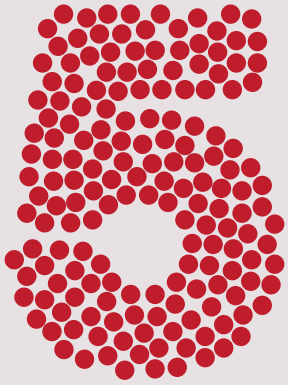
28. Mansour S, Vanderheyden M, De Bruyne B, Vandekerckhove B, Delrue L, Van Haute I, Heyndrickx G, Carlier S, Rodriguez-Granillo G, Wijns W, Bartunek J. Intracoronary Delivery of Hematopoietic Bone Marrow Stem Cells and Luminal Loss of the Infarct-Related Artery in Patients With Recent Myocardial Infarction. J Am Coll Cardiol 2006;47:1727-1730.

29. Gyongyosi M, Lang I, Dettke M, Beran G, Graf S, Sochor H, Nyolczas N, Charwat S, Hemetsberger R, Christ G, Edes I, Balogh L, Krause KT, Jaquet K, Kuck KH, Benedek I, Hintea T, Kiss R, Preda I, Kotevski V, Pejkov H, Zamini S, Khorsand A, Sodeck G, Kaider A, Maurer G, Glogar D. Combined delivery approach of bone marrow mononuclear stem cells early and late after myocardial infarction: the MYSTAR prospective, randomized study. Nat Clin Pract Cardiovasc Med 2009;6:70-81.

30. Vulliet PR, Greeley M, Halloran SM, MacDonald KA, Kittleson MD. Intra-coronary arterial injection of mesenchymal stromal cells and microinfarction in dogs. The Lancet 2004;363:783-784.

31. Kraitchman DL, Tatsumi M, Gilson WD, Ishimori T, Kedziorek D, Walczak P, Segars WP, Chen H, Fritzges D, Izbudak I, Young RG, Marcelino M, Pittenger MF, Solaiyappan M, Boston RC, Tsui BMW, Wahl RL, Bulte JWM. Dynamic Imaging of Allogeneic Mesenchymal Stem Cells Trafficking to Myocardial Infarction. Circulation 2005;112:1451-1461.


T.I.G van der Spoel



AUTOLOGOUS MESENCHYMAL STEM CELLS SHOW MORE BENEFIT ON SYSTOLIC FUNCTION COMPARED TO BONE MARROW MONONUCLEAR CELLS IN A PORCINE MODEL OF CHRONIC MYOCARDIAL INFARCTION.

Tycho I.G. van der Spoel, MD[1]
Stefan Koudstaal, MD[1]
Frebus J. van Slochteren MSc[1]
Sanne J. Jansen of Lorkeers, MD[1]
Joost P.G. Sluijter, PhD[1,2]
Imo E. Hoefer, MD PhD[3]
Paul Steendijk, PhD[4]
Maarten Jan M. Cramer, MD PhD[1]
Pieter A. Doevendans, MD PhD[1,2]
Eric van Belle, MD PhD[1]
Steven A.J. Chamuleau, MD PhD[1]

[1] Department of Cardiology, Division Heart and Lungs, University Medical Center Utrecht, the Netherlands.
[2] Interuniversity Cardiology Institute of the Netherlands (ICIN), Utrecht, the Netherlands.
[3] Department of Experimental Cardiology, University Medical Center Utrecht, the Netherlands.
[4] Department of Cardiology, Leiden University Medical Center, Leiden, the Netherlands.

ABSTRACT

Background: Cell therapy is a promising strategy to treat patients after myocardial infarction (MI). Although several cell sources have been explored, no consensus exists regarding the optimal cell type. Here, we (1) compare transendocardial injection (TE) of bone marrow-derived mononuclear cells (BMMNC) with mesenchymal stem cells (MSC) to improve systolic function, and (2) assess the effect of repetitive injections in a porcine model of chronic MI.

Methods: Nineteen animals underwent TE cell delivery using electromechanical mapping guidance at 4 and 8 weeks after MI. Animals received 10^7 autologous BMMNC or MSC, and were allocated to 3 groups: (1) placebo+placebo, (2) MSC+placebo, or (3) BMMNC+MSC delivery. Cardiac function was assessed by echocardiography and p§ressure-volume loops. Myocardial biopsies were processed for collagen content and capillary density.

Results: Ejection fraction (EF) was significantly improved after MSC injection from baseline to 4 weeks post-injection and not by BMMNC injection (Group 2 11.9±3% vs. Group 3 -1.6±6%; P=0.028). The positive effect of MSC on EF improvement was sustained 8 weeks post-injection (Group 2 17.8±3% vs. Group 1 -9.1±3%; P<0.01). No difference was observed in EF changes (P=0.28) between the single MSC and repetitive cell injected groups. Moreover, no difference in vessel density was observed (P=0.51) supporting our observations.

Conclusions: This study showed that autologous MSC rather than BMMNC injection improves systolic function in ischemic cardiomyopathy, moreover this positive effect is sustained during long-term follow-up. No additional value of repetitive injections was noted. These results are important in view of the choice of cell type in designing novel clinical stem cell trials.

INTRODUCTION

Ischemic heart failure remains a major cause of morbidity and mortality [1]. Stem cell therapy emerged as an innovative and attractive therapeutic approach for patients with chronic myocardial ischemia. The ultimate goal of this treatment is to support and enhance the endogenous repair mechanisms by replacing dysfunctional cardiomyocytes and inducing angiogenesis.

In clinical and pre-clinical studies, a modest improvement in left ventricular ejection fraction (LVEF) was observed using a single injection of bone marrow (BM) cells after myocardial infarction (MI)[2, 3]. Our recent pre-clinical meta-analysis showed that the choice of cell type is an important significant predictor of improvement in LVEF[3] suggesting a trend towards more pronounced effects of mesenchymal stem cells (MSC). Furthermore, our observations indicated that initial effects fade away over time. Based on this observation we suggested that a strategy using repetitive cell injections may be worthwhile. Till now, bone marrow mononuclear cells (BMMNC) and MSC have been well studied in patients with ischemic heart disease[4]. However, functional differences between MSC and BMMNC exist as was suggested by our group[3]. A direct comparison on functional endpoints between these cell types has not been performed so far.

Percutaneous transendocardial (TE) delivery, guided by electromechanical mapping (NOGA), was shown to be safe in patients with chronic ischemic cardiomyopathy[5] and has the advantage to detect hibernating myocardium which is the area that will probably profit most from cell delivery[6].

Our objective was to determine the most potent regenerative strategy using autologous BM cell types, i.e. BMMNC and MSC, in a large animal model of ischemia/reperfusion injury. Two aims were defined: (1) comparison of the short-term effects between BMMNC and MSC; (2) determining the long-term effects, including a strategy of repetitive injections.

METHODS

Animals

Nineteen female Dutch Landrace pigs received humane-like care in compliance with the *"Guide for the Care and Use of Laboratory Animals,"* published by the National Institutes of Health (National Institutes of Health publication 85-23, revised 1985). The study protocol was approved by the Animal Experimentation Committee of the University of Utrecht.

Study design

Animals were allocated to 1 of 3 groups: (Group 1) placebo + placebo, (Group 2) MSC + placebo or (Group 3) BMMNC + MSC. Four weeks after MI animals received injection of autologous 10^7 BMMNC, 10^7 MSC or PBS (placebo) (Invitrogen, Carlsbad, CA, USA). Eight weeks after MI the second TE injection

of 10^7 MSC or PBS was performed. Twelve weeks after the initial MI, the animals were euthanized. For aim 1, cardiac function was assessed by pressure-volume (PV) loops at 8 weeks (before the second injection), and for aim 2 at 12 weeks including histology (sacrifice). The study design is shown in Figure 1.

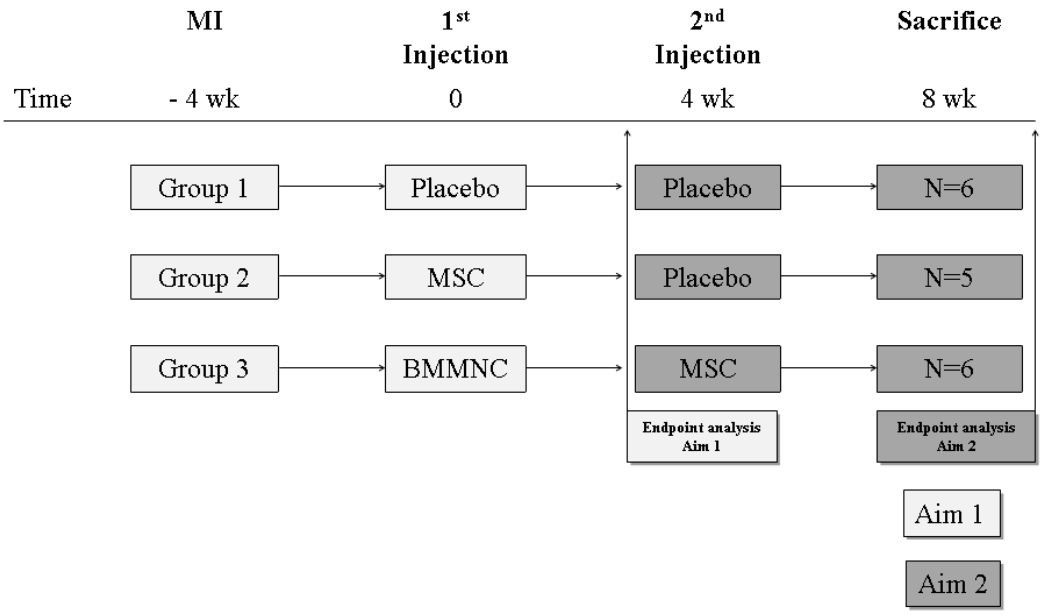


Figure 1. Study design.
BMMNC=bone marrow mononuclear cells; Echo=echocardiography; MI=myocardial infarction; MSC=mesenchymal stem cells; PV-loop=pressure-volume loop.

Premedication and anesthesia

After an overnight fast, animals were sedated with an intramuscular injection of ketamin (10 mg/kg), midazolam (0.5 mg/kg) and atropin (0.04 mg/kg). Next, thiopental (4 mg/kg) was administered intravenously before intubation. Animals were intubated with an endotracheal tube and anesthetized in the supine position. The animals were mechanically ventilated with the use of a positive-pressure ventilator with a mix of oxygen and air (FiO2 0.5). General anesthesia/analgesia was maintained with midazolam (0.5 mg kg^{-1} h^{-1}, Roche, Woerden, the Netherlands), sufentanyl citrate (2 µg kg^{-1} h^{-1}, Janssen-Cilag, Tilburg, the Netherlands) and pancuronium bromid (0.1 mg kg^{-1} h^{-1}, Organon, Oss, the Netherlands). Metoprolol (Centrafarm, Etten-Leur, the Netherlands) was administered intravenously (5 mg) to reduce the mechanical irritation of the heart. During surgery, animals were anticoagulated with heparin (ACT>250s). At the end of the experiment the animals were euthanized by pentobarbital overdose.

Myocardial ischemia/reperfusion model

During the entire procedure, electrocardiogram, arterial pressure and capnogram were continuously monitored. Prior to MI, all animals received an oral dose of amiodarone (400 mg/day; starting 10 days prior to MI) and clopidogrel (75 mg/day; starting 3 days prior to MI, Sanofi Aventis, Gouda, the Netherlands)[7]. A bolus of 500 mg acetylic salicylic acid (Centrafarm, Etten-Leur, the Netherlands) was given the day before the occlusion. MI was created by a percutaneous balloon of equivalent size to the proximal left circumflex artery (LCX). The balloon was inflated for 75 minutes at 5-8 atm[8]. Complete occlusion of the LCX was confirmed by angiography. To prevent ventricular arrhythmias, 300 mg amiodarone (Centrafarm, Etten-Leur, the Netherlands) intravenously was given. External defibrillation (150-200 Joules) was used when ventricular fibrillation occurred. After the procedure, coronary angiography was performed to confirm vessel patency. After recovery, the animals received daily an oral dose of 50 mg metoprolol, 400 mg amiodarone, 75 mg clopidogrel, and 160 mg acetylic salicylic acid until termination to prevent thrombosis and arrhythmias[7].

MSC culture and labeling

Bone marrow was aspirated (35-40 mL) from the sternum by a heparinized syringe. BMMNC were isolated by ficoll density gradient centrifugation and frozen in 10% DMSO and 90% culture medium. MSC were isolated and characterized as previously described.[9] Autologous MSC were cultured at 37°C in Alpha MEM (Invitrogen, Carlsbad, CA, USA), supplemented with 10% FBS, heparin and 1% penicillin/streptomycin. Cells were cultured, replacing medium every three days and used between passage 5-7. Before injection cells were resuspended in 2 mL PBS and viability was assessed via trypan-blue (Sigma-Aldrich, St. Louis, MO, USA) counting.

Transendocardial delivery

To enable TE injection, an 8-F sheath was placed in a carotid artery. Next, a mapping catheter (Biosense Webster, Cordis, Johnson & Johnson, USA) was placed retrogradely through the aortic valve into the left ventricle (LV). First, a 3-dimensional electromechanical map of the LV was obtained using the NOGA system (Biosense Webster, Cordis, Johnson & Johnson, USA), as described before[10, 11]. Hereafter, 10 injections of 0.2 mL were slowly placed using the MYOSTAR® injection catheter (Biosense Webster, Cordis, Johnson & Johnson, Diamond Bar, USA). Two injections were placed in the infarct zone and 8 in the border zone. Four weeks after the first injection, this procedure was repeated and the second injections were given at the same location. Injections were only given in areas with a unipolar voltage greater than 6mV[10, 11].

Echocardiography

A transthoracic echocardiogram (5-MHz probe, IE-33, Philips, Best, the Netherlands) was performed directly after MI, eight weeks after MI and at sacrifice as described before[7]. Short axis images were obtained at the papillary level, and three consecutive cardiac cycles were acquired. Wall thickness (WT) of the posterolateral wall was assessed in end-systole and end-diastole. The internal area (LVIA) was obtained without including the papillary muscles in end-systole and end-diastole. The fractional area shortening was calculated as ((LVIAed-LVIAes)/LVIAed)x100.

Pressure-Volume loop protocol

Pressure-volume loops were obtained using a 7-F conductance catheter that was inserted via a carotid artery and placed along the long axis of the LV. The catheter was connected with a signal processor (Leycom CFL, CD-Leycom, Zoetermeer, the Netherlands). The correct position of the conductance catheter was verified by angiography and by inspection of the segmental conductance signals. The conductance signals were calibrated by thermodilution and hypertonic saline dilution via a 7-F Swan-Ganz catheter that was placed into the right or left pulmonary artery [12, 13]. Data were collected during steady-state conditions with the respirator systems turned off at end-expiration. From these signals, hemodynamic indices were derived. Data analysis and calculations were performed using custom-made software (CD Leycom, Zoetermeer, the Netherlands), as previously described [14]. Parameters of global systolic and diastolic function were calculated during steady-state conditions at 4, 8, and 12 weeks after MI. Cardiac output (CO) measured by Swan-Ganz was corrected by multiplying each measurement with 0.62. This number was based on the following equation (CO Swan-Ganz at sacrifice /CO transonic aorta flow probe at sacrifice). The isovolumic relaxation time constant (Tau) was calculated by phase-plot analysis. The end-systolic pressure volume relationship was measured by its slope end-systolic elastance (Ees). Diastolic stiffness (Eed) was determined as the lineair slope of the end-diastolic pressure volume relationship. Both were calculated by single beat analysis as described earlier[15].

Histology

After euthanasia, the LV was weighed and tissue samples from the infarct and remote region of the heart were obtained. Samples were fixed in 4% formalin at room temperature or snapfrozen. Before cutting 5 micrometer sections, samples were embedded in paraffin for collagen analysis. For quantification of collagen content, picrosirius red staining and detection with circularly polarized light and digital image microscopy was used[16]. Three random images of the infarcted area were obtained. After conversion into grey value images, the average number of grey values was expressed as a grey value/mm². Snapfrozen samples were cut at 7 micrometer and stained with Lectin (Sigma Aldrich) to quantify capillary density. Nuclei were

stained with Hematoxilin and Eosin. Three random images were obtained at 10x magnification.

Statistical analysis

Values derived from echocardiography were analyzed in a blinded fashion. For statistical analysis, we used a linear mixed-effects model to account for repeated measurements on each animal. Statistical comparison of data between groups was done using an one-way ANOVA with a post-hoc Tukey test. Data are presented as mean±SE. All statistical analyses were performed using SPSS 18.1.1 and P-values <0.05 were considered statistically significant.

RESULTS

Procedural data

In total 19 animals underwent the MI procedure. One animal died due to severe heart failure evidenced by autopsy (Group 1; day 71 after MI), and one animal had to be terminated for reaching a human defined endpoint due to an abscess at the right foot not related to the study (Group 2). MSC viability (Group 2 92±4% vs. Group 3 93±1%; *P*=0.10) and number of MSC (Group 2 1.0±0.1*10^7 vs. Group 3 0.9±0.2*10^7; *P*=0.10) did not differ between the cell treated groups. BMMNC viability was 92±4% and the injected number 1.7±0.2*10^7. No cardiac tamponade was observed after any cell or placebo injection.

