SUPPLEMENTARY MATERIALS

Isolation of MPC Populations

Following sedation and under anesthesia, bone marrow aspirates (~30ml) were collected from the iliac crest with an 11-gauge biopsy-aspiration needle attached to a heparinized syringe. Similarly, when appropriate and after vascular sheaths insertion, peripheral blood (~30ml) was collected. BMMNCs and PBMNCs were isolated by density gradient separation (Histopaque, density 1.077; Sigma). For BMMNC and PBMNC isolation, BM aspirates or collected peripheral blood were deposited over equal volumes of Histopaque, followed by centrifugation at 400g for 30 minutes at room temperature. Mononuclear cells were recovered from the buffy coat by pipette aspiration and washed twice with PBS. For MSCs isolation, the adherent cells were used following plating of the mononuclear fraction of BM aspirates onto plastic culture flasks. After 72 hours, unattached cells were washed off during medium exchange. MSCs were expanded using high glucose DMEM with 10% FBS supplemented with penicillin-streptomycin at 37^{o}C and 5% CO_2. MSCs were passaged 1:3 when they reached 70-80% confluence, with 0.05% Trypsin-EDTA. Due to the time required to achieve appropriate cell numbers for intracoronary injection after harvesting and known immunomodulatory properties of MSC(1-4), the choice was made to proceed with allogenic injection of MSC. Thus, throughout the study, a total of 3 healthy, non-infarcted pigs were used as MSC donor for the MSC-group swine. Early passage MSCs (passages $\leq$5) were used in all described procedures. Cell viability prior to injection was assessed by Trypan Blue exclusion test.

The above cell processing methods were chosen so as to reflect potentially relevant SC collecting methods used in prior clinical application of cardiac cell adjuvant therapy(5-7).

Myocardial infarction creation and IC cell delivery

We based our swine model on one previously reported by other investigators(8), but modified the point of vascular occlusion (see below). Briefly, following anesthesia with Isoflurane 1.5-2%, femoral arterial sheaths were inserted per the Seldinger technique. After selective coronary cannulation, a standard 0.014-inch coronary wire (ACS Hi-Torque BMW; Guidant) was used to position an over-the-wire coronary balloon angioplasty system (Maverick Balloon, 3.5x12mm; Boston Scientific) into the left anterior descending artery (LAD), beyond the first diagonal branch in all swine. In order to induce myocardial infarction, transient occlusion using the minimal balloon inflation required was used to achieve complete arrest of antegrade flow of contrast dye. After a 60-minute occlusion time, reperfusion was performed by balloon deflation. Myocardial infarction was confirmed by ST-segment elevation. All study animals received amiodarone as follow: a 75 mg i.v. bolus given over 10 minutes prior to balloon inflation which was followed by a continuous infusion of 1mg/min for the remainder of the procedure. Study animals were brought back on day 3-4 post-MI creations for cell injection.

CD44 staining

Consecutive 10 μm cryosections were stained with H&E, or a mouse anti-CD44 primary antibody (2.5 μg/ml; Catalog #LS-B1862, Lifespan Biosciences) and Cy3-conjugated

donkey anti-mouse secondary antibody (1:200 dilution; Jackson Immunochemicals). CD44 and IR-786 were visualized simultaneously on the same slide using a custom 4-channel fluorescence microscope[1]

[[1]Reference: "Quantitation of brown adipose tissue perfusion in transgenic mice using near-infrared fluorescence imaging" Nakayama A, Bianco AC, Zhang CY, Lowell BB, Frangioni JV. Mol Imaging. 2003 Jan;2(1):37-49]

Statistical Analyses

A Linear Growth Model[1] with covariates and interaction terms was used to study the SIR parameter between cells, zone and time. In order to better assess how the SIR changes over time, a linear Growth Model with 2 covariates (cells, zone) was adjusted using the SAS procedure PROC MIXED. The CELLS and ZONE effect were included in a CLASS statement with an ID variable to identify the subject of analysis (here, swine). Time from injection was considered as a continuum (not a CLASS variable) and included in a RANDOM statement. An intercept term was also included in the RANDOM statement, just like the ZONE term to specify to the model the correlation between data from the 2 zones. The covariance structure of the VC (variance components) option was employed. A full model with all the interaction terms (CELLS | ZONE | TIME) was then adjusted.

To account for potential multiple pairwise comparisons, all interaction terms were included in the model, tested and dropped from the model when non-significant. The LSD (Least Significant Difference) procedure was used to control for Type I error due to multiple pairwise comparisons. The LSD test is a two-step test. First, the ANOVA F test is performed. If it is significant at level ALPHA, then all pairwise contrasts are carried

out, each at level ALPHA. However, if the F test is non-significant, then the procedure is then terminated.

[[1]Reference: Using SAS PROC MIXED to Fit Multilevel Models, Hierarchical Models, and Individual Growth Models, Judith Singer, Journal of Educational and Behavioral Statistics, winter 1998, vol 24, no 4 p.323-355)]

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