“Characterization and Comparison of the Angiogenic and Cardio-Regenerative Potential of Mesenchymal CD271+ and Hematopoietic CD133+ Bone Marrow Stem Cells by Means of in Vitro and in Vivo Angiogenesis Models”

Dissertation for the acquisition of the academic degree as
doctor medicinae (Dr. med.)
Medical Faculty of the University of Rostock

Sarah Hélène Sasse
Rostock

Gutachter:
Prof. Dr. med. Gustav Steinhoff, Universitätsklinik Rostock, Klinik für Herzchirurgie
Prof. Dr. med. Christian Junghanß, Universitätsklinik Rostock, Medizinische Klinik III – Klinik für Hämatologie, Onkologie und Palliativmedizin
Prof. Dr. Christof Stamm, Deutsches Herzzentrum der Charité

Jahr der Einreichung: 2020
Jahr der Verteidigung: 2024

Universität Rostock
Traditio et Innovatio
Universitätsmedizin Rostock

https://doi.org/10.18453/rosdok_id00004808

Content

1 Introduction

1.1. Clinical relevance of cardiovascular disease and heart failure

According to the *World Health Organization* (WHO), cardiovascular disease is the most common cause of death globally (206). In the course of the past decade the number of deaths due to this diagnosis has even increased. Cardiovascular diseases killed 17.7 million people in 2015, that is three in every ten deaths. Of these, 7.4 million people died of coronary heart disease and 6.7 million from stroke (205). Especially in high-income countries cardiovascular disease has a high frequency as it causes more than half of all deaths across the European Region. Therefore, it can be considered to be one of the main public health problems.

Despite of the fact that much progress has been achieved in the areas of diagnosis and therapy of cardiac diseases, the mortality rate remains high. This is mainly due to the heart's lack of ability to repair the remaining damages. Although the acute event of myocardial infarction is more often treated successfully and thus can be survived, the number of patients affected by subsequent heart failure increases.

Heart failure (ICD-10 I50.-) is a term used for a complex clinical syndrome caused by structural or functional abnormalities of the heart and therefore describes the final stage of several cardiac diseases. It is defined as the inability to provide sufficient cardiac output in order to maintain the appropriate blood circulation needed by the body (75). According to the Framingham-Offspring-Study approximately 50% of the cases can be traced back to high blood pressure as the primary cause. In the process, hypertension leads to the pathogenic sequence of coronary heart disease, myocardial infarction, and heart failure (75). In the subsequent seven to eight years after cardiac infarction, more than one third of patients can be expected to develop heart failure, whereby those with left ventricular dysfunction are at particular risk (24).

Heart failure has a current prevalence of over 5.7 million in the United States of America and over 23 million worldwide. Projections indicate that it will increase by 46% until 2030, resulting in more than eight million people affected in the USA alone (130). Every year 870,000 new cases of heart failure are reported in the USA. Therefore, the incidence approaches 10 per 1,000 in the population of people over 65 years of age (130). The lifetime risk of developing this syndrome is estimated as one in five, but also increases with age (24). In 2012, total costs of heart failure in the USA were estimated to be $30.7 billion, of which 68% was attributed to direct medical costs. Furthermore, projections expect those costs to increase by almost 127% to $69.7 billion in the period between 2012 and 2030 (130). Because of the fact that heart failure is a chronic condition with hardly any recovery, this diagnosis is accompanied by substantial risk of both morbidity and mortality. According to the report of the American Heart Association, heart failure makes the largest contribution to age-standardized years lived with disability among men and women in high-income countries (130). It has already been determined by the Framingham Heart Study in 2002 (110) that once heart failure has been diagnosed, the mortality risk steadily increases. Even though survival rates improved over the past decades, the five-year mortality from heart failure remains at least 50% (23). The Rotterdam Study of prevalent cases in Europe (17) had a cumulative survival of heart failure as little as 86% at 30 days, 63% at one year, and 35% at five years of follow-up. The median survival in this study was only 2.1 years. Stewart *et al.* even suggested that heart failure was more 'malignant' than cancer in a study comparing five-year survival rates and associated loss of expected life-years of heart failure and four common cancers in Scotland. With the exception of lung cancer, heart failure was associated with the poorest five-year survival rate (approximately 25% for both sexes) (170).

1.2. Pathogenesis and therapeutic measures

Myocardial infarction occurs when coronary blood flow is impaired, commonly due to arterial occlusion. The peripheral parts of the heart, which are hereby deprived from oxygen supply, suffer from persistent ischemic conditions. As a result, cardiac myocytes die and the related part of the heart muscle turns into an area of coagulation necrosis (21). The affected area differs in size and location depending on the individual topographical arrangement of the coronary territories, the location of occlusion, and the duration of ischemia. In consequence of inflammatory processes, the striated heart muscle is converted into nonfunctional fibrin-containing scar tissue, which cannot contribute to the pumping function of the heart anymore. Besides, myocytes distend and the actin-myosin-filaments slide apart in response to ischemia. Due to this unfavorable transmission the heart muscle's contractility decreases and is not sufficient to maintain the appropriate ejection fraction anymore (21). As an attempt to compensate this mechanism, heart muscle growth can be observed causing a pathological organ hypertrophy. Hereby, the cardiac output may potentially maintain normal for some time, but simultaneously the heart's energy consumption increases and intensifies the coronary insufficiency (21). As the remodeling is irreversible, the heart's deficiency thus is even enhanced by the counter-mechanisms (compare also Figure 1).

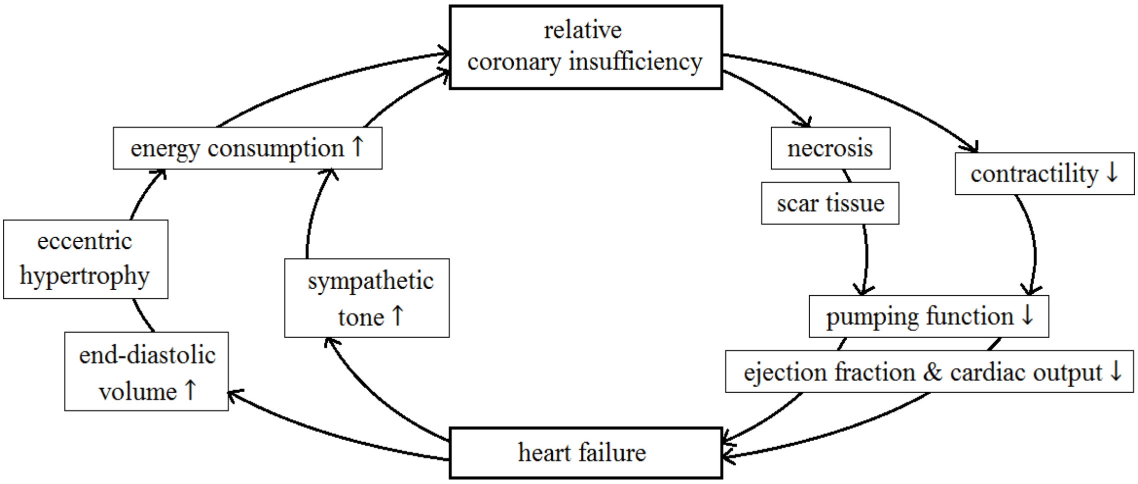


Figure 1: Vicious circle of coronary insufficiency and heart failure (21).

Concerning therapeutic options, a distinction can be drawn between causal, symptomatic, and prognosis-improving approaches. Causal therapies target mechanisms that contribute to the pathogenesis in general, which includes malfunctions like arterial hypertension, coronary heart disease, heart valve defects, and even extra-cardiac aspects like blood lipids or diabetes. The therapeutic measures range from medication and adjuvant recommendations such as nutrition, exercise, and weight reduction to interventional procedures like endoluminal vascular prosthesis or surgery like coronary bypass or heart valve replacement. Therefore, these treatments contain primary and secondary prophylaxis in order to prevent the occurrence of cardiac events, but also include acute measures that intend to keep the damage sustained from an event to a minimum.

After the onset of heart failure, the pharmacological therapy is based on five groups of medication depending on the stage of disease as classified by the *New York Heart Association* (NYHA) (compare Table 1). At all stages of heart failure *angiotensin-converting-enzyme* (ACE)-inhibitors are indicated as they have been proven to slow down the unfavorable remodeling processes (200). Additionally, beta blockers are used for all patients that are in a state after cardiac infarction, but are otherwise also substituted starting at NYHA stage II. The hereby intended effects are heart rate control, protection against toxic effects by catecholamines, prevention of down regulation of beta receptors, and anti-ischemic effects (75). Several studies have also shown that beta blocker therapy reduces mortality in patients with advanced heart failure. Likewise, aldosterone antagonists may improve mortality rates and are thus indicated from the second stage onwards. In conclusion, angiotensin-converting-enzyme-inhibitors, beta-blockers, and aldosterone antagonists may improve the patient’s prognosis and therefore prolong life.

Table 1: Medication of heart failure adapted to NYHA-classification (75)

NYHA-stage:	**I**	**II**	**II**	**IV**
ACE-inhibitors	X	X	X	X
beta blockers		X	X	X
aldosterone antagonists		X	X	X
diuretics		(X)	X	X
cardiac glycosides			X	X

In contrast, diuretics and cardiac glycosides have symptomatic effects only. Diuretics are used to counteract fluid retention, signs of congestion, and hypertension. As they reduce the heart's preload as well as the peripheral resistance and thus the heart's afterload, diuretics are generally indicated from NYHA stage III onwards (75). Moreover, the substitution of cardiac glycosides like digitalis is started at stage III as well, but is additionally used at every stage with tachyarrhythmia or atrial fibrillation. Although therapies with cardiac glycosides result in positive inotropic support for the heart, influences on the mortality of heart failure have not been proven (75). Besides from medication, other approaches can be taken into account especially in high stage heart failure. These include *cardiac resynchronization therapy* (CRT), *implantable cardioverter- defibrillators* (ICD) as well as heart transplantations (75).

Indeed, the pharmacological therapies currently in use improve the patient's quality of life as well as his survival time, but cannot compensate the loss of cardiac function. As the affected tissue cannot regenerate itself, the damage and loss of heart muscle tissue remains and likewise the accompanied negative effects. In order to cover up the deficit of regenerative potential, new treatment approaches for cardiac diseases are needed.

2 State of the art

Several studies suggest that approaches based on the application of stem cells may be the future strategy in order to compensate the regenerative deficiency of the heart. Therefore, bone marrow derived hematopoietic stem cells have already been the main focus of several studies since the beginning of the century. After promising preclinical results (140) stem cell therapies were also introduced to clinical trials in order to investigate effects on ischemic heart disease. In the course of time, numerous different research groups were able to demonstrate that autologous stem cell transplantation has positive therapeutic effects on infarcted myocardium as it improves the ventricular systolic function and regeneration of heart muscle (171), (195), (142).

The department of heart surgery and the *Reference- and Translation Center for Cardiac Stem Cell Therapies* (RTC) of the University of Rostock were also able to successfully complete several experimental and clinical studies on the effects of hematopoietic stem cells. This includes a clinical pilot trial (phase I) and subsequent controlled trial (phase II) studying the impact of intramyocardial application of bone marrow derived CD133^{+} stem cells into the myocardial border zone of patients with myocardial infarction undergoing *coronary artery bypass grafting* (CABG) surgery (169), (47), (49). Due to the promising results of these studies, the first randomized, prospective, double-blind, placebo controlled, phase III multicenter clinical trial was launched in 2009. This so called PERFECT- study as an acronym for *Intramyocardial TransPlantation of BonE MaRrow Stem Cells For ImprovEment of Post-Infarct MyoCardial RegeneraTion in Addition to CABG-Surgery* was initiated in cooperation with the RTC, Miltenyi Biotec GmbH, and other industrial and clinical associates (48). The referred studies showed that CD133^{+} application is safe and significantly improves the cardiac function concerning the ejection fraction of the left ventricle as well as the revascularization and contractility of the treated areas compared to CABG-surgery without stem cell application. This benefit has also been confirmed in long-term follow up (136).

Concerning the prospects of therapy, a differentiation between the areas of myocardial infarction has to be considered. Scar tissue cannot recover from ischemic damages even if the perfusion is restored. Therefore, therapeutic measures will not have appreciable effects on this tissue. On the other hand, so called hibernating myocardium marks the border of the infarction area and consists of ischemic but non-infarcted myocardium. These myocytes remain viable but have a decreased contractile function, which is considered to be an adaptive reaction in order to reduce oxygen demand and to allow equilibration of supply and demand. Therefore, this state can partially or completely be restored by improving blood flow and myocardial revascularization and thus offers a target for therapeutic approaches (152), (77). Whether or not the cells are viable can be detected by various imaging processes, including *magnetic resonance imaging* (MRI), dobutamine stress echocardiography, and *single photon emission computed tomography* (SPECT) (162).

Regarding the question which stem cells are particularly suitable for cardiac therapies, several aspects have to be taken into account. Stem cells in general are defined to be in an undifferentiated state. In response to external stimulation they are able to differentiate into specialized functional cells, which work tethered with resident cells in order to form an integrated system. Secondly, stem cells have to be capable of self-renewal in order to preserve the cell population. Bearing in mind the pathological processes of cardiac diseases, several desirable properties can be determined for the stem cells in use. As myocardial infarction occurs due to reduced blood flow in the coronary arteries, the formation of new blood vessels would be a preferable effect. This socalled angiogenesis leads to an improved perfusion of the area. Because of the fact that myocardial infarction results in distinct areas of necrosis, stem cells with the capability to differentiate into cardiac myocytes would also be an obvious choice as they could possibly rebuild destroyed heart muscle tissue. But in the search for suitable stem cells, various other properties are of interest as well. This among others includes cells that interfere with pathological immune mechanisms as well as cellular mediators and chemokines that cause migration and homing processes. Besides, it is of tremendous importance whether or not the cells are proofed to be safe in application. Concerning the therapeutic options, it is possible to use cultivated cells as well as primarily isolated stem cells. Furthermore, the cells can also be treated in advance in order to develop desirable properties. Likewise, the stem cells can be applied in combination with active substances, which may contribute to required effects. As the CD133 positive stem cell therapies and other approaches with hematopoietic cells provide promising results, research has to examine more cell types in order to achieve additional effects and thus to further improve the therapeutic measures. Different cell populations are distinguishable from one another by their *cluster of differentiation* (CD), which is a group of markers expressed on the cell surface. Through the use of antibodies against those surface structures, different stem cell populations can be identified and investigated separately. Bone marrow contains the greatest amount of stem cells and thus is often focused as research subject. Bone marrow derived stem cells have the ability to differentiate into multiple specific cell types and are also capable of self-renewal (140). The regenerative effects of hematopoietic stem cells are assumed to base mainly on angiogenetic processes. Several studies showed that hematopoietic stem cells can restore tissue vascularization after ischemic events as well as during physiological processes (151). After local implantation of bone marrow stem cells high proliferation of endothelial cells was detected (147). Other than that, hematopoietic cells seem not to be able to differentiate into cardiac myocytes (133). Until now, no stem cell-related cardiac complications following intramyocardial injection of bone marrow-derived stem cells have been reported so that the therapy is considered to be safe (49).

In addition to the well-studied hematopoietic stem cells, bone marrow also contains a population named after their ability to differentiate into various cell types of mesenchymal origin. These multipotent stem cells are expandable and modifiable in cell culture and offer several distinctive characteristics, which make them a promising therapeutic approach for damaged heart tissue (144). Especially the ability of these *mesenchymal stem cells* (MSCs) to differentiate into vascular endothelial cells (124) as well as cardiac myocytes (123), (181), (96) supports the concept that the application of these cells may decrease infarct size and myocardial fibrosis (53). Thereby, cardiac remodeling in total may be attenuated as well as the heart's contractility improves (53). Furthermore, MSCs have paracrine effects that inhibit apoptosis of endothelial cells in hypoxic condition, increase their survival, and thus stimulate angiogenesis processes (88). Several reports suggest that these paracrine mechanisms also include the secretion of various angiogenetic cytokines such as *vascular endothelial growth factor* (VEGF), which augment collateral perfusion by stimulating the proliferation and migration of endothelial cells and smooth muscle cells (88). In addition to their regenerative potential, studies report that MSCs may home to the site of acute cardiac injury or ischemia and localize in the infarct- and border zones (148). After several studies were able to show the potential of mesenchymal cells *in vitro* (144), (181) the mechanisms in question were also confirmed *in vivo* (148). As another advantage, MSCs do not express any antigens of histocompatibility and thus can be used in allogenic transplantation as well. Quite the contrary, these cells even have immunomodulatory paracrine effects that counteract inflammatory responses (157), (43). As inflammatory processes play a decisive role in the development of chronic heart diseases and have a negative influence on the heart function, these effects may also improve the therapy significantly. After the transplantation, MSCs may be able to stimulate the production of reparative growth factors, inhibit local inflammatory response, and replace damaged cells (135). Consequently, mesenchymal stem cells may even "create an environment that favors endogenous cardiac repair" (135).

Apart from these positive effects, difficulties with the practical application arise from the large heterogeneity of mesenchymal stem cells as well. Thus, it is uncertain to predict which exact cell type arises from the MSC population after the application. Indeed, a study by Yoon *et al.* showed that the direct transplantation of unselected bone marrow cells into the infarcted myocardium induces significant intramyocardial calcification (199). This clearly demonstrates the research demands in order to guarantee the safety of MSCs as therapeutic option. The development of methods for the identification and isolation of MSC subpopulations is crucial for a determined specific function and a successful clinical application. Besides, possible side effects such as immunogenic activity, arrhythmia, and tumorigenesis have to be investigated. Furthermore, it is difficult to compare and contrast research results with one another as different methods of isolation, expansion and thus cell characterization are used. The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposed three minimal criteria to define human MSCs. First, it is required that MSCs are adherent to plastic under standard cell culture conditions. Second, MSCs have to be able to differentiate to osteoblasts, adipocytes, and chondroblasts *in vitro*. Third, MSCs have to express several specific surface antigens (46). Concerning these antigens, it was recommended that MSCs need to express a combination of CD105, CD73, and CD90 whereas they have to lack CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA-DR surface molecules (28). Unfortunately, these proposed patterns alone are not sufficient to isolate MSCs from bone marrow.

The identification of specific markers allows the use of a homogenous subpopulation with defined properties. A currently much examined MSC subtype is characterized by the expression of the surface marker CD271. Several studies showed that this marker is one of the most specific concerning bone marrow derived MSCs (22). Thus, there may be a particular suitability of CD271 positive MSCs for preselected cell products. CD271 is also known as *low affinity nerve growth factor receptor* (LNGFR) or *neurotrophin receptor p75* (NTR) and enables prospective cell isolation of MSCs from bone marrow derived mononuclear cells (150). Neurotrophins and their receptors have been shown to be essential in the formation of the heart as they influence the survival of endothelial cells, vascular smooth muscle cells, and cardiac myocytes. Furthermore, these proteins regulate angiogenesis and vasculogenesis by autocrine and paracrine mechanisms (27). Studies indicated that the *nerve growth factor* (NGF) as one ligand of the CD271 receptor is present in the infarcted human heart under physiological conditions and that it increases cell survival as well as cardiac repair and function. Besides, this cytokine promotes neovascularization and thus myocardial blood flow (128), (108).

In conclusion, CD271 positive MSCs are a particularly promising subpopulation, which may contribute to cardiovascular regeneration and which could influence immune reactions by paracrine modulation.

3 Aim of study

This study intends to examine the therapeutic potential of mesenchymal CD271 positive stem cells in comparison to hematopoietic CD133 positive cells. At this, it should be determined if CD271$^+$ MSCs can improve the effectiveness of the stem cell therapies currently in use. We expect that the multiple characteristics and various regenerative effects of mesenchymal stem cells will result in greater therapeutic impact on ischemic heart disease. In this context, the effects of CD271$^+$ cells alone as well as in combination with CD133$^+$ are going to be explored with the main focus on angiogenic potential. The interactions between these two subpopulations and to what extent the combination may enhance heart regeneration has not been investigated yet.

First of all, it is necessary to guarantee an efficient and reproducible cell selection in order to ensure a safe and predictable application. Therefore, a standardized immuno-magnetic isolation procedure and characterization of the requested stem cell populations is going to be established as well as a reliable quality management, which has to verify aspects such as cell purity and stability. Furthermore, proliferative properties are going to be investigated by means of *colony-forming-unit fibroblast* (CFU-F) activity. The angiogenic qualities of the different cell populations are investigated based on an *in vitro* angiogenesis assay with Matrigel. For the evaluation three-dimensional microscopy, cell staining, *immunohistochemistry* (IHC), and gene expression analysis are going to be applied on the *in vitro* angiogenesis model. In order to evaluate the effect of stem cell therapies on infarcted hearts, an *in vivo* analysis is necessary. In an established mouse-model the cardiac response to the application of human CD133$^+$ and CD271$^+$ stem cells is going to be investigated. The small animal model is designed to obtain information on immune reactions and the influence of stem cell application on the heart's regenerative potential. Therefore, gene expression analysis is performed on the heart tissue.

4 Methods

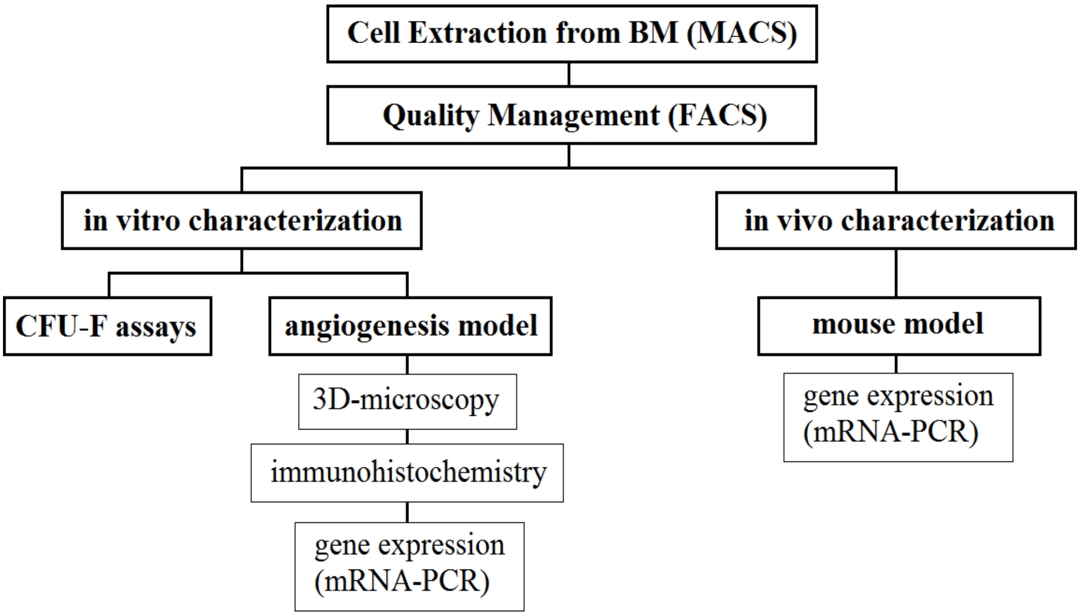


Figure 2: Conceptual scheme of study.

4.1 Manual Immuno-Magnetic Cell Separation (MACS) of human CD271$^+$ and CD133$^+$ stem cells from bone marrow[1]

For coronary artery bypass surgery, a median sternotomy is performed in order to get access to the thoracic cavity. With given informed written consent and authorization by the patient that their bone marrow may be used for research according to the Declaration of Helsinki, sternal bone marrow was aspirated from the hereby exposed sternal bone and was directly delivered to our institute stored in syringes.

The immuno-magnetic cell separation method isolates certain stem cells according to their surface antigens. Therefore, antibodies that are coated with magnetic iron particles are added to the cells and bind to their specific antigens (Figure 3). Afterwards, the cell suspension is applied to a column within a magnetic field, which holds back the iron bound stem cells (Figure 4). All unbound cells - that are thus negative for the antigen in question - pass through the magnetic field without any restriction and are separated from the others. The positive cells are then removed from the magnetic field and can be collected for further procedure (Figure 5). Performed successively with different antibodies, various types of cells can be isolated from the patient’s bone marrow.

---

[1] According to ““MiniMACS extraction protocol for human CD133$^+$ cells from bone marrow” AND “MiniMACS extraction protocol for human CD271$^+$ cells from bone marrow” by Miltenyi Biotec.

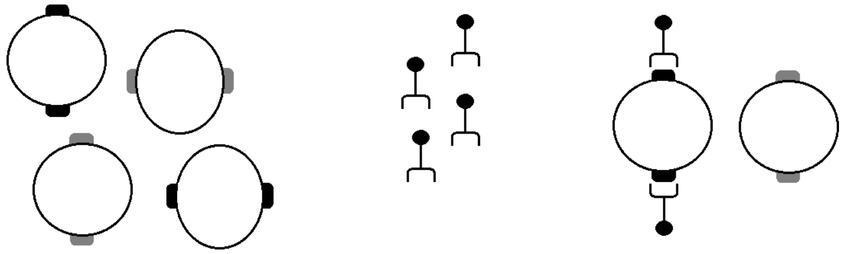


Figure 3: Schematic drawing of the stem cell labeling with antibodies. Stem cells with different surface antigens and antibodies coated with magnetic iron particles specifically bind to each other.

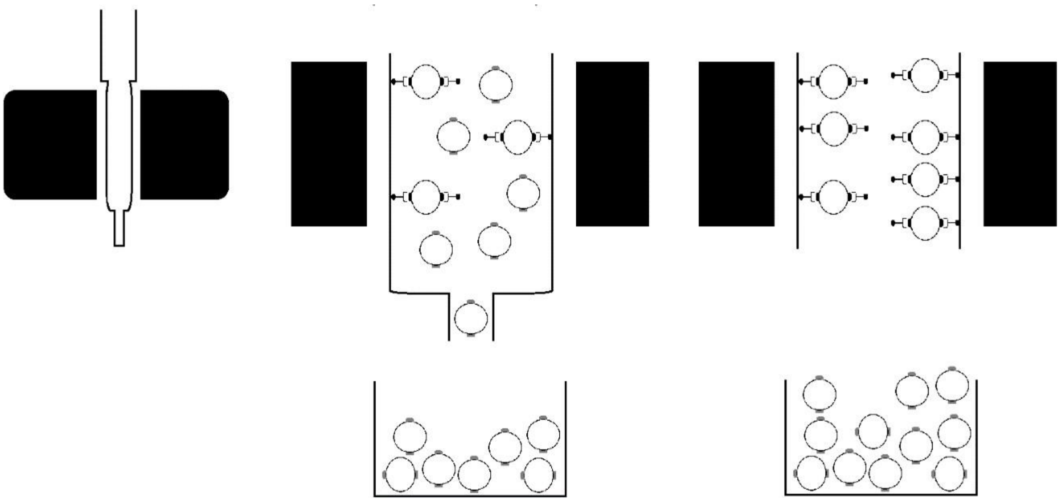


Figure 4: Magnetic cell separation. The cell suspension is applied to the MACS column within a magnetic field. Antigen positive cells stay in the column whereas antigen negative cells pass through and can be collected.

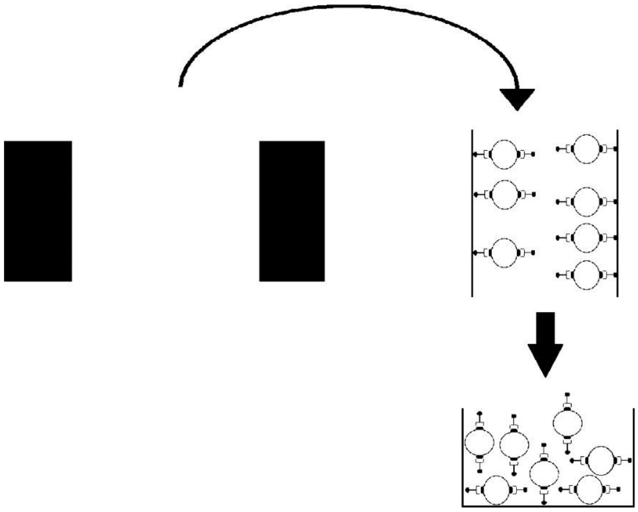


Figure 5: Release of the antibody-labeled cells. Being removed from the magnetic field, the labeled cells are no longer hold back in the column and can be collected.

Sample preparation

First of all, portions of bone marrow were supplemented by PBS/EDTA, RPMI, collagenase B stock solution, and DNase stock solution. In the next step *Lymphocyte separation medium*, also referred to as LSM or Ficoll-Plaque, was used in order to generate a density gradient within a Leukosep tube. Above the cell strainer was a white or clear layer of monocytes, lymphocytes, and platelets that has been covered by a fatty layer at the very top of the tube. The monocytes were collected and resuspended in MACS puffer, which consists of *phosphate buffered saline* (PBS), 0.5% *bovine serum albumin* (BSA), and *ethylenediaminetetraacetic acid* (EDTA).

The magnetic labeling and the separation of CD133 and CD271 positive cells were performed according to the MiniMACS extraction protocols by Miltenyi Biotec. For the isolation of the second type of stem cells the collected flow through of the first column was used, containing unlabeled negative cells. Therefore, the cell suspension was given on fresh equilibrated columns in order to remove possible remnants of the first fraction.

4.2 Quality management by fluorescence-activated cell sorting

Fluorescence-activated cell sorting (FACS) is a specialized type of flow cytometry, which allows the sorting of viable cells according to receptor expression. Therefore, the receptors in question are identified by means of specifically binding fluorescent dyes. The characterization of cells is based upon the emission of optical signals as the cells pass through a laser beam. The specific light scattering and fluorescence characteristics allow the acquisition of quantitative information about each single cell that has been analyzed. For this purpose, the prepared cell suspension is transferred into a narrow but rapid stream flow which breaks up into small droplets by means of a vibrating mechanism. This hydrodynamic focusing is adjusted in a way that each droplet contains one cell only, which allows individual cell analysis. The characterization of the cells is performed as they pass the laser beam. First, the amount of scattered light correlates with the cellular size and its complexity. Hereby, the *forward scatter* (FSC) measures at a flat angle and thus depends on the cell volume. On the other hand, the *side scatter* (SSC) measures the distribution of light at right angle, which is influenced by the cell's complexity such as surface properties, granularity, the size of nucleus, and the amount of vesicles. In the meantime, the fluorescence of the added dye is also detected during flow cytometry. By using varicolored laser beams and filter, several different dyes can be applied in each measurement, which increases the density of information. At this, the dyes can be combined with antibodies against specific surface structures like cluster of differentiation and thus mark e. g. $CD271^+$ or $CD133^+$ cells. Furthermore, dyes can also be used to quantify intracellular structures such as DNA. The specific dyes *propidium iodide* (PI) or *seven-amino-actinomycin D* (7-AAD) intercalate between nucleobases and thus enable to determine the amount of DNA within the cell as well as whether or not the cells are alive. The cell separation is finally performed using an electrical charged ring. In accordance with the prior measurement, a charge is transmitted to the droplets. Thus, the cells are sorted based upon this polarization as they pass through an electrostatic deflection system and can be collected in separate tubes (compare also Figure 6).

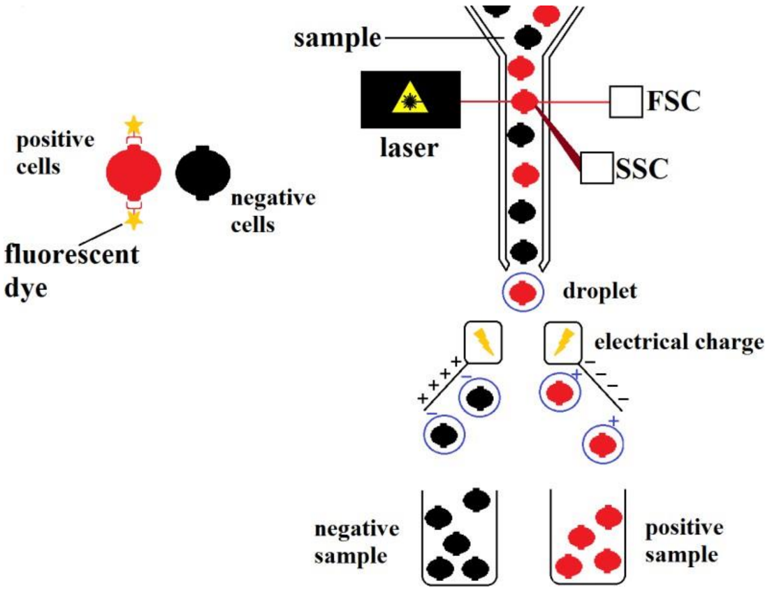


Figure 6: Schematic drawing of fluorescence-activated cell sorting.