Aim 1: Comparison between MSC and BMMNC on cardiac function

Four weeks after MI (baseline), no difference in LVEF between groups was observed (*P*= 0.30; Table 1). When comparing LVEF differences between baseline and 4 weeks after injection (Figure 2), placebo treated animals showed a reduction in LVEF whereas in MSC treated animals LVEF was significantly improved (Group 2 11.9±3% vs. Group 1 -7.8±8%; *P*=0.002). Animals treated with MSC showed a tendency for having a decrease in ΔESV (Group 2 -6.0±7mL vs. Group 1 10±10mL; *P*=0.10). Surprisingly, no significant difference in ΔLVEF between BMMNC and placebo treatment was observed (Group 3 -1.6±6% vs. Group 1 -7.8±8%; *P*=0.748). This also means that MSC injection led to a significant increase in ΔLVEF compared to BMMNC injection (Group 2 11.9±3% vs. Group 3 -1.6±6%; *P*=0.028) but also significantly improved ΔCO (Group 2 0.7±0.3 L/min vs. Group 3 -0.4±0.4 L/min; *P*=0.037) and thereby reflects an increased systolic cardiac performance. With respect to global diastolic function, no significant difference in Δend-diastolic volume between groups could be observed (Group 1 -0.2±4mL, Group 2 7.7±13mL, Group 3 -14±8mL; all *P*>0.1). In addition, dP/dt_{MIN}, Tau, end-diastolic pressure (EDP), and pressure halftime (PHT) were similar in the different treatment groups (Table 1). However, passive diastolic function was improved in the BMMNC group compared to the other groups, indicated by ΔEed (Group 1 0.08±0.05 mmHg/mL,

Group 2 0.12±0.08 mmHg/mL, Group 3 -0.08±0.05 mmHg/mL; BMMNC vs. placebo *P*=0.04, MSC vs. BMMNC *P*=0.004, MSC vs. placebo *P*=0.349).

Directly after MI, echocardiographic recordings showed that end-systolic WT was similar between groups (Group 1: 1.25±0.2 cm, Group 2: 1.38±0.1 cm, Group 3: 1.01±0.2 cm; *P*=0.78). Also no difference in end-diastolic WT was observed (Group 1 1.21±0.3 cm, Group 2 1.18±0.3 cm, Group 3 1.01±0.2 cm; *P*=0.48). Four weeks after treatment no significant effect on ΔEnd-diastolic WT (Group 1 0.03±0.06 cm, Group 2 0.01±0.03 cm, Group 3 0.10±0.05cm) and ΔEnd-systolic WT (Group 1 0.28±0.07 cm, Group 2 0.06±0.06 cm, Group 3 0.16±0.09 cm) was found.

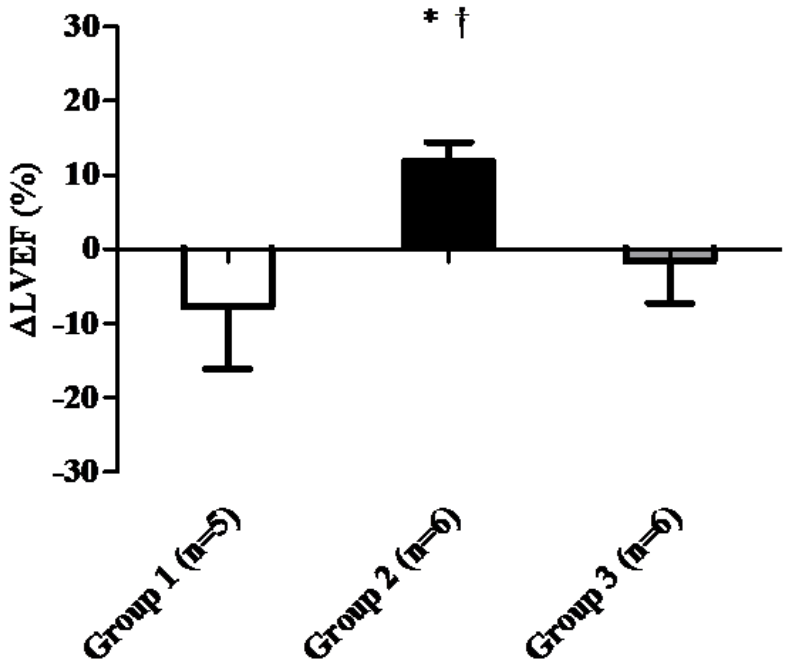


Figure 2. MSC injection improves systolic function compared to BMMNC.
Percentage of change in LVEF between baseline and 4 weeks after injection in each treatment group. *P<0.01 compared to placebo. †P=0.028 compared to BMMNC. LVEF= Left ventricular ejection fraction.

Hemodynamics	**Baseline (4 weeks after MI)**			**2e Injection (8 weeks after MI)**			**Sacrifice (12 weeks after MI)**		
Parameter	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
General									
Weight (kg)	73±3	71±2	73±1	80±3	76±2	76±1	83±3	82±3	82±1
LV weight (g)							168±7	159±5	175±9
FAS (%)				50±5	55±3	47±2	50±2	51±2	43±3
HR (beats/min)	52±2	59±4	52±6	63±8	56±1	57±7	51±7	55±1	53±4
CO (L/min)	3.5±0.3	2.8±0.2	3.0±0.4	3.2±0.2	3.5±0.3§	2.7±0.2	2.8±0.5	3.5±0.3	3.1±0.1
Systole									
ESV (mL)	41±3	37±4	49±7	50±12	31±8	44±7	51±11	23±5$^{\#}$	30±6*
ESP (mmHg)	96±7	87±11	100±5	86±7	90±8	85±4	91±7	81±5	74±4*
EF (%)	62±2	57±2	55±5	54±7	69±3$^{\#\S}$	54±5	52±3	74±3$^{\#}$	69±4*
dP/dt_{MAX} (mmHg/s)	1586±131	1390±208	1374±46	1372±152	1351±134	1096±64	1460±102	1402±40§	1033±71*
Ees (mmHg/ml)	3.9±0.5	4.2±0.6	3.7±0.1	3.7±0.7	3.7±0.4	3.2±0.2	4.1±0.9	3.7±0.5	2.5±0.3*
Diastole									
EDV (mL)	107±8	85±5	109±6	106±13	92±13	95±9	106±16	86±9	91±10
EDP (mmHg)	16±1	13±1	16±1	14±2	16±1	13±2	15±1	14±2	11±1*§
dP/dt_{MIN} (mmHg/s)	-1428±131	-1345±165	-1393±91	-1350±224	-1447±119	-1275±99	-1239±224	-1328±99	-1148±93
PHT (ms)	34±2	31±2	39±3	36±7	31±1	34±3	44±7	28±1$^{\#}$	31±1*
Tau (ms)	58±4	52±4	67±6	62±14	51±2	57±5	72±16	48±2	49±2*
Eed (mmHg/mL)	0.38±0.04	0.42±0.02	0.37±0.06	0.46±0.8*	0.54±0.06§	0.29±0.02	0.30±0.04	0.38±0.07	0.24±0.03

Table 1. Hemodynamics derived from pressure-volume loops at baseline, before the second injection and at sacrifice.
CO indicates cardiac output; HR, heart rate; EDP, end-diastolic pressure; ESP, end-systolic pressure; dP/dtMAX, maximal rate of LV pressure increase; -dP/dtMIN, maximal rate of LV pressure decrease; EDV, end-diastolic volume; Eed, myocardial stiffness; Ees, End-systolic elastance; ESV, end-systolic volume; EF, ejection fraction; FAS, fractional area shortening; LV, left ventricle; PHT, pressure halftime; Tau, isovolumic relaxation time constant. Data are presented as mean± SE. *BMMNC vs. placebo P<0.05, #MSC vs. placebo P<0.01. §MSC vs. BMMNC P<0.05.

Aim 2: Long-term effects on cardiac function

Having established the effect at four weeks after injection, we investigated the long-term effect of MSC compared to placebo, reflected by the change between baseline and 8 weeks after injection. Hemodynamic data are summarized in Table 1. PV-loop analyses revealed that global LV function was significantly improved as evidenced by an increase in ΔLVEF (Figure 3), reduction in ΔESV (Group 1 0.6±13mL vs. Group 2 -14±9 mL; *P*=0.009) and a tendency for increased ΔCO (Group 1 -0.7±0.4 L/min vs. Group 2 0.7±0.2 L/min; *P*=0.08) compared to control animals. Active relaxation indexed by ΔTau (*P*=0.07) and ΔPHT (Group 1 10±15 ms vs. Group 2 -2±3 ms; *P*=0.012) was improved after MSC injection.

No significant differences in Δend-diastolic WT (Group 1 -0.45±0.2 cm vs. Group 2 -0.48±0.1; *P*=0.74) and Δend-systolic WT (Group 1 -0.18±0.2 cm vs. Group 2 -0.26 ±0.1cm; *P*=0.70) were observed.

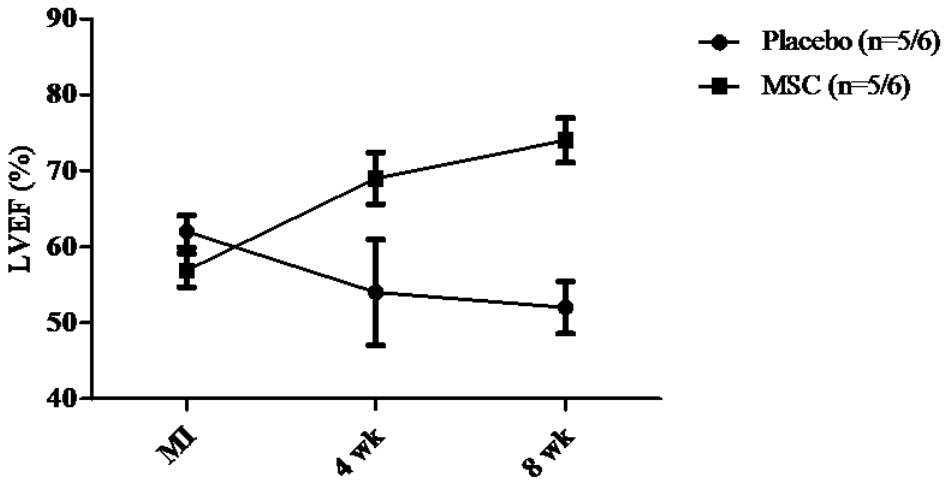


Figure 3. Improvement in LVEF is maintained during long-term follow-up after MSC injection.
LVEF at baseline, 4 weeks and 8 weeks after placebo and MSC treatment. MI=myocardial infarction, LVEF= Left ventricular ejection fraction, MSC=mesenchymal stem cells.

Effect of repeated cell injection on LV performance

Next, we studied whether a second injection of MSC could rescue the damaged myocardium. When comparing ΔLVEF between baseline and at sacrifice (Figure 4), placebo treated animals showed a reduction in ΔLVEF, whereas in cell treated animals ΔEF was significantly improved (Group 2 18±3%, Group 3 13±4% vs. Group 1 -9 ±3%; all *P*<0.01) caused by a significant reduction in ΔESV (Group 2 -14±4 mL, Group 3 -20±4 mL vs. Group 1 11±10 mL; all *P*<0.01). However, no difference in ΔEF or ΔESV between single MSC injection and repeated cell delivery could be observed (*P*=0.28 and *P*=0.79). Contractility measured by ΔdP/dtMAX

was significantly increased after single MSC injection, compared to BMMNC and MSC injection (Group 2 105±193 mmHg, Group 3 -340±63 mL; *P*=0.003). In fact, the second MSC injection on top of the first BMMNC injection (without significant difference compared to placebo) once more revealed the magnitude of effect on systolic function by MSC.

Overall, both cell groups showed an improvement in diastolic active relaxation parameters compared to placebo treated animals. This was reflected by a shortened ΔTau and decreased ΔPHT. Myocardial stiffness (Eed) was unaffected by cell therapy. No statistical difference in active and passive diastolic function between the cell treated groups could be observed, except for EDP.

No significant difference in echocardiographic parameters (Δend-systolic posterolateral WT and ΔEnd-diastolic WT) between single cell injection and repeated cell injection was observed.

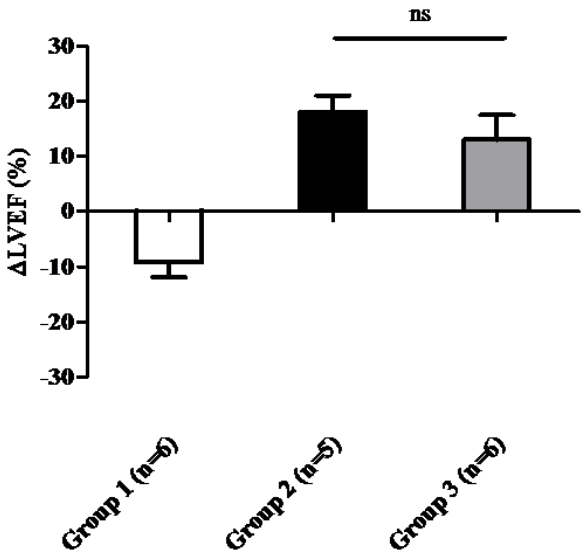


Figure 4. No beneficial effect of repeated cell delivery on LVEF 12 weeks post-MI.
No significant effect on ΔLVEF (baseline and 8 weeks after injection) between single and repeated cell injection was observed. LVEF= Left ventricular ejection fraction.

Collagen and capillary density

No significant difference in collagen density between groups was observed (Group 1 37±4, Group 2 42±2, Group 3 40±5; *P*=0.56). As shown in Figure 5, microvessel number was low in the control animals and increased upon MSC (Group 2) and repeated cell injection (Group 3). However, no significant difference between cell treated groups was observed.

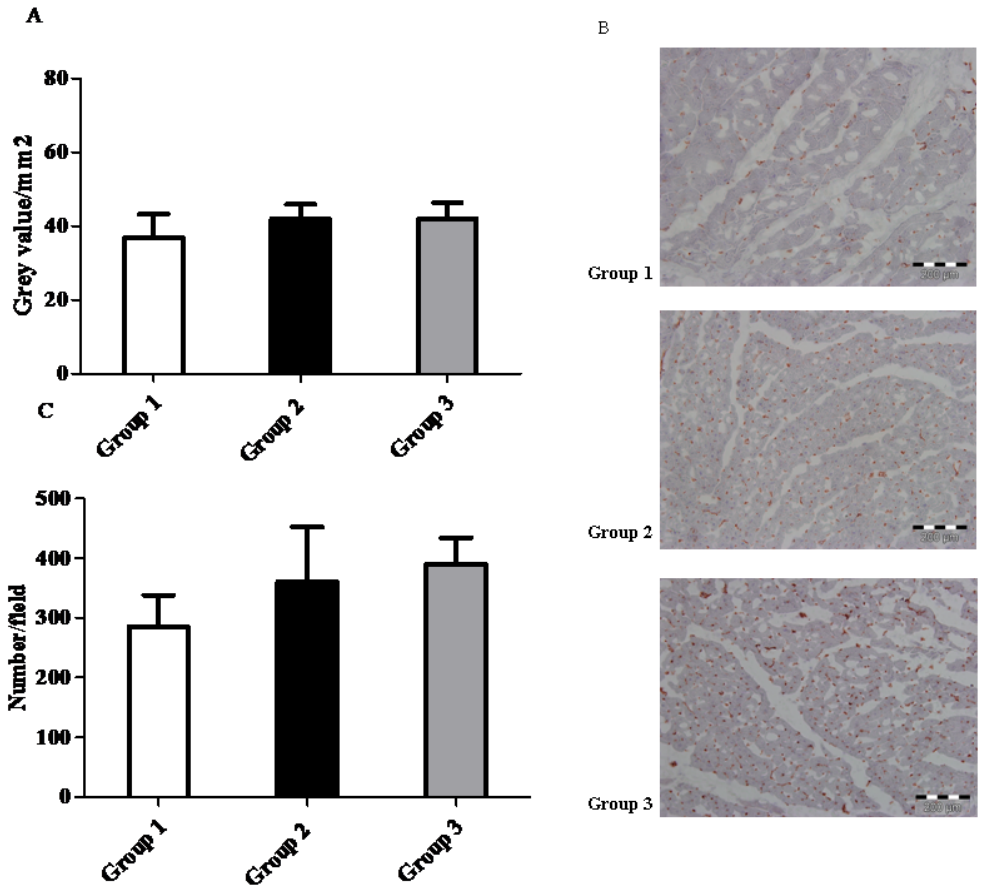


Figure 5. Similar microcirculatory remodeling after cell delivery 12 weeks post-MI.
5A. No difference in collagen density was observed. 5B. Representative images of Lectin staining at sacrifice. 5C. Microvascular formation (diameter 10-1000µm) determined by Lectin staining at sacrifice. n= 5 for group 1, n=5 for group 2, n=6 for group 3.

DISCUSSION

In this study, we performed a comparison between MSC and BMMNC via TE cell delivery in a porcine model of chronic ischemic heart disease. The main novel findings of our study are: 1) MSC are superior to BMMNC in improving systolic function; 2) The beneficial effect of MSC on LVEF was maintained during long-term follow-up; 3) Repeated cell injection does not further improve systolic function. However, MSC on top of BMMNC led to normalization of LV function, supporting the notion that MSC rather than BMMNC improve systolic function.

