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Development of a new therapeutic technique to direct stem cells to the infarcted heart using targeted microbubbles: StemBells

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ABSTRACT

Successful stem cell therapy after acute myocardial infarction (AMI) is hindered by lack of engraftment of sufficient stem cells at the site of injury. We designed a novel technique to overcome this problem by assembling stem cell-microbubble complexes, named 'StemBells'.

StemBells were assembled through binding of dual-targeted microbubbles (~3 μm) to adipose-derived stem cells (ASCs) *via* a CD90 antibody. StemBells were targeted to the infarct area *via* an ICAM-1 antibody on the microbubbles. StemBells were characterized microscopically and by flow cytometry. The effect of ultrasound on directing StemBells towards the vessel wall was demonstrated in an *in vitro* flow model. In a rat AMI-reperfusion model, StemBells or ASCs were injected one week post-infarction. A pilot study demonstrated feasibility of intravenous StemBell injection, resulting in localization in ICAM-1-positive infarct area three hours post-injection. In a functional study five weeks after injection of StemBells cardiac function was significantly improved compared with controls, as monitored by 2D-echocardiography. This functional improvement neither coincided with a reduction in infarct size as determined by histochemical analysis, nor with a change in anti- and pro-inflammatory macrophages.

In conclusion, the StemBell technique is a novel and feasible method, able to improve cardiac function post-AMI in rats.

1. Introduction

Adult mesenchymal stem cell therapy has been proposed as a promising therapy for regenerative tissue repair, for example to prevent heart failure development after acute myocardial infarction (AMI) (Shah and Shalia, 2011; Wollert et al., 2004). However, one of the major problems of stem cell therapy is a lack of engraftment of sufficient stem cells at the site of injury (van Dijk et al., 2011; Berardi et al., 2011). We hypothesized that when retention and engraftment of stem cells are increased, the therapeutic effect of stem cells will improve. Therefore, we designed a novel targeting technique to direct adipose-derived stromal/stem cells (ASCs) specifically to the activated endothelium of blood vessels within the infarct area in the heart by coating them with dual-targeted microbubbles.

We used ASCs, because adipose tissue is a rich source of mesenchymal stem cells, which can be harvested easily, show high proliferation rates in culture and have the capacity to differentiate into several cell types amongst which cardiomyocytes (Oedayrajsingh-Varma et al., 2006; van Dijk et al., 2008; Carvalho et al., 2013; Rangappa et al., 2003). Furthermore, it has been shown that ASCs have a beneficial effect on cardiac function post-AMI in several pre-clinical studies (van Dijk et al., 2011; Berardi et al., 2011; Yamada et al., 2006). Early clinical trials

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using ASC therapy post-AMI, however, show that there is a room for improvement for ASC therapy (Houtgraaf et al., 2012; Janssens et al., 2006; Jeevanantham et al., 2012; Assmus et al., 2014).

To be able to direct the ASCs to the infarct area we employed the so-called microbubbles, which are small (2–4 μm) gas-filled bubbles originally developed as contrast agents for echocardiography (Dijkmans et al., 2004). Nowadays, microbubbles can also be designed as targeting agents by conjugating antibodies, ligands or peptides to the microbubble shell (Klibanov et al., 2004; Kokhuis et al., 2013). We have constructed stem cell-microbubble complexes, named 'StemBells', by coating ASCs with microbubbles using a CD90 antibody *via* biotin-streptavidin bridging (Fig. 1A). Additionally, an antibody against ICAM-1, an adhesion molecule expressed on activated endothelium of blood vessels within the infarct area (Benson et al., 2007), was simultaneously conjugated to the microbubble shell to create a bridge and improve the attachment of the StemBells specifically in the infarct area. Application of the microbubbles has several benefits. First, it allows coupling of a targeting antibody to the ASCs without modifying the stem cell itself. Second, the microbubbles cause buoyancy and susceptibility of the ASCs to the acoustic radiation force exerted by diagnostic ultrasound, as we previously showed in an chicken embryo using intravital microscopy (Kokhuis et al., 2014). StemBells can thus be pushed from the center of the blood stream to the vessel wall by ultrasound, further enhancing the effect of targeting.

Here, we describe the development and validation of this novel StemBell technique. We demonstrate its safety, as well as its positive effect on cardiac function in a rat AMI study.

