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Intramyocardial fate and effect of iron nanoparticles co-injected with MACS® purified stem cell products

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ABSTRACT

Background: Magnetic activated cell sorting (MACS®) is routinely used to isolate stem cell subpopulations intended for the treatment of cardiovascular diseases. In strong contrast, studies examining the amount, effect and intramyocardial distribution of iron nanoparticles used for magnetic cell labelling are missing, although iron excess can cause functional disorders in the heart.

Methods and results: CD133$^+$ haematopoietic and CD271$^+$ mesenchymal stem cells were purified from bone marrow using automatically and manually MACS® based systems. Flow cytometric measurements demonstrated a rapid loss of MACS® MicroBeads from cells under culture conditions, while storage under hypothermic conditions decelerated their detachment. Moreover, an average loading of ~11 fg iron/cell caused by magnetic labelling was determined in magnetic particle spectroscopy. Importantly, hemodynamic measurements as well as histological examinations using a myocardial ischemia/reperfusion mouse model showed no influence of MACS® MicroBeads on cardiac regeneration, while the transplantation of stem cells caused a significant improvement. Furthermore, immunostainings demonstrated the clearance of co-injected iron nanoparticles from stem cells and the surrounding heart tissue within 48 h post transplantation.

Conclusions: Our results indicate that iron amounts typically co-injected with MACS® purified stem cells do not harm cardiac functions and are cleared from heart tissue within a few hours. Therefore, we conclude that MACS® MicroBeads exhibit a good compatibility in the cardiac environment.

1. Introduction

The increasing incidence of cardiovascular diseases (CVDs) demands the development of novel therapeutic approaches [1]. With the initial intention of functionally replacing injured myocardium, translation of adult stem cell-based therapies from bench to bedside has been rapidly initiated almost two decades ago [2–5]. Since then, numerous Phase I and Phase II clinical trials demonstrated the safety and feasibility of intramyocardially as well as intracoronary injected autologous adult stem cells, such as haematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs) [6–15]. These cells are easily accessible from various adult tissues and their transplantation neither poses the risk of rejection nor ethical conflicts [16–19]. Current meta-analyses reported mild but significant improvements of cardiac function after HSC and MSC transplantation [20–26]. However, to date it is commonly

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accepted that their beneficial effects are rather based on paracrine mechanisms than on direct cardiac trans-differentiation [27–31].

Magnetic activated cell sorting (MACS®) is the most frequently used technique for the isolation of specific stem cell subpopulations intended for the treatment of CVDs [32–35]. This immunomagnetic approach enables an efficient purification and a direct application of the desired target cells, thereby avoiding cultivation costs and time as well as the risk of contamination and cell alterations [36–38]. Nowadays, MACS® based automatized devices for on-site cell preparation conformant with Good Manufacturing Practice (GMP) are available for HSC, while MSC purification is still restricted to non-automatic approaches [39,40].

The binding of antibody-linked superparamagnetic iron dextran particles to specific antigens of HSCs (e.g. CD133 and CD34) and MSCs (e.g. CD271 and CD105) is an integral part of commercial MACS® purification protocols [36,41–43]. These co-injected particles can affect the quality, safety and efficiency of the final cell product [33,44,45]. Moreover, excessive iron concentrations are known to induce tissue injury caused by reactive oxygen species [46,47]. Notably, several studies have shown that deposition of iron in the myocardium can lead to serious cardiac dysfunctions and ultimately to death due to dilated cardiomyopathy and cardiac failure [48–51]. On the contrary, studies examining the amount, effect, fate and distribution of MACS® MicroBeads in the heart are still missing.

Therefore, in the present study, we investigated the detachment of iron nanoparticles from automatically and manually MACS® purified CD133$^+$ HSCs and CD271$^+$ MSCs. Furthermore, we quantified the loading of stem cells with iron nanoparticles and examined the intramyocardial fate and effect on cardiac function parameters of these co-injected particles.

2. Materials and methods

2.1. Sternal BM aspiration

BM was obtained from informed donors who gave written consent to the use of their samples for research according to the Declaration of Helsinki [52]. The ethical committee of the University of Rostock approved the presented study (registration no. A 2010 23). Sternal BM aspirates were obtained from patients undergoing coronary artery bypass graft (CABG) surgery at the Department of Cardiac Surgery (Rostock University Medical Center, Germany). Samples of 32 donors (78% male; 22% female) were used with a mean donor age of 66 ± 2 years. The mean volume of BM was 56 ± 1 ml. To prevent coagulation heparin sodium (250 i.E./ml BM) (Ratiopharm GmbH, Ulm, Germany) was used.