The samples were measured with a LSR II flow cytometer by BD Biosciences and the associated DIVA™ software. Cytometry analysis was used to evaluate the purity of the cell samples and thus the quality of the isolation procedure. Second, the viability of the cell suspensions and thus the cell loss was investigated by means of 7-AAD. The CD133$^+$ population was stained for CD133$^+$ CD34$^+$ taking into account that the majority of CD133$^+$ cells express CD34$^+$ as well (176). The CD271$^+$ population was stained for CD271$^+$ CD45$^-$ as a quality control (37). Furthermore, the surface marker CD29, CD73, CD105, and CD44, which are used to define mesenchymal stem cells (28), were also applied on the freshly isolated CD271$^+$ population. Compensation was established using single stained controls and gating was performed with matched isotype/ fluorescence minus one (FMO) controls. The Boolean gating strategy for CD133$^+$ cells was modeled on the *International Society of Hematotherapy and Graft Engineering* (ISHAGE) guidelines for CD34$^+$ cell analysis. CD271$^+$ cells were gated based on viable SSC^{low} cells. Additionally, the influence of fresh samples versus storage was examined.

The recorded FACS data was displayed in dot plot diagrams, in which the cells were sorted according to their size, complexity, and receptor expression (Figure 7). In these diagrams an adequate sorting can be accomplished by adaptation of gating regions from a broad spectrum of channels (compare Figure 8).

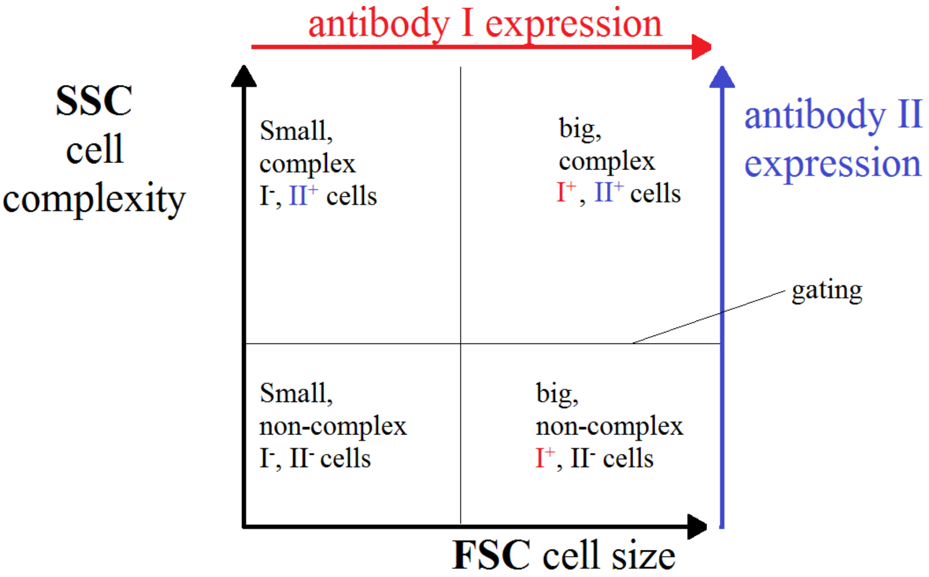


Figure 7: Schematic dot plot diagram of FACS data.

Tube: BM3

Popu1a on	Il'Events	%Parent	%Total
All E,·enls	5□.□□0	▬	100.D
L 🟦 11me	50,000	100.0	100.0
cells	<2,487	80.0	85.0
🟩 'liable	•1. 34	90 5	03 7
i-- 🟦 vlable CD45+	1.735	99.8	83.5
,.......... viable CD271•	813	1.9	I.S
L 🟪 ,iable CD271+ SSC I w	472	58.1	D.9
f....... i1a le CD271• IYmPhD	264	55.9	0.5
' 0 viable CD27 • blast I mono	185	39.2	0.4
t.. 🟦 i1able CD27 + CD45·	23	49	D 0

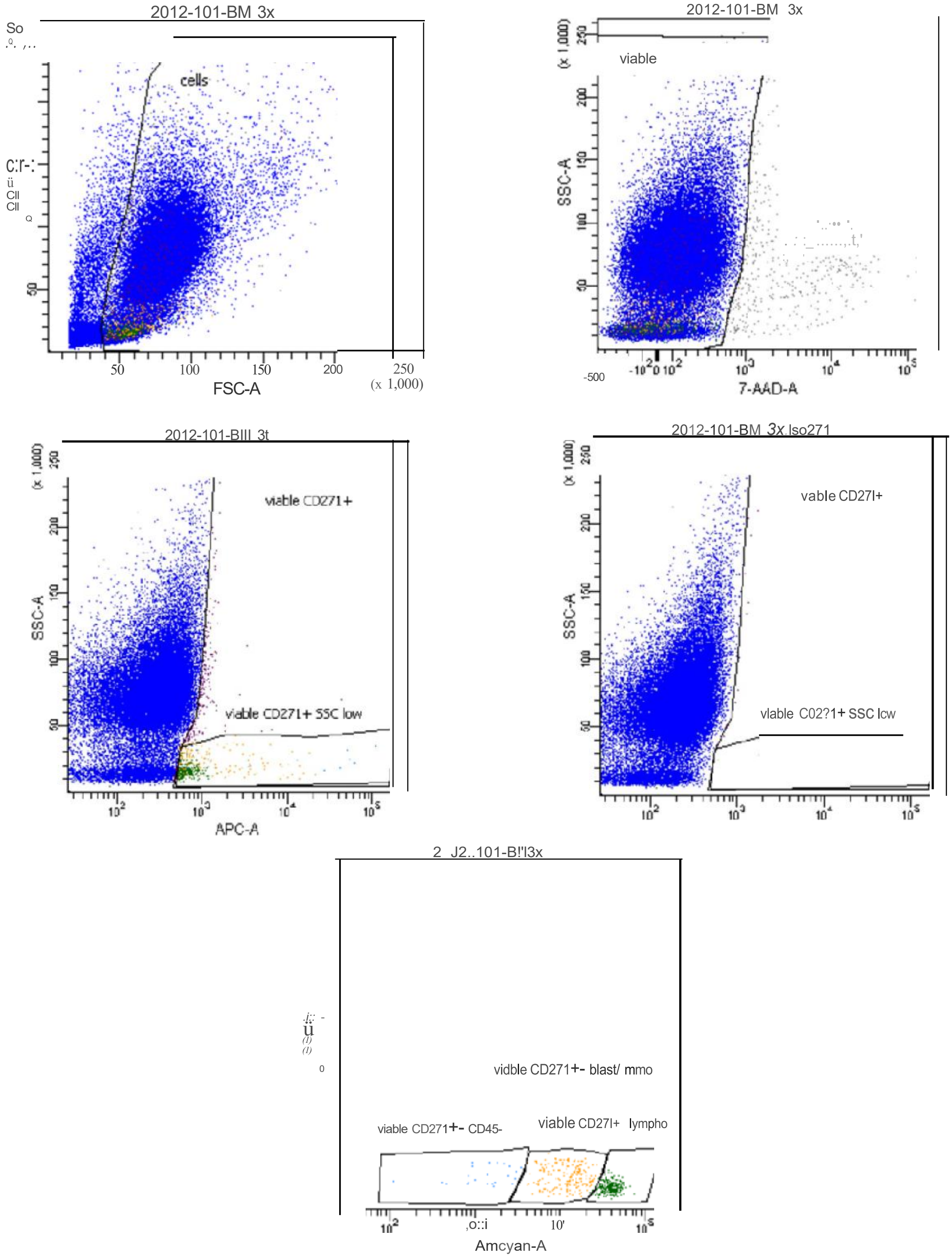


Figure 8: Exemplary gating strategy of CD271 cell analysis.

4.3 *In vitro* stem cell characterization

4.3.1. Colony-forming assays

CFU-F assays are primarily used to quantify functional mesenchymal stem cells and their proliferative potential. Mesenchymal stem cells are defined as plastic-adherent and fibroblast like clonogenic. As they proliferate, they form colonies termed *colony forming units-fibroblasts* (CFU-F). This activity has been observed in CD271$^+$ populations, but not for CD271$^-$ cells (150). Therefore, these assays were used to compare proliferative properties as well as residual CFU-F activity of the stem cell populations after being cultivated over a defined period of time. At this, three populations were evaluated in each assay:

- one sample of mononuclear cells (MNC) with one million cells,
- one sample of the CD271$^-$ CD133$^+$ fraction with one million cells,
- one sample of the CD271$^+$ fraction with 40,000 cells.

One assay contains cells of one patient only.

All cells were cultivated in *mesenchymal stem cell growth medium* (MSCGM). This medium consists of *basal medium* (MSC BM), *growth supplement* (MSC GS) and L-Glutamine. Additionally, it includes GA-1,000, which consists of *gentamicin sulfate* in combination with a*mphotericin B* as an antifungal medication. Furthermore, streptomycin and penicillin complete the medium. A six-well plate was prepared with MSCGM each. Afterwards, the calculated amount of cell suspension containing the desired cell number as indicated above was added to the medium. The assays were incubated at 37°C with 5% CO_2 for seven days. Medium exchange was performed at the seventh day with fresh MSCGM. The colonies were counted after 14 days, whereby CFU-F colonies had to be 1-8mm in diameter. Besides, cell clusters of at least 20 cells were counted as a colony as well. For better counting conditions the cells can also be fixed and labeled by Giemsa stain. For the analysis a Zeiss Axiovert 40 CFL fluorescence microscope with Axio Cam MRm and the ZEN (Zeiss efficient navigation) software was used.

4.3.2. Angiogenesis assays

In order to investigate to what extent the stem cells build networks of endothelial cells and thus to evaluate their angiogenetic capabilities, the isolated fractions of CD133$^+$ and CD271$^+$ cells were cultivated in special growth matrices and endothelial growth medium. Each patient's CD271$^+$ and CD133$^+$ cells were seeded separately in order to compare their tendency to build network structures. Additionally, one assay consisting of both cell types in equal amounts was created with the aim of investigating whether they influence each other and alter the network formation. All assays contained cells of one patient only.

Stem cells require a special surrounding to attach, grow, and proliferate, which cannot be sufficiently achieved on plastic surfaces of common lab ware. Therefore, the gelatinous protein mixture called reduced growth-factor BD Matrigel™ Matrix (Becton Dickinson) was used to create a complex extracellular environment with structural proteins like laminin, entactin, and collagen which resembles natural conditions. Thus, complex cellular behavior like network formation of endothelial cells can be induced. In the assays of consideration, greater volume of Matrigel was used in order to build a three-dimensional cell network instead of monolayer structures. For the purpose of stimulating the cell growth and endothelial differentiation EGM-2 was used as an endothelial growth medium. For the assays 100,000 cells were used in each well on a chamber slide. For every patient's sample one well with 100,000 CD271^{+} cells and one well with 100,000 CD133^{+} cells were seeded. Additionally, one well with both cell types in one to one ratio, i.e. with 50,000 CD271^{+} and 50,000 CD133^{+} cells was established. Therefore, the cell suspensions containing the intended number of cells were filled up with EGM-2, so that each sample was 100µl in total volume. Afterwards, 100µl Matrigel were added, mixed carefully and then the whole suspension could be spread into the chamber slide. The Matrigel was thawed on ice and the transfer was performed by means of cold pipettes in order to avoid early polymerization. To finally polymerize the gel, the chamber slide was incubated at 37°C for twenty to thirty minutes. Afterwards, additional 200µl EGM-2 were given on the solid Matrigel so that the cells were completely layered with medium (Figure 9). The medium has to be renewed every two days to guarantee a sufficient supply.

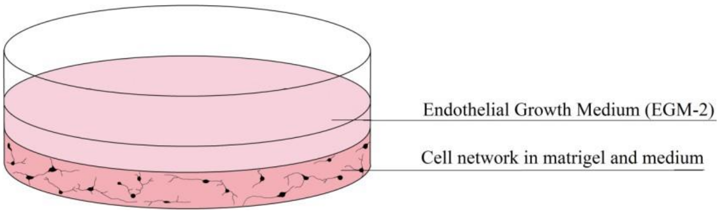


Figure 9: *In vitro* angiogenesis assay with Matrigel.

3D-microscopy analysis

After two weeks the assays were analyzed according to the criteria of tube length and number of network nodes. Therefore, z-stack images were acquired using the Zeiss high-resolution confocal microscope ELYRA S.1 and corresponding software. Z-Stack images are transversal slice images (two-dimensional) of the three-dimensional assay and thus allow representative analysis of the structures. For every well, five z-stacks with ten images each were taken into account. The evaluation was carried out using the *Image J* image analysis program. Since the networks are three-dimensional structures, the monitored tubes emerge not only in one image of the stack, but probably in several. Thus, the structures can and need to be compared as well as followed from image to image in order to prevent measuring them more than once (compare Figure 10). This can also be perceived in the merged pictures on the right (Figure 10), in which the overlay of all images of the stack was inserted. For better illustration, the different levels of the stack are marked in different colors. Likewise, the amount of nodal points was counted, respectively. In total, 900 images (n= 6) were analyzed and measured. As the analysis was performed by one person only, a constant procedure with high comparability was guaranteed.

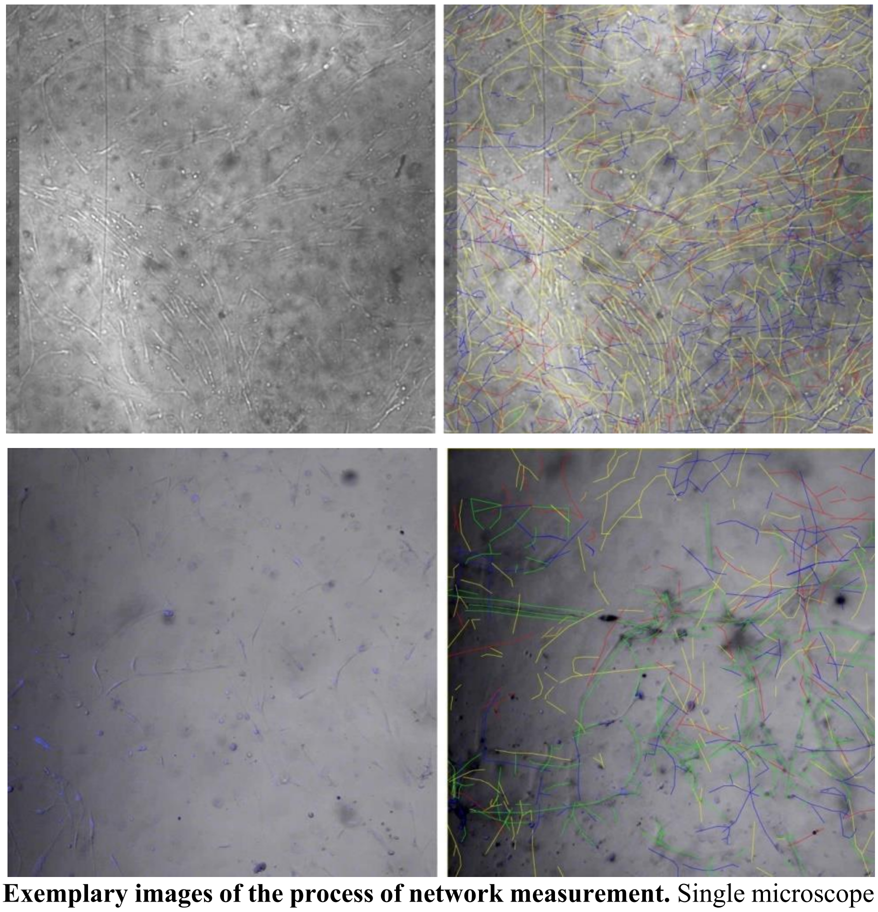


Figure 10: Exemplary images of the process of network measurement. Single microscope images on the left and combined with stack overlay on the right (10x magnification).

4.3.3. Identification of stem cell phenotype in vitro

In order to further investigate the cell networks accomplished in Matrigel (Chapter 4.3.2), immunochemistry was carried out on the angiogenesis assays. Therefore, the cells were stained with fluorescent dyes, which are supposed to make the structures more perceptible and differentiable. In this regard, the assays containing both cell types were of particular interest. By using different dyes for the stem cell subpopulations the staining should reveal whether both cell types take part in building the network structures and to which extent. Furthermore, this analysis could indicate whether the subpopulations act individually or if they are able to cooperate with each other in terms of network formation. Thus, at least two different dyes were needed in order to label the subpopulations $CD133^+$ and $CD271^+$. These dyes had to be applicable as well as non-toxic for living cells. Furthermore, the emission spectra of the fluorescent substances had to differ from each other and had to allow the combination in one assay. Obviously, the cells needed to be stained separately and thus before they were merged in the assay. In order to evaluate the cell's involvement in the network structures, the staining consequently needed to last reliably during the whole time of cultivation. Furthermore, the dyes had to label the cells significantly and specifically.

The fluorescent dyes chosen for this purpose were CFDA-SE on $CD133^+$ cells and PKH26 on $CD271^+$ cells. CFDA-SE or *carboxyfluorescein diacetate succinimidyl ester* is a lipophilic cell permeable dye that enters the cells by passive diffusion. The dye is colorless and non-fluorescent until intracellular esterase enzymes cleave the molecule's acetyl groups. This dye has also been successfully used on mesenchymal stem cell subpopulations (183). PKH26 is a red fluorescent lipophilic tracer with long aliphatic tails, which incorporates into the lipid bilayer of cytoplasmic membranes. The appearance of the labeled cells depends on the cell type, the composition of the cell membrane, and the extent to which membrane internalization occurs after the staining process. The cell labeling was performed with both reagents on the freshly isolated stem cells, which were used in the angiogenesis assays as described above (Chapter 4.3.2).

Additionally, both cell types were stained with Hoechst 33324 stain, which labels the cell nuclei. Hoechst stains (bis-benzimides) are blue fluorescent dyes that bind to the minor groove of the double-stranded DNA helix with a preference for sequences rich in adenine and thymine. Being cell-permeable and supravital the dye binds to DNA in fixed as well as in living cells without harming them. Hoechst dye was also applied on the angiogenesis assays, but other than before, the staining was performed after the cultivation period. This handling was possible as both cell types were already stained and did not need to be labeled separately anymore.

The staining methods were first established on mononuclear cells and mesenchymal stem cells as these cells are more available compared to the limited material of CD271$^+$ or CD133$^+$ cells. In microscope photography after 6, 11, and 14 days the green fluorescence of CFDA could be slightly detected around the cells, so that the dye was not only specifically within the cells (Figure 11). The PKH26 dye labels the cells more specifically than CFDA, so that in most cases only the cells were marked with fluorescence. Furthermore, the red color builds a stronger contrast to the background than the green CFDA stain (Figure 12). The application of Hoechst dye resulted in highly satisfactory staining of the cellular nuclei (compare Figure 13). The analysis of the staining results was performed using the Axiovert 40 CFL fluorescence microscope with Axio Cam MRm ZEN (Zeiss efficient navigation) software.

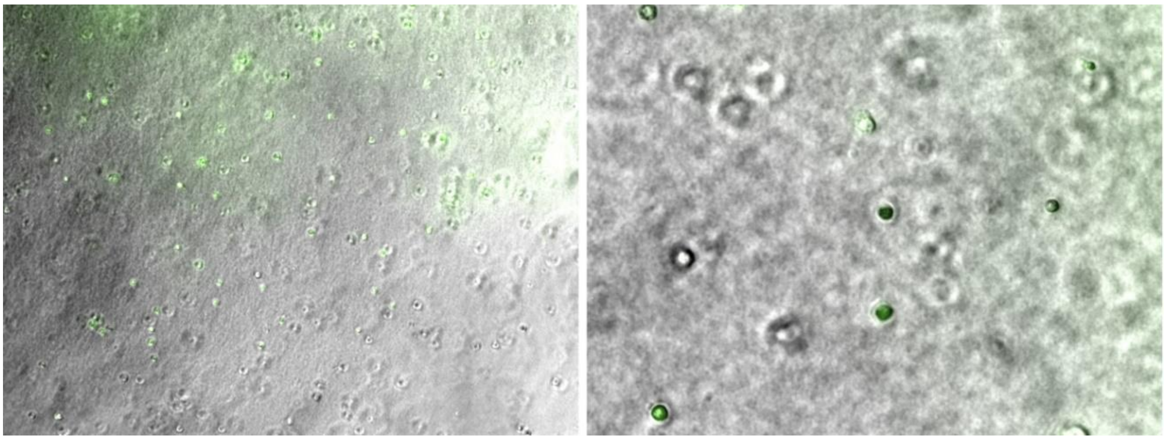


Figure 11: Images of CFDA staining on MNCs in different magnifications.

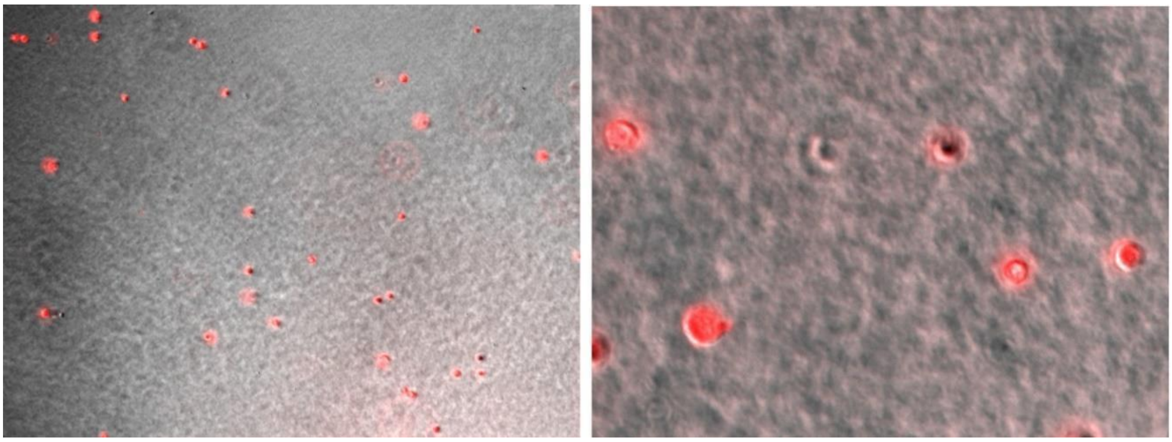


Figure 12: Images of PKH26 staining on MNCs in different magnifications.

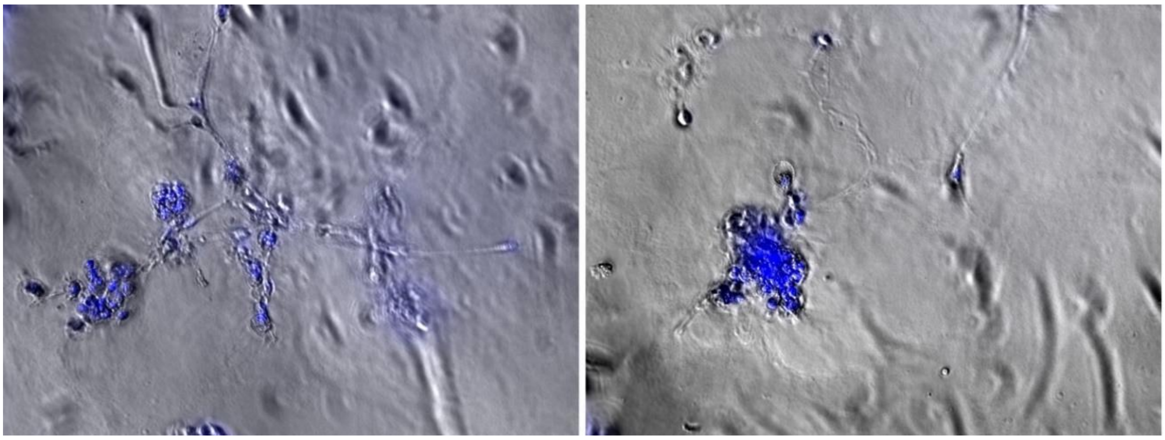


Figure 13: Images of Hoechst staining on MSCs.

In order to establish a method that further characterizes the cell networks and the stem cells contained within, staining of the surface marker CD29, CD105, CD73, and CD271 was performed. Hereby, it would be possible to obtain information on the stem cell differentiation and its properties within the network. In this context, it could be evaluated to which extend the subpopulations differ from each other in different assays. In order to establish this method, it had to be evaluated if the antibodies are able to reach the cells throughout the Matrigel. Otherwise, the network assays would need to be embedded in paraffin, sectioned, fixed on slides, and can only be stained afterwards. As initial objects of investigation the marker CD29 (127), (36), (83), CD105 (83), and CD73 (127), (28), (36) were chosen, which are known to be associated with the MSC phenotype. Furthermore, antibodies marking CD271 were tested on MSCs.

CD29 is a single chain transmembrane glycoprotein, which is also known as beta-one-integrin, fibronectin receptor beta or gpIIa. The protein is expressed on a majority of hematopoietic and non-hematopoietic cells including leukocytes, fibroblasts, epithelial cells, smooth muscle cells, and endothelial cells. Integrins in general bind to several extracellular matrix and cell surface molecules. CD29 complexes with one of nine integrin alpha subunits and belongs to the *very late antigen* (VLA) subfamily of adhesion molecules. Therefore, it is involved in a variety of cell-cell and cell-matrix interactions such as adhesion, migration, proliferation, cell signaling, proliferation, and angiogenesis (137).

CD105 is a membrane glycoprotein also known as endoglin, which is a receptor for the *transforming growth factors* (TGF) beta one and beta three and modulates their signaling. This receptor is highly expressed on angiogenetic endothelial cells and can be induced by hypoxia (127). Besides, endoglin is involved in cardiovascular development, angiogenesis, and vascular remodeling (156). Mark *et al*. were further able to show that

the transplantation of hMSC with high CD105 expression significantly improved myocardial performance in mice with a post-myocardial infarction status (127).
CD73 is an enzyme also known as five'-ectonucleotidase or NT5E that catalyzes the conversion of *five' adenosine monophosphate* (AMP) to bioactive adenosine at neutral pH. It consists of two identical 70kDa subunits, which are connected to the external part of the plasma membrane by a *glycosylphosphatidylinositol* anchor (GPI-anchor). Being expressed on the surface of endothelial cells, mesenchymal stem cells as well as subsets of lymphocytes the enzyme plays a major role in mediating activation signals. Besides, CD73 regulates cell interaction with components of the extracellular matrix and mediates proliferative and metastatic properties of cancer cells as well as tumor angiogenesis (189). This enzyme promotes endothelial migration and new vessel formation via upregulation of the *vascular endothelial growth factor* (VEGF) expression. Consequently, measures against CD73 impair angiogenesis in tumors (6).

For the culture of human mesenchymal stem cells (hMSCs) the negative fraction obtained after CD133 isolation was used. The basal growth medium MSCBM was combined with trypsin/EDTA and penicillin/streptomycin in order to prevent adverse bacterial growth. The cells were cultivated until the colonies reached confluency. The staining procedures were performed afterwards. Therefore, the antibodies were diluted with EGM-2 and incubated on the cells for half an hour (compare Table 2). Afterwards, the assays were washed with EGM-2. For every marker an isotype control was applied in the same way in order to obtain a negative control. If desired, the staining could be accompanied by Hoechst nucleus staining for improved imaging.

Table 2: Amounts of antibodies applied on 88,000 MSC per well

Antibody	Amount of dye	EGM-2	Dilution ratio
CD29-APC & iso-control	20µl	200µl	1:10
CD271-APC & iso-control	10µl	200µl	1:20
CD73-PE & iso-control	20µl	200µl	1:10
CD105 AF488 & iso-control	10µl	200µl	1:20

On the CD271^{+} and CD133^{+} angiogenesis assays only CD73 antibodies were applied. Therefore, the network assays were cultured as described in Chapter 4.3.2. Afterwards, the CD73 antibodies were diluted with EGM-2 in 1:10 ratio and added to the assays. After half an hour of incubation, the analysis could be performed.

The analysis was performed by means of the Zeiss high- resolution confocal microscope ELYRA S.1 and corresponding software. Z-stack images were used for three dimensional reconstructions.

4.3.4. Gene expression analysis by means of mRNA-PCR

The amount of *messenger ribonucleic acid* (mRNA) within a cell is directly related to the expression rate of the according gen on the *deoxyribonucleic acid* (DNA). Thus, mRNA can be used as molecular evidence whether proteins are expressed and to which extent. Aside from the results gained by surface receptor analysis, we hereby are able to achieve information on two different cellular levels on how the stem cells differentiate in the angiogenesis assays. Yet the amount of mRNA within a cell is too small to measure it quantitatively. The nucleic acid sequences can be multiplied by *polymerase chain reaction* (PCR), which only can be processed on double-strands. Hence, the mRNA has to be reverse-transcripted into *complementary deoxyribonucleic acid* (cDNA) before it can be analyzed in Real-Time PCR.

This leads to multiple steps:

1. cell culture
2. mRNA-isolation
3. reverse-transcription of the mRNA into cDNA
4. quantitative Real-Time PCR

The stem cells were tested for CD271/NGFR expression as well as endothelial and muscular properties. As marker for endothelial differentiation vWF (*von Willebrand factor*) and eNOS (*endothelial nitric oxide synthase*) were chosen. Furthermore, α-SMA also referred to as ACTA (*alpha smooth muscle actin*) served as marker for muscular parts. Aside from the stem cells in question, standard curves for each marker had to be established with positive control groups in order to classify the data. Therefore, *human umbilical vein endothelial cells* (HUVECs) in networks served as endothelial cells. The material for muscular reference was isolated from an atrial auricle of a human heart. Hypotonic preconditioned MSCs were used as additional control group for CD271/ NGFR expression.

Cell culture

The *human umbilical vein endothelial cells* (HUVECs) were isolated from the umbilical cord by staff members of the laboratory and stored in liquid nitrogen. The thawed cells were suspended in EGM-2 medium and seeded into culture flask. After several days the cells have grown sufficiently for splitting, which is the case if they achieve approximately

90% confluence. For the splitting the medium was discarded from the flask, thoroughly rinsed with PBS, and supplemented by Trypsin/EDTA. Afterwards, the cells were resuspended in EGM-2. The cell culture was established using Matrigel and EGM-2 in order to sustain the same conditions as in the angiogenesis assays and thus to make the different assays as comparable as possible. Therefore, the bottom of each well in a twelve-well-plate was covered with 200µl Matrigel. After curing, the assays were completed by 70,000 HUVECs in 1ml EGM-2 medium each. After three to four days of growing TRIzol was added to the dense networks and the RNA Isolation was carried out.

Humane mesenchymal stem cells (hMSCs) were obtained by culturing the negative fraction from bone marrow isolation in *mesenchymal stem cell growth medium* (MSCGM). In these assays only directly cultured MSCs were used, which have not been cryopreserved before. For the purpose of generating hypoosmolar conditions, a mixture of 60% MSCGM and 40% sterile water was established. In the wells of a six-well-plate 50,000 cells were seeded with 2ml hypotonic MSCGM and 200µl Matrigel. After 24 hours of incubation at 37°C, TRIzol was added to the cells and RNA isolation was carried out.

Parts of human heart atrial auricle were used as muscular tissue samples. These were received during heart surgery, in which the atrial auricles are sometimes removed in order to reduce thromboembolic risk as the blood flow through these structures tends to build blood clots. The samples were shock frozen with liquid nitrogen and ground into powder with a mortar. The tissue must not thaw during the process. Afterwards TRIzol supplementation and RNA isolation was performed.

For the genetic survey the same stem cell angiogenesis assays were used as referred to in Chapter 4.3.2. Thus, the analysis was carried out on CD271$^+$, CD133$^+$, and CD271$^+$/CD133$^+$ stem cell assays. At this, 1ml TRIzol was added to the wells and the RNA isolation was subsequently performed.

RNA-isolation

TRIzol® reagent by Life Technologies Corporation was designed for high quality RNA isolation and can be applied on cells and tissue samples. The RNA isolation was performed according to the product protocol. In order to determine the RNA concentration, the absorbance (A) at 260nm and 280nm was measured by Nanodrop 1000 spectrometry and substituted into the following formula:

$$A_{260} \text{ x dilution x } 40 = \text{RNA-concentration } [\mu\text{g RNA/ml}].$$

The RNA samples were stored at -80°C afterwards. The isolation was performed equally on all cell and tissue samples.

Reverse transcription of mRNA into cDNA

In order to obtain single-stranded coding DNA sequences from total mRNA-molecules the retroviral enzyme *reverse transcriptase* was used. For the transcription the "High Capacity cDNA Reverse Transcription Kit" by Applied Biosystems was used.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Polymerase chain reactions serve the *in vitro* amplification of specific DNA-sequences. In this study, the TaqMan™ assay by Applied Biosystems was used. As markers for endothelial differentiation vWF (*von Willebrand factor*) and eNOS (*endothelial nitric oxide synthase*) were chosen. Furthermore, α-SMA also referred to as ACTA (*alpha smooth muscle actin*) is the predominant microfilament in smooth muscles such as vascular walls and therefore is used as indicator for muscular differentiation in this study. For the CD271$^+$, CD133$^+$, and CD271$^+$/CD133$^+$ stem cell network analysis constant amounts of 40ng cDNA were used for each primer. The expression of the house keeping gene *ribosomal protein, large, P0* (RPLP0) was used as endogenous control on each cell type. In the positive control assays increasing cDNA amounts from 5ng to 160ng were tested (compare Table 3).

