Cardiosphere-derived cells improve function in the infarcted rat heart for at least 16 weeks – an MRI study

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Supplementary Information File S1 – Materials and Methods.

RNA extraction and DNase treatment

Total RNA was extracted from cultured CDCs using Trizol reagent (Sigma) according to the manufacturer's instructions. DNase treatment was performed using Turbo DNA-free (Ambion) to degrade any DNA present. The concentration and purity of RNA was determined by measuring the absorbance at 260 nm (A260) and 280 nm (A280) using a Nanodrop ND-1000 Spectrophotometer (Nanodrop Technologies Inc., USA). A ratio of A260/A280 ≈ 2.0 is generally accepted as pure RNA.

cDNA synthesis

DNase treated RNA was reverse transcribed using AB high capacity transcriptase kit (Applied Biosystem). In brief, every 1µg RNA sample was reverse transcribed using 1 µl reverse transcriptase, 2 µl random primer, 0.8 µl dNTPs (10mM each), 2 µl buffer and topped up by RNase free water to a total volume of 20 µl. The reaction mixture was subjected to incubation for 10 minutes at 25℃, 120 minutes at 37℃ and 5 seconds at 85℃.

Real-time PCR

The real-time PCR mastermix was prepared by adding 10 µl AB Sybrgreen PCR mastermix (AB International, CA), 1 µl reverse primer, 1 µl forward primer, 1 µl cDNA and 7 µl distilled water. The PCR program was set up with an initial heat activation step at 95°C for 10 minutes. Thermocycling (40 cycles) was performed with a denaturation step at 95°C for 15 seconds, an annealing step at 60°C for 30 seconds

and an extension step at 72°C for 30 seconds. Fluorescence was measured at the end of each extension step.

After amplification, a melting curve was acquired by heating the product at 4°C/second to 95°C. Fluorescence was measured through the slow heating phase. Melting curves were used to determine the specificity of PCR products.

Primer Sequences

Primer	Gene name	Forward primer 5' to 3'	Reverse primer 3' to 5'	Gene accession number
c-Kit	Stem cell factor cytokine receptor	AATCCGACAAC CAAAGCAAC	TGACATCAGAG TTGGACACCA	ENSRN-OG00000002227
GATA 4	GATA binding protein 4	CAGTCCTGCAC AGCCTACCT	CCGCAGTTGAC ACACTCTCT	NM_144730
CD90	Thy-1 cell surface antigen	CAGAATCCCAC AAGCTCCAA	GCCAGGAAGTG TTTTGAACC	NM_012673
CD105	Endoglin	GGTACAGTGCA TCGACATGG	GCTGGCCTAGC TCTATGGTG	NM_001010968
Nkx2.5	Homeobox protein NKX2-5	CATTTTATCCG CGAGCCTAC	GTCTGTCTCGG CTTTGTCCA	ENSRNOG00000020747
Tnnt 2	Troponin T type 2 (cardiac)	CGTATTCGCAA TGAACGAGA	CTGTTCTCCTC CTCCTCACG	NM_012676
MyHC	Myosin heavy chain	TATGAGACGGA CGCCATACA	CTCCAGAGAGG AGCACTTGG	NM_017239.2
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	GGGTGTGAACC ACGAGAAAT	ACTGTGGTCAT GAGCCCTTC	NM_017008

***In vivo* cine MRI**

1.5 mm true short axis ECG-gated cine images were acquired with the following parameters: field of view 51.2 mm^2, matrix size 256 x 256 zero filled to 512 x 512 giving a voxel size of 100 x 100 x 1500 µm, echo time/repetition time (TE/TR) 1.43/4.6 ms, 17.5° pulse, 25-35 frames per cardiac cycle

Stroke volume was calculated as end diastolic volume minus end systolic volume. The ejection fraction was calculated as the stroke volume divided by the end diastolic volume. The relative infarct size was calculated from the average of the

endocardial and epicardial circumferential lengths of the thinned, akinetic region of all slices, measured at diastole, and expressed as a percentage of the total myocardial surface [1]. Wall thickness of the peri-infarct (myocardium adjacent to infarct where contraction was observed) and posterior regions of the myocardium was measured at end systole in a mid-papillary slice. Myocardial mass was calculated from an average of the myocardial volumes at end diastole and end systole, multiplied by the density of myocardial tissue (1.05 mg/ml) [2].

High resolution 3D MR microscopy

MR microscopy was performed using a fast gradient echo sequence with the following parameters: TE/TR = 1.8/30 ms, flip angle 90º, field of view: 32 x 32 x 32 mm, matrix size 512 x 512 x 512, voxel size $62.5 \times 62.5 \times 62.5$ μm.

Antibodies for immunohistochemistry and flow cytometry

Antigen	Antibody	Supplier	Catalogue number	Code	Dilution
Alpha-sarcomeric actin	Mouse monoclonal	Sigma	A2172	5C5	1:200
CD31	Mouse monoclonal	Serotec	MCA1334G	TLD-2A12	1:100
CD68	Mouse monoclonal	Abcam	AB31630	ED1	1:200
CD90	Mouse monoclonal	BD Pharmingen	554892	HIS51	1:250
CD117 (c-kit)	Rabbit polyclonal	Santa Cruz	SC-5535	H300	1:50
Connexin 43	Mouse monoclonal	Sigma	C8093	CXN-6	1:200
DDR2	Goat polyclonal	Santa Cruz	SC-7555	N20	1:20
GFP	Goat polyclonal	Rockland	600-101-215		1:50
GATA-4	Rabbit polyclonal	Santa Cruz	SC-9053	H-112	1:50
Klf-4	Rabbit polyclonal	Santa Cruz	SC20691	H-180	1:50
Nanog	Rabbit polyclonal	Abcam	Ab21603		1:50
Nkx2.5	Goat polyclonal	R&D	AF2444		1:50
Oct 3/4	Rabbit polyclonal	Santa Cruz	SC-9081	H134	1:50
Smooth muscle actin	Mouse monoclonal	Sigma	A2547	1A4	1:500

Sox 2	Goat polyclonal	Santa Cruz	SC-17320	Y17	1:50
Cardiac troponin I	Rabbit polyclonal	Santa Cruz	SC-15368	H170	1:500
Cardiac troponin T	Mouse monoclonal	Abcam	Ab10214	1F11	1:200
Vimentin	Mouse monoclonal	Sigma	V2258	LN-6	1:200
von Willebrand Factor	Rabbit polyclonal	Chemicon	AB-7356		1:200
MitoTracker Red CMXRos		Jackson Immuno Research	M7512		50 nm

1. Nahrendorf M, Wiesmann F, Hiller KH, Hu K, Waller C, et al. (2001) Serial cine-magnetic resonance imaging of left ventricular remodeling after myocardial infarction in rats. J Magn Reson Imaging 14: 547-555.
2. Tyler DJ, Lygate CA, Schneider JE, Cassidy PJ, Neubauer S, et al. (2006) CINE-MR imaging of the normal and infarcted rat heart using an 11.7 T vertical bore MR system. J Cardiovasc Magn Reson 8: 327-333.