MSC treatment improves systolic function in contrast to BMMNC

We performed a head-to-head comparison of treatment with autologous BMMNC

and MSC, and demonstrated a beneficial effect for MSC on systolic function (EF 11.9±3%), whereas no positive effect of BMMNC on LVEF was found compared to placebo (EF -1.6±6%, and -7.8±8%, respectively). This observation is in line with the results of our large pre-clinical meta-analysis showing more benefit of MSC in ischemic heart disease compared to BMMNC[3]. On the contrary, Li et al did not find significant differences between MSC and BMMNC. However, they infused more BMMNC than MSC (BMMNC $4.7\pm1.7\times10^7$ vs. MSC $6.2\pm1.6\times10^5$)[17]. It is known that the number of cells is related to the magnitude of effect[3, 4]. It is important to stress that in our study we used similar numbers of both MSC and BMMNC (1×10^7). Our results may appear to be in contrast with data from previous clinical studies that did show modest but significant improvements of EF after treatment with BMMNC (approximately 3-5%)[18-20]. However, such studies were mainly performed in the setting of *acute* MI, and these effects were predominantly found in subgroups of large infarctions (baseline EF< 48%)[18]. In fact, several trials with BMMNC in *chronic* patients did not show improvement of LV systolic function[5, 21]. On the contrary, in a comparable patient cohort it was demonstrated that indeed MSC were able to improve cardiac function[22]. At present, a clinical study is initiated to directly compare these cells (TAC-HFT trail), and our pre-clinical results provide a robust rationale for such trial[23].

Long-term effects of MSC

The beneficial effects of MSC were consistent over time, since no difference was found between 4 and 8 weeks after cell injection (12% vs.18%; n.s.). It has been suggested that initial effects of cell therapy fades away over time[3, 24], although recent clinical data supports the notion of long-term effects[25]. Here, we conclude that in our model MSC treatment resulted in a stable and sustained effect on systolic function.

Repeated cell injection does not further improve cardiac function

Repetitive cell injections over time did not further improve systolic function compared to single MSC injection. This is at least in part due to the absent effect of BMMNC; this was surprising and not anticipated upon. Several studies investigated in particular the effect of repetitive cell transplantations[26]. Our observations are in line with a clinical trial investigating the effect of repeated BMMNC injections in patients with chronic heart failure showing no additional benefit of repeated BMMNC treatment on LVEF[27]. However, Yao et al. demonstrated that repeated BMMNC injection in patients with large acute MI resulted in a significant improvement in ΔLVEF compared to single cell injection[28]. This effect may be explained by the low baseline LVEF values (20-39%) which were higher in our study. Our results are inline with a recent observation[29], in which skeletal myoblasts were sequential injected in a chronic infarcted porcine myocardium. Although a different cell type was used repeated cell injections showed no additional improvement in LVEF

(repeated 15.1% vs. single 11.1%).

Histological effects of MSC injection

In an attempt to explain the observed effects on systolic function, histological analysis was performed after sacrifice (8 weeks after first injection). First, no difference in collagen density between cell groups was found, indicating that cell therapy did not have effects on scar reduction[6].

Second, measurement of capillary density showed a non-significant increased number of microvessels after MSC injection compared to placebo treatment (360 vs. 285). This is in line with the observed hemodynamic findings, and suggests a prominent role for enhanced microvasculature to explain the increased left ventricular function. This confirms previous observations[6, 30].

Finally, some MSC were observed in the infarcted tissue by fluorescent microscopy (data not shown), but it is unlikely that the observed effect was caused by differentiation of MSC into cardiac lineages as suggested by others[31]. However, MSC may lead to pro-longed secretion of paracrine factors activating capillary angiogenesis.

Study limitations

Our ischemia/reperfusion model resulted in a limited decrease in EF (appr. 50% 4 weeks after MI), but not severe heart failure. This is related to the chosen model (temporary occlusion of LCX for 75 minutes), and maybe due to the fact that animals were treated with similar medication protocols (e.g. beta blockers, which may be cardioprotective) compared to the patients suffering from MI. Nevertheless, significant effects on LVEF were observed.

The porcine model is considered the best possible model to resemble the clinical situation, although major differences exist (e.g. risk factors, comorbidity, follow-up duration), which prevent direct extrapolation to patient management. Nevertheless, our group demonstrated that large animal models can accurately predict human clinical outcome and these models are frequently used for translational purposes[3].

Nowadays, cardiac MRI is considered the gold standard to measure LVEF and volumes. However, due to practical reasons we performed echocardiographic and pressure-volume loop analysis. These techniques are still considered reliable, reproducible and a valid measure of LV function, and are therefore most often used in pre-clinical research models.

CONCLUSIONS

We clearly demonstrated that MSC are more potent in terms of improvement of LVEF than BMMNC in a chronic model for ischemic heart disease. Moreover, these effects are sustained over time. Our data do not support strategies using repetitive injections, although using different combinations of cells may be of value in more severe heart failure. These data should encourage researchers and clinicians to focus future studies on other cell types than BMMNC.

Acknowledgements

We gratefully acknowledge Cees Verlaan, Merel Schurink, Marlijn Jansen, Maringa Emons and Joyce Visser for excellent technical assistance and animal care. In addition, the authors acknowledge the technical assistance provided by Esther van Eeuwijk and Sridevi Jaksani.

Sources of funding

This work was supported by the Netherlands Heart Foundation '[2003B07304 and 2010T025]', BSIK program "Dutch Program for Tissue Engineering", '[grant 6746]', and a Bekalis price (PD). This research is part of the Project P1.04 SMARTCARE of the research program of the BioMedical Materials institute, co-funded by the Dutch Ministry of Economic Affairs.

Disclosures: none

REFERENCES

1. Rosamond W, Flegal K, Furie K, Go A, Greenlund K, Haase N, Hailpern SM, Ho M, Howard V, Kissela B, Kittner S, Lloyd-Jones D, McDermott M, Meigs J, Moy C, Nichol G, O'Donnell C, Roger V, Sorlie P, Steinberger J, Thom T, Wilson M, Hong Y, for the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart Disease and Stroke Statistics--2008 Update: A Report From the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 2008;117:e25-146.

2. Abdel-Latif A, Bolli R, Tleyjeh IM, Montori VM, Perin EC, Hornung CA, Zuba-Surma EK, Al-Mallah M, Dawn B. Adult Bone Marrow-Derived Cells for Cardiac Repair: A Systematic Review and Meta-analysis. Arch Intern Med 2007;167:989-997.

3. van der Spoel TIG, Jansen of Lorkeers S, Agostoni P, van Belle E, Gyongyosi M, Sluijter JPG, Cramer MJ, Doevendans PA, Chamuleau SAJ. Human relevance of pre-clinical studies in stem cell therapy; systematic review and meta-analysis of large animal models of ischemic heart disease. Cardiovasc Res 2011;91:649-658.

4. Jeevanantham V, Butler M, Saad A, Abdel-Latif A, Zuba-Surma EK, Dawn B. Adult Bone Marrow Cell Therapy Improves Survival and Induces Long-Term Improvement in Cardiac Parameters / Clinical Perspective. Circulation 2012;126:551-568.

5. van Ramshorst J, Bax JJ, Beeres SL, Dibbets-Schneider P, Roes SD, Stokkel MP, de Roos A, Fibbe WE, Zwaginga JJ, Boersma E, Schalij MJ, Atsma DE. Intramyocardial bone marrow cell injection for chronic myocardial ischemia: A randomized controlled trial. JAMA 2009;301:1997-2004.

6. Perin EC, Silva GV, Assad JA, Vela D, Buja LM, Sousa AL, Litovsky S, Lin J, Vaughn WK, Coulter S, Fernandes MR, Willerson JT. Comparison of intracoronary and transendocardial delivery of allogeneic mesenchymal cells in a canine model of acute myocardial infarction. J Mol Cell Cardiol 2008;44:486-495.

7. Van der Spoel TIG, Vrijsen KR, Koudstaal S, Sluijter JPG, Nijsen JFW, De Jong H, Hoefer IE, Cramer MJM, Doevendans PA, Van Belle E, Chamuleau SAJ. Transendocardial cell injection is not superior to intracoronary infusion in a porcine model of ischemic cardiomyopathy: A study on delivery efficiency. J Cell Mol Med 2012.

8. Timmers L, Henriques JPS, de Kleijn DPV, DeVries JH, Kemperman H, Steendijk P, Verlaan CWJ, Kerver M, Piek JJ, Doevendans PA, Pasterkamp G, Hoefer IE. Exenatide Reduces Infarct Size and Improves Cardiac Function in a Porcine Model of Ischemia and Reperfusion Injury. J Am Coll Cardiol 2009;53:501-510.

9. Noort WA, Oerlemans MIFJ, Rozemuller H, Feyen D, Jaksani S, Stecher D, Naaijkens B, Martens AC, Buhring HJ, Doevendans PA, Sluijter JPG. Human versus porcine mesenchymal stromal cells: phenotype, differentiation potential, immunomodulation and cardiac improvement after transplantation. Journal of Cellular and Molecular Medicine 2012;16:1827-1839.

10. Ben-Haim SA, Osadchy D, Schuster I, Gepstein L, Hayam G, Josephson ME. Nonfluoroscopic, in vivo navigation and mapping technology. Nat Med 1996;2:1393-1395.

11. Gepstein L, Hayam G, Ben-Haim SA. A Novel Method for Nonfluoroscopic Catheter-Based

Electroanatomical Mapping of the Heart : In Vitro and In Vivo Accuracy Results. Circulation 1997;95:1611-1622.

12. Baan J, van der Velde ET, de Bruin HG, Smeenk GJ, Koops J, van Dijk AD, Temmerman D, Senden J, Buis B. Continuous measurement of left ventricular volume in animals and humans by conductance catheter. Circulation 1984;70:812-823.

13. Steendijk P, Baan J. Comparison of intravenous and pulmonary artery injections of hypertonic saline for the assessment of conductance catheter parallel conductance. Cardiovasc Res 2000;46:82-89.

14. Steendijk P, BaanJr. J, Van Der Velde ET, Baan J. Effects of critical coronary stenosis on global systolic left ventricular function quantified by pressure-volume relations during dobutamine stress in the canine heart. J Am Coll Cardiol 1998;32:816-826.

15. Ten Brinke EA, Klautz RJ, Verwey HF, van der Wall EE, Dion RA, Steendijk P. Single-beat estimation of the left ventricular end-systolic pressure-volume relationship in patients with heart failure. Acta Physiologica 2010;198:37-46.

16. Sluijter JPG, Smeets MB, Velema E, Pasterkamp G, de Kleijn DPV. Increased collagen turnover is only partly associated with collagen fiber deposition in the arterial response to injury. Cardiovasc Res 2004;61:186-195.

17. Li SR, Qi XY, Hu FL, Zhang JQ, Wang TH, Dang Y, Meng CL, Liu HL, Li YX, Wu D, Dong J, Xun LY, Gao LH, Jin FC. Mechanisms of improvement of left ventricle remodeling by trans-planting two kinds of autologous bone marrow stem cells in pigs. Chin Med J (Engl) 2008;121:2403-2409.

18. Schachinger V, Erbs S, Elsasser A, Haberbosch W, Hambrecht R, Holschermann H, Yu J, Corti R, Mathey DG, Hamm CW, Suselbeck T, Assmus B, Tonn T, Dimmeler S, Zeiher AM, the REPA. Intracoronary Bone Marrow-Derived Progenitor Cells in Acute Myocardial Infarction. N Engl J Med 2006;355:1210-1221.

19. Huikuri HV, Kervinen K, Niemela M, Ylitalo K, Saily M, Koistinen P, Savolainen ER, Ukkonen H, Pietila M, Airaksinen JKE, Knuuti J, Makikallio TH. Effects of intracoronary injection of mononuclear bone marrow cells on left ventricular function, arrhythmia risk profile, and restenosis after thrombolytic therapy of acute myocardial infarction. Eur Heart J 2008;29:2723-2732.

20. Cao F, Sun D, Li C, Narsinh K, Zhao L, Li X, Feng X, Zhang J, Duan Y, Wang J, Liu D, Wang H. Long-term myocardial functional improvement after autologous bone marrow mononuclear cells transplantation in patients with ST-segment elevation myocardial infarction: 4 years follow-up. Eur Heart J 2009;30:1986-1994.

21. Perin EC, Willerson JT, Pepine CJ, Henry TD, Ellis SG, Zhao DX, Silva GV, Lai D, Thomas JD, Kronenberg MW, Martin AD, Cardiovascular Cell Therapy Research Network (CCTRN). Effect of transendocardial delivery of autologous bone marrow mononuclear cells on functional capacity, left ventricular function, and perfusion in chronic heart failure: the FOCUS-CCTRN trial. JAMA 2012;307:1717-1726.

22. Williams AR, Trachtenberg B, Velazquez DL, McNiece I, Altman P, Rouy D, Mendizabal AM, Pattany PM, Lopera GA, Fishman J, Zambrano JP, Heldman AW, Hare JM. Intramyocardial Stem Cell Injection in Patients With Ischemic Cardiomyopathy / Novelty and Significance. Circ Res

2011;108:792-796.

23. Trachtenberg B, Velazquez DL, Williams AR, McNiece I, Fishman J, Nguyen K, Rouy D, Altman P, Schwarz R, Mendizabal A, Oskouei B, Byrnes J, Soto V, Tracy M, Zambrano JP, Heldman AW, Hare JM. Rationale and design of the Transendocardial Injection of Autologous Human Cells (bone marrow or mesenchymal) in Chronic Ischemic Left Ventricular Dysfunction and Heart Failure Secondary to Myocardial Infarction (TAC-HFT) trial: A randomized, double-blind, placebo-controlled study of safety and efficacy. 161 ed. 2011. p. 487-493.

24. Meyer GP, Wollert KC, Lotz J, Steffens J, Lippolt P, Fichtner S, Hecker H, Schaefer A, Arseniev L, Hertenstein B, Ganser A, Drexler H. Intracoronary Bone Marrow Cell Transfer After Myocardial Infarction: Eighteen Months' Follow-Up Data From the Randomized, Controlled BOOST (BOne marrOw transfer to enhance ST-elevation infarct regeneration) Trial. Circulation 2006;113:1287-1294.

25. Leistner DM, Fisher-Rasokat U, Honold J, Seeger FH, Schachinger V, Lehmann R, Martin H, Burck I, Urbich C, Dimmeler S, Zeiher AM, Assmus B. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI): final 5-year results suggest long-term safety and efficacy. Clin Res Cardiol 2011;100:925-934.

26. Poh KK, Sperry E, Young RG, Freyman T, Barringhaus KG, Thompson CA. Repeated direct endomyocardial transplantation of allogeneic mesenchymal stem cells: Safety of a high dose, "off-the-shelf", cellular cardiomyoplasty strategy. International Journal of Cardiology 2007;117:360-364.

27. Diederichsen ACP, Moller JE, Thayssen P, Videbaek L, Saekmose SG, Barington T, Kassem M. Changes in left ventricular filling patterns after repeated injection of autologous bone marrow cells in heart failure patients. Scand Cardiovasc J 2010;44:139-145.

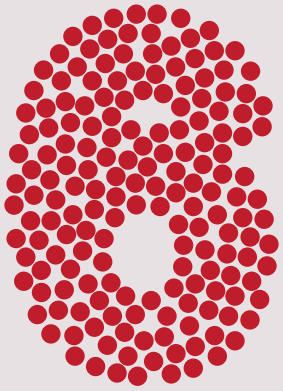
28. Yao K, Huang R, Sun A, Qian J, Liu X, Ge L, Zhang Y, Zhang S, Niu Y, Wang Q, Zou Y, Ge J. Repeated autologous bone marrow mononuclear cell therapy in patients with large myocardial infarction. Eur J Heart Fail 2009;11:691-698.

29. Gavira JJ, Nasarre E, Abizanda G, Perez-Ilzarbe M, de Martino-Rodriguez A, Garcia de Jalon JA, Mazo M, Macias A, Garcia-Bolao I, Pelacho B, Martinez-Caro D, Prosper F. Repeated implantation of skeletal myoblast in a swine model of chronic myocardial infarction. Eur Heart J 2010;31:1013-1021.

30. Schneider C, Jaquet K, Geidel S, Rau T, Malisius R, Boczor S, Zienkiewicz T, Kuck KH, Krause K. Transplantation of Bone Marrow-Derived Stem Cells Improves Myocardial Diastolic Function: Strain Rate Imaging in a Model of Hibernating Myocardium. Journal of the American Society of Echocardiography 2009;22:1180-1189.

31. Siegel G, Krause P, Wöhrle S, Nowak P, Ayturan M, Kluba T, Brehm BR, Neumeister B, Köhler D, Rosenberger P, Just L, orthoff H, Schaefer R. Bone marrow-derived human mesenchymal stem cells express cardiomyogenic proteins but do not exhibit functional cardiomyogenic differentiation potential. Stem Cells Dev 2012.


T.I.G van der Spoel



LAYER SPECIFIC RADIO FREQUENCY ULTRASOUND BASED STRAIN ANALYSIS IN A PORCINE MODEL OF ISCHEMIC CARDIOMYOPATHY VALIDATED BY A GEOMETRICAL MODEL.

Frebus J. van Slochteren MSc[1]
Tycho I.G. van der Spoel MD[1]
Hendrik H.G. Hansen MSc[2]
Peter H.M. Bovendeerd PhD[3]
Pieter A. Doevendans MD PhD[1,4]
Joost P.G. Sluijter PhD[1,4]
Steven A.J. Chamuleau MD PhD[1,4]
Chris C.L. de Korte PhD[2]

[1] Department of Cardiology, University Medical Center Utrecht, The Netherlands.
[2] Medical UltraSound Imaging Center, Department of Radiology, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands.
[3] Department of Biomedical Technology, Eindhoven University of Technology.
[4] Interuniversity Cardiology Institute of the Netherlands (ICIN), Utrecht, The Netherlands.