Table 3: PCR primer and positive controls

PCR primer	positive control group	amount of cDNA
eNOS	HUVEC	5ng, 10ng, 20ng, 40ng, 80ng, 160ng
vWF	HUVEC	5ng, 10ng, 20ng, 40ng, 80ng, 160ng
NGFR	hypotonic MSC	5ng, 10ng, 20ng, 40ng, 80ng, 160ng
α –SMA/ ACTA	atrial auricle	5ng, 10ng, 20ng, 40ng, 80ng, 160ng

PCR - analysis

The assessment was performed according to the instructions by Applied Biosystems. The measured fluorescence of the sample and the intensity of the background fluorescence given by the no-template-control (NTC) are set in relation to the emission intensity of the passive reference. The difference between both is defined as ΔR_n. At this, the *passive reference* is a dye that is added to the mastermix and is used to normalize the signal. It corrects fluctuations of the fluorescence due to different concentrations of the mastermix and adapts the signal to the respective conditions within each tube. The *no-template-*

control NTC should not show any increase of fluorescence intensity during the amplification.

$$\Delta R\mathrm{n} = \frac{\text{reporter emission intensity (sample)}}{\text{passive reference intensity (sample)}} - \frac{\text{reporter emission intensity (NTC)}}{\text{passive reference intensity (NTC)}}$$

The *threshold cycle C_t* is defined as the first cycle, in which the fluorescent signal can be detected within the background fluorescence. Thus, the threshold cycle indicates how many templates are contained within the sample. The later the threshold is reached, the less amplicon was initially in the sample. The endogenous control RPLP0 is a reference gene, which is always expressed to the same extent and thus represents the quantity of the DNA sample. Quantification based on the endogenous control allows comparing the C_T value without any standard curve. It indicates whether varying signals are due to different expression intensities of the amplicon or whether the amount of cDNA in the sample was minor to others. Furthermore, the reference gene is used to confirm that the PCR process was performed correctly. If a sample does not express a signal for the tested primer, but neither for the reference gene, these results must not be taken into account. Therefore, ΔC_T is the difference between the threshold cycles of the analyzed gene and of the endogenous control (RPLP0).

$$\Delta C_T = C_{T\,(\text{analyzed gene})} - C_{T\,(\text{endogenous control})}$$

Furthermore, this variance has to be related to the numerical value of the calibrator. Thus, $\Delta\Delta C_T$ is the difference between ΔC_T of the sample and ΔC_T given by the calibrator. In this analysis the ΔC_t for calibration was given by the positive control groups.

$$\Delta\Delta C_T = \Delta C_{T\,(\text{analyzed gene})} - \Delta C_{T\,(\text{calibrator})}$$

With the term $RQ = 2^{-\Delta\Delta Ct}$ the relative gene expression can be determined.

For the ΔΔCT calculation the efficiency of the target amplification and the efficiency of the reference must be approximately equal. At this, the efficiency is the rate at which the amplification takes place, commonly expressed as a percentage value. Ideally, the PCR amplicon doubles in quantity during each cycle of the PCR, which corresponds to 100% efficiency. The word target refers to the gene of interest whereas the reference is represented by the endogenous control (RPLP0). Furthermore, the calibrator is the sample that is used as the basis and thus allows comparison of expression results. In order to analyze the relative efficiencies of the target and reference amplifications, standard curves are assessed for all target genes.

First, ΔC_T is the difference between the threshold cycles of the analyzed gene and of the endogenous control (RPLP0).

$$\Delta C_T = C_{T\ (\text{analyzed gene})} - C_{T\ (\text{endogenous control})}$$

For the establishment of standard curves the ΔC_T values are plotted versus the logarithmic values of the input amount of DNA in order to create a semi-logarithmic regression line. The slope of this resulting line can be used as a general criterion for passing the validation. According to Applied Biosystems the absolute value of the slope has to be less than 0.1 to pass the validation. This can be confirmed for all standard curves in this study (Figures 14-17). The negative controls did not show any activity.

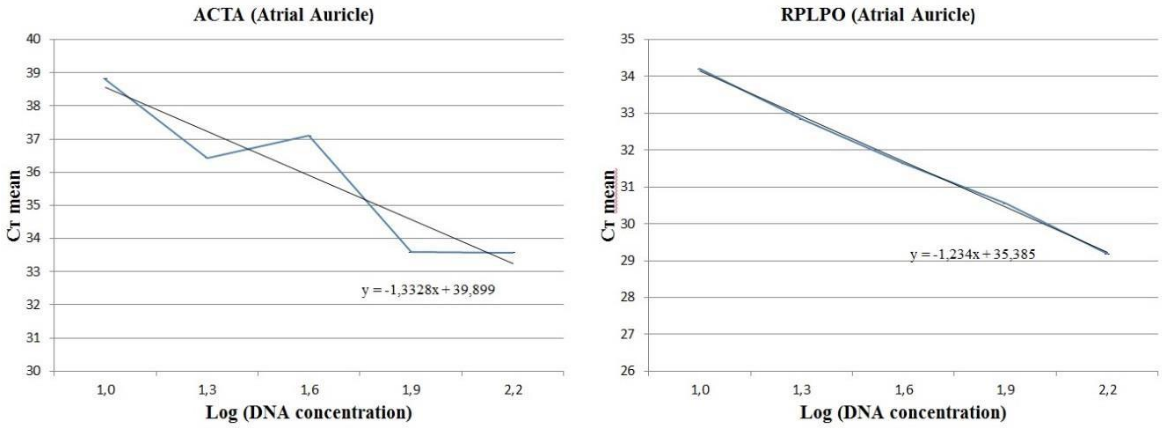


Figure 14: Standard curve of ACTA (left) and RPLP0 (right) on cells of the human atrial auricle.

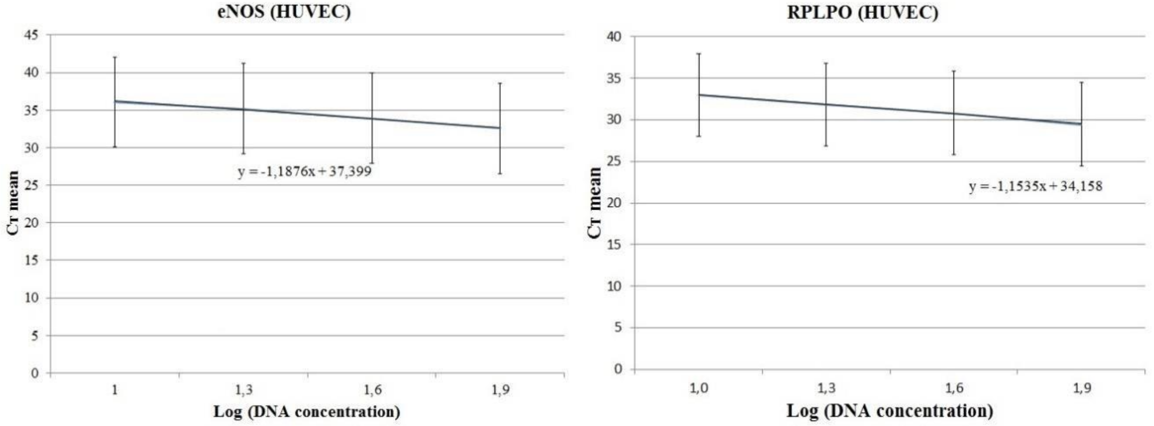


Figure 15: Standard curve of eNOS (left) and RPLP0 (right) on HUVECs.

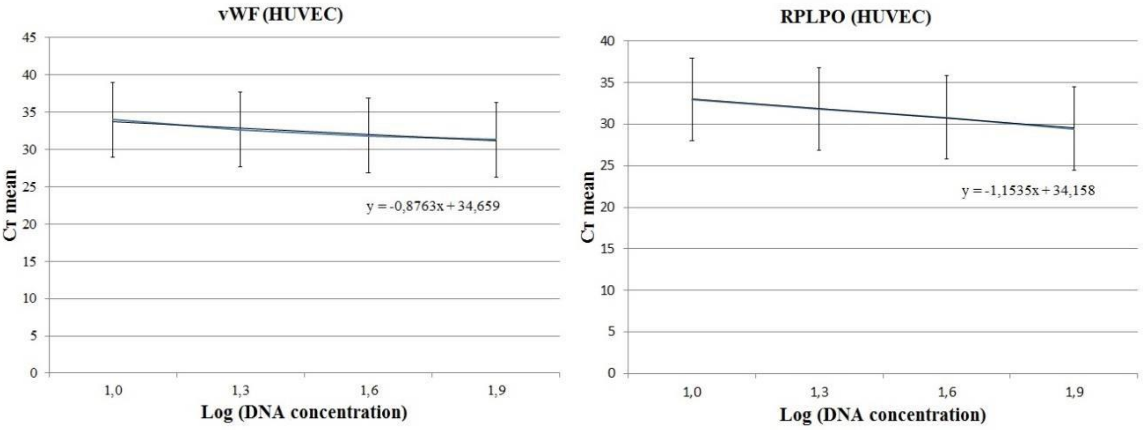


Figure 16: Standard curve of vWF (left) and RPLP0 on HUVECs.

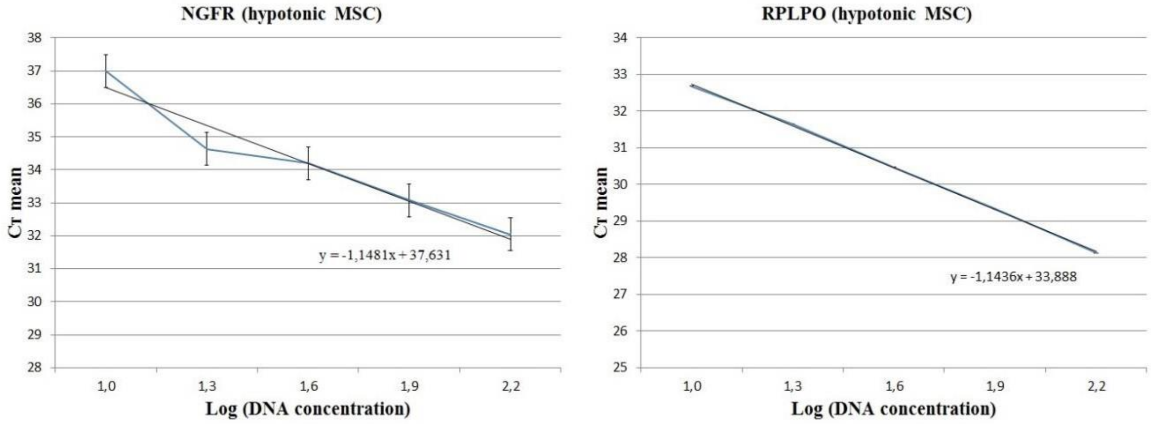


Figure 17: Standard curve of NGFR (left) and RPLP0 (right) on hypotonic preconditioned MSCs.

The validation curves demonstrate that the efficiency of the target amplification and the efficiency of the reference control gene amplification are sufficient. Therefore, the $\Delta\Delta C_T$ method can be used to calculate fold-differences in target gens between different samples.

4.4 *In vivo* stem cell characterization

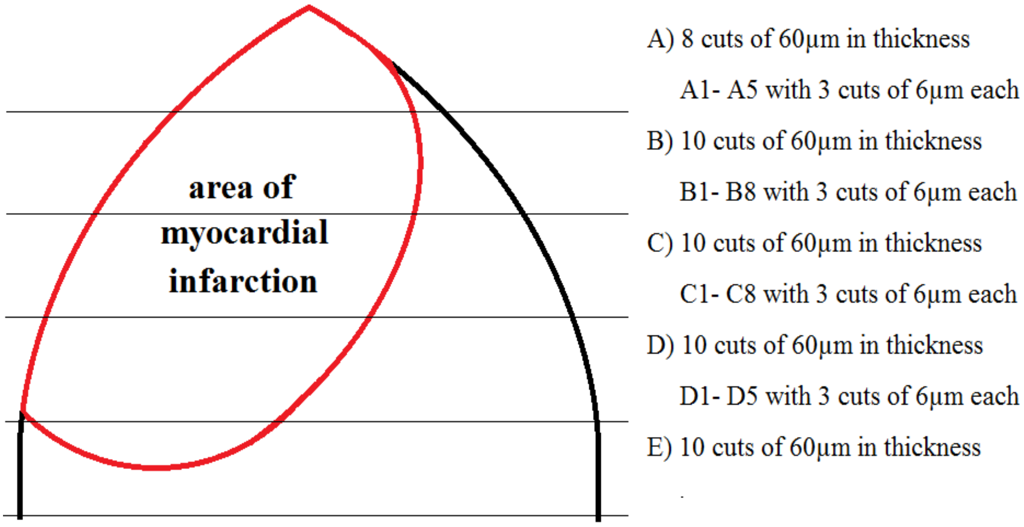
4.4.1. The myocardial ischemia mouse model

In our research group, SCID-mice (SCID *beige*; strain CB17.Cg-*Prkdc*^{scid}*Lyst*$^{bg\text{-}J}$/Crl, female, 22 ± 2 g, Charles River) were used for an induced myocardial ischemia model. SCID is an acronym for *severe combined immunodeficiency*, which is a genetic disorder affecting T- and B-lymphocyte development. These mice are not able to sustain an appropriate immune response and therefore provide a wide tolerance to the application of human cells. Other than this immunodeficiency, SCID- mice do not differ in any respect from normal mice. The experimental animals were kept with a twelve-hour day/ night cycle and were fed a sterile standard diet for rodents with free access to water.

Myocardial ischemia induced by LAD-ligation is a well-established model used in different studies all over the world. This surgical technique is highly reproducible and delivers a constant and comparable outcome. The intervention of LAD-ligation was performed by Dr. Ralf Gäbel, who has expertise in experimental surgery after the guidelines of the Federation of European Laboratory Animal Science Associations (FELASA). The surgical interventions were carried out under general anesthesia and mechanical ventilation with the Mouse Ventilator MINIVENT Type 845 Hugo Sachs Elektronik Harvard Apparatus GmbH. For this purpose, Pentobarbital (50mg/kg bodyweight) intraperitoneal was used as anesthetic whereby Fentanyl also provided a strong analgesic effect. The surgery was initiated by a left- lateral thoracotomy and a pericardial incision. Afterwards, the temporary ligation of the *left anterior descendent artery* (LAD) was accomplished. Thereby, the blood supply to the corresponding area of the myocardium was interrupted, starving the cells of nutrition and oxygen. After 45 minutes each mouse received an intramyocardial cell injection. The CD271^{+} as well as the CD133^{+} stem cells were obtained from human bone marrow and isolated by means of manual immuno-magnetic cell separation with antibodies as described above (Chapter 4.1). For the cell treatment, 10^{5} stem cells were suspended in 10µl PBS containing 0.5% BSA and 2mM EDTA and mixed with an equal amount of Matrigel^{TM}. The injections of four times 5µl were given along the border of the blanched myocardium. Subsequently, the ligation was removed and as a result, a reperfused myocardial infarction was initiated. In order to ensure that the animals suffer as little pain as possible, analgesics were used the day before the surgery as well as the day after. The same experimental set-up was applied to the control groups. The *myocardial infarction control*, also referred to as MIC, correspondingly received a left-lateral thoracotomy, a pericardial incision, and a ligation of the left anterior descendent artery (LAD). Since stem cell application was supposed to be the only difference between the test and control group, the MIC mice also received injections into the myocardial muscle using cell suspension buffer and Matrigel^{TM} for application. The control referred to as *SHAM* group was used as healthy control. In order to create comparable conditions this group underwent identical surgical procedures without LAD-ligation.

Explanted hearts

48 hours after the surgical intervention all mice received terminating applications of 5% potassium chloride, which arrests the heart in diastole. The hearts were explanted, embedded in O.C.T.™ Compound Tissue-Tek®, and snap-frozen in liquid nitrogen. Using the Leica CM1900 cryostat the infarction area of the heart tissue was divided into five horizontal levels A to E from apex to basis (compare Figure 18). Within each section a given amount of fine-cuts were produced for potential future microscopic analysis. On the other hand, the interlayer material was collected and used for subsequent RNA isolation (Chapter 4.4.2.).



A) 8 cuts of 60µm in thickness
A1- A5 with 3 cuts of 6µm each
B) 10 cuts of 60µm in thickness
B1- B8 with 3 cuts of 6µm each
C) 10 cuts of 60µm in thickness
C1- C8 with 3 cuts of 6µm each
D) 10 cuts of 60µm in thickness
D1- D5 with 3 cuts of 6µm each
E) 10 cuts of 60µm in thickness

Figure 18: Cryostat sections of the heart tissue. Horizontal levels A to E with rough cuts for RNA isolation and fine cuts for future microscope analysis.

4.4.2. Gene expression analysis by means of mRNA-PCR

The 60µm interlayers of the cryosectioned hearts were used for RNA-isolation, which was performed with TRIzol® Reagent by Life Technologies Corporation (compare Chapter 4.3.4.). Therefore, the samples were kept cold in liquid nitrogen and ground into fine powder with a mortar. The tissue must not thaw until it achieves this consistency.

The reverse transcription as well as the polymerase chain reaction were performed using the *RT² Profiler PCR Array Mouse Angiogenesis®* kit by Qiagen. These arrays are designed to analyze a large quantity of genes in the same sample. Therefore, the PCR principle is combined with the properties of microarrays in order to analyze many genes simultaneously. In these arrays the *RT^2 SYBR Green ROX qPCR Mastermix* by Qiagen was used.

The array contains several controls in order to guarantee high quality results and to evaluate the consistency between wells, plates, and samples. For the gene expression analysis each C_T value has to be normalized by means of the *reference genes* (RefG). Therefore, the ΔC_T for each *gene of interest* (GOI) has to be calculated with the following term: $\Delta C_T = C_T^{GOI} - C_T^{AVG\ RefG}$

In order to compare each gene across the different test groups the $\Delta\Delta C_T$ has to be calculated according to the following term:

$$\Delta\Delta C_T = C_T^{\text{experimental sample}} - C_T^{\text{control sample}}$$

5 Results

The statistical assessment was performed using the SigmaPlot 11.0® (Systat Software Inc., USA). All results are presented as mean values with standard deviations and are further displayed as bar charts. The statistical significance between different test groups was calculated using the *one-way analysis of variance* (ANOVA) method. Post hoc tests and pair-wise multiple comparisons were performed with the method of Holm-Sidak. In doing so, probability values p of < 0.05 were considered to be statistically significant.

The data taken into account for Chapter 5.1 and 5.2 was not only collected by me, but also includes cell numbers and quality data of isolations performed by other members of the laboratory, which have been used for other projects.

5.1 Manual Immuno-Magnetic Cell Separation (MACS) of human CD271$^+$ and CD133$^+$ stem cells from bone marrow

First of all, the question was how many stem cells can be isolated by the MACS procedure and whether or not the amount of cells is sufficient for therapeutic approaches. The average volume achieved by bone marrow aspiration was around 53.8ml. By means of manual immuno-magnetic cell separation 123.2 million mononuclear cells (MNCs) were isolated on average. From these a mean of 0.29 x 10^6 viable CD271$^+$ cells and 0.42 x 10^6 viable CD133$^+$ were separated (Figure 19).

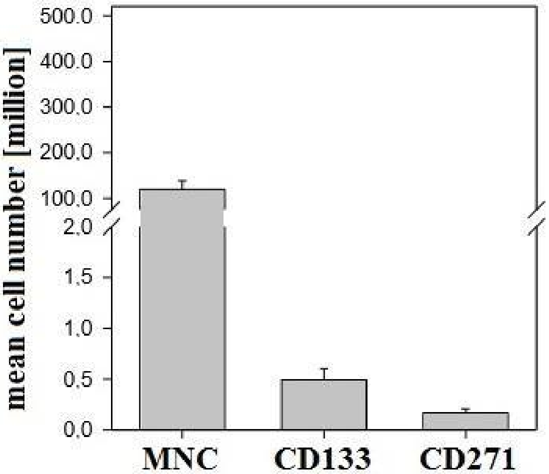


Figure 19: Mean cell numbers of MNCs, CD133$^+$, and CD271$^+$ cells achieved by MACS isolation from bone marrow. n = 45.

5.2 Quality Management by Fluorescence-Activated Cell Sorting

In order to guarantee a safe cell product for clinical application, fluorescence-activated cell sorting was used in order to investigate the quality of the isolated stem cells and to further characterize them. CD133$^+$ as well as CD271$^+$ cells showed high purity and viability after isolation. At this, CD133$^+$ cells showed a mean purity of 81.7 ± 1.8% and mean viability of 91.8 ± 1.3%. For CD271$^+$ cells a purity of 72.4 ± 3.4% with a mean viability of 81.6 ± 3.3% has been achieved (Figure 20).

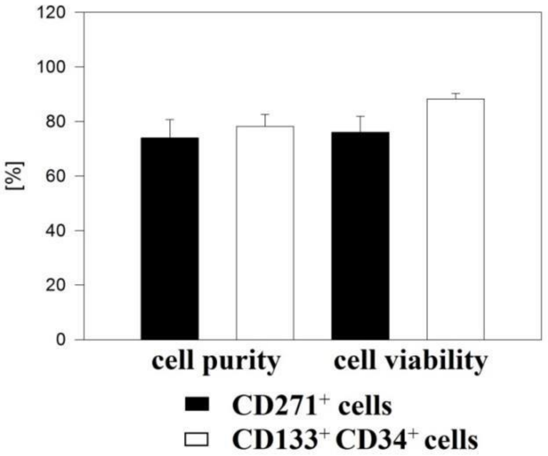


Figure 20: Purity and viability of CD271$^+$ and CD133$^+$ CD34$^+$ cells after MACS isolation.

Furthermore, analysis revealed that the amount of CD271$^+$ cells shows a linear dependence on the initial amount of MNCs that has been used for isolation (Figure 21).

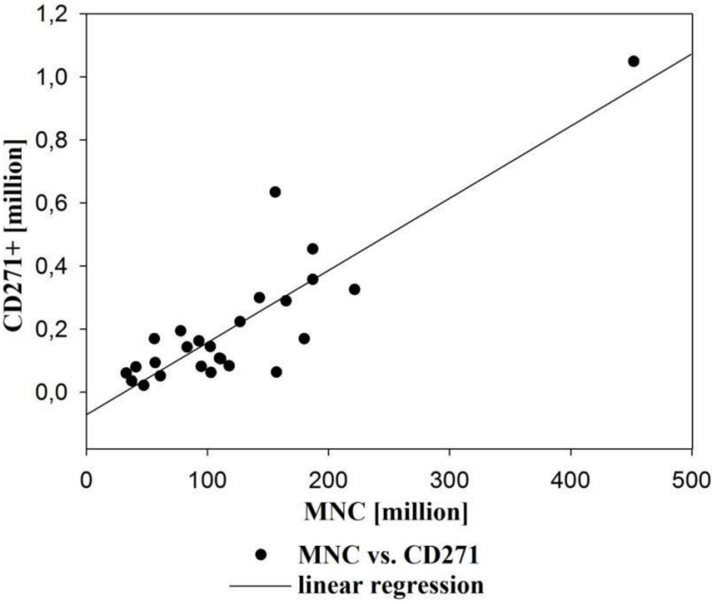


Figure 21: Dependence of CD271 positive cell number on initial amount of MNCs.
Linear regression with R = 0.871. n = 26.

Furthermore, it has been examined if CD133$^+$ and CD271$^+$ cells form separate populations in bone marrow or if the surface proteins are also expressed simultaneously by an overlap population, which is an important aspect in order to evaluate the feasibility of a combined therapeutic approach. As demonstrated below (Figure 22), 0.04 ± 0.01% of bone marrow mononuclear cells (MNCs) expressed both CD133 and CD271. This subpopulation represents 2.21 ± 0.43% of total CD133$^+$ stem cells and 4.65 ± 1.68% of total CD271$^+$ stem cells.

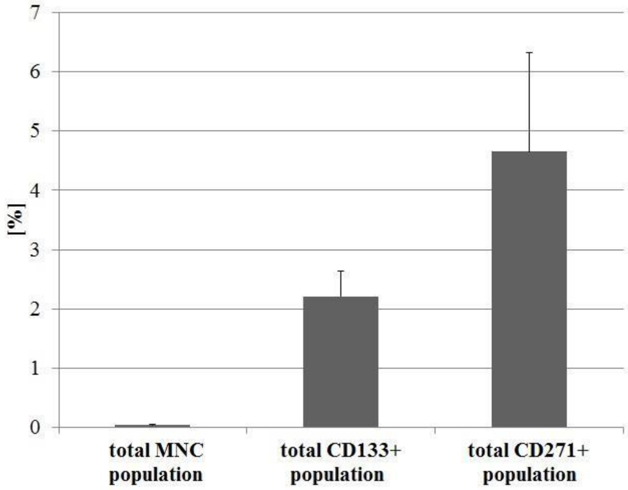


Figure 22: Percentage shares of CD271+ CD133+ overlap cells to different populations. n = 11.

Furthermore, 1.14 ± 0.50% of the isolated CD133$^+$ population were positive for CD271, and 3.00 ± 0.99% of the isolated CD271$^+$ cells were also positive for CD133 (Figure 23).

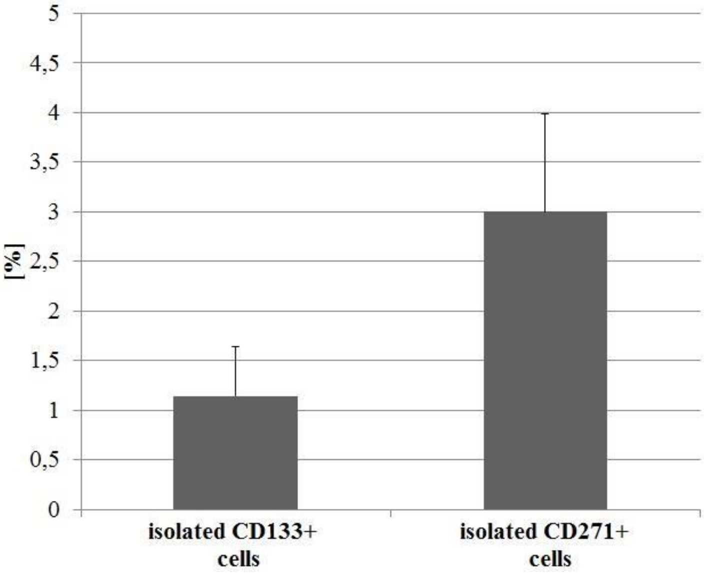


Figure 23: Percentage shares of CD271$^+$ CD133$^+$ overlap population to isolated populations. n = 11.

In order to optimize the procedure of cell isolation it had to be determined which impact the dual isolation strategy has on the cell product, which population should be isolated first or whether the order of isolation does not influence the outcome. Therefore, the CD133$^+$ population was isolated from the CD271$^-$ fraction as well as the other way around

(Figure 24). Thus, the quality of the cell populations resulting from the isolation was compared with each other.

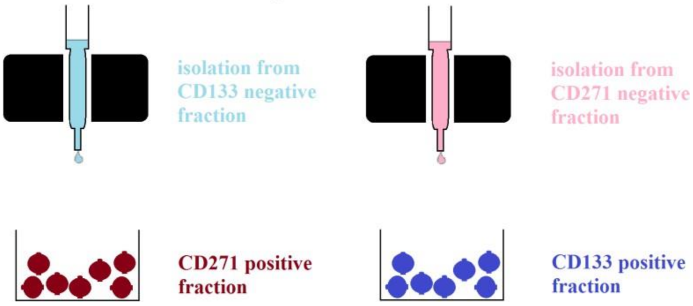


Figure 24: Dual Isolation strategy.

At this, no indication that the order of isolation has a negative or significant influence on the quality or cell number was detected (Table 4).

Additionally, the influence of fresh samples versus storage was examined. The bone marrow samples reach the laboratory depending on the time the heart surgery is performed, which cannot always be predicted precisely. Considering the fact that one single MACS-isolation takes around five hours, the possibility of sample storage would make experimental work much easier and effective. Furthermore, it is unavoidable that the cells undergo storage for at least several hours due to processing and transportation. This applies for storage of bone marrow as well as for negative fractions.

No reference was found that overnight storage of the initial material at 4°C has any significant negative influence on numbers or quality of the cells resulting from the isolation (Table 4).

Table 4: Influence of storage (4°C) and order of isolation during the dual isolation of CD271$^+$/ CD133$^+$ cells on cell numbers and quality.

Isolation / storage	**Cell number CD271**		**Purity CD271**		**Viability CD271**		**Cell number CD133**		**Purity CD133**		**Viability CD133**	
	mean	n	mean	n	mean	n	mean	n	mean	n	mean	n
Single isolation fresh BM	0.112	8	76.435	7	58.753	7	0.299	19	83.422	10	94.123	10
Storage BM	0.482	4	82.148	4	89.509	4	0.506	8	85.401	6	93.272	6
Storage CD133$^-$	0.203	4	47.500	2	77.290	2	0.338	5	73.213	3	82.895	3
Storage CD271$^-$	0.170	7	88.430	6	89.824	6	0.137	7	88.292	3	96.182	3
p	0.321		0.088		0.239		0.393		0.306		0.296	

Additionally, several surface markers were investigated to further characterize the stem cell populations. First, the surface markers CD73, CD105, and CD44 were investigated on the freshly isolated CD271$^+$ population (Figure 25). At this, CD73 was significantly more expressed in the CD271$^+$ population (9.2 ± 2.8%) compared to the whole bone marrow (0.6 ± 0.2%). Likewise, 8.3 ± 2.8% of CD271$^+$ cells expressed CD105, which was found in only 0.5 ± 0.2% of bone marrow cells. The expression of CD44 did not show any significant difference. The co-expression of two of three markers was also significantly more frequent in the CD271$^+$ population than in the whole bone marrow. However, only a small fraction of these CD271$^+$ cells co-expressed all three markers (1.2 ± 0.3% CD271$^+$ CD44$^+$ CD73$^+$ CD105$^+$).

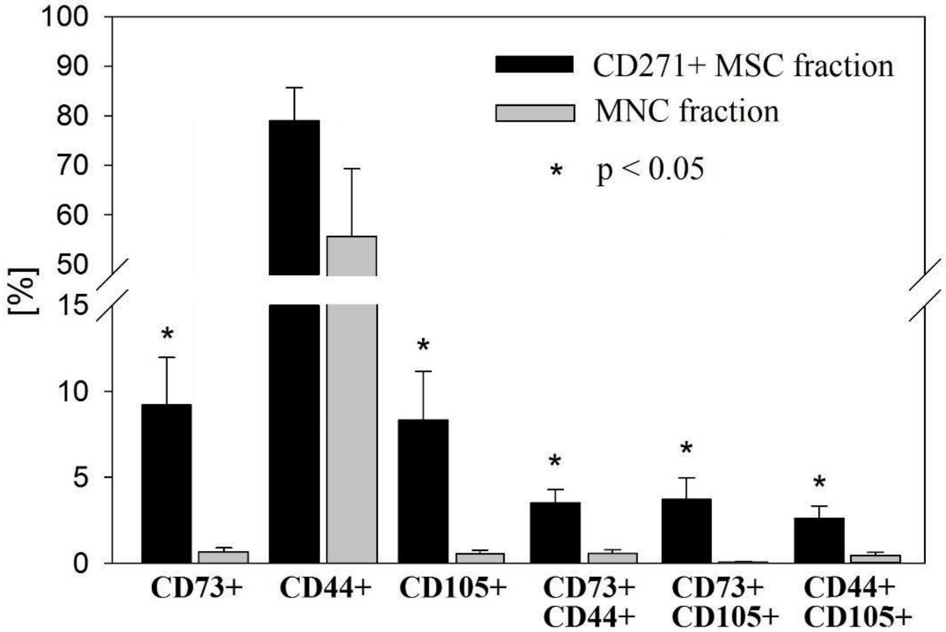


Figure 25: Expression of the surface markers CD73, CD44, and CD105 in the CD271$^+$ population compared to the whole bone marrow. n = 6.

Besides, freshly isolated CD271$^+$ cells showed a mixed expression of the pan-leukocyte marker CD45 although cultivated MSCs do not (Figure 26). In the CD271$^+$ cell population, CD45^{dim} held the biggest share with 67.1 ± 5.6% whereas CD45^{bright} represented 11.5 ± 3.8% and CD45$^-$ cells had a percentage of 8.1 ± 2.0%.