2. Methods

2.1. Isolation and culture of the stromal vascular fraction from human and rat adipose tissue

For isolation of the human stromal vascular fraction (SVF), subcutaneous abdominal adipose tissue samples were obtained as waste material after elective surgery and donated upon informed consent of the patients from three clinics in the Netherlands (Tergooi Ziekenuis, Hilversum; 'Jan van Goyen' clinic, Amsterdam; VU University Medical Center, Amsterdam). This study complied with the principles of the Declaration of Helsinki. SVF was isolated as described previously (Oedayrajsingh-Varma et al., 2006). Adipose tissue was stored in sterile phosphate-buffered saline (PBS; Braun, Melsungen, AG, USA) at 4 °C and processed within 24 h after surgery as described previously (Oedayrajsingh-Varma et al., 2006). In brief, adipose tissue was enzymatically digested using 0.1% collagenase A (Roche Diagnostics GmbH, Mannheim, Germany) in PBS containing 1% bovine serum albumin (BSA; Roche Diagnostics) for 45 min at 37 °C under intermittent shaking. To remove contaminating erythrocytes, the cells were subjected to Ficoll density centrifugation (Lymphoprep, ρ01.077 g/ml, osmolarity 280 ± 15 mOsm; Axis-Shield, Oslo, Norway).

For rat SVF isolation, adipose tissue from the inguinal fat pad of 30 male Wistar rats (Harlan Laboratories, Horst, the Netherlands; 300–400 g) was resected, pooled per 5 rats, collected in sterile PBS and processed immediately after resection as described previously (van Dijk et al., 2011). Animals were treated according to national guidelines and with permission of the Institutional Animal Care and local Animal

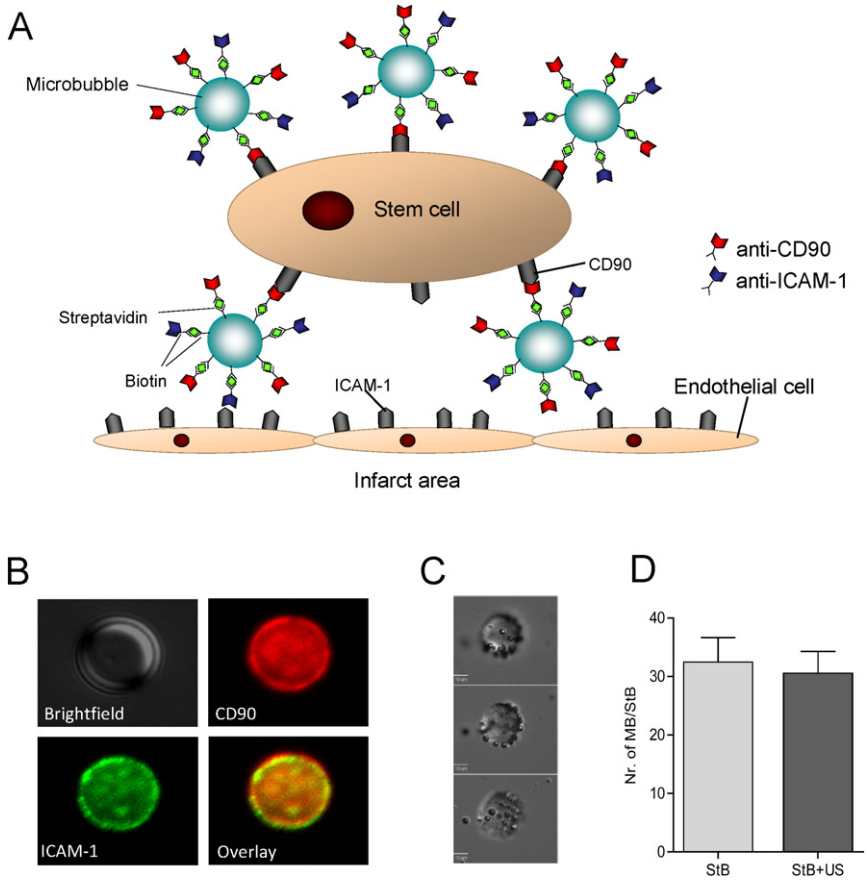

Fig. 1. StemBells A) Schematic drawing of a StemBell: a stem cell-microbubble complex coupled *via* streptavidin-biotin-antibody bridging. B) Microscopic images demonstrating the presence of two antibodies on one microbubble. Upper left panel: bright field image; upper right panel: anti-CD90 in red; lower left panel: anti-ICAM-1 in green, and lower right panel is an overlay. C) Microscopic bright field images showing three planes of a 3D stack of a StemBell. D) Quantification of the number of microbubbles per stem cell includes the effect of ultrasound. Data is shown as mean ± SEM (n = 6).