ABSTRACT

Introduction: Since the sub- endocardium is more vulnerable to increased wall stress, ischemia, and interstitial fibrosis, we hypothesized that layer specific strain might be a sensitive marker of changes in regional left ventricular (LV) performance. Radiofrequency (RF) ultrasound based analysis could provide superior deformation assessment compared to clinically available speckle based deformation imaging techniques. In this study, we investigate the ability of RF based myocardial deformation measurements to distinguish healthy from damaged myocardium in a porcine model of chronic myocardial infarction (MI).

Methods: In twenty-one pigs RF data was acquired epicardially in healthy regions, and in five pigs with a chronic MI. RF data was acquired epicardially in infarcted regions. Radial and longitudinal strains were estimated in the sub- endocardial, midwall, and sub- epicardial layers. Collagen content was quantified in healthy and infarcted regions of five pigs. We used an analytical geometrical model of the LV as a reference of normal radial deformation values in different myocardial layers when assuming tissue incompressibility.

Results: Mean ± se values of the peak radial strain estimates of the sub- endocardial, midwall, and sub- epicardial layers of the healthy and infarcted tissue were: 82.7 ± 5.2 % and 39.9 ± 10.8 % ($p=0.002$), 63.6 ± 3.3 % and 38.8 ± 7.7 % ($p=0.004$), and 34.3 ± 3.0 % and 35.1 ± 5.2 % ($p=0.9$), respectively. The difference between the sub- endocardium and the sub- epicardium was decreased 12 weeks after MI. The analytically determined normal strain values in the sub- endocardial, midwall, and sub- epicardial layers respectively were: 80.5 %, 50.6 %, and 34.9 %.

Conclusion: The estimated strain values are in the same order of magnitude as the analytically determined strain values, indicating that the estimated values are realistic, and show that endocardial radial strain in healthy tissue can be as high as 80%. Since strain assessment by RF ultrasound analysis shows most affected strain values in the sub- endocardium and midwall layers which are most sensitive for ischemia, and interstitial fibrosis, we believe that this technique can be used to quantify subtle changes in the myocardium, and sub-clinical changes in local LV performance.

INTRODUCTION

To assess early local pathological changes in the myocardium during e.g. arrhythmogenic cardiomyopathy, stable angina pectoris, or assess changes in the myocardium that are induced by cardiac regenerative therapies aiming at local enhancement of vasculogenesis, cardiomyogenesis, or matrix enhanced myocardium stabilization, the use of regional function assessment has been proposed[1, 2]. Besides providing information about the local pathological changes in the myocardium, this can help to identify dominant therapeutic mechanisms[3]. Unfortunately confounding factors affect deformation imaging[4]. In combination with limited spatial and temporal resolution of clinically available measurement techniques this prevented a widespread application of deformation imaging to assess subtle changes in the myocardium. However, when confounding factors are controlled, and higher spatial and temporal resolutions can be achieved, deformation imaging techniques can become available to assess local layer specific changes in the myocardium. Since local cardiac tissue deformation reflects the mechanical response of the myocardium to changes of contractility and stiffness, and both the before mentioned pathologies and therapy alter these tissue properties, it is most likely that deformation is altered accordingly. Moreover, the sub-endocardium is more vulnerable to increased wall stress, ischemia, and interstitial fibrosis. Since local extracellular matrix deposition enhances tissue stiffening and impairs deformation, we hypothesized that layer specific strain might be a sensitive marker of sub-clinical changes in regional LV performance. In this study, we investigate the ability of radiofrequency (RF) based myocardial deformation measurements to distinguish healthy from chronically infarcted myocardium in a porcine model of chronic myocardial infarction (MI). Local tissue deformation and local collagen content were quantified in different layers of the healthy and infarcted myocardium. Thereby local radial (thickening) and lateral (shortening) deformation indices that can be used to assess subtle pathological changes are derived. An analytical geometrical model of the left ventricle based on dimensions derived from the experiments served as a reference of normal radial deformation values that can be expected in the different myocardial layers when assuming tissue incompressibility[5].

METHODS

Porcine myocardial infarction model

Twenty-one female Dalland Landrace pigs (weighing 69 ± 4 kg) received care in accordance with the *Guide for the Care and Use of Laboratory Pigs* prepared by the Institute of Laboratory Animal Resources. Experiments were approved by the Animal Experimentation Committee of the Utrecht University, the Netherlands. Closed-chest MI was created by a percutaneous balloon of equivalent size to the proximal left circumflex artery (LCX). Prior to MI all animals received an oral dose

of amiodarone (400 mg/day; start 10 days prior to MI) and clopidogrel (75 mg/day; start 3 days prior to MI, Sanofi Aventis, Gouda, the Netherlands). A bolus of 500 mg acetylic salicylic acid (Centrafarm, Etten-Leur, the Netherlands) was given the day before the occlusion. The balloon was inflated for 75 minutes at 5-8 atm[6]. Complete occlusion of the LCX was confirmed by angiography. To prevent ventricular arrhythmias, 300 mg amiodarone (Centrafarm, Etten-Leur, the Netherlands) was given intravenously. External defibrillation (150-200 Joules) was used if ventricular fibrillation occurred. After the procedure, coronary angiography was performed to confirm vessel patency. After recovery, the animals received daily an oral dose of 50 mg metoprolol, 400 mg amiodarone, 75 mg clopidogrel, and 160 mg acetylic salicylic acid until termination to prevent thrombosis and arrhythmias.

Experimental protocol

Twelve weeks after MI a thoracotomy was performed for epicardial measurements of ultrasound RF data in long-axis cross sections from the LV lateral and anterior wall as depicted in figure 1. Data recorded from the LCX territory of the lateral wall are hereafter referred to as infarcted, and since the left anterior descending coronary artery (LAD) is untreated, data recorded from the anterior wall are referred to as healthy. Locations where data were recorded were marked by two epicardial stitches. To avoid errors in deformation estimation caused by motion of the heart in a direction perpendicular to the ultrasound imaging plane (out of plane motion), the apex was loosely fixed by using a Starfish Cardiac Positioner (Medtronic Inc., Minneapolis, MN, USA), and the ultrasound probe was manually moved with the tissue. After data acquisition hearts were excised and tissue samples marked by the two stitches were taken for collagen quantification.

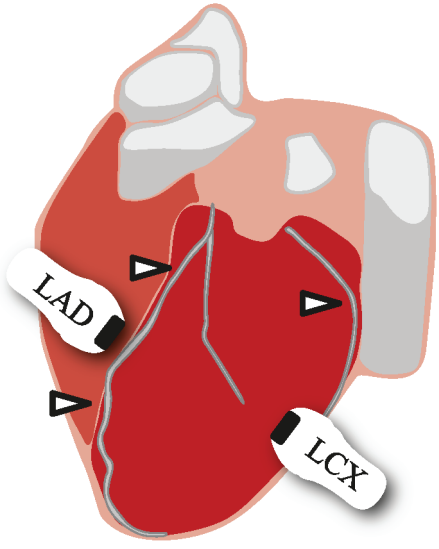


Figure 1. Measurement positions at the left ventricular lateral (LCX) and anterior wall (LAD).

Functional parameters

End diastolic and end systolic volumes, and ejection fraction (EF) where measured at baseline and after four weeks by pressure-volume loops using a conductance catheter.

RF echocardiographic data acquisition and deformation estimation

RF data were acquired with a Medison Accuvix V10 ultrasound scanner with a radiofrequency data interface in combination with a 3.8 centimeter wide linear array probe (L5-13) with a center frequency of 8.7 MHz. The RF data were digitized at a sampling rate of 61.6 MHz. To determine the layer specific deformation (strain) first the motion of small tissue regions (0.6 mm x 1mm) was tracked over one cardiac cycle starting in the ultrasound image frame that revealed minimum wall thickness (end diastole). To estimate the tissue motion (displacement) from one ultrasound frame to the next an iterative search algorithm was used[7, 8]. Both the motion in the direction of the ultrasound beam (radial tissue direction) as well as perpendicular to the ultrasound beam (longitudinal tissue direction) was determined. In each of five iterations RF data for a certain tissue region in one ultrasound frame were shifted over the RF data within a larger tissue region (search region) in the next ultrasound frame and the 2D cross-correlation value was calculated. The 2D shift that resulted in the highest cross-correlation value (best match) was a value for the occurred 2D motion for that part of the tissue. The accuracy of the motion estimates was increased in each iteration. In the first four iterations this was achieved by halving the window sizes of both the initial window and the search window. In the final iteration the motion estimates were improved by sub sample aligning of the RF data of the initial window and search window[7, 9]. The motion estimates of preceding iterations were used as a starting point for the search in each new iteration. In the first iteration the cross-correlations were calculated using the envelope of the RF data to get a robust intial motion estimate. After each iteration motion estimates were median filtered to remove outliers. The window size of the median filter was 9 × 9 motion estimate values. The iterative procedure resulted in motion estimates for tissue segments of 0.125 mm x 0.200 mm. To derive the radial and longitudinal strains a 2D least-squares strain estimator (LSQSE) was applied to the radial and longitudinal motion field, respectively[10, 11]. Strain values were averaged in three myocardial layers of equal thickness: sub- endocardial, midwall, and sub- epicardial. Local radial and lateral peak strain values were used to quantify local cardiac biomechanics.

Analytical strain

We assumed that myocardial tissue is composed of incompressible spongy solid material filled with incompressible intracoronary blood as defined by Huyghe et al.[5] Then tissue deformations in the three orthogonal directions of the cardiac coordinate system are related. We used an analytical elaboration of this relation to calculate

the expected values of the strains in the different layers of the myocardium. From two truncated ellipsoids, we constructed an LV shape with dimensions similar to the average values measured from the five animals used during the experiments (figure 3). End diastolic inner diameter (D_{edi}) = 4.5 cm, end diastolic outer diameter (D_{edo}) = 6.5 cm, and end diastolic length (l_{ed}) = 7.5 cm. A longitudinal shortening (εl) of -0.22 (12) was applied, and an epicardial diameter change $(\lambda_{c,epi})$ of 0.95 (13). The longitudinal (λ_l) and circumferential (λ_c) stretch ratios can be calculated from the length and the volume of the truncated ellipsoidal geometry.

[1] [2] [3]

$$V = \frac{2}{3} \cdot \pi \cdot \left(D/2\right)^2 \cdot l \qquad \lambda_l = \frac{l_{end\ systole}}{l_{end\ diastole}} \qquad \lambda_c = \left(\frac{V_{end\ diastole} \cdot l_{end\ systole}}{V_{end\ systole} \cdot l_{end\ diastole}}\right)^{-1/2}$$

In which l is the length of the ventricle, D is the diameter of interest (endocardial, midwall, or epicardial), and V is the volume inside the diameter of interest. These parameters are derived at end diastole and end systole. The assumption of incompressibility of the myocardium imposes the relations between the three orthogonal stretch ratios λ_l, λ_c, and λ_r (radial) to be

[4] $$\lambda_r = \frac{1}{\lambda_l \cdot \lambda_c}$$

Now λ_r can be calculated for different layers in the myocardium by altering the diameter D and length l The diameters and lengths used for the calculation V of in different myocardial layers are listed in table 1.

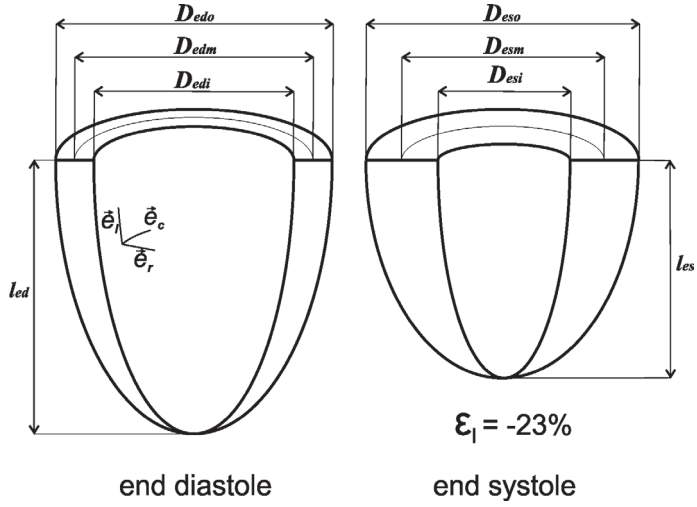


Figure 3. Geometrical model with the essential parameters for the analytical determination of layer specific radial strain values.

		end diastolic	end systolic
Epicardium			
length		l_{ed}	$l_{es} = l_{ed} + l_{ed} \cdot \varepsilon_l$
diameter		D_{edo}	$D_{eso} = D_{edo} - D_{edo} \cdot \lambda_{c,epi}$
myocardial volume	wall	$V_w = \frac{2}{3} \cdot \pi \cdot l_{ed} \cdot \left(\left({}^{D_{edo}}/_{2} \right)^2 - \left({}^{D_{edi}}/_{2} \right)^2 \right)$	
Endocardium			
length		l_{ed}	$l_{es} = l_{ed} + l_{ed} \cdot \varepsilon_l$
cavity volume			$V_{esi} = \frac{2}{3} \cdot \pi \cdot \left({}^{D_{eso}}/_{2} \right)^2 \cdot l_{es} - V_w$
diameter		D_{edi}	$D_{esi} = 2 \cdot \sqrt{{}^{\frac{3}{2} \cdot V_{esi}}/_{\pi \cdot l_{es}}}$
Midwall			
length		l_{ed}	$l_{es} = l_{ed} + l_{ed} \cdot \varepsilon_l$
wall thickness		$WT_{ed} = \frac{(D_{edo} - D_{edi})}{2}$	$WT_{es} = \frac{(D_{eso} - D_{esi})}{2}$
diameter		$D_{edm} = D_{edo} - WT_{ed}$	$D_{esm} = D_{eso} - WT_{es}$

Table 1. Parameters used for layer specific strain calculation

Histology

After euthanasia tissue samples of the exact areas that were used for RF data acquisition were taken from the infarcted and remote regions of the heart of five pigs. Samples were fixed in 4% formalin, and embedded in paraffin. Sections of 5 micrometers were cut, and collagen content was quantified by picrosirius red staining with circularly polarized light and digital image microscopy as described before[14]. Multiple images of the infarcted and healthy tissue were obtained at 1.25x magnification, and merged together to reconstruct the exact tissue area that was used for RF data acquisition (figure 2). After conversion into grey value images the image intensity was quantified in the endocardial, midwall, and epicardial layers, and collagen content was expressed as a fraction of the area using Image J (version 1.44p). Gaps in the tissue, induced by the cutting of the 3 x 1 cm samples, and the intraluminal areas of blood vessels were excluded from the analysis.

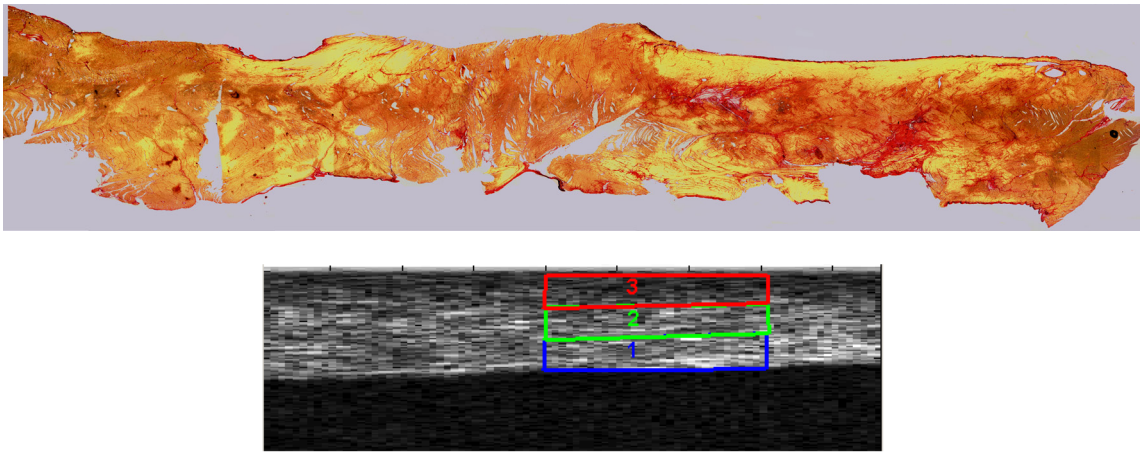


Figure 2. Histological section of infarcted region with corresponding ultrasound image. Regions were deformation was assessed are depicted by the rectangles: sub- endocardium (blue), midwall (green), sub- epicardium (red).

Statistics

Data are presented as mean ± se. An independent sample t-test was used to compare parameters measured from healthy and infarcted tissue for each layer. P values < 0.05 were considered significant. Pearson linear correlations were calculated to determine the coherence between area collagen and echocardiographic strain measurements.

RESULTS

Myocardial infarction reduced the ejection fraction of the pigs from 62 ± 2 % at baseline to 52 ± 3 % after twelve weeks. End systolic volume increased from 41 ± 3 mL at baseline to 51 ± 11mL after 12 weeks, and end diastolic volume did not change 107 ± 8 mL vs. 106 ± 16mL. Mean heart rate during measurements was 51 ± 7 beats per minute.