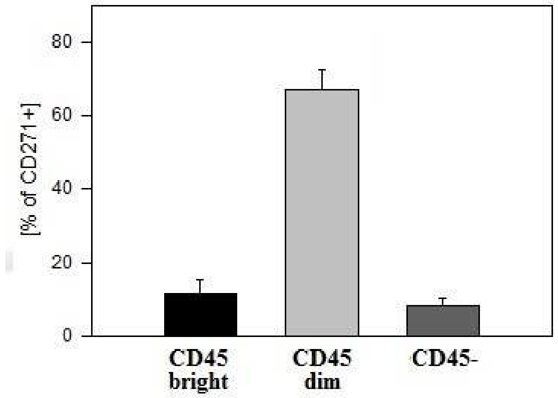


Figure 26: CD45 expression on freshly isolated CD271$^+$ cells.

5.3 *In vitro* Stem Cell Characterization

5.3.1. Colony-forming assays (CFU-F)

The stem cells were investigated concerning proliferative properties and their tendency to form colonies termed *colony forming units-fibroblasts* (CFU-F) after being cultivated over a defined period of time (Figure 27). For mononuclear cells a mean CFU-F formation of 10.7 ± 4.0 colonies per million cells was detected. Besides, this formation was further enriched by CD271$^+$ purification. For purified cells a mean CFU-F formation of 375.0 ± 93.6 colonies per million CD271$^+$ cells was determined. On the other hand, CD271$^-$ cells – including CD133$^+$ fractions - did not show any colony formation.

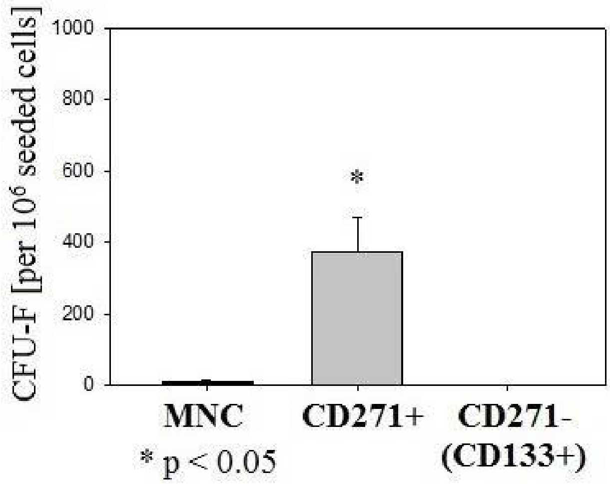


Figure 27: CFU-F formation in MNCs, CD271$^+$, and CD271$^-$ cells. n = 8.

5.3.2. Angiogenesis assays (3D-microscopy Analysis)

In order to investigate to what extend the isolated fractions of CD133$^+$ and CD271$^+$ cells build networks of endothelial cells, cultivation in special growth matrices was used. The angiogenesis assays were analyzed according to the criteria of tube length and number of network nodes (Figure 28). At this, each patient's CD271$^+$ and CD133$^+$ cells were seeded separately as well as in a combined assay. The CD271$^+$ cells formed well-defined, three dimensional networks in Matrigel, whereas CD133$^+$ cells only built minimal networks in three out of six experiments at all. The dual assays consisting of CD271$^+$ and CD133$^+$ cells also showed well-defined, three dimensional networks. CD271$^+$ cells generated networks with mean lengths of 81.20 ± 86.02mm, combined CD271$^+$/CD133$^+$ assays formed networks with 60.35 ± 44.81mm in length, and CD133$^+$ cells only build 1.5 ± 4.74mm of network structure. Thus, in terms of tube length CD271$^+$ and CD271$^+$/CD133$^+$ assays were both significant ($p < 0.001$) compared to CD133$^+$.

Moreover, networks formed by CD271$^+$/CD133$^+$ cells contained the highest number of nodal points (mean 893.2 ± 838.4), which is significant ($p < 0.001$) compared to CD133$^+$ cells alone. Likewise, the amount of nodal points built in CD271$^+$ networks was also significant ($p = 0.04$) compared to CD133$^+$ (with 639.9 ± 901 nodal points for CD271$^+$ and 18.1 ± 75.4 for CD133$^+$ cells, respectively).

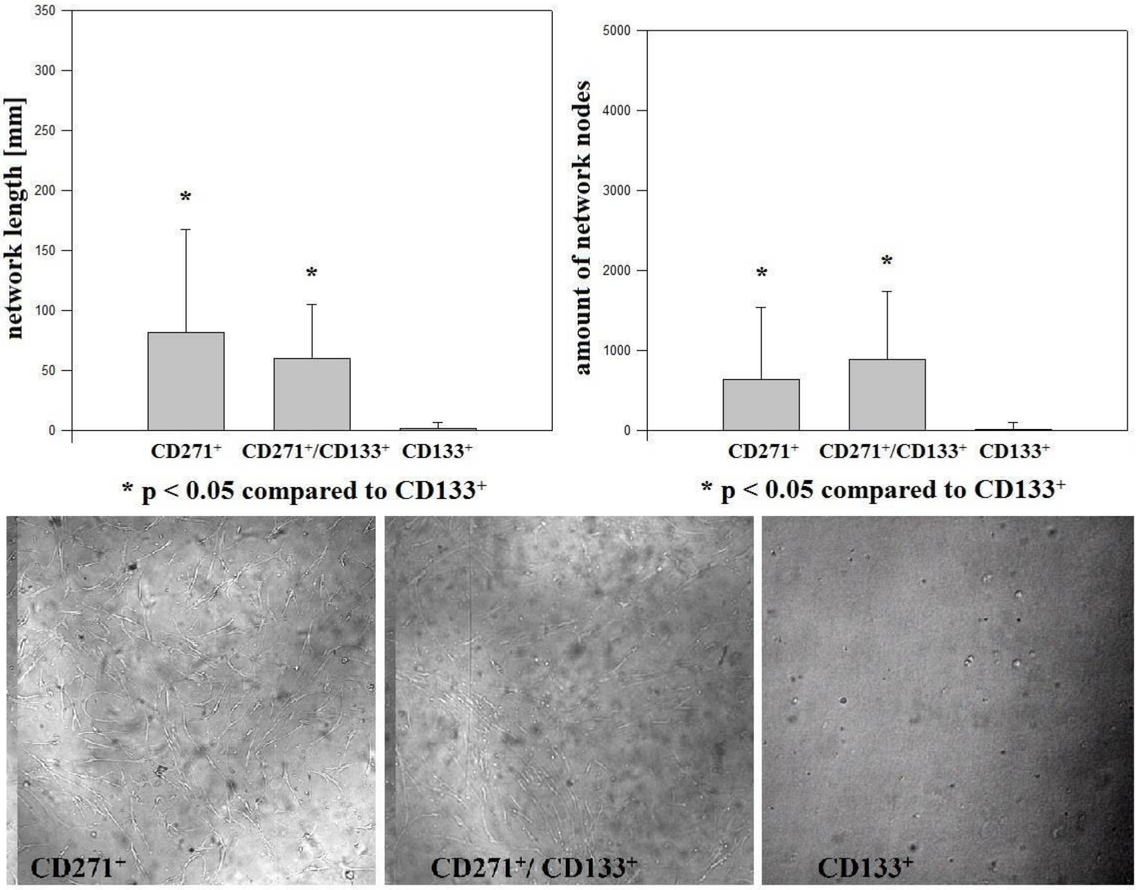


Figure 28: Network length (left) and network nodes (right) in Matrigel angiogenesis assays with CD271$^+$, CD271$^+$/ CD133$^+$, and CD133$^+$ cells. n = 6. Below exemplary images of the networks (10x magnification).

5.3.3. Identification of stem cell phenotype in vitro

In order to further define the cell networks accomplished in Matrigel, immunochemistry was performed on the assays. The CD133$^+$ and CD271$^+$ cells have been specifically stained with CFDA and PKH 26 within the angiogenesis assays. At this, both fluorescent dyes can be detected within the network structures (Figure 29).

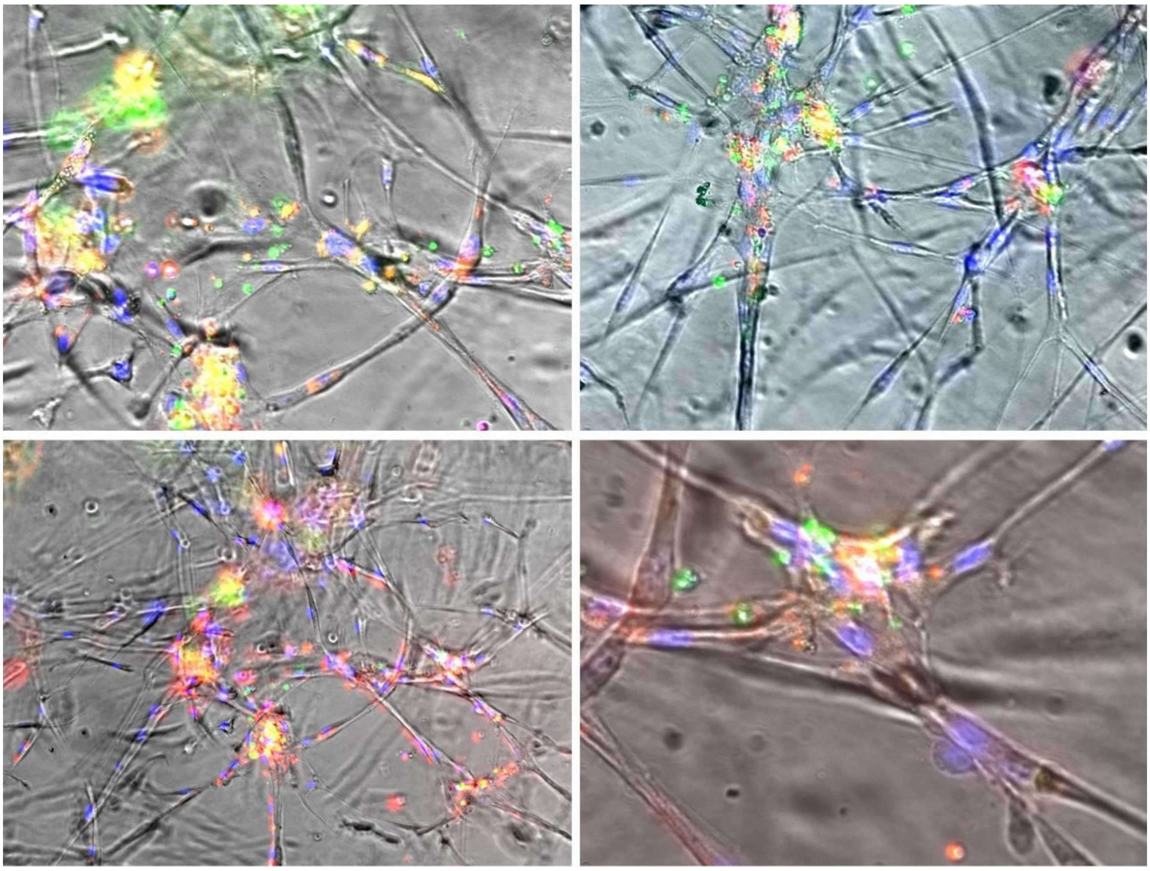


Figure 29: Exemplary images of CD271$^+$/ CD133$^+$ dual angiogenesis assay with 80,000 CFDA stained CD133$^+$ cells (green) and 20,000 PKH26 stained CD271$^+$ cells (red) with additional Hoechst nuclear stain (blue). Top left and right 20x magnification, lower left 10x magnification, lower right 40x magnification.

As another illustration, only CFDA fluorescent signal of CD133$^+$ cells is shown in Figure 30, which shows that the signal really comes from within the network sturctures.

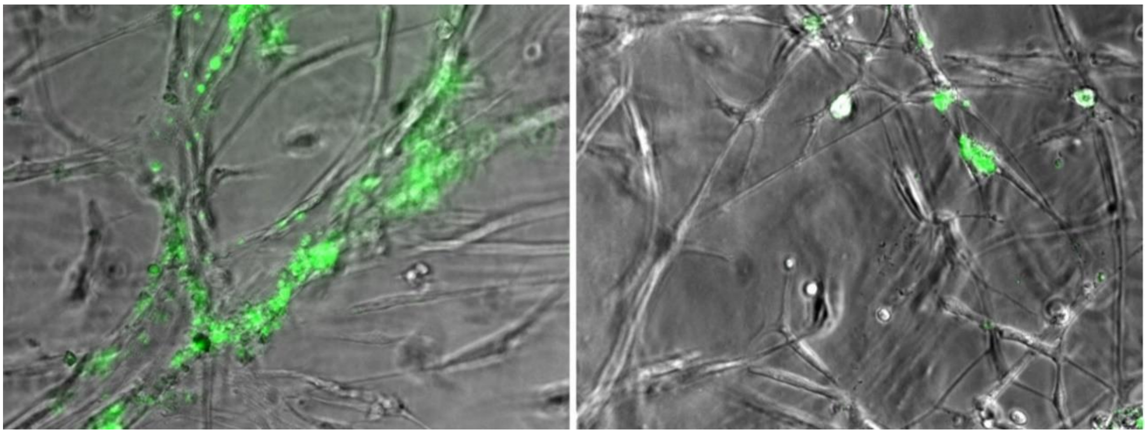


Figure 30: CD271$^+$/ CD133$^+$ dual angiogenesis assay with 80,000 CFDA stained CD133$^+$ cells (green) and 20,000 CD271$^+$ cells (40x magnification).

In order to establish a method that further characterizes the cell networks and the stem cells contained within, staining of the surface marker CD29, CD105, CD73, and CD271 was performed. At this, the MSCs were positive for CD73-PE and the staining was specific. The performed staining with the isotype was negative and serves as a satisfactory control. The same applies to the staining with CD29-APC (compare exemplary reconstruction Figure 31). The mesenchymal cells were positive for this marker and the isotype control was negative. Conversely, the staining with CD271-APC and its isotype control were both negative. Furthermore, the staining with CD105-AF 488 was positive on MSCs, but the isotype control was not very specific. Thus, this marker always requires a control group in order to get reliable results.

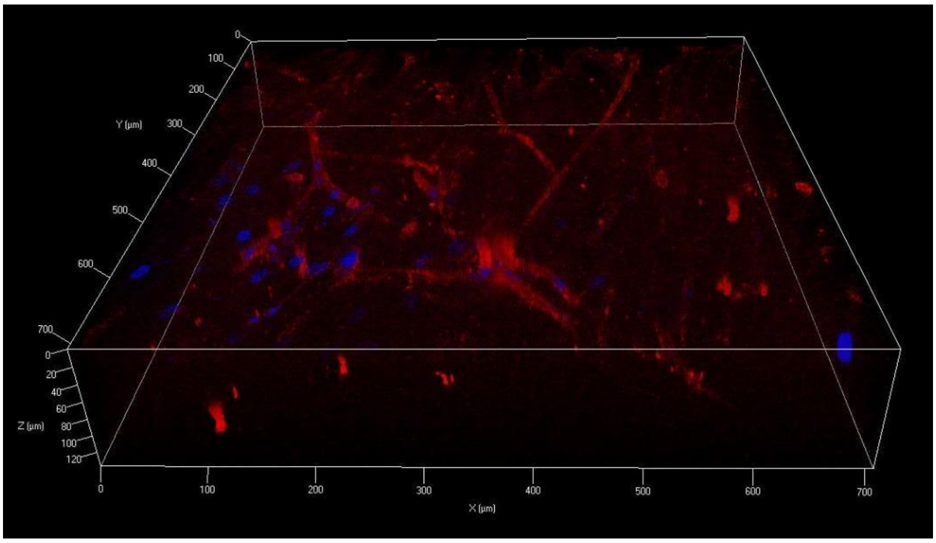


Figure 31: Three dimensional reconstruction of a MSC network stained with CD29 APC (red) and Hoechst (blue).

Based on these results, CD73-PE antibodies were tested on the CD271$^+$ and CD133$^+$ angiogenesis assays. At this, the staining was specific and limited to the network structures. CD271$^+$ (Figure 32) and dual CD271$^+$/ CD133$^+$ networks (Figure 33) showed CD73-PE expression. In those assays in which no networks were built – like in most of the assays with CD133$^+$ cells alone - no fluorescent signal of CD73PE was detected. In those rare assays where CD133$^+$ cells built network structures, the fluorescence was also found within the cellular extensions.

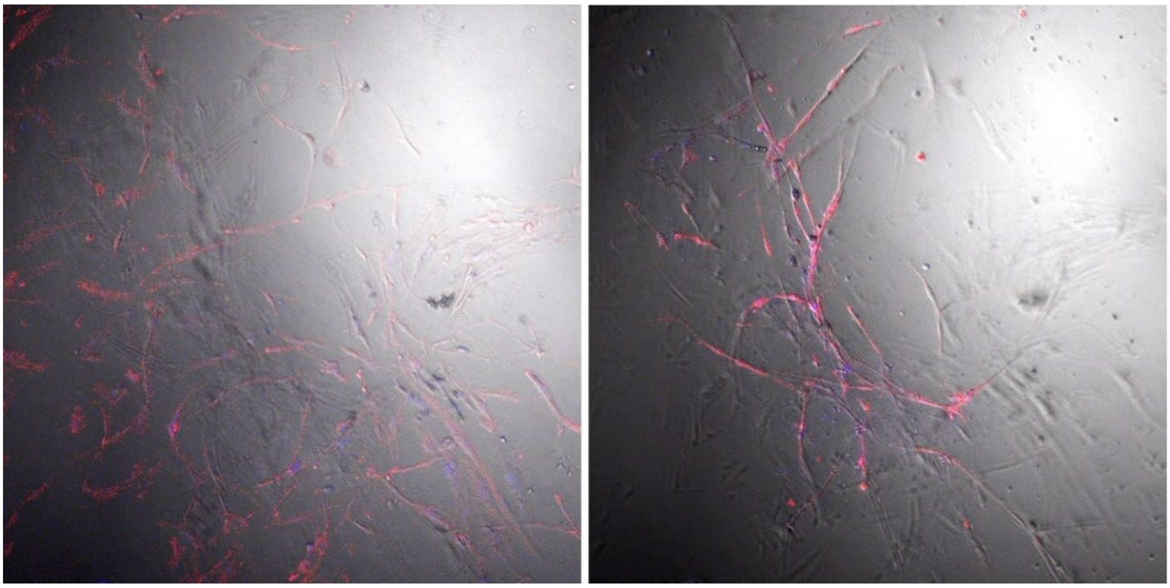


Figure 32: CD271$^+$ stem cell angiogenesis assays stained with CD73PE (red) and Hoechst (blue). (10x magnification).

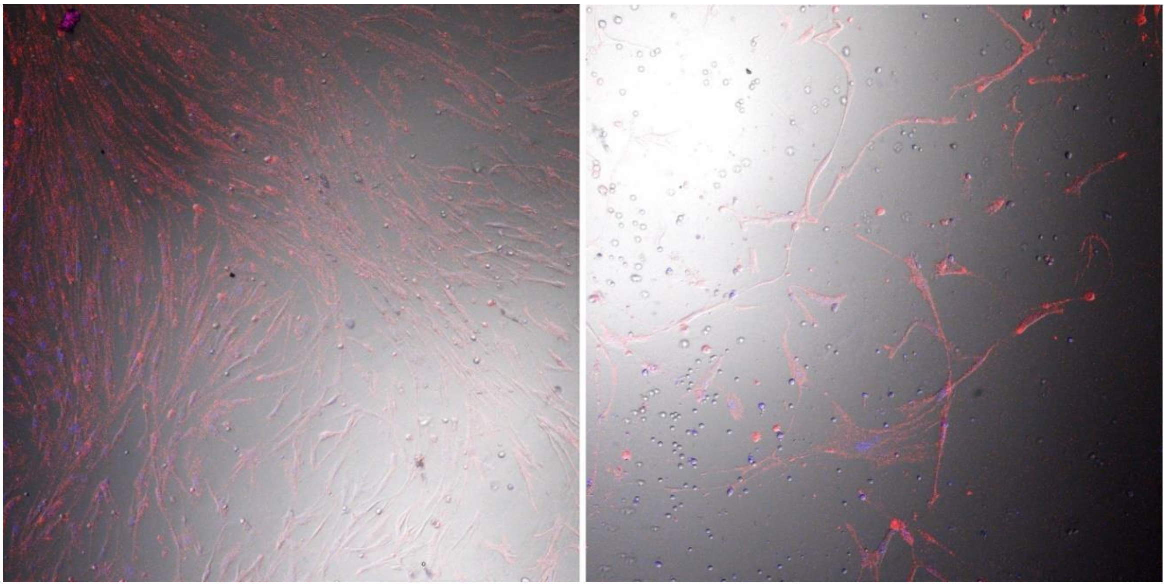


Figure 33: CD271+ and CD133+ dual stem cell angiogenesis assays stained with CD73PE (red) and Hoechst (blue). (10x magnification).

5.3.4. Gene expression analysis by means of mRNA-PCR

Since mRNA can be used as molecular evidence whether or not proteins are expressed and to which extent, PCR was used in order to achieve further information on how the stem cells differentiate in the angiogenesis assays. At this, CD271/NGFR expression as well as marker for endothelial (eNOS, vWF) and muscular properties (ACTA) were investigated (Figure 34).

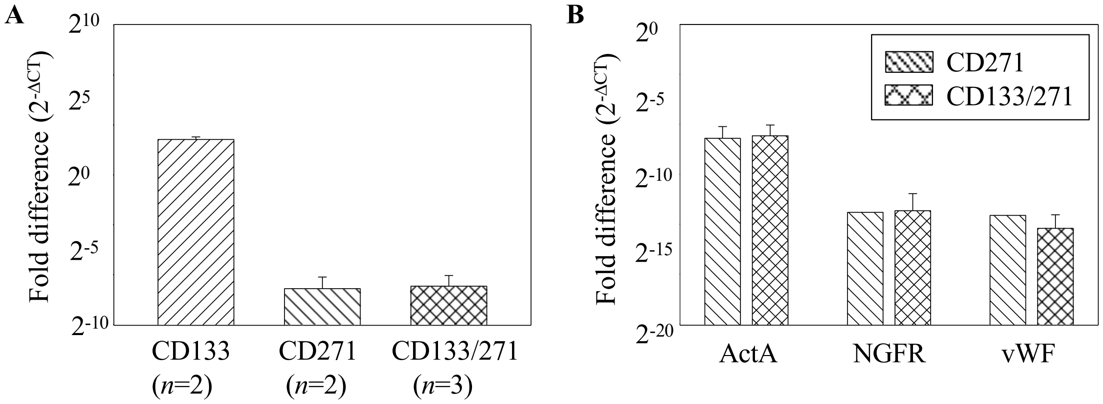


Figure 34: A Expression of ACTA as a marker for muscular differentiation with a 10-fold higher expression in CD133$^+$ monoculture compared to CD271$^+$ influenced assays. B Expression of ACTA, NGFR and vWF in CD271$^+$ monoculture as well as in CD271/CD133 coculture without any significant differences and no expression on CD133$^+$.

5.4 *In vivo* Stem Cell Characterization

5.4.1. Gene expression analysis by means of mRNA-PCR

To the present state of research, animal experiments are necessary to evaluate the effect of stem cell therapies on infarcted hearts. Due to the fact that complex pathophysiological processes have to be taken into account, an entire organism with intact circulation, vegetative nervous system, and cellular interaction is essential to guarantee reliable research results. Using a small animal model the stem cells were investigated on their potency to differentiate and the associated gene expression patterns.

The $\Delta\Delta C_T$ method was also used to calculate fold-differences in target gens compared to the SHAM control in the *in vivo* assays.

The CD133 stem cell treated group showed a significantly lower expression of the *tyrosine kinase with immunoglobulin-like and EGF-like domains* Tie1 (MIC 1.669, CD133 0.690, CD271 0.957 fold difference vs. SHAM) and the *transforming growth factor-beta* TGFβ3 (MIC 1.670, CD133 1.103, CD271 2.421) whereas the expression of the *chemokine (C-X-C motif) ligand 1* Cxcl1 (MIC 0.415, CD133 1.384, CD271 0.753) and *chemokine (C-X-C motif) ligand 2* Cxcl2 (MIC 0.523, CD133 2.758, CD271 0.601) were significantly increased (Figure 35).

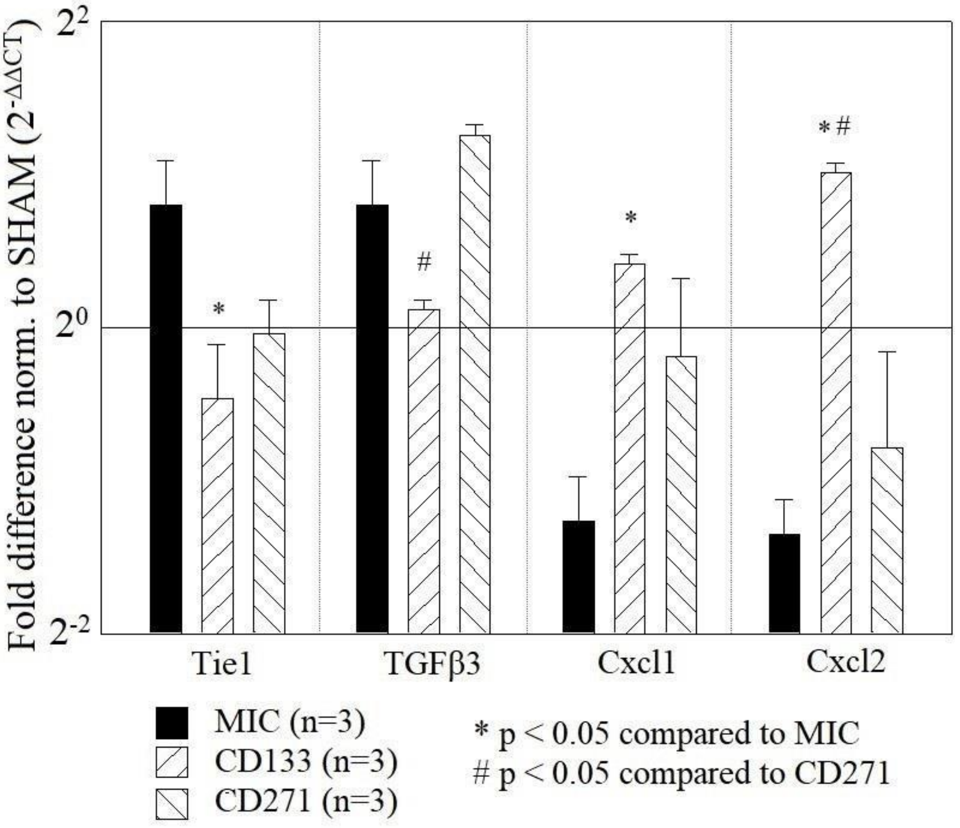


Figure 35: Relative expression of Tie1, TGFβ3, Cxcl1 and Cxcl2 illustrated as fold differences of the MIC control as well as CD133 and CD271 stem cell treated groups normalized to SHAM control.

The CD133 stem cell treated group showed a significantly higher expression of the *interleukin-1β* IL1β (MIC 0.522, CD133 1.736, CD271 0.598 fold difference vs. SHAM) and *interleukin-6* IL6 (MIC 0.522, CD133 2.738, CD271 0.945) as well as the *chemokine (C-C motif) ligand 11* Ccl11 (MIC 0.520, CD133 3.460, CD271 1.508) and the *tissue inhibitor of metalloproteinases* Timp1 (MIC 1.045, CD133 2.763, CD271 1.909). The *vascular endothelial growth factor* Vegfc was significantly increased in the CD271 stem cell treated group (MIC 1.656, CD133 0.869, CD271 3.017) (Figure 36).

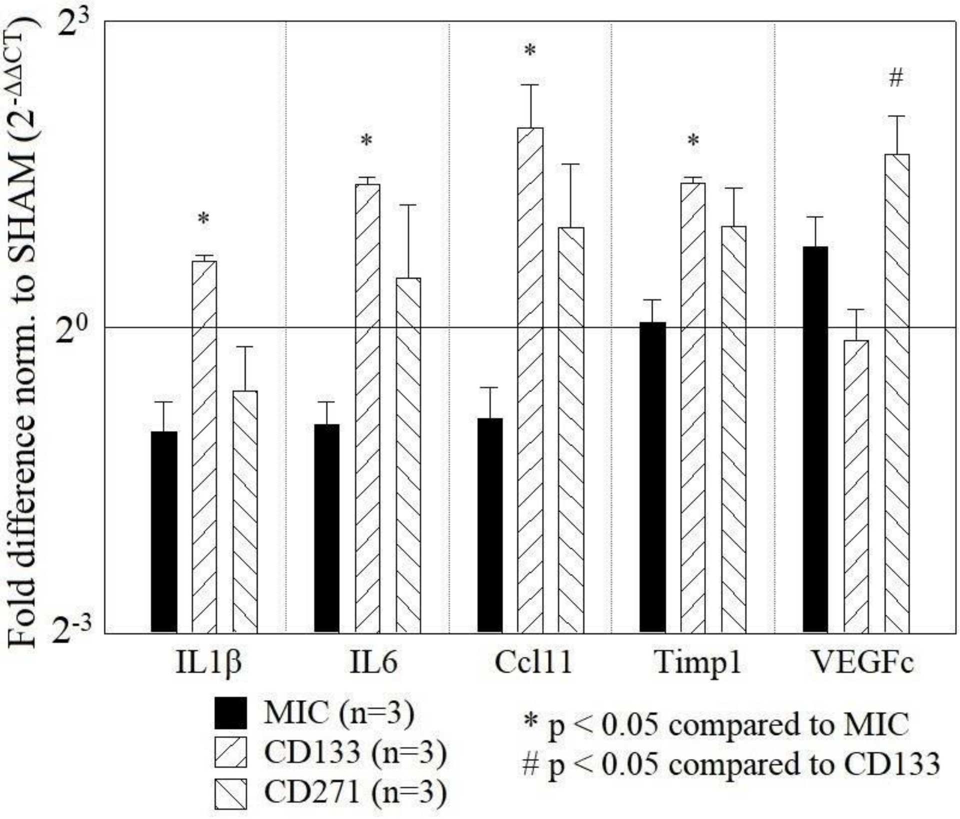


Figure 36: Relative expression of IL1β, IL6, Ccl11, Timp1 and VEGFc illustrated as fold differences of the MIC control as well as CD133 and CD271 stem cell treated groups normalized to SHAM control.

The CD271 stem cell treated group showed a significantly higher expression of *angiogenin* Ang (MIC 1.309, CD133 1.384, CD271 3.034). *Midkine* Mdk (MIC 1.319, CD133 2.765, CD271 3.015) as well as *ephrin B2* Efnb2 (MIC 2.642, CD133 1.381, CD271 1.515) showed an increased expression in both stem cell treated groups. On the other hand, the *ephrin type B receptor 4* Ephb4 (MIC 2.118, CD133 1.388, CD271 1.193) was less expressed in the CD271 stem cell treated group (Figure 37).

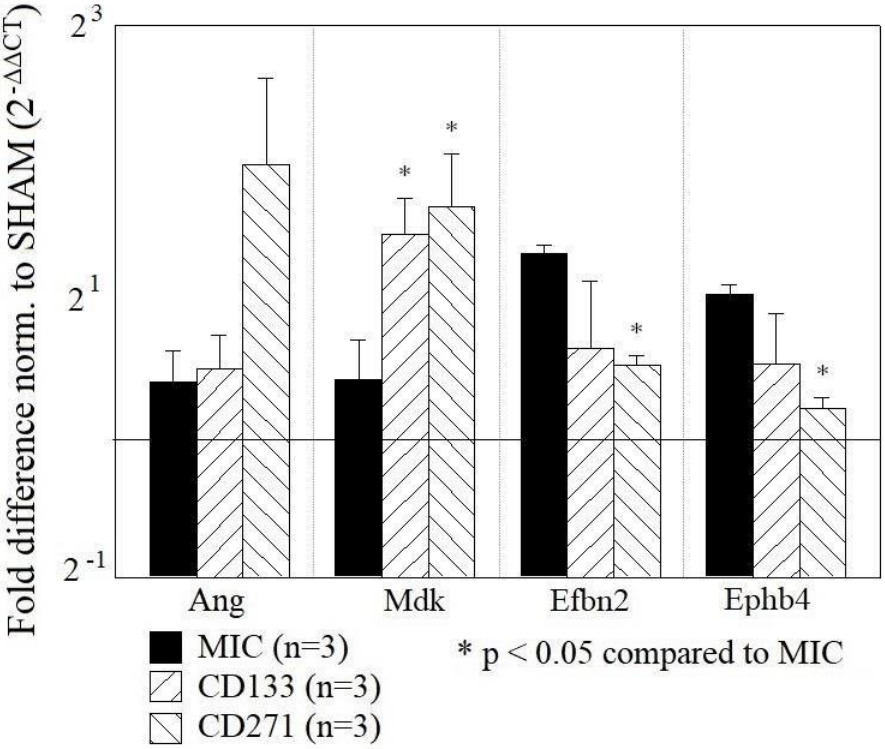


Figure 37: Relative expression of Ang, Mdk, Efnb2 and Ephb4 illustrated as fold differences of the MIC control as well as CD133 and CD271 stem cell treated groups normalized to SHAM control.