2.2. Automatic CD133$^+$ cell isolation

The CE-certified CliniMACS® Prodigy BM-133 system (consisting of CliniMACS® Prodigy device, CliniMACS® Prodigy Tubing Set (TS) 100, CliniMACS® CD133 Reagent and CliniMACS® phosphate buffered saline (PBS)/ethylenediaminetetraacetic acid (EDTA) buffer) was provided by Miltenyi Biotec. Sodium chloride (NaCl) solution (0.9%) and human serum albumin (HSA) solution (20%) were certified as medical products and received from Fresenius Kabi (Bad Homburg, Germany) and CSL Behring (Marburg, Germany), respectively.

The automatic manufacturing of the CD133$^+$ cell product using the CliniMACS® Prodigy BM-133 system was performed in a cell manufacturing facility in the Department of Cardiac Surgery in compliance with EU guidelines for Good Manufacturing Practices (GMP) (license DE_MV_01_MIA_2016_0001/310.0003.02). The preinstalled BM-CD133 enrichment program was selected, TS 100 was installed and the integrity test was performed by the device. In order to ensure automatic plasma generation, sternal BM was filled up with a fixed volume of 70 ml using PBS/EDTA buffer supplemented with HSA solution before it was applied to the system. At the end of the manufacturing process the CD133$^+$ cell product was welded off and transferred to the labs of the RTC for further investigations.

2.3. Manual CD133$^+$ and CD271$^+$ cell isolation

Mononuclear cells (MNCs) were isolated from sternal BM aspirates by density gradient centrifugation on Pancoll human separation solution (PAN-Biotech, Aidenbach, Germany). CD133$^+$ cells and CD271$^+$ cells were magnetically enriched using the CD133 MicroBead Kit and the CD271 MicroBead Kit (APC) from Miltenyi Biotec following the manufacturer's instructions.

2.4. In vitro labelling of MACS® MicroBeads

MACS® MicroBeads were visualized directly after manual CD133$^+$ and CD271$^+$ cell isolation. Therefore, 5 × 10^4 cells were treated with Labeling Check Reagent-FITC (Miltenyi Biotec) following the manufacturer's instructions. Subsequently, cell membrane was stained by incubating samples with CellMask™ Plasma Membrane Stains (1:100, Thermo Fisher Scientific, Schwerte, Germany) for 10 min in the dark. Afterwards, samples were fixed with 4% formaldehyde solution (Merck Millipore, Darmstadt, Germany), spun down on to a coverslip and washed with PBS (PAN-Biotech). Then, coverslips were mounted with Fluoroshield™ with DAPI (Sigma-Aldrich, Taufkirchen, Germany) on microscope slides.

2.5. Assay to address cell viability and the presence of MACS® MicroBeads

Cell viability, percentage of MACS® MicroBeads$^+$ cells and mean fluorescence of MACS® MicroBeads labelled cells were analyzed at different time points (0, 2.5, 18, 24, 48, 72 h) using flow cytometry. To warrant conditions realistic for a clinical scenario, automatically purified CD133$^+$ cells were stored at room temperature (RT) in NaCl solution supplemented with 10% autologous plasma for 2.5 h (sufficient time frame for cell application under optimal conditions [40]). This served to ensure stable temperature conditions during the entire manufacturing process. After 2.5 h, cells were stored at 4 °C up to 72 h to reduce cell metabolism as well as oxygen demand [53,54]. In order to assess the influence of different culture conditions on MACS® MicroBead detachment, manually purified cells were either stored at 4 °C in NaCl solution supplemented with 2% HSA solution or at 37 °C, 20% O_2 and 5% CO_2 in specific cell culture medium (CD133$^+$ cells in StemSpan™ H3000 (STEMCELL Technologies, Köln, Germany); CD271$^+$ cells in MSCGM™ (Lonza, Köln, Germany)) supplemented with 1% penicillin/streptomycin (Thermo Fisher Scientific).

For flow cytometric analysis, samples were taken at respective time points, suspended in MACS® buffer (PBS supplemented with 2 mM EDTA (Thermo Fisher Scientific) and 0.5% bovine serum albumin (BSA, Sigma-Aldrich)). Human FcR Blocking Reagent (Miltenyi Biotec) was added to reduce unspecific bindings. To investigate the presence of MACS® MicroBeads on stem cells, samples were treated with specific antibodies (HSCs with CD133/2 (293C3)-PE (Miltenyi Biotec); MSCs with CD271 (LNGFR)-APC (Miltenyi Biotec)) and Labeling Check Reagent-FITC for 10 min at 4 °C. The same samples were used for viability testing by adding 7-Amino-Actinomycin (7-AAD) staining solution (Becton Dickinson, Heidelberg, Germany). After washing with PBS samples were