RF based strain estimation

Typical strain curves measured from healthy and infarcted tissue are shown in figure 4. Both in healthy and infarcted tissue a gradient exists between the endocardial, midwall, and epicardial strain.

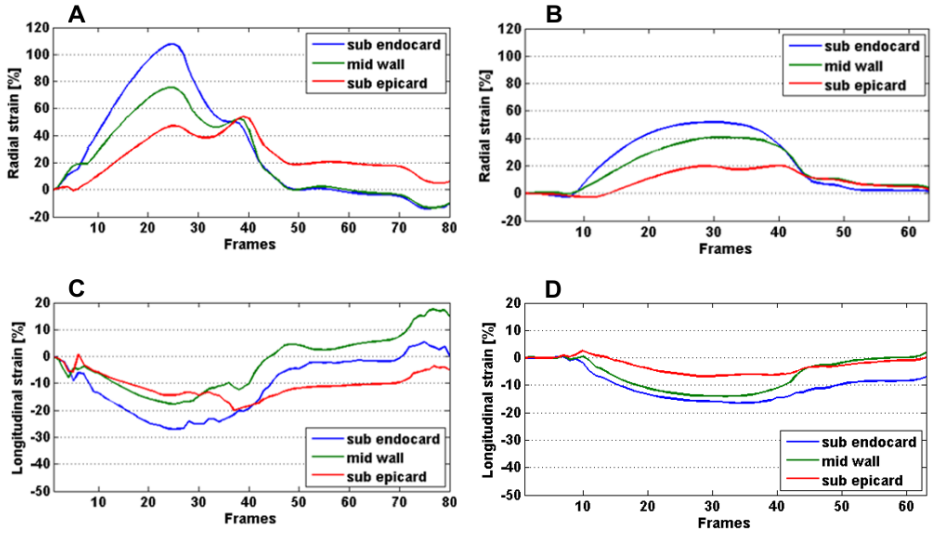


Figure 4. Radial and longitudinal strain curves of healthy (A+C) and infarcted (B+D) tissue.

In the depicted strain curves obtained from the healthy tissue postsystolic thickening and shortening can be observed at frame 35 approximately. This phenomenon was not observed in all samples. The nonzero strain estimates at end diastole imply drift of the tracking algorithm. This is most pronounced in the longitudinal strain. Mean RF based strain values of all 5 pigs are shown in figure 5 and table 2. Mean peak radial strain values of the healthy sub- endocardial, midwall, and sub- epicardial layers of all pigs are 82.7 ± 5.2 %, 63.6 ± 3.3 %, and 34.3 ± 3 %, respectively. The transmural difference between the sub- endocardium and the sub- epicardium is 48.4 ± 4.8 %. In the infarcted wall the mean peak strain values are: 39.9 ± 10.8 %, 38.8 ± 7.7 %, and 35.1 ± 5.2 %. The difference between the endocardium and the epicardium is 4.8 ± 11.8 %. The mean peak radial strain values in the sub- endocardial and mid wall regions are significantly lower, and the gradient between the endocardium and the epicardium is decreased 12 weeks after myocardial infarction. Mean peak longitudinal strain values of the healthy layers are: -31.9 ± 3.8 %, -26.9 ± 3.7 %, and -17.1 ± 2.1 %, with a transmural difference of 14.8 ± 2.9%. A smaller transmural gradient can be observed in comparison to the gradient of the peak radial strain values. In infarcted regions the mean peak longitudinal strain values are: -22.7 ± 3.5 %, -20.4 ± 6.4 %, and -9.5 ± 2.6 % with a gradient of 13.2 ± 4.4%. Both, the mean peak longitudinal strain values and the transmural gradient are diminished 12 weeks after myocardial infarction.

	Peak radial strain				Peak longitudinal strain		
	Infarct (n=5)	Remote (n=21)	p	Model	Infarct (n=5)	Remote (n=21)	p
Sub- endocard	40 ± 10.8%	82.7 ± 5.2%	0.002	80.5%	-22.7 ± 3.5%	-31.9 ± 3.8%	0.27
Midwall	38.8 ± 7.73%	63.6 ± 3.3%	0.004	50.6%	-20.4 ± 6.4%	-26.9 ± 3.7%	0.44
Sub- epicard	35.1 ± 5.24%	34.3 ± 3%	0.9	34.9%	-9.5 ± 2.6%	-17.1 ± 2.1%	0.12
Difference	4.8 ± 11.8%	48.4 ± 4.8%	0.001	45.6%	13.2 ± 4.4%	14.8 ± 2.9%	0.8

Table 2. Radial and longitudinal strain in sub- endocardial, midwall, and sub- epicardial layers and the gradient between the sub- endocardial and sub- epicardial layers.

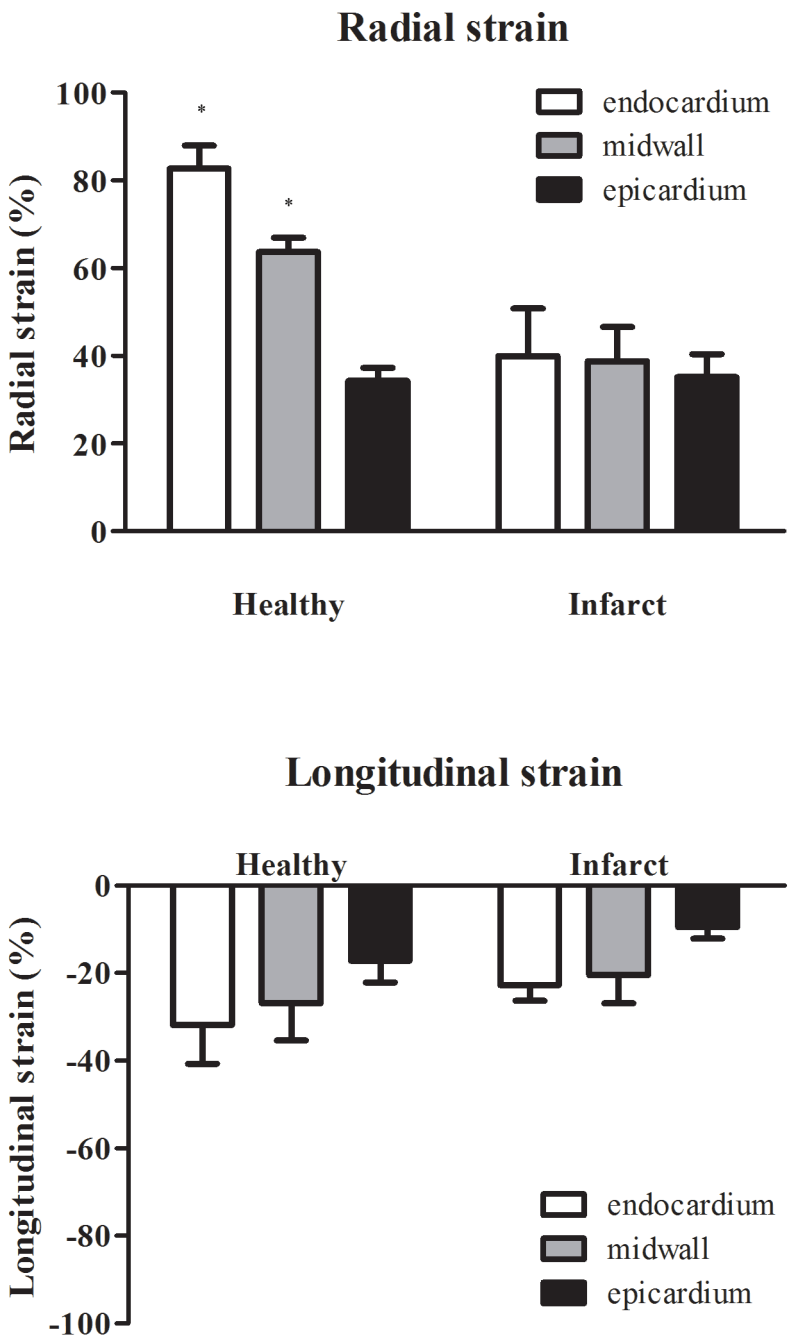


Figure 5. Peak radial and longitudinal strain values. Data is presented as mean ± se. *P<0.01.

Analytical Solution

The analytically determined strain values in the endocardial, midwall, and epicardial layers respectively are 80.5 %, 50.6 %, and 34.9 %. These are in the same order of magnitude as the estimated strain values, indicating that our assumptions are correct, and showing that endocardial radial strain in healthy tissue can be as high as 80%. In figure 5 the radial strain values that result from the analytical solution are projected alongside the measured radial strain values of healthy tissue.

Histology

A difference was found between the mean percentage of collagen in the endocardial, midwall, and epicardial layers of the healthy and infarcted tissue by picrosirius red staining. Collagen content was 3.36 ± 1.7 %, 1.34 ± 0.6 %, and 1.84 ± 0.46 % in the endocardial, midwall, and epicardial layers of the heathy myocardium respectively, and 8.8 ± 3.4 %, 6.5 ± 3.1 %, and 4.8 ±1 %, in the same layers in the infarcted myocardium. These values are depicted in figure 7. As expected higher strain values were found in areas with a low collagen content, and lower strain values were found in regions with higher collagen content, but correlations between collagen content and the strain values were poor (figure 8). The R^2 values of the correlation between collagen content and strain in the infarcted and healthy tissue are 0.045 and 0.227 respectively, for radial strain and 0.112, and 0.219, respectively for longitudinal strain.

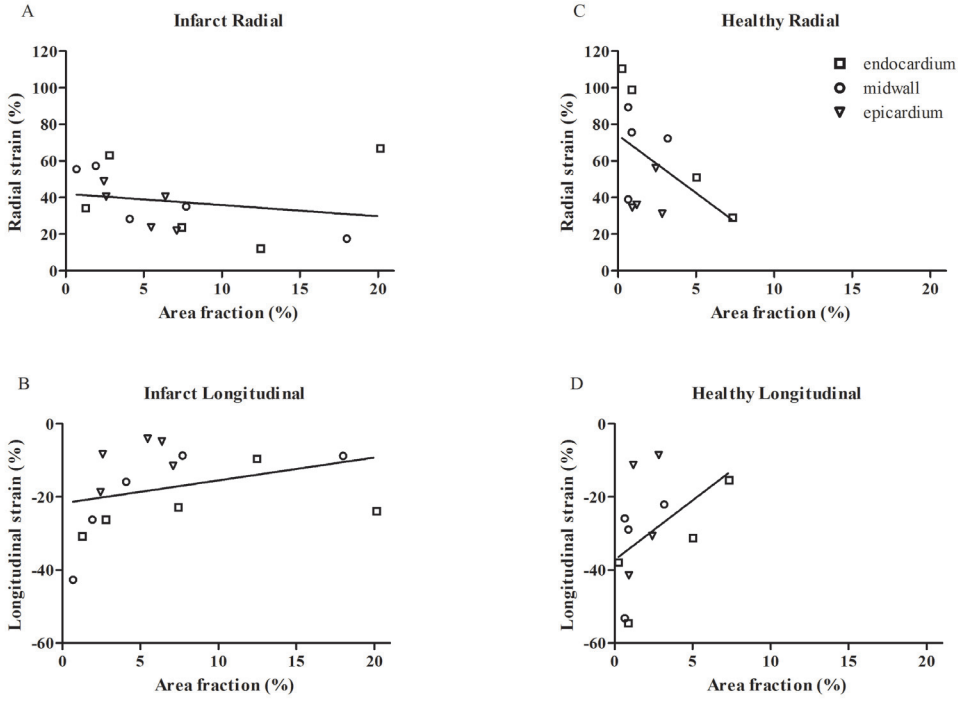


Figure 8: Correlation between the percentage of the area staining positive for collagen, and radial and lateral strain. Radial strain in the infarcted tissue (A), longitudinal strain in the infarcted tissue (B), radial strain in the healthy tissue (C), and longitudinal strain in the healthy area (D). The results of the endocardial, midwall, and epicardial layers are shown by individual markers.

DISCUSSION

In this study both measurements and geometric modeling show that (1) a transmural gradient exists in the radial and longitudinal strain, that (2) radial strain in the endocardium can range up to 80%, and that (3) the transmural gradient diminishes after MI. To our best knowledge this was demonstrated for the first time by the use of RF ultrasound analysis. The high frequency of the RF ultrasound signal, and the detailed tracking and strain calculation algorithm allows strain estimation in different myocardial layers with sub- millimeter resolution. The lower spatial and temporal resolution of clinically available speckle tracking and MRI tagging techniques, or the difficulties to optimally align the ultrasound beam for tissue Doppler imaging hampers accurate quantification of radial strain in different layers of the myocardium. The peak radial strain values in the three transmural layers that were found with this analysis are in correspondence with the condition of conservation of volume as validated by the geometrical model. Moreover, the mean values of the radial and longitudinal strain over the entire wall respectively are 60.2 ± 2.8% and -25.3 ± 2.9 %. These values correspond to the values measured by clinically available techniques that are available in literature[12].

RF based strain estimation

As can be observed in figure 4, the longitudinal strain curves contained most noise, and show nonzero values at end diastole, suggesting residual stretch or shortening in these layers. This is caused by the lack of phase information available for tissue tracking in this direction. We chose not to apply drift compensation to compensate for this, since drift was minimal in the RF datasets used for this study.

Peak strain values

Strain estimation is done in sub- endocardial, midwall, and sub- epicardial layers. These three layers could be identified clearly, and are large enough to estimate a representative value of the area. Although strain curves were different between animals, peak strain values and differences between layers were consistent as is shown in figure 5. In detail, mean peak radial strain values from the sub-endocardium and midwall were significantly different between healthy and infarcted tissue which led to a significant decreased gradient between the endocardium and the epicardium 12 weeks after myocardial infarction. In general, the longitudinal shortening is caused by shortening of the longitudinally oriented myofibers, whereas the circumferential shortening is caused by shortening of circumferentially oriented fibers. As a diffusion tensor imaging study by Geerts et al.[15] has shown, the myofibers in the myocardial wall are predominantly oriented longitudinally in the sub- endo-, and sub- epicardium, and predominantly circumferential in the midwall. Radial strain is related to longitudinal and circumferential strain through tissue incompressibility and therefore quantifies tissue thickening caused by contracting

myofibers in perpendicular directions in, and in the vicinity of the measured region. After MI, the peak radial strain is most decreased in the midwall and sub- endocardial layers. It is known that extracellular matrix deposition enhances tissue stiffening and impairs deformation, and consequently radial thickening[16]. This can be caused by tissue perfusion being most severely impaired in the sub-endocardium, which directly reduces the radial strain in the endocardial layer, or by the decreased strain in the midwall and sub- epicardial layers causing a decreased radial motion in these layers, and thereby decrease the radial strain in the endocardial layer due to the incompressibility of the tissue. The fact that the longitudinal strain is impaired in all three layers, but this reduction is most pronounced in the sub- epicardium is in line with this latter hypothesis, but is more likely to be caused by the least accurate motion assessment in this direction, or by the epicardial ultrasound probe placement on the infarcted area. Taking into consideration all before mentioned mechanisms it must be concluded that decreased radial strain in the sub- endocardium is most likely caused by sub- endocardial collagen deposition. Since there is no data available of the circumferential strain in the different layers, and the orientation of collagen does not add additional information in non transmural infarcts, we cannot further unravel the relation between collagen deposition, collagen orientation, and strain in different directions in the different layers of the myocardium.

RF based and analytical strains

A high correspondence was found between the RF based peak radial strain values and the theoretically expected radial strain values as assessed by modeling. The truncated ellipsoidal geometrical model was based on the mean dimensions of the five animals used during the experiments, a longitudinal strain of -22%, an epicardial diameter change of -5%, and tissue incompressibility. Since the longitudinal strain corresponds to literature, and results in a normal mean circumferential strain value of -16% (data not shown), it is assumed that the resulting radial strain values are also correct. The assumption of 100% tissue incompressibility might, however, be an over simplification since this neglects the squeezing of blood from the tissue during systole[17]. The -5% epicardial diameter change was based on echocardiographic findings by Emilsson et al.[13]

RF based strain and histology

Highest collagen content is found in the endocardial layers (figure 7). Most likely this reflects the most tissue damage caused by the biggest perfusion deficiency in the sub- endocardial layer. The relatively low collagen content in the infarcted tissue in comparison to other studies using a ligation technique[18] indicates the appearance of both transmural and non-transmural infarcts and thereby variance between the pigs in our population caused by the ischemia reperfusion technique. The absence of uniform transmural infarcts makes it impossible to elaborate further on the relation between

local wall strain and local collagen deposition and orientation. The correlation between the layer specific percentage of collagen and the radial and longitudinal peak strain values (figure 8) shows that a lower collagen content relates to higher deformation, and therefore indicates an inversely proportional relation between local adverse remodeling and strain. Regions with high collagen content and high strain estimates, however, show that this relation is not irrefutable (figure 8). This is most likely caused by normal strain values in small areas of non-transmural infarcts which often occurred in the porcine MI model. The collagen content of the tissue sample is than severely increased, while the measured deformation is normal. This is most likely caused by tethering of adjacent tissue.