6 Discussion

6.1 Manual Immuno-Magnetic Cell Separation (MACS) and Quality Management by Fluorescence- Activated Cell Sorting (FACS) of Human CD271$^+$ and CD133$^+$ Stem Cells from Bone Marrow

Quality management

The results from the manual immuno-magnetic cell separation in combination with the FACS analysis revealed that bone marrow contains only a small amount of CD133$^+$ and even less CD271$^+$ cells (Figure 19). At this, the initial amount of MNCs allows an early assessment of the number of CD271$^+$ cells that can be expected from the isolation since they show a linear dependence (Figure 21). However, the initial cell numbers and percentages highly differ between patients. This may be influenced by the medical condition of the donor - including age, diseases, and previous treatments – as well as by the applied methods such as the technique of puncture, storage, processing of bone marrow, and cell isolation.

The fluorescence-activated cell sorting was performed in order to analyze various aspects of the isolated stem cell subpopulations. First, the purity of the cell samples and thus the quality of the isolation procedure needed to be evaluated. Second, the viability of the cell suspensions and thus the cell loss was investigated. At this, CD133$^+$ as well as CD271$^+$ cells showed high purity and viability after isolation (Figure 20).

Different studies that deal with stem cell applications are not always consistent since multiple isolation protocols are in use. An exemplary description and analysis of this central problem can be found in the study performed by Seeger *et al.* (165) who compared the different study protocols of the ASTAMI trial (121) using Lymphoprep and the REPAIR-AMI trial (160) using Ficoll. For instance, bone marrow derived MNCs that have been isolated by the ASTAMI protocol showed a significantly reduced invasion capacity as well as an abolished capacity to augment neovascularization in an experimental hindlimb ischemia model (165). In conclusion, the decision which protocol is going to be performed may have considerable impact on the outcome and the success of the therapy. Furthermore, it is vital to guarantee an efficient and reproducible cell product in order to ensure predictable and therefore safe applications. We consider our protocol to highly meet these expectations.

Overlap population and order of isolation

It was examined if CD133$^+$ and CD271$^+$ form separate populations in bone marrow or if the surface proteins are also expressed simultaneously by an overlap population. This is an important aspect in order to evaluate the feasibility of a combined therapeutic approach. In conclusion, CD271+ CD133+ cells represent a higher proportion of CD133+ as well as CD271+ cells than of total mononuclear cells (Figure 22). Nevertheless, only a small percentage of isolated cells express both surface markers (Figure 23). During magnetic cell separation no selective enrichment or depletion of double or single positive cells took place. Therefore, the separation of the two populations is considered to be sufficient for this study and the investigation of their separate and combined effects.

Besides, it has been investigated if the order of isolation has any impact on the achieved cell numbers or their quality. At this, no negative or significant influence has been detected (Table 4). Therefore, it is recommendable to first isolate the CD271$^+$ fraction, since the cell numbers are in general smaller than those of CD133$^+$ populations. Hereby, the second isolation would not be performed, if the achieved CD271$^+$ cell number was not sufficient for the intended assay.

Storage of material

Another point of practical relevance is the question if the different materials may only be processed in fresh condition – i.e. directly after the isolation - or if the materials may also be stored. As mentioned before, the bone marrow samples reach the laboratory depending on the time the heart surgery is performed, which is not always predictable precisely. The possibility of sample storage makes experimental work much easier and effective. Furthermore, it is unavoidable that cells undergo storage for at least several hours due to processing and transportation. This applies on multicenter studies in particular. Therefore, the possibility of storage makes stem cells much more practicable for therapeutic approaches. In clinical setting, autologous stem cells may be transplanted 24 to 30 hours after bone marrow aspiration (122). Thus, establishing standard conditions for storage are essential in order to ensure an optimal cell product.

In the mid-1980s, Lasky *et al.* have already shown that the viability and colony forming potential of progenitor cells in unpurified bone marrow are fairly well maintained for up to three days even if stored at room temperature (107). Likewise, a study by Kao *et al.* confirmed that bone marrow products maintain optimal cell viability under storing conditions regardless if at 4 or 20°C for 72 hours. In contrast, peripheral blood stem cell

products showed better viability at 4°C. However, they also reported a mean viable CD34$^+$ cell recovery of 75% for both product types stored at 4°C (95). According to Antonenas *et al*. the mean loss of viable CD34$^+$ stem cells in bone marrow stayed below 10% after 24 hours of storage at refrigerated temperature (8). A recent study by our study group closely investigated the impact of short-term storage on human CD133$^+$ cells (122). At this, standardized non-freezing storage conditions were used for up to 72 hours. It was stated that cell number as well as metabolic activity decreased after 30 hours, although no significant alterations were observed in cell viability at this point in time. However, storage of 72 hours also resulted in a significant decrease of viability. Likewise, colony forming capability and cell proliferation remained high up to 30 hours of storage, but also decreased after 72 hours. Other than that, the expression of CD133 as well as CXCR4, a surface receptor that interacts with SDF-1 and contributes to chemotaxis, was preserved at all time points and no significant changes were detected concerning endothelial-like differentiation. With these results it was showed that the isolated CD133$^+$ cells can be stored with high viability and functionality (122).
Furthermore, different clinical trials including the ASTAMI trial *(Autologous Stem cell Transplantation in Acute Myocardial Infarction* trial*)* (121) and REPAIR-AMI trial (*Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction* trail) (160), also used bone marrow derived mononuclear cells that have been stored for one day and detected high viabilities. However, Seeger *et al*. reported that the expression of CXCR4 has been significantly downregulated after storage (165).

Our results do not show any reference of a negative influence of storage on cell numbers or quality (Table 4). This applies for storage of bone marrow as well as negative fractions. Nevertheless, the time of storage should be limited in order to avoid cell loss. However, a limited number of damaged or dead transplanted cells may have only negligible impact in relation to the amount of neutrophils that infiltrate the ischemic cardiac tissue during reperfusion. In contrast, Lux *et al*. considered the possibility that storage might even have positive side effects. They assumed that “in a stored BM-MNC product, decomposing cells may be taken up by neighbor phagocytes, thus preventing the release of toxic cellular content and further cell damage” (122).

Additionally, stem cells can also be isolated from cryopreserved materials, but this may go along with an overall loss of viable cells due to the freezing and thawing process. At this, Sartor *et al.* reported a mean recovery of 90% for viable CD34$^+$ cells from cryopreserved bone marrow, but with a high range from 68 to 100% (158). Additionally, multiple severe clinical side effects including cardiovascular ones have been described by various studies (41), (173), (93). Other studies stated that cryopreserved mesenchymal stem cells show a lower initial CD271 surface expression level compared to freshly expanded bone marrow derived MSCs (10), (76). However, PCR analysis has not confirmed a decrease of CD271 expression on the mRNA level (76). According to a study by Mamidi *et al.*, successive or multiple rounds of cryopreservation did not impact the main characteristics of MSCs including morphology, growth properties, differentiation potential, and gene expression patterns (125). Therefore, cryopreservation seems to influence the surface expression of CD271, but does not necessarily alter intracellular expression levels. Nevertheless, these surface alterations may impact the functionality of CD271$^+$ cells in their therapeutic application. Therefore, liquid storage should be preferred over cryopreservation.

Surface expression analysis

Cultivated mesenchymal stem cells are often characterized by the expression of several surface markers including CD73, CD105, and CD44 as well as the absence of others, including CD45 and CD34 (144). In our analysis the expression of CD73 and CD105 was significantly increased on CD271$^+$ cells compared to other bone marrow cells (Figure 25). Gäbel *et al.* have already detected a favorable survival pattern, improved healing performance, and a more robust preservation of cardiac function for CD105$^+$ cells in infarcted hearts (61). Therefore, the increased expression on the CD271$^+$ population can be expected to have a positive influence on the regenerative potential.

Other than that, the expression of CD44 did not show any significant differences (Figure 25). These results correspond with the findings of Qian *et al.* who detected primary mesenchymal stem and progenitor cells in the CD44$^-$ cell fraction. Furthermore, they showed that these cells acquire the CD44 expression after *in vitro* culture (149).

Whereas most of cultivated MSCs do not express CD45, the largest proportion of freshly isolated CD271$^+$ cells expressed CD45^{dim} (Figure 26) – a surface marker that has been correlated to mesenchymal colony formation by Cuthbert *et al.* (37). The percentage of CD45$^-$ cells was small and showed considerable fluctuations between patients. In conclusion, the most frequent population in purified CD271$^+$ cells presumably consists of mesenchymal progenitors.

In conclusion, the established isolation protocol used for this study is highly suitable to gain high quality stem cells, which can be used for research intentions as well as therapeutic approaches. As such, bone marrow derived CD271$^+$ stem cells have previously been successfully investigated by our research group as a suitable cell type to prevent arrhythmia after myocardial infarction (154).

Preconditioning

Since the percentage shares of total bone marrow cells or MNCs that are positive for CD271 is very small, it is quite challenging to achieve the cell numbers intended for treatment. Thus, methods that increase the cellular outcome would be favorable for research as well as therapeutic applications. Hypoosmolar preconditioning may be suitable for such intentions. Studies by Herrmann S and Ramos *et al.* indicated that hypoosmolar preconditioning can be used to enhance CD271 expression on cultured cells (153) and fresh MNCs (76). At this, 60% hypoosmolar adjusted medium increased CD271 expression on MSCs, HUVECs, and MNCs without any loss of viability (76). This effect on MNCs has further been shown to be time-dependent since exposure for half an hour as well as five hours was not sufficient to induce the expression as opposed to exposure for 24 hours. Herrmann S also showed that hypoosmolar preconditioning of whole bone marrow does not increase CD271 expression and thus indicated the need of MNC isolation before the application of the preconditioning treatment. Besides, Herrmann S indicated that hypoosmolar treatment may increase the ability of MNCs and MSCs to build network structures or at least to increase MNC proliferation. At this, the combination of hypoosmolality and NGF treatment resulted in the best outcome concerning tube length and amount of network nodes (76). Thus, the preconditioning may even improve the effects of the stem cell treatment. However, it needs to be further evaluated whether these cells really show the same effects as CD271$^+$ cells that have been isolated without preconditioning. Therefore, *in vitro* and *in vivo* studies should be carried out to guarantee a safe and efficient product.

6.2 *In vitro* Stem Cell Characterization

Colony forming assays

Mesenchymal stem cells are defined as plastic-adherent and fibroblast-like clonogenic. As they proliferate, they form colonies termed *colony forming units-fibroblasts* (CFU-F). Therefore, these assays were used to compare proliferative properties and residual CFU-F activity of the stem cell populations after being cultivated over a defined period of time. Quirici *et al.* stated that this activity can only be observed in CD271$^+$ populations, but not in CD271$^-$ cells (150). In accordance with their results, colony-formation was only detected in mononuclear cells and CD271$^+$ fractions, but neither in CD271$^-$ nor in CD133$^+$ populations (Figure 27). The ability to proliferate is a major contributor to the impacts and effectiveness of stem cells in clinical applications. This applies to various functions such as differentiation into specialized mature cells as well as self-renewal or angiogenic processes.

Although CD133$^+$ hematopoietic stem cells do not show colony formation in CFU-F assays, they have been demonstrated to proliferate and generate offspring with endothelial characteristics in so called CFU-EC assays (122). These differentiated *colony-forming units-endothelial cells* acquire the functional phenotype of endothelial cells and also show typical gene expression including von Willebrand-factor (122) and angiogenic factors such as FGF3, PDGFB, and others (139). Several studies have shown that bone marrow derived *endothelial progenitor cells* (EPCs) are recruited into the circulatory system in response to vascular damage or ischemia (15). Benndorf *et al.* indicated a correlation between the number of CFU-EC and the number of circulating CD133$^+$/CD34$^+$/CD146$^+$ cells and thereby provided evidence that these cells represent functional endothelial progenitor cells (15). In accordance with this, Ong *et al.* detected a significant augmentation of EC-colonies after hypoxic preconditioning of CD133$^+$ cells and thus assumed that hypoxia may lead to the differentiation of CD133$^+$ cells towards endothelial lineage (139). Additionally, Attar *et al.* detected a strong correlation of the number of *circulating angiogenic cells* (CACs) with the number of CD133$^+$/CD309$^+$ cells (11).

In conclusion, both CD271$^+$ and CD133$^+$ cells have been shown to possess proliferative, colony-building properties. However, all mesenchymal progenitors originated from CD271$^+$ cells.

Angiogenesis assays

However, the results of the CD271$^+$ and CD133$^+$ stem cell *in vitro* angiogenesis assays indicate that the *in vitro* network formation relies on CD271$^+$ cells. Since CD133$^+$ cells alone rarely build network structures at all, it is remarkable that they will nevertheless contribute to the network, if they are cultivated in combination with CD271$^+$ cells (Figure 28). The fluorescence images provided evidence for this contribution as both fluorescent signals were detected from within the networks (Figure 29). Even more, no obvious differences of proportional contribution to the network structures were apparent. Besides, the CD271$^+$ CD133$^+$ dual angiogenesis assays were smaller in terms of tube length, but were better connected as they nonetheless had more nodal points (Figure 28). Therefore, the combination seems to be more effective concerning networking abilities *in vitro* than each cell type on its own.

Identification of stem cell phenotype in vitro

With the antibody staining we established a method to illustrate and characterize cellular networks. This method will enable further investigations on the differentiation and interaction of cells as well as their mutual impact. As initial objects of investigation the marker CD29, CD105, and CD73 were chosen in order to establish the method on MSCs. All three markers were detectable on MSC networks, which has been expected since they are known to be associated with the MSC phenotype. Additionally, CD73 PE was successfully applied on CD271$^+$ and CD133$^+$ angiogenesis assays. Apparently, the antibodies were able to reach the stem cells throughout the Matrigel as they specifically mark cell structures after direct application (Figure 32-33). Thus, this method does not need embedding in paraffin prior to the staining and enables us to analyze assays directly. Hereby, it is possible to obtain information on the stem cell differentiation and its properties within the network.

CD73-PE

As mentioned before, CD73 is oftentimes mentioned relating to the MSC phenotype (127), (28). In our analysis the expression of CD73 was significantly increased on CD271$^+$ cells compared to other bone marrow cells (Figure 25). However, only 9.2 ± 2.8% of freshly isolated CD271$^+$ cells express CD73. Nevertheless, networks built by CD271$^+$ as well as CD271$^+$/CD133$^+$ cells strongly expressed the marker on their surface in the angiogenesis assays (Figure 32-33). Furthermore, CD133$^+$ cell networks also expressed CD73 whereas the marker was not detected on CD133$^+$ cells that did not form network structures. In conclusion, CD73 seems be increasingly expressed during the differentiation process. Thus, the question is which properties or processes can be associated with the CD73 expression other than the descriptive MSC phenotype.

The surface marker CD73 is also referred to as five'-ectonucleotidase or NT5E and catalyzes the conversion of *five' adenosine monophosphate* (AMP) to bioactive adenosine. Aside from mesenchymal cells, the marker is further known to be expressed on the surface of endothelial cells and is involved in mediating various activation signals. Several studies have identified CD73 to be involved in cell interaction with the extracellular matrix and thus to regulate proliferation, migration of endothelial and cancer cells (64). According to Ghiringhelli *et al.* CD73-generated adenosine even plays a key role in tumor immunoescape (67).

For this study the involvement of the marker in angiogenic processes is of particular importance. At this, CD73 does not only seem to be associated with cells that are involved in angiogenesis, but appears to play a crucial role in the process itself. A study by Wang *et al.* found that capillary-like structures were formed more in CD73(+/+) *pulmonary microvascular endothelial cells* (PMECs) than in CD73(-/-) PMECs *in vitro*. In accordance with that they also observed that the extent of tumor angiogenesis as well as the tumor size was greater in CD73 (+/+) mice compared to CD73(-/-) mice *in vivo*. Furthermore, CD73 expression decreased the adhesion of endothelial cells to collagen IV and promoted migration. Thus, they concluded that CD73 contributes to endothelial cells forming new vessels especially in cancer conditions (189). A study by Allard *et al.* demonstrated that both tumor and host-derived CD73 are involved in these processes. In fact, tumor-derived CD73 enhances the production of *vascular endothelial growth factor* (VEGF) by tumor cells whereas host-derived CD73 is required for *in vivo* angiogenic responses. Furthermore, they also confirmed that endothelial cells require CD73 expression for tube formation and migration (6). Consequently, measures against CD73 have been shown to impair angiogenesis in tumors (6), (9). However, Böring *et al.* stated that CD73 deficiency has no effect on angiogenesis in their mice model of hind limb ischemia (19). Thus, it is not certain if the angiogenic effect applies on tumor tissue only and might be different in non-tumor environment. Apart from the influence on endothelial cells, Klein *et al.* identified $CD73^+$ multipotent cells within the adult human arterial adventitia that exhibit typical mesenchymal stem cell characteristics. Moreover, they showed that these cells differentiate to pericytes/ smooth muscle cells, which cover the wall of newly formed endothelial capillary-like structures *in vitro* (101).

As mentioned before, the CD73 marker seems to be upregulated during the differentiation process in our *in vitro* angiogenesis assays. In contrast to that, a study on bone marrow-derived MSCs by Delorme *et al.* mentioned a high enrichment in CFU-Fs for CD73 but a downregulation of CD73 in differentiated cells (43). On the other hand, Bi *et al.* stated that microvesicles that have been release by bone marrow-derived mesenchymal stem cells expressed CD29, CD44, CD73 and CD105 (16). Jiang *et al.* investigated MSC-derived endothelial cells and detected no significant change in the phenotype concerning CD73 (90). Additionally, Shamis *et al.* performed a phenotypic analysis of human embryonic and induced pluripotent stem cells during the course of differentiation towards fibroblasts that have been shown to secrete proangiogenic mediators and stimulate endothelial cell sprouting in an *in vitro* angiogenesis model. At this, they detected that both cell types progressively acquired pericytes lineage markers including CD73 (166).

In conclusion, the increased expression of CD73 especially on CD271$^+$ cells may have positive impact on the angiogenic potential of the stem cell treatment.

Other mesenchymal marker: CD29 and CD105

As mentioned before **CD29** is a single chain transmembrane glycoprotein, which is also known as beta-one-integrin, fibronectin receptor beta or gpIIa. It is expressed on a majority of hematopoietic and non-hematopoietic cells including fibroblasts, epithelial cells, smooth muscle cells, and endothelial cells. Integrins in general bind to several extracellular matrix and cell surface molecules. CD29 in particular complexes with one of nine integrin alpha subunits and belongs to the *very late antigen* subfamily (VLA) of adhesion molecules. Therefore, it is involved in a variety of cell-cell and cell-matrix interactions such as adhesion, migration, proliferation, cell signaling, proliferation, and angiogenesis (137).

Kubota *et al.* investigated the role of CD29 on HUVECs and showed that antibodies against CDw49b and CD29 inhibit capillary formation up to 70 per cent. This effect has further been shown to be dose-dependent. Additionally, they realized that HUVECs that have been pretreated with those antibodies do not form capillary-like structures with a hollow lumen encircled with endothelial cells, which has been observed after culture in Matrigel without antibodies. Therefore, they concluded that CDw49b/CD29 integrins are necessary for the interaction between endothelial cells with the extracellular matrix and thus for the differentiation of endothelial cells during angiogenesis (105). Tijore *et al.* further reported the recruitment of CD29 in human MSCs during commitment towards cardiomyogenic lineage (180). Additionally, Li *et al*. identified the adhesion molecule to be critical for inducing therapeutic angiogenesis in cell-based therapies since it is involved in the regulation of cell survival as well as differentiation after the implantation into ischemic tissue (115).

In conclusion, the expression of CD29 could be an interesting target for further analysis on the CD271 and CD133 angiogenesis networks as well as freshly isolated cells.

CD105 is a membrane glycoprotein that is also referred to as endoglin and that is classified as an accessory receptor for *transforming growth factor beta* (TGF-β), a pleiotropic cytokine regulating cellular proliferation, differentiation, migration, and adhesion (134). Studies have shown that the expression of endoglin is upregulated in actively proliferating endothelial cells (134). In accordance with that, endoglin is highly expressed in the neo-vasculature of ischemic tissues and tumors. At this, Sanchez-Elsner *et al*. have shown a transcriptional regulation that is induced by hypoxia (156). A study by Alev *et al*. has demonstrated a dynamic expression pattern of endoglin and suggested an important role in the transition from endothelial progenitors to functional cells during early vascular development (5). Furthermore, endoglin has been identified as a component of the endothelial nitric oxide synthase pathway and thus NO-dependent vasodilation (89). The involvement of endoglin in angiogenic processes is further confirmed by studies on knockout mice that died from defective vascular development. At this, Li DY *et al*. showed that a loss of endoglin results in poor vascular smooth muscle development and arrested endothelial remodeling (112). Li C *et al.* further investigated the interaction of endoglin with TGFβ1 and detected that the inhibition of endoglin translation enhances the ability of TGFβ1 to suppress growth and migration of cultured HUVECs. As a result, they indicated that endoglin antagonizes the inhibitory effects of TGFβ1on vascular endothelial cells and thus is required for new vessel formation (111). Additionally, endoglin plays an important role in tumor-related angiogenesis and thus has been identified as a promising target for tumor imaging, prognosis as well as therapeutic approaches (50).

With regard to bone marrow derived mesenchymal stem cells, Bi *et al.* have already detected that released microvesicles express CD105 as well as CD29 and CD73 (16). In our analysis the expression of CD105 was significantly increased on CD271^{+} cells compared to other bone marrow cells. A study by Delorme *et al*. also described a low enrichment for CD105 in bone marrow-derived mesenchymal stem cell CFU-Fs (43). Furthermore, Gäbel *et al.* have already detected a favorable survival pattern, improved healing performance, and a more robust preservation of cardiac function for CD105$^+$ cells in infarcted hearts (60). Studies were further able to show that the transplantation of hMSCs with high CD105 expression significantly improves myocardial performance in mice with a post-myocardial infarction status (127).

In conclusion, the increased expression of CD105 on the CD271$^+$ population can be expected to have a positive influence on the regenerative potential and its therapeutic approach.

CD271

Interestingly, staining with CD271-APC was negative on mesenchymal stem cell networks, although Hermann stated that culture expanded MSCs displayed an initial CD271 expression of 65% ± 12% under standard culture conditions (76). Furthermore, we also detected CD271 expression on mRNA-level of MSCs (Figure 17). Thus, MSCs might lose the surface receptor during their differentiation.

Relative Quantitation of Gene Expression

The CD133$^+$ cell culture resulted in a 10-fold higher level of ACTA gene expression in comparison to the CD133$^+$/ CD271$^+$ co-culture (Figure 34). However, Lu et al. observed that following culture in endothelial cell-promoting environment CD133$^+$ cells did not produce endothelial-like cells that expressed α-SMA, but only HUVEC and CD34$^+$ progenitor cells (120). As mentioned before, the majority of CD133$^+$ cells express CD34$^+$ as well (176). Remarkably, CD133$^+$ α-SMA$^+$ phenotype has been found only in association with blood vessels of cancer-associated fibroblasts (185), confirming the linkage of the α-SMA expression to endothelial origin and cell activation. A possible reason for less *ACTA* mRNA levels in a CD271+ cell co-culture might be by the phenotypical alterations of CD133$^+$ hematopoietic stem cells when applied together with MSC and/or due to the maintained undifferentiated state of MSC in co-culture model (97), (84). Hence, these in vitro co-culture conditions may have a reducing effect on the α-SMA mRNA levels in total.

6.3 *In vivo* Stem Cell Characterization

6.3.1 Angiogenesis

Vascular endothelial growth factor

The vascular endothelial growth factor (VEGF) is one of the best studied signal proteins associated with the formation of blood vessels. Various studies proved that VEGF promotes angiogenesis and improves cardiac function after myocardial infarction. In 1996 Li *et al*. have already described the expression kinetic of VEGF and its receptors in angiogenesis associated with myocardial infarction (113). Therefore, VEGF is considered a promising factor for clinical applications and thus is utilized in many various studies concerning targeted delivery of proangiogenic factors to the ischemic myocardium as well as tumor therapies. To name a few, Yang *et al*. investigated the effects of modified VEGF, which has been transferred to the ischemic myocardium by intravenous injections and observed a decreased scar size, enhanced angiogenesis and improved cardiac function (198). Cheng C *et al*. further showed that the expression level of VEGF positively relates to the increasing density of microvessels in the infarct area (32). Liu *et al*. observed enhanced cardiac function, increased perfusion and angiogenesis *in vivo* using MSCs as a VEGF delivery system (117). Similar to that, Zhu *et al*. used local intramyocardial injections of VEGF165 along with a biodegradable hydrogel to induce angiogenesis and improve cardiac function in a rat model using artery ligation. Furthermore, they noted reduced collagen content and infarction area (202). But also epicardial implantation of VEGF and cardiac stem cells using a poly (l-lactic acid) mat showed therapeutic effects on angiogenesis and cardiomyogenesis if applied to acutely infarcted myocardium. This resulted in reduced cardiac remodeling and enhanced global cardiac function (33).

Thus, the significantly increased expression of VEGF in the CD271$^+$ stem cell treated group (Figure 36) will result in improved angiogenesis as well as cardiac function in the infarcted area. In fact, our study group was able to demonstrate that co-transplantation of CD271$^+$ mesenchymal stem cells and CD133$^+$ hematopoietic stem cells lead to enhanced synergistic effects on cardiac remodeling. At this, hematopoietic stem cells were associated with blood vessel formation, whereas CD271$^+$ cells were identified to contribute to transdifferentiation (109).

Angiogenin (Ang)

Angiogenin (Ang) encodes a member of the *pancreatic ribonuclease A* (RNase A) superfamily and has been identified as a potent inducer of neovascularization. Therefore, it interacts with endothelial and smooth muscle cells to induce a wide range of physiological and pathological cellular processes including cell migration, invasion, proliferation, survival, differentiation, and formation of tubular structures (196), (167). Angiogenin exerts its function through various signal pathways. First, the interaction between angiogenin and cell-surface receptors is assumed to lead to degradation of the extracellular matrix and activation of matrix metalloproteinases that promote cell migration and invasion (167). Second, released angiogenin undergoes receptor-mediated endocytosis from the cellular surface to the inside of endothelial cells (129) as well as smooth muscle cells (197). One of the involved angiogenin-binding proteins has been identified as smooth muscle alpha actin (129). Under growth conditions angiogenin is then translocated into the cellular nucleus, which has been determined as an essential step in the process of angiogenesis (197). Hence, angiogenin plays a role in regulating gene expression, stimulates ribosomal RNA procession, and contributes to mRNA transcription under growth conditions (167). As rRNA transcription determines the translation potential of a cell, angiogenin is required for cell proliferation. Kishimoto *et al*. further showed that nuclear angiogenin is also necessary for angiogenesis induced by other angiogenic factors and thus may serve as a crossroad in the whole angiogenetic process. This includes angiogenesis induced by *acidic* and *basic fibroblast growth factors* (aFGF and bFGF), *epidermal growth factor* (EGF), and *vascular endothelial growth factor* (VEGF). In return, inhibitors of the nuclear translocation of angiogenin as well as the downregulation of angiogenin in endothelial cells lead to decreased rRNA transcription, cell proliferation, and angiogenic activities of these factors (99). Furthermore, the nuclear regulations of angiogenin also induce the synthesis of *nitric oxide* (NO) by triggering *nitric oxide synthase* (NOS) activity (182).

Apart from that, angiogenin accumulates in the cytoplasm under stress conditions where it interacts as a multifunctional ribonuclease. The catalytic region of angiogenin mediates stress-induced translational repression by cleaving cellular tRNAs under conditions such as nutrition deficieny, hypoxia, and hypothermia (57). The resulting tiRNA (tRNA-derived, stress induced small RNA) reprogram protein synthesis and thereby promote stress response programs, damage repair as well as cell survival (167), (86), (38). Besides, cytoplasmic angiogenin optimizes stress fiber assembly and focal adhesion formation to accommodate cell migration (167) and plays an important role in endogenous protective pathways of motoneurons exposed to hypoxia (164). Angiogenin circulates in human plasma and has been detected in plenty of different cell types and organs, including the heart. Interestingly, “Ang does not trigger ribosome biogenesis in confluent endothelial cells that constitute blood vessels” (167), as its receptor has only been detected on sparsely cultured endothelial membranes, but not on confluent ones (167). However, Pan *et al.* showed angiogenin involvement in wound neovascularization and positive correlation of high angiogenin expression with high vascular density in human burn wound (141). Sheng *et al.* (167) thus assumed:

> When injured clots disrupt endothelial cell confluence, high concentration of ANG in blood vessels could facilitate rapid blood vessel growth and tissue repair, suggesting that ANG may promote wound healing while losing vascular integrity.

As mentioned before, angiogenin promotes angiogenesis in response to stress conditions and stimuli like hypoxia. This has for example been demonstrated by Burgmann *et al.* who detected higher angiogenin concentrations in patients with more severe peripheral arterial occlusive disease (PAOD) (26). Increased angiogenin levels have also been detected in acute coronary syndrome and related to adverse prognosis following the event (179). Thus, Tello-Montoliu *et al.* suspected that high levels could be surrogate markers of unstable plaque and also may be a risk marker for future events. This hypothesis does not apply to our experiments as hypoxia was induced by arterial ligation. Other experiments showed angiogenin concentrations to be significantly increased in patients with heart failure with preserved ejection fraction compared to healthy or hypertensive controls and therefore it was suspected as predictor for the disease (79). However, the angiogenin increase under these conditions may presumably be due to the previous damage of the ACS or the other reasons that led to the condition of heart failure in the first place. The more the tissue suffered from hypoxia during myocardial infarction, the more stress response is induced and thus angiogenin expression increased. Therefore, angiogenin is rather a predictor for the severity of the stress condition and the tissue’s ability to respond to this. Elevated angiogenin expressions may be indicators for endothelial damage caused by impaired vascular perfusion or compensatory revascularization, or both. In our experiments the MIC control group and the stem cell treated groups underwent the same procedure of arterial ligation. As the CD271$^+$ treated group shows the highest expression of angiogenin with significant difference compared to other groups (Figure 37), the treatment could have resulted in increased proangiogenic condition with presumably higher angiogenesis. Being one of the most potent angiogenic factors, the upregulation expression may result in a better outcome. This has been confirmed during our study group's investigation of the effect of stem cell transplantation on neovascularization within infarcted area. At this, infiltration of the infarcted scar with new blood vessels was improved in stem cell treated hearts compared to MIC. Likewise, the capillary density in the border zone was significantly higher in the stem cell treated hearts (109).

Ephrin B2 (Efnb2) and ephrin type B receptor 4 (EphB4)

Ephrin receptors and their corresponding ephrin ligands constitute the youngest family of essential vascular tyrosine kinases that are involved in vascular development (12). They are divided into five ephrin A and three ephrin B ligands. Hereby, type A ligands are *glycosylphosphatidylinositol* (GPI)-anchored peripheral membrane molecules and B ligands consist of transmembrane molecules whose cytoplasmatic domain is capable of engaging in various signaling activities. Their corresponding receptors are categorized as eight ephrin type A and six ephrin type B receptors, which are classical receptor tyrosine kinases. As receptors as well as ligands are transmembrane molecules, the ephrin pathway requires juxtapositional contact of neighboring cells but is also capable of inducing reciprocal bidirectional signaling between interacting cells (12). *In vitro* studies reveal that EphB4 is involved in regulating embryonic stem cell responses to mesodermal induction signals and that it is essential for cardiomyocyte and vascular differentiation (192).

The ligand-receptor pair consisting of ephrin B2 and ephrin type B receptor 4 (EphB4) has been identified as essential element for the development of the cardiovascular system including arteriovenous differentiation (12), (188). Ephrin B2 as well as EphB4-deficiency in mice results in lethal disorder of vascular differentiation and arteriovenous remodeling in the early embryonic development (1), (188). Furthermore, gene targeting experiments revealed that the ligand ephrin B2 is expressed predominantly on arterial endothelial cells whereas EphB4 can be detected on venous endothelial cells (1), (12), (188). Therefore, the ephrin signaling has been the first evidence for an asymmetric expression pattern in arteries and veins and initiated further investigations in this field (12). Besides, these insights resulted in reconsideration of the origin of angiogenesis as well as the identity of capillary vessels (62), (168) and further evoked the question whether the expression of these markers is regulated by intrinsic or environmental aspects. "The intrinsic regulation of ephrin B2 expression would support an arterial origin of ephrin B2-positive angiogenic endothelial cells" (12), which was indicated for example by Gale *et al*. (62) and Shin *et al* (168). On the other hand, a postcapillary venule origin of angiogenesis as suspected by Burger *et al*. (25) would be supported by a microenvironmental regulation of ephrin B2 expression (12). In fact, both regulation mechanisms seem to be partially involved.