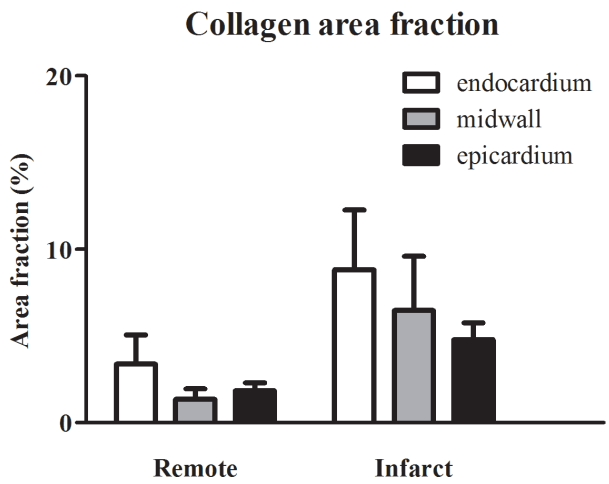


Figure 7. Percentage of endocardial, midwall, and epicardial areas that were stained by picrosirius red

Limitations

A limitation of the experimental setup of this study is the myocardial ischemia-reperfusion model that results in an inhomogeneous population in terms of infarct size, and transmurality, and thereby prevents comparison between subjects. Furthermore, positioning the ultrasound probe on the epicardium might have influenced the measurements. However, the resulting strain values are normal and very consistent, we minimized manipulation of the heart by positioning of the probe on the epicardial surface, and we applied the Starfish Cardiac Positioner to loosely fixate apical motion so that radial and longitudinal deformation was not impaired. Therefore, we believe that epicardial probe positioning has not influenced the results to a large extent. In this study, we have only assessed peak radial and longitudinal strain values since these are easy assessable and represent local cardiac function.

Comparison between different areas of the myocardium (LAD, LCX) being treated differently (healthy, infarct) might not be appropriate since both areas might respond differently to MI. It is therefore important to note that this study is primarily intended to evaluate the use of RF ultrasound in assessing sub-clinical alterations in local tissue. Besides local contractility, peak strain values can also be influenced by confounding factors: preload, afterload, and tethering by adjacent tissue. Since the resulting strain profiles are very consistent we believe that these factors have not influenced the results severely.

Clinical implications of RF ultrasound

In the present study, we have shown in a representative large animal model, that RF ultrasound based strain analysis can detect radial strain differences between sub- endocardial, midwall, and sub- epicardial layers. This non invasive local cardiac function assessment can be used to detect subtle changes in regional wall function. Besides detection of local changes of cardiac mechanics in patients treated with cardiac regenerative therapy, this assessment might also be of interest for e.g: dobutamine stress testing in patients with stable angina pectoris, selection of patients with any cardiomyopathy including arrhythmogenic right ventricular dysplasia/ cardiomyopathy. This study was performed in open chest animals with a linear array probe with a high frequency, and consequently an excellent ultrasound penetration, and field of view. Unfortunately, this is not feasible for a transthoracic approach. Moreover, no phase information is available in the RF data for longitudinal strain estimation which makes lateral strain estimates less accurate. To overcome this problem angular compounding ultrasound can be used[8]. This approach makes use of the possibility of deriving motion in any desired direction by projecting along the beam motion estimates from acquisitions in which the ultrasound beam is transmitted at multiple angles. Since motion is estimated more accurately in the beam direction, the longitudinal motion estimated by angular compounding can also be more accurate. Further studies are therefore necessary. Ideally future studies are done in a fully equipped animal to be able to optimally correct for confounding factors, using a 3D probe to overcome epicardial imaging, and assess deformation in all three directions of the cardiac coordinate system simultaneously.

CONCLUSION

Layer specific peak radial and longitudinal strain differences can be assessed by RF strain estimation and shows a clear difference between healthy and infarcted tissue. This novel technique provides a valuable way to assess the effects of subtle local pathological changes and locally oriented therapies.

Sources of funding

This work was supported by the Netherlands Heart Foundation '[2003B07304 and 2010T025]', BSIK program "Dutch Program for Tissue Engineering", '[grant 6746]', and a Bekalis price (PD). This research is part of the Project P1.04 SMARTCARE of the research program of the BioMedical Materials institute, co-funded by the Dutch Ministry of Economic Affairs and the Nederlandse Hartstichting.

Acknowledgements

We gratefully acknowledge Cees Verlaan, Merel Schurink, Marlijn Jansen, Maringa Emons and Joyce Visser for excellent technical assistance and animal care.
Conflict of Interest: none declared.

REFERENCES

1. Tendera M, Wojakowski W. How to measure the effects of the intracoronary stem cell therapy ? European Journal of Echocardiography. 2010.

2. Traverse JH, Henry TD, Moye La. Is the measurement of left ventricular ejection fraction the proper end point for cell therapy trials? An analysis of the effect of bone marrow mononuclear stem cell administration on left ventricular ejection fraction after ST-segment elevation myocardial infarction when evaluated by cardiac magnetic resonance imaging. American heart journal. 2011;162:671-7.

3. van Slochteren FJ, Teske AJ, van der Spoel TIG, Koudstaal S, Doevendans PA, Sluijter JPG, et al. Advanced measurement techniques of regional myocardial function to assess the effects of cardiac regenerative therapy in different models of ischaemic cardiomyopathy. European Journal of Cardiovascular Imaging. 2012;13:808-812.

4. Bijnens B, Claus P, Weidemann F, Strotmann J, Sutherland GR. Investigating Cardiac Function Using Motion and Deformation Analysis in the Setting of Coronary Artery Disease. Circulation. 2007;116:2453-64.

5. Huyghe JM, Arts T, Campen DHV, Reneman RS. Porous medium finite element model of the beating left ventricle Porous medium finite element model of the beating left ventricle. American Journal Of Physiology Heart and Circulation Physiology. 1992;262:1256-67.

6. van der Spoel TIG, Vrijsen KR, Koudstaal S, Sluijter JPG, Nijsen JFW, de Jong HW, et al. Transendocardial cell injection is not superior to intracoronary infusion in a porcine model of ischemic cardiomyopathy: A study on delivery efficiency. Journal of cellular and molecular medicine. 2012.

7. Lopata RGP, Nillesen MM, Hansen HHG, Gerrits IH, Thijssen JM, de Korte CL. Performance evaluation of methods for two-dimensional displacement and strain estimation using ultrasound radio frequency data. Ultrasound in medicine & biology. 2009;35:796-812.

8. Hansen HHG, Lopata RGP, Idzenga T, de Korte CL. Full 2D displacement vector and strain tensor estimation for superficial tissue using beam-steered ultrasound imaging. Physics in Medicine and Biology. 2010;55:3201-18.

9. Alam SK, Ophir J. Reduction of signal decorrelation from mechanical compression of tissues by temporal stretching: applications to elastography. Ultrasound in medicine & biology. 1997;23:95-105.

10. Kallel F, Ophir J. A least squares strain estimator for elastograpy. ultrasonic imaging. 1997;19:195-208.

11. Lopata RGP, Hansen HHG, Nillesen MM, Thijssen JM, de Korte CL. Comparison of One-Dimensional and Two-Dimensional Least-Squares Strain Estimators for Phased Array Displacement Data. ultrasonic imaging. 2009;16:1-16.

12. Rosner A, Bijnens B, Hansen M, How OJ, Aarsæther E, Sutherland GR, et al. Left ventricular size determines tissue Doppler-derived longitudinal strain and strain rate. European Journal of

Echocardiography. 2009;10:271-7.

13. Emilsson K, Brudin L, Wandt B. The mode of left ventricular pumping: is there an outer contour change in addition to the atrioventricular plane displacement? Clinical physiology. 2001;21:437-46.

14. Sluijter JPG, Smeets MB, Velema E, Pasterkamp G, de Kleijn DPV. Increase in Collagen Turnover But Not in Collagen Fiber Content Is Associated with Flow-Induced Arterial Remodeling. Journal of Vascular Research. 2004;41:546-55.

15. Geerts L, Bovendeerd P, Nicolay K, Arts T. Characterization of the normal cardiac myofiber field in goat measured with MR-diffusion tensor imaging. American journal of physiology Heart and circulatory physiology. 2002;283:H139-45.

16. Holmes JW, Borg TK, Covell JW. Structure and Mechanics of Healing Myocardial Infarcts. Annual Reviews of Biomedical Engineering. 2005;7:223-53.

17. Spaan JA. Coronary diastolic pressure-flow relation and zero flow pressure explained on the basis of intramyocardial compliance. Circulation Research. 1985;56:293-309.

18. Holmes JW, Nunez JA, Covell JW. Functional implications of myocardial scar structure. American Journal Of Physiology Heart and Crculation Physiology. 1997;41:2123-30.


T.I.G van der Spoel

DISCUSSION

DISCUSSION

Ischemic heart disease is still a major cause of mortality and morbidity in the Western world[1], despite the recent improvements in medical and reperfusion strategies, which urges the need for new therapeutic strategies. Small and large animal studies have demonstrated that cardiac cell therapy can improve cardiac function and limits infarct size after MI. Till now, approximately 2600 patients have been treated with bone marrow cells following acute MI or chronic ischemic cardiomyopathy with conflicting results. Although these results (limited but significant effect on left ventricular ejection fraction(LVEF)) do not match the expectations, they are in line with earlier observation of established treatments.[2] It is essential to advance our understanding of the underlying mechanisms following cell therapy that stimulated the observed effects and thereby to refine therapeutic approaches. Moreover, more technical challenges are first to overcome, including low survival and cell retention rates, since these are persistent obstacles after cell delivery.

In this thesis, we have investigated the optimization of delivery strategies for cardiac cell therapy via pre-clinical experimental studies to further enhance cardiac repair in patients with ischemic heart disease. Three approaches were explored namely:

- detailed analysis of current literature
- clinical available delivery techniques
- application of state-of-the-art nuclear imaging and a novel echocardiographic technique

In this chapter, the major findings, conclusions, and future directions will be discussed.

MAJOR FINDINGS OF THIS THESIS

In **Chapter 2**, a review is presented about non-invasive imaging and percutaneous delivery methods used for cardiac repair. First, the advantages and disadvantages of *in vivo* cell tracking were discussed. Next, a summary of various cell delivery techniques was provided including an overview of delivery efficiencies to the heart as observed in patients and large animal studies. Efficiency results are still inconclusive due to differences in study design, cell type, animal model, labelling method and delivery techniques. Understanding the biodistribution and subsequent fate of stem cells after delivery is critical to determine the optimal transplantation technique in view of clinical trials. To observe biodistribution nuclear imaging is preferred although it should be realized that cells are a live and productive. Cardiac MRI, however, is more suitable to evaluate cardiac function and to trace stem cells during long-term follow-up. We therefore, postulated that a randomized experimental study

investigating percutaneous delivery efficiency using nuclear imaging to assess short-term biodistribution was necessary which we conducted in **Chapter 4**.

In **Chapter 3** results were presented of a systematic review and meta-analysis performed on large animals to evaluate the effect of cell therapy in ischemic heart disease. From 52 studies, including 888 animals, efficacy and mortality data were extracted. At longest follow-up, LVEF improved by 7.5% compared to placebo treated animals. No difference in mortality between groups was observed. Cell type (mesenchymal stem cells (MSC) showed more effect than bone marrow mononuclear cells (BMMNC)) was a significant predictor of functional outcome. To confirm these results a comparison was performed in **Chapter 5**. Also, type of infarction was a significant predictor of LVEF. A trend was seen for high cell number (higher than 10^7), late injections (later than one week), and chronic MI models. Less benefit of cell therapy was seen during long-term follow-up. Strategies to get sustained effect were explored in **Chapter 5**. No differences in functional improvement between delivery techniques including: retrograde coronary transvenous injection, intracoronary infusion, transendocardial injection and surgical injection was observed. Interestingly, it was suggested that transendocardial delivery was superior to intracoronary infusion in efficiency.[3] This was investigated in **Chapter 4** comparing these strategies.

In summary, our analysis confirmed that cell therapy was safe and improved LVEF as was observed in a clinical setting. Moreover, this study provided the indication that indeed large animal models can accurately predict human clinical outcome upon cell transplantation therapy.

In **Chapter 4**, a comparison between transendocardial, intracoronary and surgical cell delivery was performed using Indium-oxine labeled MSC to observe short-term cell biodistribution at four hours after transplantation. The results of this study demonstrated no differences in safety profile between precutaneous delivery techniques. Interestingly, delivery efficiency was similar between groups (11-16%). For each technique, about 45% of radioactive cells accumulated in non-target organs especially the lungs. Intracoronary infusion showed lower variation compared to transendocardial injection. This implicates that intracoronary cell infusion is a more robust and less operator experience dependent technique. After termination, myocardial biopsies were taken and we confirmed the presence of MSC in the myocardium although the location of cells differed among percutaneous techniques (local vs. widespread distribution). In conclusion, no significant differences in delivery efficiency to the heart or in myocardial damage were observed between transendocardial injection and intracoronary infusion was observed.

In **Chapter 5**, a comparison between MSC and BMMNC was performed in a post MI model to evaluate short and long-term efficacy including a repeated strategy.

Myocardial function was assessed at different time-points by pressure-volume loops and echocardiography. Regarding safety and procedural data no difference between groups was observed. Results showed that the differences in LVEF (follow-up minus baseline) after MSC injection was significantly enhanced compared to bone marrow treatment (11.9% vs. -1.6%) and this effect was maintained during follow-up. No additional benefit on LVEF of repeated cell injections compared to single cell injection was observed (13% vs. 18%). Thus, injection of MSC was more effective compared to BMMNC treatment and the positive effect was sustained during follow-up.

The aim of **Chapter 6** was to provide more functional insight on top-off prognostic relevant LVEF in local wall changes caused by MI as a first step using a novel echocardiographic deformation technique. In pigs, radiofrequency (RF) ultrasound epicardial images were acquired twelve weeks after MI in normal and infarcted regions. Radial and longitudional strains were estimated at three levels namely; endocardium, midwall and epicardium. Strains were compared to histology and results were validated by an analytical model. Overall, peak strain values were significantly lower in the infarcted tissue compared to healthy tissue (endocardium 38.8% vs. 72.7%; P=0.002, midwall 34.8% vs. 40.0%; P=0.004) except for the subepicardium (35.1% vs.38.8%; P=0.9). In conclusion, layer specific peak radial and longitudinal strain differences can be assessed by RF strain estimation and shows a clear difference between healthy and infarcted tissue. We believe that this technique can be used to assess and detect subtle changes in the myocardium which may guide us to the underlying mechanisms of cell therapy.

GENERAL CONCLUSIONS

In this thesis, we addressed several unresolved issues regarding cardiac cell therapy as also outlined by the ESC Task Force.[4] The main conclusions of this thesis are:

- Large animal studies are valid to predict human clinical outcome (**Chapter 3**).
- Cardiac cell therapy is safe and leads to a preserved systolic function (**Chapter 3**).
- Transendocardial cell injection was not superior to intracoronary cell infusion in delivery efficiency (**Chapter 4**).
- Intracoronary and transendocardial delivery are safe delivery methods. (**Chapter 4**).

- MSC are superior compared to BMMNC in terms of functional outcome (**Chapter 3** and **Chapter 5**).

- MSC treatment is safe and resulted in a stable and sustained effect on systolic function. (**Chapter 5**).

- No additional value of repeated cell injection including BMMNC on cardiac function. (**Chapter 5**).

- Radiofrequency ultrasound is a novel and promising technique to assess and quantify local changes in the myocardium (**Chapter 6**).

FUTURE DIRECTIONS FOR CARDIAC CELL THERAPY

This thesis evaluated three strategies to optimize stem cell delivery methods for ischemic heart disease: systematic analysis of current literature, clinically available transplantation methods and state-of-the-art imaging techniques.

What can we learn from large animals?

By thorough analysis of pre-clinical data (**Chapter 3**), we demonstrated that cell therapy improves cardiac function and that pre-clinical data can be extrapolated to humans. Moreover, we generated new clues for future clinical stem cell trials. Based on our in-depth meta-analysis, several recommendations can be formulated. First, other stem cells than bone marrow mononuclear cells should be explored. Given the results from **Chapter 5** and the encouraging results from the SCIPIO and CADUCEUS trials[5, 6] future research should focus on MSC and cardiac stem cells. Secondly, before designing novel pre-clinical trials researchers should take into account that the type of vessel to create an acute MI, chronic MI or heart failure influences functional outcome. Moreover, the site of ligation/constriction of the vessel should be similar in all animals which was not always the case due to anatomical variations. Third, cells should not be administered between 2-7 days after MI because it was associated with lower functional outcome possibly due to inflammatory phase subdued one week after MI.[7] Fourth, to maintain a beneficial effect of cell therapy over time novel strategies should be explored. To overcome this problem several potential materials (hydrogels and scaffolds) have been developed and tested in experimental models.[8-10] However, before taking these approaches to the clinic, delivery efficiency should be tested and compared to current methods of delivery to prove additional value. Finally, we believe that although animal model was a non-significant predictor of functional outcome the use of pigs for functional studies is preferred since a large amount of data is available for comparison.

An interesting observation is that most of the studies included in our meta-analysis were published before 2006 indicating that some of our recommendations could have

been made years ago. We therefore, recommend that before designing new clinical and pre-clinical stem cell studies careful exploration of available experimental data is necessary. To help researchers in this process a novel database was created together with the CAMARADES group.[5] In 2011, we joined the CAMARADES group which is an international collaboration between cardiologists and neurologists. Our goal is to improve the design, conduct, analysis and reporting of animal studies thereby improving translational potential of these studies to the clinical field.[5] Moreover, performing pre-clinical meta-analysis can also be very helpful in evaluating the effect of other therapies e.g. medication (beta-blockers or immunosuppression). In our opinion, pre-clinical studies should be executed according to high quality standards. The following items should be included in a guideline for pre-clinical research to improve extrapolation of animal studies directly to man: 1) randomized study design, 2) blinded functional analysis, 3) sample size during the study and 4) mortality.