However, "ephrin B2 has a stimulatory effect on the migration of microvascular endothelial cells *in vitro*" (126). Gale *et al*. suggested that "ephrin B2 and EphB4 are involved in establishing arterial versus venous identity and perhaps in anastomosing arterial and venous vessels at their junctions" (62). Several endothelial cell interaction experiments support a model of reverse propulsive and repulsive effector function of ephrin interactions in the vascular system. At this, ephrin B2 forward signaling through the EphB4 receptor inhibits cell adhesion, lateral cell migration, VEGF-gradient driven chemotaxis, capillary-like network formation, and sprouting angiogenesis as well as it restricts intermingling of cells and supports cellular segregation. In turn, EphB4 reverse signaling by the transmembrane ephrin B2 promotes adhesion, migration, chemotaxis, capillary network formation, and sprouting angiogenesis (71). As mentioned before, ephrin B2 is mainly expressed on arterial and EphB4 on venous endothelial cells. As both molecules are transmembrane structures, the ephrin B2-mediated propulsive and EphB4-mediated repulsive signaling results in an artery to vein "push and pull" model of invasive angiogenesis that mediates spatial positional signals during angiogenesis and vessel assembly and controls boundary formation (59). Furthermore, Gale *et al*. noticed that during the proceeding development "ephrin B2 expression progressively extends from the arterial endothelium to surrounding smooth muscle cells and to pericytes, suggesting that ephrin-B2 may play an important role during formation of the arterial muscle wall" (62). Indeed, vascular EphB/ ephrinB expression is not limited to endothelial cells, but can also be found in smooth muscle cells and pericytes (12).

Stromal ephrin B2 expression seems to have a permissive role on vascular network formation as ephrin B2 positive endothelial cells proliferate in their presence and moreover induce the recruitment and proliferation of cells that express α-SMA. On the contrary, stromal cells expressing EphB4 inhibit vascular formation, the proliferation of ephrin B2 positive endothelial cells, and the recruitment of α-SMA expressing cells (201). Furthermore, quiescent endothelial cells that have contact to smooth muscle cells express ephrin B2 in a strictly luminal expression whereas confluent monolayer endothelial cells translocate ephrin B2 to interendothelial cell junctions (104). At this, "prolonged stimulation with EphB4-Fc leads to endocytosis of the receptor–ligand complex, as has been reported during the EphB/ ephrinB-mediated termination of adhesion after contact-mediated cell–cell repulsion" (104). Conversely, the endothelial expression of ephrin B2 limits the migration of smooth muscle cells and thereby promotes segregation of both cell types (103). The expression of EphB4 on vascular smooth muscle cells was demonstrated to be also involved in blood pressure regulation and small artery contractility. Wang *et al*. found out that the SMC-specific deletion of EphB4 in generated knockout mice results in hypotension and reduced contractility (191). Besides, Erber *et al.* showed that EphB4 converts the vascularization program from spouting angiogenesis to circumferential vessel growth (54).

Additionally, studies revealed that ephrin type B receptors are also expressed in cardiomyocytes. Experiments with EphB4 deficient embryonic stem cells indicated that the receptor affects the expression of the most primitive mesodermal gene products involved in hemangioblast and myocyte formation. At this, "EphB4 was not essential for completion of any of the differentiation programs assessed; rather, it modulated the rate and magnitude by which they were accomplished" (192). Chen *et al.* also showed that EphB4 forward signaling is essential in the development of cardiac progenitor cells and for the early stage of cardiac lineage development in mice (30). Furthermore, EphB4 seems to be involved in modulating the electrical coupling of cardiomyocytes through effects on gap junctional intracellular communication. At this, inducing high levels of Eph receptor activation desynchronized the contraction of adjacent clusters of cardiomyocytes, which had contracted synchronously before (85).

The expression of ephrin elements is regulated by various aspects. "Overexpression of the VEGF-A 164 isoform in cardiac muscle increased the number of ephrinB2-positive capillaries in the heart while reducing the number of EphB4-positive venules" (69). Besides, ephrin B2 is a direct Notch target gene (68) and is biomechanically induced by cyclic (103) and shear stress (104). Furthermore, the contact with quiescent SMCs also upregulates the ephrin B2 expression in endothelial cells (104). Basic fibroblast growth factor also enhances its expression, whereas it is attenuated by angiopoetin 1 (74). Besides, hypoxia is reported to upregulate the expression of ephrin B2 and EphB4. Vihanto *et al.* detected an induced upregulation of EphB4 in ischemic mouse skin (186). Limbourg *et al.* used a mouse model with limb ischemia induced by arterial ligation. They detected no expression of EphB4 in steady-state arteries, but observed strong perivascular expression of EphB4 after the ligation, which they considered to be an induction of a proangiogenic milieu (116). Mansson-Broberg *et al*. further investigated the ephrin receptor/ ligand pair in a myocardial infarction mouse model with ligation of the left anterior descending artery. During the two weeks following the procedure, EphB4-ephrinB2 showed a biphasic and opposing expression pattern. Furthermore, an intraperitoneal treatment with ephrinB2 resulted in increased capillary density in the periinfarcted area, whereby the ligand was detected bound to cells along the capillaries. Hence, modulation of ephrin B2 specifically induces vascular sprouting in ischemic myocardium and endothelial proliferation. The achieved therapeutic angiogenesis is of similar order as resulting from other tyrosine kinase angiogenic ligands (126).

As a result, the significant upregulation of the ephrin B2 and the ephrin type B receptor 4 in the myocardial infarction control group (MIC) compared to the SHAM control (Figure 37) can be considered as a hypoxia induced response to the suffered ischemia due to LAD ligation. As mentioned before, ephrin B2 specifically induces vascular sprouting in ischemic myocardium and endothelial proliferation without targeting healthy muscle. This constitutes a proangiogenic process that contributes to angiogenesis in the hypoxic area and thus increases the capillary density in the periinfarcted area.

In contrast to that, the expression in the stem cell treated groups is only slightly upregulated (Figure 37). Reviewing the considerations above, this may have plenty of possible reasons. Being referred to as a proangiogenic factor, one would first tend to assume that the upregulation of Efnb2 and EphB4 consequently results in preferable angiogenetic conditions. On the other hand, the regulation and function of EphB4 is complex as has been pointed out above and the differences may indirectly result from various regulating factors. As hypoxia itself induces the expression, the non-significant alteration in the stem cell treated groups may indicate that the stem cell therapy reduces the hypoxic stress. Besides, Mansson-Broberg *et al*. identified Ephrin B2 to significantly increase capillary density (126). Other than it would be expected by ephrin B2 expression, the investigations of our study group concerning the effect of stem cell transplantation on neovascularization within infarcted area showed significantly increased capillary density three weeks after the infarction compared to the untreated myocardial infarction (109). Besides, the stem cell therapy showed better vessel infiltration of scar tissue than MIC in the long-term-follow-up (109), which possibly matches that Erber *et al*. showed that EphB4 converts the vascularization program from spouting angiogenesis to circumferential vessel growth (54).

Midkine

Midkine (Mdk) is a secreted growth factor and a heparin-binding cytokine that links to oversulfated structures in heparan sulfate. It is not related to most other growth factors and belongs to a small protein family. The midkine cytokine functions in a variety of biological processes such as promotion of growth, differentiation, migration, and survival of several target cells. Therefore, the protein is involved in inflammatory reactions, formation of scar tissue and adhesions as well as neuronal growth processes and angiogenesis. On the other hand, the cytokine is also known to be involved in various diseases including inflammatory processes such as rheumatoid arthritis as well as renal diseases, multiple sclerosis, and malignant tumors, in which they influence invasive behavior, the resistance to chemotherapy as well as tumor angiogenesis. With regard to inflammatory properties, it has been shown that midkine attracts and activates neutrophils and macrophages. Furthermore, the cytokine is expressed at barriers of the body, such as the skin and the large airways, and contains potent bactericidal and fungicidal properties (132).

In general, midkine expression in adult tissue is weak or undetectable. Nevertheless, it is induced upon injury and is thus considered to be mainly involved in tissue reparative processes. Indeed, midkine has been reported to ameliorate ischemic injury in heart and brain tissue (132). At this, hypoxia has been identified as a promoter of midkine expression (65). The effects of an increased expression are versatile.

On the one hand, midkine shows an acute cytoprotective effect at least in part via an anti-apoptotic mechanism (81), (146). On the other hand, the cytokine promotes endothelial cell proliferation and enhances angiogenesis (94). Weckbach *et al.* identified endothelial cells to be the source of soluble midkine in the vascular system under hypoxic conditions and further demonstrated the pivotal role of midkine for the process of angiogenesis. In a hind limb ischemia model they observed almost no angiogenic response or endothelial cell proliferation in midkine-deficient mice (193). Additionally, midkine also recruits inflammatory cells including macrophages and polymorphonuclear neutrophils (PMNs) to the damaged areas, which further promotes angiogenesis (94). As a result, midkine also prevents cardiac tissue remodeling.

In the late 90s Obama *et al.* have already shown that after ligation of the left anterior descending coronary artery, midkine immunoreactivity in myocytes and endothelial cells is highly increased. Interestingly, this only applied on cells in a non-infarcted cardiac region, whereas myocytes of the infarcted area showed only little immunoreactivity

(138). A study by Takenaka *et al.* investigated the long-term effects of midkine in the myocardial infarction mouse model induced by ligation of the left coronary artery. They also detected that midkine expression progressively increased after infarction and that midkine-deficient mice have a higher mortality. Furthermore, exogenous midkine improved survival and ameliorated ventricular function and fibrosis as well as enhanced angiogenesis in the peri-infarct zone (177). Similar results were also achieved in rat models by Fukui *et al.* (58) and Sumida *et al.* (175) Likewise, it has been shown that intravenous bolus treatment with midkine (94) as well as intramuscular injections of midkine into the peri-infarcted area (81) significantly reduces infarct size. Therefore, midkine may also improve long-term outcomes of myocardial infarction.

Harada *et al.* recently investigated the effects of exogenous midkine administration on congestive heart failure of rabbits. They showed that the application of midkine also prevents cardiac remodeling in pacing-induced heart failure and thus in a state of non-ischemic etiology. The midkine treated animals demonstrated a lower mortality rate and better compensation than the control group. Furthermore, left ventricular end-diastolic dimension decreased whereas left ventricular systolic function increased and histological analysis revealed a decreased collagen deposition area. These results were traced back to the ability of midkine to prevent apoptosis and to minimize myocyte loss (73). On the other hand, midkine levels have been determined to rise with advancing NYHA class of heart failure and to be dependent on heart and kidney function (145). Thus, it has been suggested as a marker for risk stratification of patients with chronic heart failure (100).

Furthermore, midkine's chemoattractant activity also recruits inflammatory cells into the vascular wall, which triggers neointima formation and consequently vascular stenosis. Indeed, midkine-deficient mice have been shown to exhibit less inflammatory cell infiltration and to strongly suppress neointima formation whereas exogenous administration of midkine restored this process (80).

Several studies also investigated signaling pathways and molecular mechanisms through which midkine becomes effective. Honda *et al.* assumed that midkine might be a key mediator of cardiorenal interactions by inducing *epidermal growth factor receptor* (EGFR), which plays a pivotal role in the development of cardiac hypertrophy in chronic kidney disease (78). Furthermore, van der Horst *et al.* reported that midkine can abrogate the *vascular endothelial growth factor A* (VEGF-A) induced proliferation of human microvacular endothelial cells *in vitro*. According to their study midkine downregulated VEGF-A induced neovascularization and vascular permeability *in vivo*. Additionally, phosphorylation of *vascular endothelial growth factor receptor 2* (VEGFR-2) and its downstream signaling molecules was impaired (184). Other than this, Williams *et al*. focused on the protein expression patterns that are associated with abluminal sprouting and longitudinal splitting of capillaries during angiogenesis. Sprouting angiogenesis influenced the expression of genes related to extracellular matrix remodeling, such as *matrix metalloproteinase 2* (MMP-2), *tissue inhibitor of metalloproteinase* (TIMP), and thrombospondin, whereas splitting angiogenesis affects the expression of neuropilin, midkine, and restin. Upregulation of *vascular endothelial growth factor* (VEGF), angiopoetin-2 and platelet/ endothelial cell adhesion molecule.1 (PECAM-1) was seen in both forms (194).

Sumi *et al*. further investigated the interactions between smooth muscle cells and endothelial cells during angiogenesis and the role midkine plays in these. By means of an artificial blood vessel model they were able to show that midkine induces stratification of endothelial cells and promotes their proliferation. Likewise, coculturing endothelial cells with smooth muscle cells in the presence of midkine as well as coculturing with the conditioned medium of midkine treated smooth muscle cells also increased endothelial cell proliferation. Nevertheless, midkine did not have any effect on smooth muscle cells or endothelial cells when they were cultured separately. As a result, Sumi *et al*. concluded that midkine targets smooth muscle cells, which secrete factor(s) acting on endothelial cells (174).

As a result, the upregulation of midkine in both stem cell treated groups (Figure 37) presumably has an acute cytoprotective and anti-apoptotic effect and thus minimizes myocyte loss. Furthermore, the cytokine promotes endothelial cell proliferation, recruits inflammatory cells to the side of action and enhances angiogenesis. As a result, midkine may ameliorate the ischemic injury, the ventricular function and fibrosis as well as prevent cardiac tissue remodeling, and improve the long-term outcomes.

This also correlates with our results concerning the effect of stem cell transplantation on neovascularization within the infarcted area (109). Here we were able to show that the established stem cell therapies improve neovascularization within the infarcted area and results in significantly higher capillary density in the infarct border. Furthermore, the stem cell treatment decreased collagen deposition at the infarction border in comparison to untreated hearts. Our results indicate that the predominant mechanism of CD133$^+$ hematopoietic stem cells is based on their ability to regulate angiogenesis. On the other hand, the plasticity of CD271$^+$ mesenchymal stem cells triggers the intrinsic program of cardiogenic lineage specification by providing cardiomyocyte-derived cues. At this, a gap junction-dependent crosstalk between myocytes and stem cells has been identified as the basis for the intercellular communication (109).

6.3.2 Inflammatory Chemokines

Chemokines are attractants and regulators of cellular activities. Originally described as chemotactic cytokines, chemokines stimulate the recruitment of various cells into different tissue compartments. Furthermore, they modulate a vast array of cellular functions by influencing gene expression rates in infiltrating as well as resident cells.

In controlling tissue injury, neutrophilic recruitment is an essential step. Studies showed that tissue mast cells and macrophages synthesize chemokines such as Cxcl1 and Cxcl2 to mediate neutrophil influx (42). Besides, "neutrophils are able to self-regulate their own recruitment and responses during immune complex mediated inflammation through a Cxcl2 driven feed forward loop" (114). In accordance with that, neutralization of Cxcl2 in the interstitium effectively reduced neutrophil responses (114). Chemokines have been further determined to play a role in establishing a favorable microenvironment for tumor-associated angiogenesis (66), (172). At this, Cxcl2 has been suggested to be a surrogate marker for the functional process of neovascularization as angiogenic effects correlate with Cxcl2 levels in inflammatory granulation tissue (14).

Myocardial infarction represents a process of noninfectious inflammation, in which Cxcl2 expression contributes to neutrophil-mediated tissue injury. Al-Amran *et al.* demonstrated that down-regulation of Cxcl2 results in decrement in myocardial neutrophil infiltration, which is associated with less reactive oxygen species and reactive nitrogen species formation after global ischemia reperfusion. Therefore, the Cxcl2 expression contributes to inflammatory and oxidative processes during myocardial ischemia as well as neutrophil-dependent myocardial apoptosis (4). On the contrary, Tarzami *et al.* established a pathway by which Cxcl2 and MCP1 modulate cardiac myocyte viability during myocardial ischemia (178). They showed that hypoxia induces a significant increase of those chemokines in cell culture as well as *in vivo*. Furthermore, Cxcl2 antibodies also inhibited the hypoxia-induced expression of MCP1, which illustrates that Cxcl2 is a critical regulator of MCP1. As a result of MCP1 expression, they observed pronounced decrease of hypoxic cell death and thus assumed that MCP1 mediates a survival pathway in cardiac myocytes (178).

In our study, the expression of the cytokines Cxcl 1 and Cxcl2 has been significantly increased in the CD133 treated test group, whereas the CD271 group showed less expression (Figure 35). The same applies to the expression of the cytokines Interleukin-1β (IL1B) (Figure 36) and Interleukin-6 (IL6) (Figure 36) as well as the tissue inhibitor of metalloproteinases (Timp1) (Figure 36). These interleukins are mediators of inflammatory processes. An increased expression as observed in the CD133 test group may result in an increased inflammatory and oxidative process and could enhance the myocardial injury.

The *tyrosine kinase with immunoglobulin-like and EGF-like domains* Tie 2 has been identified as an important angiopoietin receptor. The modulation of this receptor activity is crucial for angiogenesis, vessel maturation and remodeling as well as vascular permeability and cell integrity. At this, the Angpt/ Tie2 signaling controls later steps during the angiogenic cascade and contributes to the acquisition of quiescent endothelial cells (106). This may be partly explained by the fact that angiopoietins induce translocation and activation of the Tie receptors in subcellular compartment depending on the cellular microenvironment (51). In contrast, the endothelial tyrosine kinase receptor Tie1 remains less characterized, largely owing to its orphan receptor status. However, it has been showed that Tie1 is essential for vascular development. Tie1-deficient mice died during late gestation as their vasculature fails to mature (106). The deletion of the receptor in endothelial cells inhibited sprouting angiogenesis in adult mice and thereby reduced intratumoral microvessel density and tumor growth. Furthermore, the deletion resulted in improved perfusion of the residual, more mature vessels. Besides, Tie1 deletion led to increased endothelial adherens junctions and perivascular coverage with improved vascular integrity and vessel perfusion (106). However, Tie 1 does not express its effects independently but works as a context-dependent regulator of the receptor Tie2. Tie1 is expressed in angiogenic tip and remodeling stalk cells during angiogenesis and is downregulated in the adult quiescent vasculature (159). At this, Tie expression contributes to shaping the tip cell phenotype by negatively regulating Tie2 surface presentation and Angpt2 signaling. On the other hand, Tie1 sustains Tie2 signaling in remodeling stalk cell vasculature (159). Both Tie1 and Tie2 undergo proteolytic cleavage in response to VEGF and inflammatory cytokines but with different kinetics (51). Interestingly, Tie1 was rapidly cleaved at the onset of acute inflammation in mice (51). Thus, the reduced expression in the CD133 treated group could also be an inflammatory reaction (Figure 35).

6.3.3 Remodeling and fibrosis

As mentioned before, ischemic conditions in the heart muscle are followed by inflammatory processes. Due to the massive sudden loss of cardiomyocytes and missing regenerative capabilities the striated heart muscle is converted into nonfunctional fibrin-containing scar tissue, which cannot contribute to the pumping function of the heart anymore. The cellular events involved in this conversion can be divided in three overlapping processes: inflammation, proliferation, and maturation. During the inflammatory phase cytokines released by necrotic cardiomyocytes recruit leukocytes and phagocytes into the affected area. After the removal of dead material the proliferative phase is induced, in which activated myofibroblasts as well as endothelial and stromal cells move in. The maturation phase results in the development of scar tissue and the remodeling of the heart muscle. Several studies revealed that signaling pathways involving transforming growth factor-beta play a decisive role in the regulation of these inflammatory and reparative cellular events (1).

The transforming growth factor-beta has been identified as a pleiotropic and multifunctional growth factor that regulates a wide range of cellular response including cardiomyocytes, fibroblasts, immune cells, and vascular cells. At this, effects such as cardiomyocyte survival patterns, promotion of monocyte recruitment and chemotaxis as well as suppression of adhesion in endothelial cells have been suspected. However, the most investigated effect and the central role of TGFβ is the conversion of myofibroblasts (55). TGFβ3 in particular has been associated with cardiac fibroblast proliferation, collagen biosynthesis and thus in the development of interstitial fibrosis in cardiac hypertrophy. Many existing *in vitro* studies confirm the involvement of TGFβ in the activation of cardiac fibroblasts. Nevertheless, the *in vivo* functioning has not completely been explained because of the complexity and the context-dependent signaling cascades (55). A study by Dewald *et al.* demonstrated that TGF-β1 and β2 mRNA levels show an early peak after 6-72 hours of reperfusion in a mouse infarct model whereas TGFβ3 exhibit a delayed response peaking after three to seven days of reperfusion (44). A recent study by Gao *et al.* investigated the effect of TGFβ on human-induced pluripotent stem cells. At this, derived endothelial cells, smooth muscle cells, pericytes, and fibroblasts were cultured with the cytokine, which resulted in an increased expression of myofibroblast markers whereas marker for their former differentiation significantly declined (i.e. endothelial cell, smooth muscle cell, pericyte, and fibroblast). Furthermore, they transplanted the cells into hearts of mice after myocardial infarction and investigated the effect of the simultaneous treatment with a TGFβ inhibitor. The treatment resulted in a reduced expression of myofibroblast markers and was associated with significant improvement of fibrosis and cardiac function. Thus, they concluded that TGFβ inhibition may significantly improve the efficacy of transplanted cells for cardiac repair (63).

The *chemokine (C-C motif) ligand 11* (Ccl11), also referred to as Eotaxin, has originally been described as a chemotactic and activating protein for eosinophils that displays its effects through the *chemokine receptor 3* (CCR3) (34). Therefore, it is known to be involved in inflammatory processes such as allergic reactions, asthma, atopic dermatitis, and parasitic infections (3), (92). Besides these well studied roles, the chemokine has recently also been related to tissue fibrosis and remodeling processes. Kodali *et al.* reported that Ccl11 and CCR3 are expressed in vascular smooth muscle cells of the media and neointima of injured arteries. Thus, they assumed that Eotaxin is involved in mediating SMC migration in the injured arterial wall (102). On the other hand, Salcedo *et al.* demonstrated that Eotaxin induces chemotaxis of human microvascular endothelial cells and thus may also directly contribute to endothelial cell migration (155). This theory was further supported as the chemotactic activity was consistent with the endothelial expression of CCR3. Furthermore, they observed a Ccl11-induction during blood vessel formation, which was accompanied by a predominantly eosinophilic inflammatory infiltration (155). As Eotaxin and its receptor are upregulated in human atherosclerosis, the chemokine seems to also participate in vascular inflammation (70).

Myocardial infarction is also associated with an inflammatory reaction, which results in scar tissue formation. Consequently, Ccl11 could play an important role in ischemia-induced neovascularization and vessel wall remodeling. Indeed, Chen *et al.* showed an increased expression of Eotaxin in the media of aorta vessels, which have been exposed to prolonged ischemia (29). Besides, Ccl11 has been reported to correlate with cardiac mast cell infiltration of the heart (204). In turn, cardiac mast cell density has been related to collagen deposition and fibrosis in myocardial ischemia, cardiovascular disease, reperfusion injury, cardiomyopathy, and congestive heart failure (13), (56), (143), (204).

Therefore, the observed upregulation of Ccl11 in the CD133$^+$ stem cell treated test group (Figure 36) presumably results in an enhanced inflammatory reaction and higher degree of fibrosis after myocardial infarction whereas the CD271$^+$ treated group showed a lower expression. On the other hand, the significant lower expression of the fibrotic marker TGFb3 after the treatment with CD133$^+$ cells in contrast to MIC or the CD271 treated group (Figure 35) may result in reduced development of fibrotic tissue and thus could preserve muscle function. However, the long-term-follow-up experiments performed by our study group revealed that the stem cell treatment even decreased collagen deposition at the infarction border in both stem cell groups compared to the MIC control (109). Furthermore, we were able to demonstrate that the stem cell therapy shows better vessel infiltration of scar tissue than MIC (109), which could also be related to Ccl11-induced migration of endothelial and smooth muscle cells.

Abstract

Bone marrow derived stem cells are characterized by varying functions and properties. This study intends to examine the therapeutic potential of mesenchymal CD271$^+$ stem cells in comparison to hematopoietic CD133$^+$ cells with regard to their application in myocardial infarction. At this, it should be determined if CD271$^+$ MSCs can improve the effectiveness of the stem cell therapies currently in use. We expect that the multiple characteristics and various regenerative effects of mesenchymal stem cells will result in greater therapeutic impact on ischemic heart disease. In this context, the effects of CD271$^+$ cells alone as well as in combination with CD133$^+$ have been explored with the main focus on angiogenic potential. The interactions between these two subpopulations and to what extent the combination may enhance heart regeneration has not been investigated yet. Our standardized immuno-magnetic isolation procedure has been proofed to be efficient, reproducible, feasible, and safe. The resulting cell product meets the expectations concerning cell purity, quality, and viability. *In vitro* proliferation assays showed that the application of CD271$^+$ stem cells results in improved angiogenic potential. Besides of CFU-F assays, angiogenesis assays with Matrigel have been evaluated by three-dimensional microscopy, immunohistochemistry, and gene expression analysis. At this, the combination of CD133$^+$ and CD271$^+$ cells seems to be the most feasible and effective approach. In order to evaluate the effect of stem cell therapies on infarcted hearts, an *in vivo* analysis has been performed in a mouse-model with ligation of the left anterior descendent artery. The performed gene expression analysis supports the assumption of various angiogenic effects of bone marrow derived stem cells in the infarcted heart.

Appendix

References

(1) Abdelaziz N, Colombo F, Mercier I, Calderone A (2001). „Nitric Oxide Attenuates the Expression of Transforming Growth Factorbeta(3) mRNA in Rrat Cardiac Fibroblasts via Destabilization.“ *Hypertension*. 38: 261-266, 2001.

(2) Adams RH, Wilkinson GA, Weiss C, Diella F, Gale NW et al. (1999). "Roles of EphrinB Ligands and EphB Receptors in Cardiovascular Development: Demarcation of Arterial/Venous Domains, Vascular Morphogenesis, and Sprouting Angiogenesis." *Genes& Development.* 1999 Feb;13(3):295-306.

(3) Ahmadi Z, Hassanshahi G, Khorramdelazad H, Zainodini N, Koochakzadeh L (2016). "An Overlook to the Characteristics and Roles Played by Eotaxin Network in the Pathophysiology of Food Allergies: Allergic Asthma and Atopic Dermatitis." *Inflammation.* 2016 Jun;39(3):1253-67.

(4) Al-Amran FF, Shahkolahi M (2014). "Oxytocin Ameliorates the Immediate Myocardial Injury in Heart Transplant through Down Regulation of the Neutrophil Dependent Myocardial Apoptosis." *Heart Views*. 2014 Apr;15(2):37-45.

(5) Alev C, McIntyre BA, Ota K, Sheng G (2010). "Dynamic Expression of Endoglin, a TGF-Beta Co-Receptor, during Pre-Circulation Vascular Development in Chick." *International Journal of Developmental Biology*. 2010;54(4):737-42.

(6) Allard B, Turcotte M, Spring K, Pommey S, Royal I, Stagg J (2014). "Anti-CD73 Therapy Impairs Tumor Angiogenesis." *International Journal of Cancer*. 2014 March;134(6):1466-73.

(7) Andrade-Gordon P, Derian CK, Maryanoff BE, Zhang HC, Addo MF et al. (2001). "Administration of a Potent Antagonist of Protease-Activated Receptor-1 (PAR-1) Attenuates Vascular Restenosis Following Balloon Angioplasty in Rats." *Journal of Pharmacology and Experimental Therapeutics.* 2001 Jul;298(1):34-42.

(8) Antonenas V, Garvin F, Webb M, Sartor M, Bradstock KF, Gottlieb D (2006). "Fresh PBSC Harvest, but not BM, Show Temperature-Related Loss of CD34 Viability during Storage and Transport." *Cytotherapy.* 2006;8(2):158-65.

(9) Antonioli L, Blandizzi C, Malavasi F, Ferrari D, Haskó G (2016). "Anti-CD73 Immunotherapy: A Viable Way to Reprogram the Tumor Microenvironment." *Oncoimmunology*. 2016 Jul;5(9):e1216292.

(10) Aoyagi K, Yamazaki Y, Matsuo A, Shimakura Y, Takeda A, Uchinuma E (2010). "Significance of CD271 in Bone Marrow Mesenchymal Stem Cells-Changes in Cryopreservation." J*ournal of Craniofacial Surgery*. 2010 May;21(3):666-78.

(11) Attar A, Aghasadeghi K, Parsanezhad ME, Namavar Jahromi B, Habibagahi M et al. (2015). "Absence of Correlation between Changes in the Number of Endothelial Progenitor Cell Subsets." *Korean Circulation Journal*. 2015 Jul;45(4):325-32.

(12) Augustin HG, Reiss Y (2003). "EphB Receptors and EphrinB Ligands: Regulators of Vascular Assembly and Homeostasis." *Cell and Tissue Research*. 2003 Oct;314(1):25-31.

(13) Batlle M, Pérez-Villa F, Lázaro A, Garcia-Pras E, Ramirez J et al (2007). "Correlation between mast cell density and myocardial fibrsis in congestive heart failure patients." *Transplantation Proceedings*. 2007 Sep;39(7):2347-9.

(14) Belo AV, Leles F, Barcelos LS, Ferreira MA, Bakhle YS el al. (2005). "Murine Chemokine CXCL2/KC is a Surrogate Marker for Angiogenic Activity in the Inflammatory Granulation Tissue." *Microcirculation.* 2005 Oct-Nov;12(7):597-606.

(15) Benndorf RA, Gehling UM, Appel D, Maas R, Schwedhelm E et al. (2007). "Mobilization of Putative High-Proliferative-Potential Endothelial Colony Forming Cells during Antihypertensive Treatment in Patients with Essential Hypertension." *Stem Cells and Development*. 2007 Apr;16(2):329-38

(16) Bi XY, Huang S, Chen JL, Wang F, Wang Y, Guo Z (2014). ["Exploration of Conditions for Rreleasing Microvesicle from Human Bone Marrow Mesenchymal Stem Cells. Article in Chinese] *Zhongguo Shi Yan Xue Ye Xue Za Zhi*. 2014 Apr;22(2):491-5.

(17) Bleumink GS, Knetsch AM, Sturkenboom MC, Straus SM, Hofman A et al. (2004). "Quantifying the Heart Failure Epidemic: Prevalence, Incidence rate, Lifetime Risk and Prognosis of Heart Failure. The Rotterdam Study." *European Heart Journal*. 2004 Sep; 25(18):1614-9.

(18) Borges E, Jan Y, Ruoslahti E (2000). "Platelet-Derived Growth Factor Receptor-β and Vascular Endothelial Growth Factor Receptor-2 Bind to the β_3 Integrin through its Extracellular Domain." *Journal of Biological Chemistry*. 2000 Dec;275(51):39867-73.

(19) Böring YC, Flögel U, Jacoby C, Heil M, Schaper W, Schrader J (2013). “Lack of Ecto-5'-Nucleotidase (CD73) Promotes Arteriogenesis.” *Cardiovascular Research*. 2013 Jan;97(1):88-96.

(20) Boron WF, Boulpaep EL (2005) [2003]. *Medical Physiology – a Cellular and Molecular Approach.* Updated edition, Philadelphia: Elsevier Inc.

(21) Bühling KJ, Lepenies J, Witt K (2008) [1995]. *Intensivkurs - Allgemein und spezielle Pathologie.* 4th edition, München: Elsevier GmbH, Urban & Fischer Verlag.

(22) Bühring HJ, Battula VL, Treml S, Schewe B, Kanz L et al. (2007). “Novel Markers for the Prospective Isolation of Human MSC.” *Annals of the New York Academy of Science.* 2007 June; 1106:262-71.

(23) Bueno H, Ross JS, Wang Y, Chen J, Vidán MT et al. (2010). “Trends in Length of Stay and Short-term Outcomes Among Medicare Patients Hospitalized for Heart Failure, 1993-2006.” *JAMA*. 2010 June; 303(21):2141-7.

(24) Bui AL, Horwich TB, Fonarow GC (2011). “Epidemiology and Risk Profile of Heart Failure.” *National Reviews Cardiology*. 2011 Jan; 8(1): 30–41.

(25) Burger PC, Chandler DB, Klintworth GK (1983). “Corneal Neovascularization as Studied by Scanning Electron Microscopy of Vascular Casts.” *Laboratory Investigation*. 1983 Feb;48(2):169-80.

(26) Burgmann H, Hollenstein U, Maca T, Zedwitz-Liebenstein K, Thalhammer F et al. (1996). “Increased Serum Laminin and Angiogenin Concentrations in Patients with Peripheral Arterial Occlusive Disease.” *Journal of Clinical Pathology.* 1996 Jun;49: 508–510.

(27) Caporali A, Emanueli C (2009). “Cardiovascular Actions of Neurotrophins.” *Physiological Revues*. 2009 Jan; 89:1-27.

(28) Cashman TJ, Gouon-Evans V, Costa KD (2013). “Mesenchymal Stem Cells for Cardiac Therapy: Practical Challenges and Potential Mechanisms.” *Stem Cell Reviews and Reports*. 2013 June; 9(3):254-65.