Stem cell delivery strategies: what's next?

Since no difference in functional outcome (**Chapter 3**) and delivery efficiency (**Chapter 4**) was observed we recommend that the choice of delivery method could depend on medical indication and practical aspects (e.g. operator experience and coronary anatomy). For instance, in patients with occluded coronary arteries we suggest the use of transendocardial injection and patients with a patent coronary artery intracoronary infusion. Overall, efficiency to the heart was low which paves the way for development of new catheters (side holes[11], helical needle[12] or double lumen) or strategies (image fusion) to improve targeted cell retention. In our studies, we used the NOGA system to guide transendocardial cell injection but this technique can not detect non-transmural scars and infarct grey zones, fusion with cardiac MRI may provide important additional information to facilitate cell injections.[13] In **Chapter 3** and **Chapter 5**, we demonstrated that MSC treatment improves systolic function compared to BMMNC treatment. However, these results need to be confirmed in a randomized controlled clinical trial. Repeated cell injection did not further improve cardiac function compared to single cell injection. This may be caused by a less potent cell type that was injected and higher baseline LVEF of our animals. It is therefore not excluded that this strategy could work in animals with a LVEF<45% using a more potent cell type e.g. cardiac stem cells or MSC. In our study, MI was created by temporary occlusion of the circumflex artery for 75 minutes. Four weeks after MI, we observed a moderate decrease in LVEF in all animals possibly due to the standard medication regime (e.g. beta-blockers) that was prescribed to the animals.[14] Future studies should explore the protective effect of beta-blockers during ischemia-reperfusion injury.

Novel techniques to assess cardiac function

In **Chapter 6**, we have demonstrated that radiofrequency ultrasound based strain analysis can be used to detect local changes in endocardium, midwall and epicardium deformation between healthy and infarcted tissue. This technique could be usefull in patients with stable angina pectoris to observe ischemia, patients screened for arrhythmogenic right ventricular dysplasia/cardiomyopathy and in patients treated with stem cells. Novel studies should confirm this hypothesis. However, before advancing to the clinical arena this technique should be incorporated in a 3D probe to overcome epicardial imaging, and assess deformation in all three directions of the cardiac coordinate system simultaneously. More techniques are available and currently explored in large animal studies.[15]

In conclusion, this thesis provides some answers to the unresolved issue as outlined by the ESC Task Force[4] thereby advancing cardiac cell therapy to the next level. Nevertheless, much more research is necessary since there is still room for improvement of the magnitude of effect of cardiac cell therapy.

REFERENCES

1. Roger VL, Go AS, Lloyd-Jones DM, Adams RJ, Berry JD, Brown TM, Carnethon MR, Dai S, de Simone G, Ford ES, Fox CS, Fullerton HJ, Gillespie C, Greenlund KJ, Hailpern SM, Heit JA, Ho PM, Howard VJ, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Makuc DM, Marcus GM, Marelli A, Matchar DB, McDermott MM, Meigs JB, Moy CS, Mozaffarian D, Mussolino ME, Nichol G, Paynter NP, Rosamond WD, Sorlie PD, Stafford RS, Turan TN, Turner MB, Wong ND, Wylie-Rosett J. Heart Disease and Stroke Statistics 2011 Update: a report from the American Heart Association. Circulation 2011;123:e18-e209.

2. Reffelmann T, Konemann S, Kloner RA. Promise of Blood- and Bone Marrow-Derived Stem Cell Transplantation for Functional Cardiac Repair: Putting It in Perspective With Existing Therapy. J Am Coll Cardiol 2009;53:305-308.

3. Hou D, Youssef EA, Brinton TJ, Zhang P, Rogers P, Price ET, Yeung AC, Johnstone BH, Yock PG, March KL. Radiolabeled cell distribution after intramyocardial, intracoronary, and interstitial retrograde coronary venous delivery: implications for current clinical trials. Circulation 2005;112:I150-I156.

4. Bartunek J, Dimmeler S, Drexler H, Fernández-Avilés F, Galinanes M, Janssens S, Martin J, Mathur A, Menasche P, Priori S, Strauer B, Tendera M, Wijns W, Zeiher A. The consensus of the task force of the European Society of Cardiology concerning the clinical investigation of the use of autologous adult stem cells for repair of the heart. Eur Heart J 2006;27:1338-1340.

5. Bolli R, Chugh AR, D'Amario D, Loughran JH, Stoddard MF, Ikram S, Beache GM, Wagner SG, Leri A, Hosoda T, Sanada F, Elmore JB, Goichberg P, Cappetta D, Solankhi NK, Fahsah I, Rokosh DG, Slaughter MS, Kajstura J, Anversa P. Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial. The Lancet 1926;378:1847-1857.

6. Makkar RR, Smith RR, Cheng K, Malliaras K, Thomson LE, Berman D, Czer LS, Marban L, Mendizabal A, Johnston PV, Russell SD, Schuleri KH, Lardo AC, Gerstenblith G, Marban E. Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial. The Lancet 2012;379:895-904.

7. Deten A, Volz HC, Briest W, Zimmer HG. Cardiac cytokine expression is upregulated in the acute phase after myocardial infarction. Experimental studies in rats. Cardiovasc Res 2002;55:329-340.

8. Imanishi Y, Miyagawa S, Maeda N, Fukushima S, Kitagawa-Sakakida S, Daimon T, Hirata A, Shimizu T, Okano T, Shimomura I, Sawa Y. Induced Adipocyte Cell-Sheet Ameliorates Cardiac Dysfunction in a Mouse Myocardial Infarction Model. Circulation 2011;124:S10-S17.

9. Singelyn JM, Sundaramurthy P, Johnson TD, Schup-Magoffin PJ, Hu DP, Faulk DM, Wang J, Mayle KM, Bartels K, Salvatore M, Kinsey AM, DeMaria AN, Dib N, Christman KL. Catheter-Deliverable Hydrogel Derived From Decellularized Ventricular Extracellular Matrix Increases Endogenous Cardiomyocytes and Preserves Cardiac Function Post-Myocardial Infarction. J Am Coll Cardiol 2012;59:751-763.

10. Gaetani R, Doevendans PA, Metz CHG, Alblas J, Messina E, Giacomello A, Sluijter JPG. Cardiac tissue engineering using tissue printing technology and human cardiac progenitor cells. Biomaterials 2012;33:1782-1790.

11. http://www.c3bs.com/. 2012.

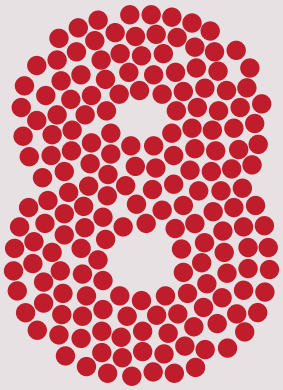
12. http://www.biocardia.com/. 2012.

13. Wijnmaalen AP, van der Geest RJ, van Huls van Taxis C, Siebelink HM, Kroft LJM, Bax JJ, Reiber JHC, Schalij MJ, Zeppenfeld K. Head-to-head comparison of contrast-enhanced magnetic resonance imaging and electroanatomical voltage mapping to assess post-infarct scar characteristics in patients with ventricular tachycardias: real-time image integration and reversed registration. Eur Heart J 2011;32:104-114.

14. Ibanez B, Prat-Gonzalez S, Speidl WS, Vilahur G, Pinero A, Cimmino G, Garcia MJ, Fuster V, Sanz J, Badimon JJ. Early Metoprolol Administration Before Coronary Reperfusion Results in Increased Myocardial Salvage. Circulation 2007;115:2909-2916.

15. Van Slochteren FJ, Teske A, Van der Spoel TIG, Koudstaal S, Doevendans PA, Sluijter JPG, Cramer MJ, Chamuleau SAJ. Advanced measurement techniques of regional myocardial function to assess the effects of cardiac regenerative therapy in different models of ischaemic cardiomyopathy. Eur Heart J Cardiovasc Imaging 2012.


T.I.G van der Spoel



SUMMARY IN DUTCH - SAMENVATTING IN HET NEDERLANDS

SAMENVATTING

Het acute hartinfarct is een van de belangrijkste oorzaken van hartfalen en sterfte in de Westerse wereld. De behandeling van zogenaamd chronisch ischemisch hartfalen is voornamelijk symptomatisch van aard (revascularisatie middels dotteren en medicatie). Een mogelijk structurele oplossing is het versterken van de hartspier door het doen ontstaan van nieuwe hartspiercellen en vaten met behulp van stamcel-implementatie. Proefdierstudies hebben aangetoond dat cardiale celtherapie de pompfunctie van het hart kan verbeteren na een hartinfarct. Inmiddels zijn nu ook al ongeveer 2600 patiënten na een acuut hartinfarct of met chronisch ischemisch hartfalen behandeld met stamcellen.

De initiële resultaten laten een beperkt, maar wel significant gunstig effect op de pompfunctie van het hart zien. Dit blijft helaas achter bij de hoog gespannen verwachtingen, maar komt wel overeen met eerdere observaties van de op dit moment meest gangbare behandelingen (o.a medicatie). De uitdaging voor onderzoek in dit veld ligt in het verder verbeteren van de resultaten van celtherapie

Het is daarvoor van belang om meer te weten te komen over het onderliggende mechanisme van cardiale celtherapie dat zorg draagt voor het gunstige effect op de knijpkracht van het hart zodat we onze therapeutische mogelijkheden kunnen verbeteren.

Alvorens hieraan te kunnen beginnen moeten eerst technische problemen worden verholpen, zoals de geringe overleving en lage cel retentie na transplantatie. Dit proefschrift richt zich op het optimaliseren van transplantatie strategieën voor cardiale cel therapie in ischemische hartziekten. De beschreven onderzoeken bevatten drie pijlers:

- Gedetailleerde analyse van de huidige literatuur
- Evaluatie van klinisch toepasbare en beschikbare transplantatie technieken
- Efficiëntie en functionele analyse middels beeldvormende technieken (o.a. nucleair en echocardiografie)

In **hoofdstuk 1** worden vanuit een klinisch perspectief de belangrijkste basis principes en de eerste resultaten van stamceltherapie beschreven. Aansluitend wordt in **hoofdstuk 2** een overzicht gegeven van alle grote proefdier- en patiëntenstudies die cel retentie middels beeldvormend onderzoek hadden bepaald. Momenteel worden bij patiënten met chronisch hartfalen 2 niet-operatieve technieken gebruikt: (1) het inspuiten van stamcellen via de kransslagaders, of (2) direct in de hartspier. Om retentie direct na injectie in het lichaam te bepalen werd in de meeste gevallen nucleaire beeldvorming gebruikt. Belangrijk is wel dat de stamcellen na binding met een radioactief label in leven moeten blijven en productief moeten zijn. Cardiale magnetic resonance imaging (MRI) is een techniek waarbij gebruik gemaakt wordt

van magneetvelden zodat niet alleen de pompfunctie van het hart maar ook overleving van stamcellen op de lange termijn kan worden beoordeeld. Helaas kan niet gekeken worden naar het gehele lichaam in één oogopslag.

In **hoofdstuk 3** worden de resultaten gepresenteerd van een systematische review en meta-analyse naar de effecten van celtherapie in grote proefdiermodellen (varkens, honden, schapen) van ischemische hartziekten. In totaal werden 52 studies (888 dieren) geanalyseerd waarbij werd gekeken naar sterfte en toename in pompfunctie na celtherapie. Stamceltherapie leidde tot een verbetering van 7.5% in pompfunctie t.o.v placebo behandeling. Er werd geen verschil in sterfte geobserveerd. Zowel cel type als type infarct zijn belangrijke voorspellers voor verbetering in pompfunctie. Zo lieten mesenchymale stamcellen (MSC) een groter effect zien dan beenmerg mononucleaire cellen (BMMNC). Om deze resultaten te bevestigen werd een vergelijkende studie tussen deze 2 celtypen opgezet (**hoofdstuk 5**). Er werden geen verschillen in knijpkracht van het hart gezien tussen de verschillende transplantatie technieken. Echter, in de literatuur werd gesuggereerd dat katheter geleide directe injectie van stamcellen in het hart leidt tot een hogere cel retentie in het hart t.o.v injectie van stamcellen via de kransslagaders. Dit werd in een gerandomiseerde studie onderzocht in **hoofdstuk 4**. Onze meta-analyse toonde aan dat stamceltherapie veilig was en leidde tot een significante verbetering in pompfunctie van het hart. Bovendien toonden we aan dat grote proefdieren (o.a. varkens) de uitkomst van patiëntenstudies kunnen voorspellen.

In **hoofdstuk 4** werd een vergelijking gemaakt tussen 2 niet-operatieve katheter technieken. Deze studie werd in varkens uitgevoerd omdat er grote overeenkomsten zijn tussen het varkens hart met dat van de mens. Een hartinfarct werd kunstmatig opgewekt. Daarna (4 weken) werd onderzocht of de ingebrachte cellen in het geïnfarceerde deel van het hart zijn blijven zitten of dat ze zich hebben verplaatst naar andere organen (‘retentie’). Het kunnen volgen en visualiseren van getransplanteerde stamcellen is van uitermate belang om de meest efficiënte katheter injectietechniek te kunnen bepalen voor klinische toepassingen van stamceltherapie. Onze studie heeft aangetoond dat er geen verschil is in celretentie tussen de twee technieken in vergelijking tot de gouden standaard (open hart chirurgie).

In **hoofdstuk 5** van dit proefschrift werd een vergelijking gemaakt tussen MSC en BMMNC op korte en lange termijn na chronische ischemie, dus vier weken na de kortdurende afsluiting van de kransslagader. De hartfunctie werd beoordeeld op verschillende tijdspunten met echocardiografie en invasieve druk en volume metingen. Injectie van MSC was effectiever in het verbeteren van de pompfunctie in vergelijking tot BMMNC behandeling en dit positieve effect hield aan tijdens follow-up (8 weken na behandeling).

Klassiek kijken we bij stamcel onderzoek voornamelijk naar de verbetering in 'ejectiefractie' van het linkerventrikel, ofwel de knijpkracht van het hart. Het doel van **hoofdstuk 6** was om juist meer inzicht te verkrijgen in regionale wandbewegingen na een hartinfarct zodat we beter de locatie kunnen vinden waar de stamcellen terecht moeten komen en daarmee het effect van onze therapie kunnen vergroten.

Ten tweede kunnen we het onderliggende mechanisme beoordelen wat deze knijpkracht verbetering geeft. Radiofrequente echobeelden werden direct op het hart gemaakt van een gezond en een infarct gebied. Er werden lengte en dwarsdoorsnede opnames gemaakt van de verschillende lagen in het hartspierweefsel; van het binnenste, midden en buitenste gedeelte van de hartspier. De resultaten werden vergeleken met hartbiopsies (gouden standaard) en gevalideerd door een computer model. Onze studie heeft aangetoond dat deze nieuwe techniek inderdaad een duidelijk verschil kan maken tussen gezond en infarct weefsel. Deze techniek kan dus pathologische veranderingen in de wand van het hart detecteren en het effect van locaal toegepaste therapieën observeren. **In hoofdstuk 7** worden niet alleen de belangrijkste bevindingen en conclusies besproken maar ook de gevolgen hiervan voor toekomstig onderzoek.

Tot slot, dit proefschrift biedt een aantal antwoorden op onopgeloste kwesties met name op het gebied van transplantatie technieken. Het is ook in lijn met de aanbevelingen van de Europese Task Force op het gebied van stamceltherapie. Daarnaast geeft het richting voor verder onderzoek. Echt, er is veel meer onderzoek nodig aangezien er nog veel ruimte is voor verbetering ten aanzien van het positieve effect van stamceltherapie.

AUTHORS AND AFFILIATIONS

P. Agostoni MD PhD
Department of Cardiology
University Medical Center Utrecht
Utrecht, The Netherlands

E. Van Belle MD PhD
Department of Cardiology
University Medical Center Utrecht
Utrecht, The Netherlands

P.H.M. Bovendeerd PhD
Department of Biomedical Technology
Eindhoven University
Eindhoven, The Netherlands

S.A.J. Chamuleau MD PhD
Department of Cardiology
University Medical Center Utrecht
Utrecht, The Netherlands

M.J.M. Cramer MD PhD
Department of Cardiology
University Medical Center Utrecht
Utrecht, The Netherlands

P.A.F.M. Doevendans MD PhD
Department of Cardiology
University Medical Center Utrecht
Utrecht, The Netherlands

M.J. Goumans PhD
Department of Molecular Cell Biology
Leiden University Medical Center
Leiden, The Netherlands

M. Gyöngyösi MD PhD
Department of Cardiology
Medical University of Vienna
Vienna, Austria.

H.H.G. Hansen MSc
Department of Radiology
Radboud University Nijmegen
Medical Center
Nijmegen, The Netherlands

I.E. Hoefer MD PhD
Experimental Cardiology Laboratory
University Medical Center Utrecht
Utrecht, The Netherlands

S.J. Jansen of Lorkeers MD
Department of Cardiology
University Medical Center Utrecht
Utrecht, The Netherlands

H.W. De Jong PhD
Department of Radiology and Nuclear Medicine
University Medical Center Utrecht
Utrecht, The Netherlands

C.C.L. de Korte PhD
Department of Radiology
Radboud University Nijmegen
Medical Center
Nijmegen, The Netherlands

S. Koudstaal MD
Department of Cardiology
University Medical Center Utrecht
Utrecht, The Netherlands

J.C. Lee MSc
Faculty of Medicine
University of Malaya
Kuala Lumpur, Malaysia

J. Liu MD PhD
Experimental Cardiology Laboratory
University Medical Center Utrecht
Utrecht, The Netherlands

H. Nathoe MD PhD
Department of Cardiology
University Medical Center Utrecht
Utrecht, The Netherlands

J.F. Nijsen PhD
Department of Radiology and Nuclear Medicine
University Medical Center Utrecht
Utrecht, The Netherlands

F.J. van Slochteren MSc
Department of Cardiology
University Medical Center Utrecht
Utrecht, The Netherlands

J.P.G. Sluijter PhD
Experimental Cardiology Laboratory
University Medical Center Utrecht
Utrecht, The Netherlands

P. Steendijk PhD
Department of Cardiology
Leiden University Medical Center
Leiden, The Netherlands

K.R. Vrijsen MSc
Experimental Cardiology Laboratory
University Medical Center Utrecht
Utrecht, The Netherlands

LIST OF PUBLICATIONS

Van Slochteren FJ, Teske AJ, **Van der Spoel TI**, Koudstaal S, Doevendans PA, Sluijter JP, Cramer MJ, Chamuleau SA. Advanced measurement techniques of regional myocardial function to assess the effects of cardiac regenerative therapy in different models of ischaemic cardiomyopathy. Eur Heart J Cardiovasc Imaging. 2012;13:808-18.

Van der Spoel TI, Vrijsen KR, Koudstaal S, Sluijter JP, Nijsen JF, De Jong HW, Hoefer IE, Cramer MJ, Doevendans PA, Van Belle E, Chamuleau SA. Transendocardial cell injection is not superior to intracoronary infusion in a porcine model of ischemic cardiomyopathy: A study on delivery efficiency. J Cell Mol Med. 2012 June 14.

Gründeman PF, **Van der Spoel TI**, Steendijk P, Van Slochteren F, Cramer MJ, Doevendans PA, Pasterkamp G. Surgical left ventricular radius enlargement by patch insertion on the beating heart: a new experimental aneurysm model. Interact Cardiovasc Thorac Surg. 2012;15:10-3.

Van der Spoel TI, Jansen of Lorkeers SJ, Agostoni P, Van Belle E, Gyöngyösi M, Sluijter JP, Cramer MJ, Doevendans PA, Chamuleau SA. Human relevance of pre-clinical studies in stem cell therapy: systematic review and meta-analysis of large animal models of ischaemic heart disease. Cardiovasc Res. 2011;91:649-58.

Van der Spoel TI, Lee JC, Vrijsen K, Sluijter JP, Cramer MJ, Doevendans PA, Van Belle E, Chamuleau SA. Non-surgical stem cell delivery strategies and in vivo cell tracking to injured myocardium. Int J Cardiovasc Imaging. 2011;27:367-83.

Koudstaal S, **Van der Spoel TIG**, Van Slochteren FJ, Vrijsen K, Sluijter JPG, Cramer MJM, Doevendans PA, Chamuleau SAJ. Regeneratie van het beschadigde hart. Hart Bulletin 2011;42:79-83.

Huijgen WHF, Gründeman PF, **Van der Spoel TIG**, Cramer MJM, Steendijk P, Klautz RJM, Van Herwerden LA. Resizable Ventricular Patch Plasty in the Porcine Left Ventricle: A Pilot Study. Innovations. 2010;5:16-21.

Liu J, Sluijter JP, Goumans MJ, Smits AM, **Van der Spoel T**, Nathoe H, Doevendans PA. Cell therapy for myocardial regeneration. Curr Mol Med. 2009;9:287-98.

Ashikaga H, **Van der Spoel TI**, Coppola BA, Omens JH. Transmural myocardial mechanics during isovolumic contraction. JACC Cardiovasc Imaging. 2009;2:202-11.

Van der Spoel TI, Liu J, Sluijter JPG, Goumans MJ, Nathoe H, Van Belle E, Chamuleau SAJ, Doevendans PA. Human cardiac progenitor cells differentiate into cardiomyocytes. CML Cardiology 2009;274:4.

Rhodius RJ, Bauwens AMM, **Van der Spoel TIG**, Van de Graaf EA, Cramer MJM. De dyspnoepolikliniek: In: Boot B.S., Kerstjens H.A.M., redacteuren. Zorg Rondom COPD. 1edruk. Houten: Bohn Stafleu Van Loghum; 2007.

Van der Spoel TIG, Cramer MJM, Bauwens AMM, Kelder JC, Van de Graaf EA. Dyspnoe-poli: multidisciplinaire aanpak van onbegrepen kortademigheid. Hartbulletin 2006;37:82-86.

Dankwoord

Dit proefschrift is het resultaat van bijna 4 jaar onderzoek. Zonder de hulp, inzet en steun van vele was het niet mogelijk om dit proefschrift te voltooien.

Prof. dr. P.A.F.M. Doevendans, beste ***Pieter***, na het doen van mijn keuzeonderzoek was ik op zoek naar een nieuwe uitdaging. Onderzoek doen in het buitenland leek me wel wat. San Diego is dat niet wat voor jou! Het was een fantastische tijd. Toen ik daar onderzoek aan het doen was en jij op bezoek kwam bij mijn supervisoren vroeg je 'is dat mode die gaten in je broek'. Ik waardeer je eerlijkheid en wil je bedanken voor het in mij gestelde vertrouwen, de vrijheid om mijzelf te ontwikkelen en de steun die ik heb mogen ervaren tijdens het aanvragen van de dr. E. Dekker beurs. Ik hoop dat we nog veel zullen samenwerken in de toekomst.

Prof. dr. E. van Belle, dear ***Eric***, thank you for the pleasant collaboration and critical revision of my manuscripts. I really enjoyed the discussions on data interpretation.

Dr. S.A.J. Chamuleau, beste ***Steven***, de afgelopen vier jaar heb ik je leren kennen als een betrokken, bevlogen en geduldig wetenschapper die mij heeft geleerd om goed wetenschappelijk onderzoek op te zetten en uit te voeren. Je hebt mij leren focussen (een begrip dat ik nooit meer vergeet) en kritisch resultaten te beoordelen. Samen hebben we de eerste proefdieren geopereerd. Je enthousiasme werkte zeer aanstekelijk en de vrijheid die ik van jou kreeg waardeer ik enorm. Je betrokkenheid voor mijn werkzaamheden ging zelfs zover dat je vanuit het ziekenhuis waar je lag opgenomen, opbelde met de vraag of ik nog hulp nodig had met een patiëntpresentatie. Verder wil ik je bedanken voor de gastvrijheid en het warme welkom van Carolien en de kinderen. Sinterklaas en Zwarte Piet spelen in maison Chamuleau deed ik graag. Daarnaast hebben we veel plezier gehad en goede gesprekken gevoerd tijdens congressen (New Orleans, Stockholm) en symposia (Madrid). Ik ben vereerd je eerste promovendus te zijn en hoop in de toekomst onze samenwerking en vriendschap te kunnen voortzetten. O ja, die tenniswedstrijd wanneer zouden we die gaan spelen? Volgende week maandag misschien?

Dr. J.P.G. Sluijter, beste ***Joost***, ik kan me nog goed herinneren dat ik als PhD student je kamer binnenkwam en vroeg of het mogelijk was om 20 miljoen cellen te genereren. De vraag stellen is hem beantwoorden. Jij leerde me alles over de basale mechanismen van stamcellen maar ook het maken van een blot, doen van kleuringen en kweken van cellen. Ik waardeer je flexibiliteit, geduld en laagdrempeligheid tot overleg. Snel en gronding nakijken van mijn manuscripten of proeven was nooit een probleem. Ik ben dankbaar voor de ruimte en mogelijkheden die je mij hebt geboden binnen het stamcellab. Graag blijf ik contact houden en met je samenwerken in de toekomst.

Dr. M.J.M. Cramer, beste ***Maarten-Jan***, mijn eerste ervaring met het doen van wetenschappelijk onderzoek heb ik bij jou opgedaan. Je enorme enthousiasme en persoonlijke betrokkenheid heb ik zeer gewaardeerd.

Dr. P.F. Gründeman, beste ***Paul***, mijn eerste kennismaking met het GDL en wetenschappelijke output zijn bij u begonnen. Uw enorme wetenschappelijke kennis en neus voor innovatie maakte u tot een interessante gesprekspartner.

Prof. dr. G. Pasterkamp, beste ***Gerard***, dank voor de mogelijkheden om mijn onderzoek te mogen uitvoeren in uw laboratorium.

Dr. I. E. Hoefer, beste ***Imo***, peentjes zweten was het wanneer ik weer een DEC aanvraag naar je moest sturen. Niets ontging je kritische blik. Jij leerde mij om een goed wetenschappelijk onderbouwd maar ook werkbaar protocol te schrijven. Dank hiervoor.

Dr. P. Agostoni, beste ***Pierfrancesco***, dank voor je hulp bij het maken van onze meta-analyse en de voorbereidingen van onze NOGA procedures.

De leden van de leescommissie, ***Prof. dr. P.R. Luijten, Prof. dr. M.C. Verhaar, Prof. dr. J. Piek en Prof. dr. D.P. de Kleijn*** wil ik graag bedanken voor het beoordelen van mijn proefschrift.

Het doen van proefdieronderzoek wordt begeleid door een fantastisch team van biotechnici. Beste ***Cees, Maringa, Joyce, Marlijn, Ben, Merel en Evelyn***, ontzettend bedankt voor jullie hulp bij mijn experimenten. Avonden waren we op stap met mijn proefdieren voor nucleaire of MRI scans. Jullie flexibiliteit, humor en energie zijn ongekend. Veel dank voor het meedenken en de komische momenten die we hebben gehad.

Beste GDL collega's, beste ***Hester, Jannie, Jannico, Nico en Romy***, dank voor jullie hulp bij mijn experimenten. De verzorging en administratie van de grote proefdieren werd gedaan door Anja, Helma,Wendy, Tamara en Jeroen waarvoor dank.

De stralingsdeskundigen van het UMCU (***Kitty, Marlies en Michel***) en de UU (***Cees Vos***) ben ik zeer dankbaar voor hun hulp en kritische beoordeling van mijn protocollen.

Dr. F. Nijsen, dr. F. van 't Schip en dr. H.W. de Jong, beste ***Frank, Fred en Hugo***, jullie keken raar op toen ik bij jullie aanklopte om een retentiestudie op te zetten maar tegelijkertijd vonden jullie het een enorme uitdaging. Een gedegen en doortastende

aanpak met daarbij een tomeloze inzet heeft geleid tot een goed lopend labeling- en scan protocol, waardoor het project succesvol werd afgerond. Veel dank hiervoor. Beste ***John, Sieneke en Nikki***, avonden zijn wij bezig geweest met het scannen van dieren en het labelen van stamcellen. Jullie enthousiasme en doorzettingsvermogen kent geen grenzen, zelfs niet onder winterse omstandigheden. John, ontzettend bedankt voor je hulp ondanks het verlies van een dierbare. Sieneke, veel succes met je carrière in het noorden. Nikki, we gaan nog een keer naar de FC samen, echt waar.

Beste ***Krijn***, het voorbereidende werk voor de nucleaire studie heb ik grotendeels met jou samen gedaan. Daarnaast heb je mij wegwijs gemaakt binnen het lab waarvoor mijndank. ***Alain***, dank voor de gezellige tijd en leermomenten (een visarend heeft toch een witte kop?) tijdens onze USA roadtrip. ***Marish***, dank voor je input tijdens de workmeetings maar ook voor je hulp in de kliniek. Inmiddels de trotse vader van een zoon, fantastisch om wederom met je samen te werken. ***Dries***, thanks for your contribution in the labeling experiments. ***Zhiyong***, you are an asset to our team with your microRNA knowledge. ***Paul, Roberto, Esther en Corina***, dank voor jullie hulp bij mijn experimenten en jullie input tijdens de werkbesprekingen. Oud collega's ***Sridevi en Willy*** dank voor jullie hulp bij de SMARTCARE en RIPASSA studie. Nieuwe collega's ***Frederieke, Vera en Janine*** veel succes met jullie promotieonderzoek.

Het secretariaat cardiologie (***Sylvia, Tamara en Jantine***) en experimentele cardiologie (***Ineke en Marjolein***) dank voor de secretariële ondersteuning.

(Oud) collega´s van de experimentele cardiologie en chirurgie: ***Daphne, René, Fatih, Ellen, Sanne, Pleunie, Dave, Guus, Geert, Vincent, Marten, Joyce***, dank voor de gezellige borrels, congressen, symposia en steun tijdens de ESC Challange. Beste ***Loes en Arjan***, dank voor jullie hulp bij het bestellen van wires en materialen die ik nodig had voor mijn nucleaire studie.

Mijn collega arts-onderzoekers van de cardiologie: ***Manon, Judith, Jetske, Mieke, Willemien, Ing Han, Geert, Irene, Marieke, Margot, Sjoukje, Roos, Sofieke, Anouar en Gijs*** wil ik bedanken voor de gezellige borrels, goede feestjes, etentjes en natuurlijk de mooie ski trip.

Mijn studenten ***Sanne en Verena***, ik ben zeer dankbaar voor het harde werk dat jullie hebben gedaan. Ik heb veel van jullie geleerd en ben blij dat jullie voor het onderzoek hebben gekozen. Tot in de kliniek! Beste ***René***, na een periode als TG student bij ons te hebben gewerkt ben je nu bezig met je eigen onderzoek. Veel succes!

Veel dank gaat uit naar mijn kamergenoten in de Bunker (het kloppende hart van de cardiologie in het UMCU), ***Frebus en Stefan***. Beste ***Frebus***, we zijn tegelijkertijd gestart met ons promotieonderzoek. Onze samenwerking resulteerde in een aantal mooie projecten. Ik heb veel van je geleerd (o.a lindy Hop) en met je gelachen. Ik waardeer je humor en collegialiteit, en ben blij dat je mijn paranimf wilt zijn.

Beste ***Stefan***, jij bent iemand met een brede interesse, van schaatsen en gitaar spelen tot bestuur en wetenschap. Als student leerde ik je kennen en in korte tijd heb je een enorme ontwikkeling doorgemaakt. Ik ben trots op het feit dat je nu je eigen weg hebt gekozen door o.a. onderzoek te doen in Liverpool. Veel succes met de afronding van je proefschrift.

Beste ***Michiel***, we kennen elkaar al van jongs af aan en komen uit dezelfde regio. Een gemeenschappelijke passie voor auto's en goede humor zorgden ervoor dat wij het direct goed met elkaar konden vinden. Bedankt voor je steun en interesse in mijn werkzaamheden. Fantastisch dat jij op deze belangrijke dag mijn paranimf wilt zijn.

Lieve ***Céleste***, jij bent de enige die mijn boekje van begin tot eind heeft gelezen. De kaft is prachtig geworden. Met je humor, enorme creativiteit en positieve uitstraling weet je de reclame wereld te veroveren. Ik ben trots op alles wat je hebt bereikt. Beste ***Benjamin***, jij bent de ondernemer in het gezin. Fantastisch dat je van een klein bedrijfje in Amsterdam een snel groeiende onderneming hebt weten te maken. Ik waardeer je doorzettingsvermogen en die Porsche komt er vanzelf wel.

Lieve ***Pap en Mam***, jullie liefde, vertrouwen en onvoorwaardelijk steun in mij zijn niet in woorden uit te drukken. Ik ben dankbaar voor alles wat jullie voor me hebben gedaan.

Lieve ***Patricia***, ik ben blij dat wij elkaar hebben leren kennen. Jij bent mijn steun en toeverlaat maar ook mijn tegenwicht. Ik kijk uit naar onze toekomst samen.

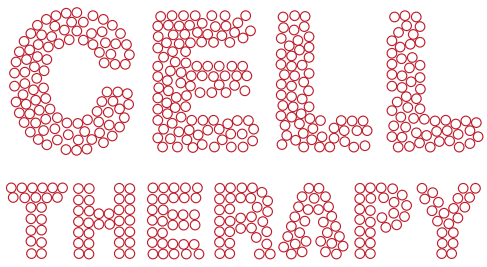
Curriculum Vitae

Tycho van der Spoel was born on June 3th 1982 in Hilversum, the Netherlands. After graduating from the ksg De Breul (Zeist) in 2000 he studied Medicine at the Erasmus University in Rotterdam. During this period he visited the University of California, San Diego for an experimental research project. After graduating from medical school in 2008, he started working as a non-training resident at the Department of Internal Medicine in the Albert Schweitzer Hospital, the Netherlands. In oktober 2008 he started his PhD research in the Stem Cell Group embedded within the Experimental Cardiology Laboratory, University Medical Center Utrecht, under supervision of Prof. dr. P.A.F.M. Doevendans and Prof. dr. E. van Belle. In 2010 he received the dr. E. Dekker grant from the Netherlands Heart Foundation to continue his research for 2 years. After finishing his PhD research in 2012 he started with his training in Internal Medicine at the Diakonessen Hospital in Utrecht, supervised by Dr. A.F. Muller. In 2015 he will continue his Cardiology training at the Utrecht University Medical Center, under supervision of Dr. J.H. Kirkels.


T.I.G van der Spoel


T.I.G van der Spoel

OPTIMIZATION OF DELIVERY STRATEGIES FOR CARDIAC



IN ISCHEMIC HEART DISEASE