(29) Chen J, Akyurek LM, Fellstrom B, Hayry P, Paul LC (1998). “Eotaxin and Capping Protein in Experimental Vasculopathy.” *American Journal of Pathology*. 1998 Jul;153: 81–90.

(30) Chen K, Bai H, Liu Y, Hoyle DL, Shen WF et al. (2015). “EphB4 Forward-Signaling Regulates Cardiac Progenitor Development in Mouse ES Cells.” *Journal of Cellular Biochemistry*. 2015 Mar; 116(3):467-75.

(31) Chen WC, Lee BG, Park DW, Kim K, Chu H, et al. (2015). “Controlled Dual Delivery of Fibroblast Growth Factor-2 and Interleukin-10 by Heparin-Based Coacervate Synergistically Enhances Ischemic Heart Repair.” *Biomaterials*. 2015 Dec;72:138-51.

(32) Cheng C, Li P, Wang YG, Bi MH, Wu PS (2016). „Study on the Expression of VEGF and HIF-1α in Infarct Area of Rats with AMI.“ *European Review for Medical and Pharmacological Sciences.* 2016;20(1):115-9.

(33) Chung HJ, Kim JT, Kim HJ, Kyung HW, Katila P et al. (2015). “Epicardial Delivery of VEGF and Cardiac Stem Cells Guided by 3-dimensional PLLA Mat Enhancing Cardiac Regeneration and Angiogenesis in Acute Myocardial Infarction.” *Journal of Controlled Release*. 2015 May 10;205:218-30.

(34) Combadiere C, Ahuja SK, Murphy PM. (1995). “Cloning and Functional Expression of a Human Eosinlophil CC Chemokine Receptor.” *Journal of Biological Chemistry*. 1995 Jul 14;270(28):16491-4.

(35) Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS) Trial Study Group (1987). “Effects of Enalapril on Mortality in Severe Congestive Heart Failure.” *The New England Journal of Medicine*. 1987 June; 316:1429.

(36) Curran JM, Pu F, Chen R, Hunt JA (2011). “The Use of Dynamic Surface Chemistries to Control MSC Isolation and Function.” *Biomaterials*. 2011 Jul;32(21):4753-60.

(37) Cuthbert R, Boxall SA, Tan HB, Giannoudis PV, McGonagle D, Jones E (2012). “Single-Platform Quality Control Assay to Quantify Multipotential Stromal Cells in Bone Marrow Aspirates Prior to Bulk Manufacture or Direct Therapeutic Use.” *Cytotherapy*. 2012 April;14(4):431-40.

(38) Czech A, Wende S, Ignatova Z (2013). “Reversible and Rapid transfer-RNA Deactivation as a Mechanism of Translational Repression in Stress.” *PLoS Genetics*. 2013 Aug;9(8):e1003767.

(39) Dabek J, Ligus J, Szota J (2010). “Oligonucleotide Microarray and QRT-PCR Study of Adhesion Protein Gene Expression in Acute Coronary Syndrome Patients.” *Inflammation*. 2010 Dec; 33(6):398-407.

(40) Dai LC, Wang X, Yao X, Lu YL, Ping JL, He JF (2007). “Antisense Oligonucleotide Targeting Midkine Suppresses *in vivo* Angiogenesis.” *World Journal of Gastroenterology*. 2007 Feb;13(8):1208-13.

(41) Davis JM, Rowley SD, Braine HG, Piantadosi S, Santos GW (1990). “Clinical Toxicity of Cryopreserved Bone Marrow Graft Infusion.” *Blood*. 1990 Feb;75(3):781-6.

(42) De Filippo K, Dudeck A, Hasenberg M, Nye E, van Rooijen N et al. (2013). “Mast Cell and Macrophage Chemokines CXCL1/CXCL2 Control the Early Stage of Neutrophil Recruitment during Tissue Inflammation.” *Blood*. 2013 Jun;121(24):4930-7.

(43) Delorme B, Ringe J, Gallay N, Le Vern Y, Kerboeuf D et al. (2008). “Specific Plasma Membrane Protein Phenotype of Culture-Amplified and Native Human Bone Marrow Mesenchymal Stem Cells.” *Blood*. 2008 Mar 1;111(5):2631-5.

(44) Dewald O, Ren G, Duerr GD, et al. (2004). “Of Mice and Dogs: Species-Specific Differences in the Inflammatory Response Following Myocardial Infarction. *American Journal of Pathology*. 2004;164:665-77.

(45) Di Nicola M, Carlo-Stella C, Magni M, Milanesi M, Longoni PD et al. (2002). “Human Bone Marrow Stromal Cells Suppress T-Lymphocyte Proliferation Induced by Cellular or Nonspecific Mitogenic Stimuli.” *Blood.* 2002 May; 99: 3838–3843.

(46) Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F et al. (2006). “Minimal Criteria for Defining Multipotent Mesenchymal Stromal Cells. The International Society for Cellular Therapy Position Statement.” *Cytotherapy*. 2006 *8*, 315–317.

(47) Donndorf P, Kundt G, Kaminski A, Yerebakan C, Liebold A, Steinhoff G, Glass A (2011). “Intramyocardial Bone Marrow Stem Cell Transplantation during Coronary Artery Bypass Surgery: a Meta-analysis.” *Journal of Thoracic and Cardiovascular Surgery*. 2011 Oct; 142(4): 911-20.

(48) Donndorf P, Kaminski A, Tiedemann G, Kundt G, Steinhoff G (2012). “Validating Intramyocardial Bone Marrow Stem Cell Therapy in Combination with Coronary Artery Bypass Grafting, the PERFECT Phase III Randomized Multicenter Trial: Study Protocol for a Randomized Controlled Trial.” *Trials*. 2012 July. 13:99.

(49) Donndorf P, Steinhoff G (2013). “CD133-Positive Cells for Cardiac Stem Cell Therapy: Current Status and Outlook.” *Advances in Experimental Medicine and Biology*. 2013;777:215-27.

(50) Duff SE, Li C, Garland JM, Kumar S (2003). "CD105 is Important for Angiogenesis: Evidence and Potential Applications." *FASEB Journal*. 2003 Jun;17(9):984-92.

(51) Eklund L, Kangas J, Saharinen P (2017). "Angiopoietin-Tie Signaling in the Cardiovascular and Lymphatic Systems." *Clinical Science*. 2017;131(1):87–103

(52) Elmadhun NY, Burgess TA, Bianchi C, et al. (2014). "Microvascular Notch Signaling is Upregulated in Response to Vascular Endothelial Growth Factor and Chronic Myocardial Ischemia." *Circulation Journal.* 2014;78(3):743-51.

(53) Elnakish MT, Kuppusamy P, Khan M (2013). "Stem Cell Transplantation as a Therapy for Cardiac Fibrosis." *Journal of Pathology*. 2013 Jan; 229: 347–354.

(54) Erber R, Eichelsbacher U, Powajbo V, Korn T, Djonov V et al. (2006). "EphB4 Controls Blood Vascular Morphogenesis During Postnatal Angiogenesis." *EMBO Journal*. 2006 Feb; 25(3):628-41.

(55) Frangogiannis NG (2017). „The Role of Transforming Growth Factor (TGF)-beta in the Infarcted Myocardium." *Journal of Thoracic Disease*. 2017;9:S52–S63.

(56) Frangogiannis NG, Perrard JL, Mendoza LH, Burns AR, Linsey ML et al. (1998). "Stem Cell Factor Induction is Associated with Mast Cell Accumulation after Canine Myocardial Ischemia and Reperfusion." *Circulation*.1998 Aug 18;98(7):687-98.

(57) Fu H, Feng J, Liu Q, Sun F, Tie Y, et al. (2009). "Stress Induces tRNA Cleavage by Angiogenin in Mammalian Cells." *FEBS Letters*. 2009 Jan;583(2):437-42.

(58) Fukui S, Kitagawa-Sakakida S, Kawamata S, Matsumiya G, Kawaguchi N et al. (2008). "Therapeutic Effect of Midkine on Cardiac Remodeling in Infarcted Rat Hearts." *The Annals of Thoracic Surgery*. 2008 Feb;85:562–570.

(59) Füller T, Korff T, Kilian A, Dandekar G, Augustin HG (2003). "Forward EphB4 Signaling in Endothelial Cells Controls Cellular Repulsion and Segregation from EphrinB2 Positive Cells." *Journal of Cell Science*. 2003 Jun;116(Pt 12):2461-70.

(60) Gäbel R (2011). "Intramyokardiale Applikation humaner CD133/CD271 positiver Stammzellen nach Herzinfarkt am Mausmodell." Request for permission of animal experiments to the State Office for Agriculture, Food Safety, and fisheries. 2011 Okt.

(61) Gäbel R, Furlani D, Sorg H, Polchow B, Frank J et al. (2011). "Cell Origin of Human Mesenchymal Stem Cells Determines a Different Healing Performance in Cardiac Regeneration." *PLoS One*. 2011 Feb; 6(2):e15652.

(62) Gale NW, Baluk P, Pan L, Kwan M, Holash J et al. (2001). "Ephrin-B2 Selectively Marks Arterial Vessels and Neovascularization Sites in the Adult, with Expression in Both Endothelial and Smooth-Muscle Cells." *Developmental Biology*. 2001 Feb;230(2):151-60.

(63) Gao L, Yang L, Wang L, Geng Z, Wei Y et al. (2018). „Relationship Between the Efficacy of Cardiac Cell Therapy and the Inhibition of Differentiation of Human iPSC-Derived Nonmyocyte Cardiac Cells Into Myofibroblast-Like Cells." *Circulation Research.* 2018 Dec 7;123(12):1313-1325.

(64) Gao ZW, Dong K, Zhang HZ (2014). "The Roles of CD73 in Cancer." *BioMed Research International*. 2014;2014:460654.

(65) Gela A, Jovic S, Nordin SL, Egesten A (2014). "Midkine in Host Defense." *British Journal of Pharmacology*. 2014 Feb;171(4):859-69.

(66) Gerber PA, Hippe A, Buhren BA, Muller A, Homey B (2009). "Chemokines in Tumor-Associated Angiogenesis." *Journal of Biological Chemistry*. 2009 Dec;390(12):1213-23.

(67) Ghiringhelli F, Bruchard M, Chalmin F, Rébé C (2012). "Production of Adenosine by Ectonucleotidases: a Key Factor in Tumor Immunoescape." *Journal of Biomedicine and Biotechnology*. 2012;2012:473712.

(68) Grego-Bessa J, Luna-Zurita L, del Monte G, Bolós V, Melgar P et al. (2007). "Notch Signaling is Essential for Ventricular Chamber Development." *Developmental Cell*. 2007 Mar;12(3):415-29.

(69) Gridley T (2010). "Notch Signaling in the Vasculature." *Current Topics in Developmental Biology*. 2010;92:277-309.

(70) Haley KJ, Lilly CM, Yang JH, Feng Y, Kennedy SP, et al. (2000). "Overexpression of Eotaxin and the CCR3 Receptor in Human Atherosclerosis: Using Genomic Technology to Identify a Potential Novel Pathway of Vascular Inflammation." *Circulation.* 2000 Oct; 102: 2185–2189.

(71) Hamada K, Oike Y, Ito Y, Maekawa H, Miyata K et al. (2003). "Distinct Roles of Ephrin-B2 Forward and EphB4 Reverse Signaling in Endothelial Cells." *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2003 Feb;23(2):190-7.

(72) Han KH, Kim AK, Kim MH, Kim DH, Go HN, Kim DI (2016). "Enhancement of Angiogenic Effects by Hypoxia-Preconditioned Human Umbilical Cord-Derived Mesenchymal Stem Cells in a Mouse Model of Hindlimb Ischemia." *Cell Biology International*. 2016 Jan;40(1):27-35.

(73) Harada M, Hojo M, Kamiya K, Kadomatsu K, Murohara T et al. (2016). “Exogenous Midkine Administration Prevents Cardiac Remodeling in Pacing-Induced Congestive Heart Failure of Rabbits.” *Heart Vessels*. 2016 Jan;31(1):96-104.

(74) Hayashi S, Asahara T, Masuda H, Isner JM, Losordo DW (2005). “Functional Ephrin-B2 Expression for Promotive Interaction Between Arterial and Venous Vessels in Postnatal Neovascularization.” *Circulation*. 2005 May;111(17):2210-8. Epub 2005 Apr 25.

(75) Herold G, et al. (2012). *Innere Medizin*. Köln: Gerd Herold.

(76) Herrmann S (2012). “Hypoosmolar Preconditioning as a Means to Enhance CD271 Expression on Human Cells.” Bachelor Thesis in Medical Biotechnology at the University of Rostock. 2012 Jul.

(77) Heusch G, Schulz R (1996). “Hibernating Myocardium: a Review.“ *Journal of Molecular and Cellular Cardiology*. 1996 Dec; 28(12):2359-72.

(78) Honda Y, Shishido T, Takahashi T, Watanabe T, Netsu S et al. (2016). “Midkine Deteriorates Cardiac Remodeling via Epidermal Growth Factor Receptor Signaling in Chronic Kidney Disease.” *Hypertension*. 2016 May;67(5):857-65.

(79) Hong J, Lei Z, Ying Y, Ming L, Xuejuan J et al. (2014). “A Pilot Study of Angiogenin in Heart Failure With Preserved Ejection Fraction: a Novel Potential Biomarker for Diagnosis and Prognosis?” *Journal of Cellular and Molecular Medicine.* 2014 Nov; 18(11): 2189–2197.

(80) Horiba M, Kadomatsu K, Nakamura E, Muramatsu H, Ikematsu S et al. (2000). “Neointima Formation in a Restenosis Model is Suppressed in Midkine-Deficient Mice.” *Journal of Clinical Investigation*. 2000 Feb;105:489–495.

(81) Horiba M, Kadomatsu K, Yasui K, Lee JK, Takenaka H, et al. (2006). “Midkine Plays a Protective Role against Cardiac Ischemia/Reperfusion Injury through a Reduction of Apoptotic Reaction.” *Circulation*. 2006 Oct; 14(16):1713-20.

(82) Hue S, Kared H, Mehwish Y, Mouhamad S, Balbo M, Levy Y (2012). “Notch Activation on Effector T Cells Increases their Sensitivity to Treg Cell-Mediated Suppression through Upregulation of TGF-betaRII Expression.” *European* Journal of *Immunology.* 2012 Jul;42(7):1796-803.

(83) Hung SC, Chen NJ, Hsieh SL, Li H, Ma HL, Lo WH (2002). “Isolation and Characterization of Size-Sieved Stem Cells from Human Bone Marrow.” *Stem Cells*. 2002;20(3):249-58.

(84) Hung SC, Kuo PY, Chang CF, Chen TH, Ho LLT (2006). "Alpha-Smooth Muscle Actin Expression and Structure Integrity in Chondrogenesis of Human Mesenchymal Stem Cells. *Cell Tissue Research*. 2006;324:457–466.

(85) Ishii M, Mueller I, Nakajima T, Pasquale EB, Ogawa K (2011). "EphB Signaling Inhibits Gap Junctional Intercellular Communication and Synchronized Contraction in Cultured Cardiomyocytes." *Basic Research in Cardiology*. 2011 Nov; 106(6):1057-68.

(86) Ivanov P, Emara MM, Villen J, Gygi SP, Anderson P (2011). "Angiogenin-Induced tRNA Fragments Inhibit Translation Initiation." *Molecular Cell*. 2011 Aug;43(4):613-23.

(87) Jackson KA, Majka SM, Wang H, Pocius J, Hartley CJ, et al. (2001). "Regeneration of Ischemic Cardiac Muscle and Vascular Endothelium by Adult Stem Cells." *Journal of Clinical Investigation*. 2001 June; 107:1395–1402.

(88) Jain M, Pfister O, Hajjar RJ, Liao R (2005). "Mesenchymal Stem Cells in the Infarcted Heart." *Coronary Artery Disease*. 2005 March; 16(2): 93–97.

(89) Jerkic M, Rivas-Elena JV, Prieto M, Carron R, Sanz-Rodriguez F et al. (2004). "Endoglin Regulates Nitric Oxide-Dependent Vasodilatation." *FASEB Journal.* 2004;18: 609-611.

(90) Jiang XX, Chen JS, Su YF, Liao C, Liu B, Mao N (2007). ["Endothelial Cells Derived from Mesenchymal Stem Cells Harbor Immunoregulatory Effects." Article in Chinese]. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2007 Feb;15(1):175-8.

(91) Joffe SW, Webster K, McManus DD, Kiernan MS, Lessard D et al. (2013). "Improved Survival After Heart Failure: A Community-Based Perspective." *Journal of the American Heart Association*. 2013 June; 2(3): e000053.

(92) Jose PJ, Adcock IM, Griffiths-Johnson DA, Berkman N, Wells TN et al. (1994). "Eotaxin: Cloning of an Eosinophil Chemoattractant Cytokine and Increased mRNA Expression in Allergen-Challenged Guinea-Pig Lungs." *Biochemical and Biophysical Research Communications*. 1994 Nov;205(1):788-94.

(93) Júnior AM, Arrais CA, Saboya R, Velasques RD, Junqueira PL, Dulley FL (2008). "Neurotoxicity Associated with Dimethylsulfoxide-Preserved Hematopoietic Progenitor Cell Infusion." *Bone Marrow Transplantation*. 2008 Jan;41(1):95-6.

(94) Kadomatsu K, Bencsik P, Görbe A, Csonka C, Sakamoto K et al. (2014). “Therapeutic Potential of Midkine in Cardiovascular Disease.” *British Journal of Pharmacology*. 2014 Feb;171(4):936-44.

(95) Kao GS, Kim HT, Daley H, Ritz J, Burger SR, Kelley L et al. (2011.) “Validation of Short-Term Handling and Storage Conditions for Marrow and Peripheral Blood Stem Cell Products.” *Transfusion.* 2011 Jan;51:137–147; 2011.

(96) Kawada H, Fujita J, Kinjo K, Matsuzaki Y, Tsuma M et al. (2004). “Nonhematopoietic Mesenchymal Stem Cells Can be Mobilized and Differentiate Into Cardiomyocytes After Myocardial Infarction.” *Blood.* 2004 Dec; *104*(12):3581–3587.

(97) Kinner B, Zaleskas JM, Spector M (2002). “Regulation of Smooth Muscle Actin Expression and Contraction in Adult Human Mesenchymal Stem Cells.” *Experimental Cell Research.*2002 Aug 1;278(1):72-83.

(98) Kinnaird T, Stabile E, Burnett MS, Lee CW, Barr S, et al. (2004). “Marrow-Derived Stromal Cells Express Genes Encoding a Broad Spectrum of Arteriogenic Cytokines and Promote *in vitro* and *in vivo* Arteriogenesis Through Paracrine Mechanisms.” *Circulation Research*. 2004 March; 94 (5):678–685.

(99) Kishimoto K, Liu S, Tsuji T, Olson KA, Hu GF (2005). “Endogenous angiogenin in endothelial cells is a general requirement for cell proliferation and angiogenesis.” *Oncogene.* 2005 Jan; 24(3):445-56.

(100) Kitahara T, Shishido T, Suzuki S, Katoh S, Sasaki TJ et al. (2010). “Serum Midkine as a Predictor of Cardiac Events in Patients with Chronic Heart Failure.” *Journal of Cardiac Failure*. 2010 Apr;16(4):308-13.

(101) Klein D, Weisshardt P, Kleff V, Jastrow H, Jakob HG, Ergün S (2011). “Vascular Wall-Resident CD44+ Multipotent Stem Cells Give Rise to Pericytes and Smooth Muscle Cells and Contribute to New Vessel Maturation.” *PLoS One.* 2011;6(5):e20540.

(102) Kodali RB, Kim WJ, Galaria II, Miller C, Schecter AD et al. (2004). “CCL-11 (Eotaxin) Induces CCR3-dependent Smooth Muscle Cell Migration.” *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2004 July;24(7):1211-6.

(103) Korff T, Braun J, Pfaff D, Augustin HG, Hecker M (2008). “Role of EphrinB2 Expression in Endothelial Cells During Arteriogenesis: Impact on Smooth Muscle Cell Migration and Monocyte Recruitment.” *Blood.* 2008 Jul 1;112(1):73-81. doi: 10.1182/blood-2007-12-128835.

(104) Korff T, Dandekar G, Pfaff D, Füller T, Goettsch W et al. (2006). “Endothelial EphrinB2 is Controlled by Microenvironmental Determinants and Associates Context-dependently with CD31.” *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2006 Mar;26(3):468-74.

(105) Kubota Y, Kawa Y, Mizoguchi M (1996). “CDw49b/CD29 Integrin Complex Mediates the Differentiation of Human Endothelial Cells into Capillary-Like Structures *in vitro*.” *Journal of Dermatological Science*. 1996 Apr;12(1):36-43.

(106) La Porta S, Roth L, Singhal M et al. (2018). “Endothelial Tie1-Mediated Angiogenesis and Vascular Abnormalization Promote Tumor Progression and Metastasis.” *Journal of Clinical Investigation*. 2018 Feb 1;128(2):834-845.

(107) Lasky LC, McCullough J, Zanjani ED (1986). “Liquid Storage of Unseparated Human Bone Marrow. Evaluation of Hematopoietic Progenitors by Clonal Assay.” *Transfusion*. 1986 Jul-Aug;26(4):331-4.

(108) Lecht S, Foerster C, Arien-Zakay H, Marcinkiewicz C, Lazarovici P, Lelkes PI (2010). “Cardiac Microvascular Endothelial Cells Express and Release Nerve Growth Factor But Not Fibroblast Growth Factor-2.” *In vitro Cellular & Developmental Biology – Animal*. 2010 May*;* 46(5), 469–476.

(109) Lemcke H, Gaebel R, Skorska A, Voronina N, Lux CA, Petters J, Sasse S et al. (2017).“Mechanisms of Stem Cell Based Cardiac Repair-Gap Junctional Signaling Promotes the Cardiac Lineage Specification of Mesenchymal Stem Cells.” Scientific Reports. 2017 Aug 29;7(1):9755.

(110) Levy D, Kenchaiah S, Larson MG, Benjamin EJ, Kupka MJ et al. (2002). “Long-Term Trends in the Incidence of and Survival with Heart Failure” The Framingham Heart Study, *The New England Journal of Medicine*. 2002 Oct; 347(18):1397-402.

(111) Li C, Hampson IN, Hampson L, Kumar P, Bernabeu C, Kumar S (2000). “CD105 Antagonizes the Inhibitory Signaling of Transforming Growth Factor β1 on Human Vascular Endothelial Cells.” *FASEB Journal*. 2000;14*:* 55-64.

(112) Li DY, Sorensen LK, Brooke BS, Urness LD, Davis EC et al. (1999). “Defective Angiogenesis in Mice Lacking Endoglin.” *Science*. 1999;284: 1534-153.

(113) Li J, Brown LF, Hibberd MG, Grossman JD, Morgan JP et al. (1996). „VEGF, flk-1, and flt-1 Expression in a Rat Myocardial Infarction Model of Angiogenesis.“ *American Journal of Physiology*. 1996 May;270(5 Pt 2):H1803-11.

(114) Li JL, Lim CH, Tay FW, Goh CC, Devi S et al. (2016). “Neutrophils Self-Regulate Immune Complex-Mediated Cutaneous Inflammation through CXCL2.” *Journal of Investigative Dermatology*. 2016 Feb;136(2):416-24.

(115) Li TS, Ito H, Hayashi M, Furutani A, Matsuzaki M, Hamano K (2005). “Cellular Expression of Integrin-beta 1 is of Critical Importance for Inducing Therapeutic Angiogenesis by Cell Implantation.” Cardiovascular Research. 2005 Jan 1;65(1):64-72.

(116) Limbourg A, Ploom M, Elligsen D, Sörensen I, Ziegelhoeffer T, et al. (2007). “Notch Ligand Delta-like 1 is Essential for Postnatal Arteriogenesis.” *Circulation Research*. 2007 Feb;100(3):363-71.

(117) Liu G, Li L, Huo D, Li Y, Wu Y et. Al. (2017). “A VEGF Delivery System Targeting MI Improves Angiogenesis and Cardiac Function Based on the Tropism of MSCs and Layer-by-Layer Self-assembly.” *Biomaterials.* 2017 May;127:117-131.

(118) Liu P, Zhou B, Gu D, Zhang L, Han Z (2009). “Endothelial Progenitor Cell Therapy in Atherosclerosis: a Double-Edged Sword?” *Ageing Research Reviews*. 2009 Apr;8(2):83-93.

(119) Löffler G, Petrides PE, Heinrich PC (2007) [1975]. *Biochemie & Pathobiochemie*. 8th edition, Heidelberg: Springer Medizin Verlag.

(120) Lu X, Dunn J, Dickinson AM., Gillespie JI, Baudouin SV (2004). „Smooth Muscle α-actin Expression in Endothelial Cells Derived from CD34+ Human Cord Blood Cells.” Stem Cells and Development. 2004;13:521–527.

(121) Lunde K, Solheim S, Aakhus S, Arnesen H, Abdelnoor M, Forfang K (2005). “Autologous Stem Cell Transplantation in Acute Myocardial Infarction: The ASTAMI Randomized Controlled Trial. Intracoronary Transplantation of Autologous Mononuclear Bone Marrow Cells, Study Design and Safety Aspects.” *Scandinavian Cardiovascular Journal*. 2005 Jul;39(3):150-8.

(122) Lux CA, Mark P, Klopsch C, Laupheimer M, Tu-Rapp H et al. (2015). “Impact of Short-Term Liquid Storage on Human CD133+ Stem Cells.” *Cell Transplantation*. 2015;24(11):2409-22.

(123) Makino S, Fukuda K, Miyoshi S, Konishi F, Kodama H, et al. (1999). “Cardiomyocytes Can be Generated from Marrow Stromal Cells *in vitro*.” *Journal of Clinical Investigation*. 1999 March; 103:697–705.

(124) Makkar RR, Price MJ, Lill M, Frantzen M, Takizawa K et al. (2005). “Intramyocardial Injection of Allogenic Bone Marrow-Derived Mesenchymal Stem Cells Without Immunosuppression Preserves Cardiac Function in a Porcine Model of Myocardial Infarction.” *Journal of Cardiovascular Pharmacololgy and Therapeutics*. 2005 Dec; 10(4), 225–233.

(125) Mamidi MK, Nathan KG, Singh G, Thrichelvam ST, Mohd Yusof NA et al. (2012). “Comparative Cellular and Molecular Analyses of Pooled Bone Marrow Multipotent Mesenchymal Stromal Cells during Continuous Passaging and after Successive Cryopreservation.” *Journal of Cellular Biochemistry*. 2012 Oct;113(10):3153-64.

(126) Mansson-Broberg A, Siddiqui AJ, Genander M, Grinnemo KH, Hao X et al. (2008). “Modulation of EphrinB2 Leads to Increased Angiogenesis in Ischemic Myocardium and Endothelial Cell Proliferation.” *Biochemical and Biophysical Research Communications*. 2008 Aug; ;373(3):355-9.

(127) Mark P, Kleinsorge M, Gaebel R, Lux CA, Toelk A et al. (2013). “Human Mesenchymal Stem Cells Display Reduced Expression of CD105 after Culture in Serum-Free Medium.” *Stem Cells International*. 2013 Sep;2013:698076.

(128) Meloni M, Caporali A, Graiani G, Lagrasta C, Katare R, et al. (2010). “Nerve Growth Factor Promotes Cardiac Repair Following Myocardial Infarction.” *Circulation Research*. 2010 Apr; *106* (7):1275–1284.

(129) Moroianu J, Riordan JF (1994). “Nuclear Translocation of Angiogenin in Proliferating Endothelial Cells is Essential to its Angiogenic Activity.” *Proceedings of the National Academy of Sciences of the USA*. 1994 Mar;91(5):1677-81.

(130) Mozaffarian D, Benjamin EJ, Go AS, Donna DK, Blaha MJ et al. (2015). “Heart Disease and Stroke Statistics – 2015 Update, a Report From the American Heart Association.” *Circulation*. 2015 Jan; 131:00-00.

(131) Mülhardt C (2009). *Der Experimentator- Molekularbiologie/ Genomics*. 6th edition, Heidelberg: Springer Verlag.

(132) Muramatsu T, Kadomatsu K (2014). “Midkine: an Emerging Target of Drug Development for Treatment of Multiple Diseases.” *British Journal of Pharmacology*. 2014 Feb;171(4):811-3.

(133) Murry CE, Soonpaa MH, Reinecke H, Nakajima H, Nakajima HO et al. (2004). “Haematopoietic Stem Cells Do Not Transdifferentiate Into Cardiac Myocytes in Myocardial Infarcts.” *Nature*. 2004 April;428: 664–668.

(134) Nassiri F, Cusimano MD, Scheithauer BW, Rotondo F, Fazio A et al. (2011). "Endoglin (105): a Review of its Role in Angiogenesis and Tumor Diagnosis, Progression and Therapy." *Anticancer Research*. 2011 Jun;31(6):2283-90.

(135) Nesselmann C, Ma N, Bieback K, Wagner W, Ho A et al. (2008). "Mesenchymal Stem Cells and Cardiac Repair." *Journal of Cellular and Molecular Medicine*. 2008 Oct; 12:1795- 810.

(136) Nesteruk J, Voronina N, Kundt G, Donndorf P, Klopsch C (2017). "Stem Cell Registry Programme for Patients with Ischemic Cardiomyopathy Undergoing Coronary Artery Bypass Grafting: What Benefits Does it Derive?" *ESC Heart Failure*. 2017 May;4(2):105-111.

(137) Noto K, Kato K, Okumura K, Yagita H (1995). "Identification and Functional Characterization of Mouse CD29 with a mAb." *International Immunology*. 1995 May;7(5):835-42.

(138) Obama H, Biro S, Tashiro T, Tsutsui J, Ozawa M et al. (1998). "Myocardial Infarction Induces Expression of Midkine, a Heparin-Binding Growth Factor with Reparative Activity." *Anticancer Research*. 1998 Jan-Feb;18(1A):145-52.

(139) Ong LL, Li W, Oldigs JK, Kaminski A, Gerstmayer B et al. (2010). "Hypoxic/ Normoxic Preconditioning Increases Endothelial Differentiation Potential of Human Bone Marrow CD133+ cells." *Tissue Engineering Part C: Methods*. 2010 Oct;16(5):1069-81.

(140) Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, et al. (2001). "Bone Marrow Cells Regenerate Infarcted Myocardium." *Nature*. 2001 April; 410:701–705.

(141) Pan SC, Wu LW, Chen CL, Shieh SJ, Chiu HY *(2012).* "Angiogenin Expression in Burn Blister Fluid: Implications for its Role in Burn Wound Neovascularization." *Wound Repair and Regeneration.*2012, 20(5):731–739.

(142) Patel AN, Geffner L, Vina RF, Saslavsky J, Urschel HC Jr et al. (2005). "Surgical Treatment for Congestive Heart Failure with Autologous Adult Stem Cell Transplantation: a Prospective Randomized Study." *The Journal of Thoracic and Cardiovascular Surgery.* 2005 Dec; 130(6):1631-8.

(143) Patella V, Marinò I, Arbustini E, Lamparter-Schummert B, Verga L et al. (1998). "Stem Cell Factor in Mast Cells and Increased Mast Cell Density in Idiopathic and Ischemic Cardiomyopathy". *Circulation.* 1998 Mar;97(10):971-8.

(144) Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, et al. (1999). "Multilineage Potential of Adult Human Mesenchymal Stem Cells." *Science.* 1999 April; *284*, 143–147.

(145) Przybylowski P, Malyszko J, Malyszko JS (2010). "Serum Midkine is Related to NYHA Class and Cystatin C in Heart Transplant Recipients." *Transplantation Proceedings*. 2010 Nov;42(9):3704-7.

(146) Qi M, Ikematsu S, Ichihara-Tanaka K, Sakuma S, Muramatsu T, Kadomatsu K (2000). "Midkine Rescues Wilms' Tumor Cells from Cisplatin-Induced Apoptosis: Regulation of Bcl-2 Expression by Midkine." *Journal of Biochemistry*. 2000 Feb;127:269–277.

(147) Quaini F, Urbanek K, Graiani G, Lagrasta C, Maestri R, et al. (2004). "The Regenerative Potential of the Human Heart." *International Journal of Cardiology*. 2004 June;95 1:S26–28.

(148) Quevedo HC, Hatzistergos KE, Oskouei BN, Feigenbaum GS, Rodriguez JE et al. (2009). "Allogeneic Mesenchymal Stem Cells Restore Cardiac Function in Chronic Ischemic Cardiomyopathy via Trilineage Differentiating Capacity." Proceedings of the National Academy of Sciences. 2009 Aug; 106(33): 14022–14027.

(149) Qian H, Le Blanc K, Sigvardsson M (2012). "Primary Mesenchymal Stem and Progenitor Cells from Bone Marrow lack Expression of CD44 Protein." *Journal of Biological Chemistry*. 2012 Jul;287(31):25795-807.

(150) Quirici N, Soligo D, Bossolasco P, Servida F, Lumini C, Deliliers GL (2002). "Isolation of Bone Marrow Mesenchymal Stem Cells by Anti-Nerve Growth Factor Receptor Antibodies." *Experimental Hematology.* 2002 July; 30(7):783-91.

(151) Rafii S, Lyden D (2003). „Therapeutic Stem and Progenitor Cell Transplantation for Organ Vascularization and Regeneration." Nature Medicine. 2003 June; 9(6):702-12.

(152) Rahimtoola SH (1993). "The Hibernating Myocardium in Ischemia and Congestive Heart Failure." *European Heart Journal*. 1993 July; 14 Suppl A:22-6.

(153) Ramos A, Ho WC, Forte S, Dickson K, Boutilier J et al. (2007). "Hypo-osmolar Stress Induces p75NTR Expression by Activating Sp1-Dependent Transcription." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*. 2007 Feb 7;27(6):1498-506.

(154) Sadraddin H, Gaebel R, Ahmad B, Skorska A, Lux CA, Sasse S, Vasudevan P, Steinhoff G, David R. "CD271^{+} Human Mesenchymal Stem Cells Show

Antiarrhythmic Effects in a Novel Murine Infarction Model." *Submitted to Biomaterials.*

(155) Salcedo R, Young HA, Ponce ML, Ward JM, Kleinman HK et al. (2001). "Eotaxin (CCL11) Induces *in vivo* Angiogenic Responses by Human CCR3+ Endothelial Cells." *Journal of Immunology*. 2001 Jun;166:7571–7578.

(156) Sánchez-Elsner T, Botella LM, Velasco B, Langa C, Bernabéu C (2002). "Endoglin Expression is Regulated by Transcriptional Cooperation Between the Hypoxia and Transforming Growth Factor-beta Pathways." *Journal of Biological Chemistry*. 2002 Nov 15;277(46):43799-808.

(157) Sánchez-Guijo F, Caballero-Velázquez T, López-Villar O, Redondo A, Parody R et al. (2014). "Sequential Third-Party Mesenchymal Stromal Cell Therapy for Refractory Acute Graft-Versus-Host Disease." *Biology of Blood and Marrow Transplantation*. 2014 Oct; 20(10):1580-5.

(158) Sartor M, Antonenas V, Garvin F, Webb M, Bradstock KF (2005). "Recovery of Viable $CD34^+$ Cells from Cryopreserved Hemopoietic Progenitor Cell Products." *Bone Marrow Transplantation*. 2005 Aug;36(3):199-204.

(159) Savant S, La Porta S, Budnik A et al. (2015). "The Orphan Receptor Tie1 Controls Angiogenesis and Vascular Remodeling by Differentially Regulating Tie2 in Tip and Stalk Cells." *Cell Reports*. 2015;12(11):1761–1773.

(160) Schächinger V, Erbs S, Elsässer A, Haberbosch W, Hambrecht R et al. (2006). "Intracoronary Bone Marrow-Derived Progenitor Cells in Acute Myocardial Infarction." *New England Journal of Medicine.* 2006 Sep;355(12):1210-21.

(161) Scharfenberg D (2009). "Protokoll Analyse der Genexpression mittels Real-Time PCR." University of Rostock. 2009 Feb.

(162) Schinkel AF, Valkema R, Geleijnse ML, Sijbrands EJ, Poldermans D (2010). "Single-Photon Emission Computed Tomography for Assessment of Myocardial Viability." *EuroIntervention*. 2010 May;6 Suppl G:G115-22.

(163) Schmidt RF, Lang F, Heckmann M et al. (2010) [1936]. *Physiologie des Menschen mit Pathophysiologie.* 31st edition, Heidelberg: Springer Medizin Verlag.

(164) Sebastià J, Kieran D, Breen B, King MA, Netteland DF et al. (2009). "Angiogenin Protects Motoneurons Against Hypoxic Injury." *Cell Death & Differentiation*. 2009 Sep; 16(9):1238-47.

(165) Seeger FH, Tonn T, Krzossok N, Zeiher AM, Dimmeler S (2007). "Cell Isolation Procedures Matter: a Comparison of Different Isolation Protocols of Bone Marrow

Mononuclear Cells used for Cell Therapy in Patients with Acute Myocardial Infarction.” *European Heart Journal*. 2007 Mar;28(6):766-72.

(166) Shekhonin BV, Guriev SB, Irgashev SB, Koteliansky VE (1990). “Immunofluorescent Identification of Fibronectin and Fibrinogen/Fibrin in Experimental Myocardial Infarction.” *Journal of Molecular and Cellular Cardiology*. 1990 May;22(5):533-41.

(167) Sheng J, Xu Z (2016). “Three Decades of Research on Angiogenin: a Review and Perspective.” *Acta Biochimica* et *Biophysica Sinica.* 2016 May;48(5):399-410.

(168) Shin D, Garcia-Cardena G, Hayashi SI, Gerety S, Asahara T et al. (2001). “Expression of EphrinB2 Identifies a Stable Genetic Difference between Arterial and Venous Vascular Smooth Muscle as well as Endothelial Cells, and Marks Subsets of Microvessels at Sites of Adult Neovascularization.” *Developmental Biology*. 2001 Feb;230(2):139-50.

(169) Stamm C, Westphal B, Kleine HD, Petzsch M, Kittner C et al. (2003). “Autologous Bone-Marrow Stem-Cell Transplantation for Myocardial Regeneration.” *Lancet.* 2003 Jan; 361, 45-46.

(170) Stewart S, MacIntyre K, Hole DJ, Capewell S, McMurray JJ (2001). “More 'Malignant' than Cancer? Five-Year Survival Following a First Admission for Heart Failure.” *European Journal of Heart Failure*. 2001 June; 3(3):315-22.

(171) Strauer BE, Brehm M, Zeus T, Köstering M, Hernandez A et al. (2002). “Repair of Infarcted Myocardium by Autologous Intracoronary Mononuclear Bone Marrow Cell Transplantation in Humans.” *Circulation.* 2002 Oct; 106(15):1913-8.

(172) Strieter RM, Polverini PJ, Arenberg DA, Kunkel SL (1995). “The Role of CXC Chemokines as Regulators of Angiogenesis.” *Shock*. 995 Sep;4(3):155-60.

(173) Stroncek DF, Fautsch SK, Lasky LC, Hurd DD, Ramsay NK, McCullough J (1991). “Adverse Reactions in Patients Transfused with Cryopreserved Marrow.” *Transfusion.* 1991 Jul-Aug;31(6):521-6.

(174) Sumi Y, Muramatsu H, Takei Y, Hata K, Ueda M, Muramatsu T (2002). “Midkine, a Heparin-Binding Growth Factor, Promotes Growth and Glycosaminoglycan Synthesis of Endothelial Cells through its Action on Smooth Muscle Cells in an Artificial Blood Vessel Model.” *Journal of Cell Science*. 2002 Jul;115(Pt 13):2659-67.

(175) Sumida A, Horiba M, Ishiguro H, Takenaka H, Ueda N et al. (2010). “Midkine Gene Transfer after Myocardial Infarction in Rats Prevents Remodeling and

Ameliorates Cardiac Dysfunction." *Cardiovascular Research*. 2010 Apr 1;86(1):113-21.

(176) Sutherland DR, Anderson L, Keeney M, Nayar R, Chin-Yee I (1996). "The ISHAGE Guidelines for CD34+ Cell Determination by Flow Cytometry. International Society of Hematotherapy and Graft Engineering." *Journal of Hematotherapy*. 1996 June;5(3): 213-26.

(177) Takenaka H, Horiba M, Ishiguro H, Sumida A, Hojo M et al. "Midkine Prevents Ventricular Remodeling and Improves Long-Term Survival after Myocardial Infarction." *American Journal of Physiology - Heart and Circulatory Physiology*. 2009 Feb;296:H462–H469.

(178) Tarzami ST, Cheng R, Miao W, Kitsis RN, Berman JW (2002). "Chemokine Expression in Myocardial Ischemia: MIP-2 Dependent MCP-1 Expression Protects Cardiomyocytes from Cell Death." *Journal of Molecular and Cellular Cardiology*. 2002 Feb;34(2):209-21.

(179) Tello-Montoliu A, Marín F, Patel J, Roldán V, Mainar L et al. (2007). "Plasma Angiogenin levels in Acute Coronary Syndroms: Implications for Prognosis." *European Heart Journal*. 2007 Dec; 28(24):3006-11.

(180) Tijore A, Hariharan S, Yu H, Lam CR, Wen F et al. "Investigating the Spatial Distribution of Integrin β_1 in Patterned Human Mesenchymal Stem Cells Using Super-Resolution Imaging." ACS Applied Materials & Interfaces. 2014 Sep 24;6(18):15686-96.

(181) Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD (2002). "Human Mesenchymal Stem Cells Differentiate to a Cardiomyocyte Phenotype in the Adult Murine Heart." *Circulation.* 2002 Jan; 105:93–98.

(182) Trouillon R, Kang DK, Park H, Chang SI, O'Hare D (2010). "Angiogenin Induces Nitric Oxide Synthesis in Endothelial Cells Through PI-3 and Akt Kinases." *Biochemistry*. 2010 Apr, 49: 3282–3288.

(183) Urbani S, Caporale R, Lombardini L, Bosi A, Saccardi R (2006). "Use of CFDA-SE for Evaluating the *in vitro* Proliferation Pattern of Human Mesenchymal Stem Cells." *Cytotherapy*. 2006;8(3):243-53.

(184) Van der Horst EH, Frank BT, Chinn L, Coxon A, Li S et al. (2008). "The Growth Factor Midkine Antagonizes VEGF Signaling *in vitro* and *in vivo*." *Neoplasia*. 2008 Apr;10(4):340-7.

(185) Vered M, Shnaiderman-Shapiro A, Zlotogorski-Hurvitz A, Salo T, Yahalom R (2019). “Cancer-associated Fibroblasts in the Tumor Microenvironment of Tongue Carcinoma is a Heterogeneous Cell Population. Acta Histochemica. 2019;121:151446.

(186) Vihanto MM, Plock J, Erni D, Frey BM, Frey FJ, Huynh-Do U (2005). “Hypoxia Up-Regulates Expression of Eph Receptors and Ephrins in Mouse Skin.” *FASEB Journal*. 2005 Oct;19(12):1689-91.

(187) Vogel W, Grünebach F, Messam CA, Kanz L, Brugger W, Bühring HJ (2003). “Heterogenity Among Human Bone Marrow-Derived Mesenchymal Stem Cells and Neural Progenitor Cells.” *Haematologica*. 2003 Feb;88(2):126-33.

(188) Wang HU, Chen ZF, Anderson DJ (1998). “Molecular Distinction and Angiogenic Interaction Between Embryonic Arteries and Veins Revealed by Ephrin-B2 and its Receptor Eph-B4.” *Cell*. 1998; 93:741–753.

(189) Wang L, Tang S, Wang Y, Xu S, Yu J et al. (2013). “Ecto-5'-Nucleotidase (CD73) Promotes Tumor Angiogenesis.” *Clinical &Experimental Metastasis*. 2013 June;30(5):671-80.

(190) Wang XQ, Duan XM, Liu LH, Fang YQ, Tan Y (2005). “Carboxyfluorescein Diacetate Succinimidyl Ester Fluorescent Dye for Cell Labeling.” *Acta Biochimica et Biophysica Sinica (Shanghai)*. 2005 Jun; 37(6):379-85.

(191) Wang Y, Thorin E, Luo H, Tremblay J, Lavoie JL et al. (2015). “EPHB4 Protein Expression in Vascular Smooth Muscle Cells Regulates Their Contractility, and EPHB4 Deletion Leads to Hypotension in Mice.” *The Journal of Biological Chemistry*. 2015 May;290(22):14235-44.

(192) Wang Z, Cohen K, Shao Y, Mole P, Dombkowski D, Scadden DT (2004). “Ephrin Receptor, EphB4, Regulates ES Cell Differentiation of Primitive Hemangioblasts, Blood, Cardiomyocytes, and Blood Vessels” *Blood*. 2004 Jan; 103(1):100-9.

(193) Weckbach LT, Groesser L, Borgolte J, Pagel JI, Pogoda F et al. (2012). “Midkine Acts as Proangiogenic Cytokine in Hypoxia-Induced Angiogenesis.” *American Journal of Physiology – Heart and Circulatory Physiology*. 2012 Aug;303(4):H429-38.

(194) Williams JL, Weichert A, Zakrzewicz A, Da Silva-Azevedo L, Pries AR et al. (2006). “Differential Gene and Protein Expression in Abluminal Sprouting and Intraluminal Splitting Forms of Angiogenesis.” *Clinical Science (London).* 2006 May;110(5):587-95.

(195) Wollert KC, Meyer GP, Lotz J, Ringes-Lichtenberg S, Lippolt P et al. (2004). “Intracoronary Autologous Bone-Marrow Cell Transfer after Myocardial Infarction: the BOOST Randomized Controlled Clinical trial.” *Lancet.* 2004 July;364(9429):141-8.

(196) Xu ZP, Tsuji T, Riordan JF, Hu GF (2002). “The Nuclear Function of Angiogenin in Endothelial Cells is Related to rRNA Production.” *Biochemical and Biophysical Research Communications*. 2002 Jun;294(2):287-92.

(197) Xu ZP, Tsuji T, Riordan JF, Hu GF (2003). “Identification and Characterization of an Angiogenin-Binding DNA Sequence that Stimulates Luciferase Reporter Gene Expression.” *Biochemistry*. 2003 Jan;42(1):121-8.

(198) Yang Y, Shi C, Hou X, Zhao Y, Chen B et al. (2015). “Modified VEGF Targets the Ischemic Myocardium and Promotes Functional Recovery after Myocardial Infarction.” *Control Release*. 2015 Sep 10;213:27-35.

(199) Yoon YS, Park JS, Tkebuchava T, Luedeman C, Losordo DW (2004). “Unexpected Severe Calcification after Transplantation of Bone Marrow Cells in Acute Myocardial Infarction.” *Circulation*. 2004 June; 109 (25): 3154–3157.

(200) Yusuf S, et al. (1991). “Effect of Enalapril on Survival in Patients with Reduced Left Ventricular Ejection Fractions and Congestive Heart Failure.” *The New England Journal of Medicine*. 1991 Aug; 325(5):293-302.

(201) Zhang XQ, Takakura N, Oike Y, Inada T, Gale NW, et al. (2001). “Stromal Cells Expressing Ephrin-B2 Promote the Growth and Sprouting of Ephrin-B2(+) Endothelial Cells.” *Blood*. 2001 Aug;98(4):1028-37.

(202) Zhu H, Jiang X, Li X, Hu M, Wan W et al. (2016). „Intramyocardial Delivery of VEGF165 via a Novel Biodegradable Hydrogel Induces Angiogenesis and Improves Cardiac Function after Rat Myocardial Infarction.” *Heart and Vessels*. 2016 Jun;31(6):963-75.

(203) Zipper H, Brunner H, Bernhagen J, Vitzthum F (2004). “Investigations on DNA Intercalation and Surface Binding by SYBR Green I, its Structure Determination and Methodological Implications.” *Nucleic Acids Research: Oxford Journals*. 2004 Jul; 32(12):e103.

(204) Zweifel M, Matozan K, Dahinden C, Schaffner T, Mohacsi P (2010). “Eotaxin/CCL11 Levels Correlate with Myocardial Fibrosis and Mast Cell Density in Native and Transplanted Rat Hearts.” *Transplantation Proceedings*. 2010 Sep;42(7):2763-6.

Websides:

(205) World Health Organization (WHO) www.who.int. Fact Sheet The top 10 causes of death.
Copyright © WHO 2018
URL (01.02.2018):
http://www.who.int/mediacentre/factsheets/fs310/en/

(206) World Health Organization (WHO) www.who.int. Fact Sheet Cardiovascular diseases (CVDs). Copyright © WHO 2018
URL (01.02.2018):
http://www.who.int/mediacentre/factsheets/fs317/en/

List of abbreviations

Annotation: In different studies and sources abbreviations of genes can be found in capital letters (e. g. VEGF) or small letters (e. g. vegf). At this, capital letters refer to human gene expression whereas small letters represent the same gene in mice, which are the most common test subjects.

α-SMA: alpha smooth muscle actin
A_{260}: absorbance at 260nm
ACE: angiotensin-converting-enzyme
ACS: acute coronary syndrome
ACTA: alpha smooth muscle actin
AF488: Alexa fluor 488, fluorophore
aFGF: acidic fibroblast growth factor
AMP: adenosine monophosphate
Ang: angiogenin
APC: allophycocyanin fluorophore
ASTAMI trial: Autologous Stem cell Transplantation in Acute Myocardial Infarction trial
bFGF: basic fibroblast growth factor
BM: bone marrow
BSA: bovine serum albumin
C_H: constant region of an antibody heavy chain
C_L: constant region of an antibody light chain
C_t: threshold cycle
CABG: coronary artery bypass grafting
Calmodulin: calcium modulated protein
CaMKII: Ca^{2+}/calmodulin-dependent protein kinase II
Ccl: chemokine (C-C motif) ligand
CCR: chemokine receptor
CD: cluster of differentiation
CD41: Integrin alpha(IIb)
CD51: see Itgav
CD61: see Itgb3
CD133$^+$: stem cell fraction being positive for the cluster of differentiation No. 133
CD271$^+$: stem cell fraction being positive for the cluster of differentiation No. 271

Cdh5: cadherin 5

cDNA: complementary deoxyribonucleic acid

CFDA-SE: carboxyfluorescein diacetate succinimidyl ester, dye

CFU-EC: colony- forming units- endothelial cells

CFU-F: colony-forming-unit fibroblast

CFSE: carboxyfluorescein succinimidyl ester

cGMP: cyclic guanosine monophosphate

CIBIS-II: Cardiac Insufficiency Bisoprolol Trial II; a study concerning beta blocker therapy

Col18a1: collagen, type XVIII, alpha 1

Col4a3: collagen, typ IV, alpha 3

CONSENSUS: Cooperative North Scandinavian Enalapril Survival Study

COPERNICUS: Carvedilol Prospective Randomized Cumulative Survival Study

COX: cyclooxygenase

CPC: cardiac progenitor cell

CRT: cardiac resynchronisation therapy

Csf3: (granulocyte) colony stimulating factor 3

Ctgf: connective tissue growth factor

CVP: central venous pressure

Cxcl: chemokine (C-X-C motif) ligand

ΔR_n: represents the fluorescence intensity during PCR amplification

DAPI: 4',6-diamidino-2-phenylindole, fluorescent stain

DEPC: Diethylpyrocarbonate

Dll4: delta-like 4

DNA: deoxyribonucleic acid

dNTPs: nucleoside triphosphates

dsDNA: double stranded deoxyribonucleic acid

dt: thymine oligonucleotidic primer sequence

DTT: dithiotheitol, reducing agent

EAM: experimental autoimmune myocarditis

EBM: endothelial base medium

EC: endothelial cells

ECM: extracellular matrix

Edn1: endothelin 1

EDTA: ethylenediaminetetraacetic acid

EDV: end diastolic volume

Efna1: ephrin A1

Efnb2: ephrin B2

e. g.: for example

EGF: epidermal growth factor

EGM: endothelial growth medium

Egr1: *early growth response factor 1*

Eng: endoglin

eNOS: endothelial nitric oxide synthase

Epas1: endothelial Per-Arnt-Sim domain protein 1

EPC: endothelial progenitor cell

EphB4: ephrin type B receptor 4

Erbb2: erb-b2 receptor tyrosine kinase 2

ERK: endothelial cell mitogen-activated protein (MAP) kinase

ESPVR: end systolic pressure-volume relation

F2: coagulation factor II/ Thrombin

F3: coagulation factor III

F2R: *coagulation factor II receptor*

Fab: fragment antigen binding

FACS: fluorescence- activated cell sorting

FAK: focal adhesion kinase

FBS: fetal bovine serum

Fc: crystallizable fragment

FCR: crystallizable fragment receptor on the cell surface

FGF: fibroblast growth factor

Fgfr: fibroblast growth factor receptor

Figf: vascular endothelial growth factor D (Vegf-d)

FITC: fluorescein isothiocyanate

Flt1: vascular growth factor receptor 1 (Vegfr1)

FMO: fluorescence minus one

Fn1: Fibronectin 1

FRET: Förster resonance energy transfer

FSC: forward scatter

GA: gentamicin sulfate with amphotericin B

GC: guanylate cyclase

G-CSF: granulocyte colony- stimulating factor

GDC: genomic DNA control

GOI: gene of interest

GP: glycoprotein

GPI: glycosylphosphatidylinositol

Gro: growth-regulated protein

GTP: guanosine triphosphate

h: human

H: heavy chain (of antibodies)

Hgf: hepatocyte growth factor

Hif1a: hypoxia inducible factor 1, alpha subunit

HKG: housekeeping genes

HSPG: heparan sulfate proteoglycan

HUVEC: human umbilical vein endothelial cells

ICAM: *intercellular adhesion molecule*

ICD: implantable cardioverter-defibrillator

i.e.: that means

Ifng: interferon gamma

IGF: insulin-like growth factor

IHC: immunohistochemistry

IL: interleukin

Il1b: interleukin 1 beta

Il6: interleukin 6

i.p.: intraperitoneal

Itgav: integrin alpha v, CD51

Itgb3: integrin beta 3, CD61

Jag1: jagged 1

K: potassium

Kdr: vascular growth factor receptor 2 (Vegfr2)

L: light chains (of antibodies)

LAD: left anterior descendent artery

Lect1: leukocyte cell derived chemotaxin 1

Lep: leptin

LNGFR: low affinity nerve growth factor receptor

LSM: lymphocyte separation medium

MACS: magnetic cell separation

Madh5: mothers against decapentaplegic homologue 5

Mapk14: mitogen-activated protein kinase 14

MCP1: monocyte chemoattractant protein 1

Mdk: midkine

MEK: mitogen-activated protein kinase kinase

MERIT-HF: Metoprolol Randomized Intervention Trial in Congestive Heart Failure

MIC: myocardial infarction control

MK-AS: antisense oligonucleotide targeting midkine

Mmp: matrix metallopeptidase

MNC: mononuclear cells

MRI: magnetic resonance imaging

mRNA: messenger ribonucleic acid

MSC: mesenchymal stem cell

MSCGM: mesenchymal stem cell growth medium

MSC BM: mesenchymal stem cell basal medium

MSC GS: mesenchymal stem cell growth supplement

Na: sodium

Nrp2: neuropilin 2

NGF: nerve growth factor

NGFR: nerve growth factor receptor

NMP: nucleoside monophosphate

No.: number

NO: nitric oxide

Nos3: endothelial cell nitric oxide synthase 3 (see also eNOS)

NT5E: 5'-ectonucleotidase or CD73

NTC: no-template-control

NTP: nucleoside triphosphate

NTR: neurotrophin receptor

NYHA: New York Heart Association

p.: page

PAI-1: *plasminogen activator inhibitor 1*

PAOD: peripheral arterial occlusive disease

PAR: protease-activated receptor

PAS: Per-Arnt-Sim

PBS: phosphate buffered saline

PBSC: peripheral blood stem cell

PCR: polymerase chain reaction

PDGF: *platelet-derived growth factor*

PDGF-Rβ: platelet derived growth factor receptor beta

PE: phycoerythrin fluorophore

PerCP-Cy5.5: Peridinin chlorophyll protein-Cyanine 5.5 tandem dye

PERFECT- study: Intramyocardial Trans**P**lantation of Bon**E** Ma**R**row Stem Cells **F**or Improv**E**ment of Post-Infarct Myo**C**ardial Regenera**T**ion in Addition to CABG-Surgery

PI: propidium iodide, fluorescent stain

PKC: protein kinase C

Pdgfa: platelet derived growth factor alpha

Pecam1: platelet/ endothelial cell adhesion molecule 1

Pgf: placental growth factor

Plau: plasminogen activator

Plg: plasminogen

PMEC: pulmonary microvascular endothelial cell

PMN: polymorphonuclear neutrophil

p.op.: postoperative

PPC: positive polymerase chain reaction control

P/S: penicillin/streptomycin

Ptgs1: prostaglandin-endoperoxide synthase 1

Ptk2: protein tyrosine kinase 2

qRT-PCR: quantitative real- time polymerase chain reaction

RAAS: renin-angiotensin-aldosterone system

RALES: randomized aldactone evaluation study

RBC lysis buffer: red blood cell lysis buffer

RefG: reference genes

REPAIR-AMI trial: Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction trial

RNA: ribonucleic acid

RNase: ribonuclease

RPLP0: ribosomal protein, large, P0

RPMI: Roswell Park Memorial Institute medium

rRNA: ribosomal ribonucleic acid

RT: reverse transcription

RT: room temperature

RTC: Reference- and Translation Center for Cardiac Stem Cell Therapies University Rostock

RTC: reverse-transcription controls (for PCR analysis)

S1pr1: sphingosine-1-phosphate receptor 1

Serpine1: serine (or cysteine) peptidase inhibitor, clade E, member 1

Serpinf1: serine (or cysteine) peptidase inhibitor, clade F, member 1

SCID: severe combined immunodeficiency

SMA: smooth muscle α-actin

SMAD5: SMAD family member 5, see also Madh5

SMC: smooth muscle cell

Sm-mhc: smooth muscle myosin heavy chain

SOLVD: studies of left ventricular dysfunction

SPECT: single photon emission computed tomography

Sphk1: sphingosine kinase 1

SSC: side scatter

Taq: Thermus aquaticus

Tbx1: T-box 1

Tek: endothelial-specific receptor tyrosine kinase

TGF: transforming growth factor

Tgfa: transforming growth factor alpha

Tgfb: transforming growth factor beta 1

Tgfbr1: transforming growth factor, beta receptor I

Thbs1: thrombospondin 1

Thbs2: thrombospondin 2

Tie1: tyrosine kinase with immunoglobulin-like and EGF-like domains 1

Timp: tissue inhibitor of metalloproteinase

TNC: total nuclear cells

TN-C: tenascin-C
Tnf: tumor necrosis factor
Tnfsf12: tumor necrosis factor (ligand) superfamily, member 12
tRNA: transfer ribonucleic acid
tiRNA: **t**RNA-derived, stress **i**nduced small ribonucleic acid
Tymp: thymidine phosphorylase
uPA: urokinase
USA: United States of America
VEGF: vascular endothelial growth factor
VEGFR: vascular endothelial growth factor receptor
VLA: very late antigen, subfamily of adhesion molecules
vWF: von Willebrand factor
WHO: World Health Organization
7-AAD: seven-amino-actinomycin D, stain

Units of measurement:
°C: degree Celsius
Da: Dalton (atomic mass unit)
g: gram (weight unit)
g: gravity (unit of acceleration)
l: liter (volume unit)
M: mole (amount of substance)
pH: numeric scale used to display the acidity or alkalinity of aqueous solutions
µ: micro 10^{-6}
m: milli 10^{-3}
k: kilo 10^{3}

Cluster of differentiation:
CD29: beta-one-integrin or fibronectin receptor beta or gpIIa
CD73: 5'-ectonucleotidase, NT5E
CD105: endoglin
CD271: low affinity nerve growth factor receptor (LNGFR)
CD133: prominin-1

Table 5: Used materials and devices

Lab Tek® chamberslide™ system	Thermo Fisher Scientific
MACS equipment	Miltenyi Biotec
96 PCR plate	Applied Biosystems
MACS puffer; made of PBS/ EDTA, 2mM buffer, 0,5% albumin from bovine serum (BSA)	Sigma-Aldrich
PBS/ EDTA	Sigma-Aldrich
LSM 1077 (Lymphocyte separation medium)	PAA laboratories
RPMI 1640 with Glutamine (Roswell Park Memorial Institute medium)	PAN Biotec
MSCGM (MSC growth medium)	Lonza
EBM-2 (endothelial base medium)	Lonza
EGM-2 (endothelial growth medium)	Lonza
BD Matrigel Basement Membrane Matrix	BD Biosciences
CFDA-SE dye	Sigma-Aldrich
PKH-26 red fluorescent Cell Linker (MINI 26) Including Diluent C	Sigma-Aldrich
Hoechst 33342 stain	Thermo Scientific
TRIzol Reagent	Life Technologies
O.C.T.™ Compound Tissue-Tek®	Sakura Finetek Europe B.V.
Axiovert 40 CFL fluorescence microscope with Axio Cam MRm ZEN (Zeiss efficient navigation) software	Carl Zeiss AG

High- resolution confocal microscope ELYRA S.1	Carl Zeiss AG
Image J, image analysis program	Wayne Rasband
LSR II flow cytometer	BD Biosciences
LSR II, FACS DivaTM software	BD Biosciences
Nanodrop 1000, spectrophotometer	PEQLAB Biotechnologie
MJ mini^{TM} personal thermal cycler, PCR cycler	Bio-Rad Laboratories
StepOnePlus® Real-Time PCR System	Applied Biosystems
CM1900 cryostat	Leica Microsystems
Kits	
CD271 Micro Bead Kit, (APC) human	Miltenyi Biotec
CD133 Micro Bead Kit, human	Miltenyi Biotec
Anti-CD133-PE (293C2)	Miltenyi Biotec
Isotype control mouse IgG 2b-PE	Miltenyi Biotec
Mouse IgG1 isotype APC	Miltenyi Biotec
Anti-CXCR4-APC (12G5)	Becton Dickinson
Isotype control mouse IgG 2a κ-APC	BioLegend
Anti-CD45-allophycocyanin-H7 Anti-CD45-Horizon-V500	BD Bioscience
Anti-CD34-FITC (AC136)	Miltenyi Biotec
Human CD105 Alexa-Fluor 488	AbD Serotec
Human CD73-phycoerythrin a	BD Bioscience

anti-CD44-Peridinin-chlorophyll-protein-Cyanine-5.5 tandem dye	BD Bioscience
7-aminoactinomycin	BD Bioscience
anti-CD29 allophycocyanin	BD Bioscience
High Capacity cDNA Reverse Transcription Kit Multi Scribe; with RNAse Inhibitor	Applied Biosystems
TaqMan® Universal PCR Master Mix	Applied Biosystems
RPLPO- VIC, PCR house keeping gene	Applied Biosystems
NGFR- FAM, PCR Primer	Applied Biosystems
vWF – FAM, PCR Primer	Applied Biosystems
eNOS –FAM, PCR Primer	Applied Biosystems
α-SMA / ACTA –FAM, PCR Primer	Applied Biosystems
RT^2 First Strand Kit	Qiagen
RT^2 Profiler PCR Array Mouse Angiogenesis PAMM 024ZC-12	Qiagen
RT^2 SYBR Green ROX qPCR Mastermix	Qiagen

List of tables

List of figures

Curriculum vitae

Persönliche Daten

Name	Sarah Hélène Sasse
Geburtsdatum / -ort	19.04.1990 Frankfurt am Main

Beruflicher Werdegang

04/2018 – 2024	**Ärztin in Weiterbildung zum Facharzt für Viszeralchirurgie** Diakonie Krankenhaus Bremen unter Leitung von Prof. Dr. S. M. Freys
10/2016 – 02/2018	**Ärztin in Weiterbildung zum Facharzt für Viszeralchirurgie** am Klinikum Südstadt Rostock unter Leitung von Prof. Dr. K. Ludwig

Ausbildung

WS 2009 – SS/2016	**Studium der Humanmedizin** Medizinischen Fakultät der Universität Rostock
2009	**Abitur** Humboldt Gymnasium, Bad Homburg

Publikationen

Dez 2019	**Sasse S**, Skorska A, Lux CA, Steinhoff G, David R, Gaebel R (2019). “Angiogenic Potential of Bone Marrow Derived CD133+ and CD271+ Intramyocardial Stem Cell Trans-Plantation Post MI.” Cells. 2019 Dec 27;9(1).
Nov 2019	Sadraddin H, Gaebel R, Skorska A, Lux CA, **Sasse S**, Ahmad B, Vasudevan P, Steinhoff G, David R (2019). “CD271+ Human Mesenchymal Stem Cells Show Antiarrhythmic Effects in a Novel Murine Infarction Model.” Cells. 2019 Nov 20;8(12).
Sep 2017	Bernhardt J, **Sasse S**, Ludwig K, Meier PN (2017). "Update in Natural Orifice Translumenal Endoscopic Surgery (NOTES).” Curr Opin Gastroenterol. 2017 Sep;33(5):346-351.
Aug 2017	Lemcke H, Gaebel R, Skorska A, Voronina N, Lux CA, Petters J, **Sasse S**, Zarniko N, Steinhoff G, David R (2017). "Mechanisms of stem cell based cardiac repair - gap junctional signaling promotes the cardiac lineage specification of mesenchymal stem cells.“ Sci Rep. 2017 Aug 29;7(1):9755.