Preconditioning of skeletal myoblast-based engineered tissue constructs enables functional coupling to myocardium in vivo

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Objective: Skeletal myoblasts fuse to form functional syncytial myotubes as an integral part of the skeletal muscle. During this differentiation process, expression of proteins for mechanical and electrical integration is seized, which is a major drawback for the application of skeletal myoblasts in cardiac regenerative cell therapy, because global heart function depends on intercellular communication.

Methods: Mechanically preconditioned engineered tissue constructs containing neonatal mouse skeletal myoblasts were transplanted epicardially. A Y-chromosomal specific polymerase chain reaction (PCR) was undertaken up to 10 weeks after transplantation to confirm the presence of grafted cells. Histologic and electrophysiologic analyses were carried out 1 week after transplantation.

Results: Cells within the grafted construct expressed connexin 43 at the interface to the host myocardium, indicating electrical coupling, confirmed by sharp electrode recordings. Analyses of the maximum stimulation frequency (5.65 $\pm$ 0.37 Hz), conduction velocity (0.087 $\pm$ 0.011 m/s) and sensitivity for pharmacologic conduction block (0.736 $\pm$ 0.080 mM 1-heptanol) revealed effective electrophysiologic coupling between graft and host cells, although significantly less robust than in native myocardial tissue (maximum stimulation frequency, 11.616 $\pm$ 0.238 Hz, $P < .001$; conduction velocity, 0.300 $\pm$ 0.057 m/s, $P < .01$; conduction block, 1.983 $\pm$ 0.077 mM 1-heptanol, $P < .001$).

Conclusions: Although untreated skeletal myoblasts cannot couple to cardiomyocytes, we confirm that mechanical preconditioning enables transplanted skeletal myoblasts to functionally interact with cardiomyocytes in vivo and, thus, reinvigorate the concept of skeletal myoblast-based cardiac cell therapy. (J Thorac Cardiovasc Surg 2015;149:348-56)

See related commentary on pages 357-9.

Cardiovascular disease and specifically ischemic heart disease are the most common causes of natural death worldwide.[1] In cases of massive myocardial infarction (MI) and acute heart failure, implantation of ventricular assist devices and ultimately heart transplantation are currently the only therapeutic options available. Endogenous cardiomyocyte regeneration after MI is negligible[2] and not sufficient to compensate for the detrimental effects of MI on heart function. Thus, the application of cells or tissues to support cardiac regeneration or repair damaged heart muscle is an attractive therapeutic option.

Skeletal myoblasts (SMs) represent an extensively studied progenitor cell type from skeletal muscle. They can be acquired with minimum invasiveness as biopsies from patients regardless of age[3] and comorbidities[4] allowing extensive in vitro cell culture expansion. SMs also show remarkable resistance to ischemia[5] and are committed to a myogenic differentiation. Preclinical cell transplantation studies using SMs have confirmed efficacy for improving postinfarction left ventricular function.[6] SMs were the first cell type applied in clinical trials on cell-based cardiac regenerative therapies[7] and have been shown to improve the development of heart function after MI.[8] However, cases of arrhythmia after intramyocardial transplantation of SMs have been observed.[9,10]

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ETDBS

Abbreviations and Acronyms

AP = action potential
bFGF = basic fibroblast growth factor
DAPI = 4′,6-diamidino-2-phenylindole
DMEM = Dulbecco's modified Eagle's medium
EHS = embryonic heart slices
ETC = engineered tissue construct
FBS = fetal bovine serum
MI = myocardial infarction
NP = nonpreconditioned
P = preconditioned
PBS = phosphate-buffered saline
PCR = polymerase chain reaction
SM = skeletal myoblast

To address these electrophysiologic incompatibilities between transplanted cells and host myocardium, genetic manipulation of SMs before transplantation have been carried out in animal studies,[11,12] although they lack potential for clinical translation.

Different methods and platforms for the delivery of cells have been developed for various cell types, with supplemental pharmaceutical agents, and artificial or biological materials. Intramyocardial injection, although the most direct method of delivering cells to the heart, has from low cellular retention in the receiving tissue.[13,14] However, cell retention, survival, and integration are improved when cells are transplanted within engineered tissue constructs (ETCs) compared with injections as cell suspensions.[15,16] The application of ETCs allows precise control of cell numbers, shape, size, and structure of the graft with reduced cell loss by washout.[17]

Previously, we have shown that mechanical preconditioning of collagen-based and SM-containing ETCs led to the preservation of gap junction protein expression, which normally subsides during the differentiation of SMs to myotubuli and myofibers.[18] In addition, we confirmed that mechanical preconditioning led to preservation of the electromechanical competence of SMs, as they coupled to cardiomyocytes in vitro.[19]

The aim of the present study was to confirm electrical coupling between transplanted SM-containing ETCs and host myocardium in vivo by a novel application of the viable heart slice technique.[20]

METHODS

Cell Isolation

SMs were isolated as described before.[18] The resulting primary cells were resuspended in isolation medium, consisting of Ham's F10 medium (Invitrogen, Darmstadt, Germany), 20% fetal bovine serum (FBS; Invitrogen), 2.5 ng/mL basic fibroblast growth factor (bFGF; PeproTech, Hamburg, Germany), 0.5 μg/mL Fungizone (Invitrogen), and 1% penicillin/streptomycin (Invitrogen), transferred to coated (5 μg/cm^2 collagen type I; Invitrogen) dishes at a density of 10^5 cells/cm^2 and purified by preplating after serial transfers at 1, 2, 18, and 48 hours. After the last incubation step, nonadherent cells were discarded and the remaining adherent cells were collected, resuspended in growth medium (40% Ham's F10 medium, 40% Dulbecco's modified Eagle's medium (DMEM; Invitrogen), 20% FBS, 2.5 ng/mL bFGF, 0.5 μg/mL Fungizone, and 1% penicillin/streptomycin) and plated on collagen-coated dishes. Cell culture passages were performed at 80% confluence, including 15 minutes of preplating before transfer. After 1 week of expansion, the cells were used for the fabrication of ETCs.

Engineered Tissue Constructs

ETCs were prepared as previously published.[18,21] Briefly, SMs were counted and labeled with Vybrant DiI Cell-Labeling Solution (Invitrogen). The matrix, consisting of isolation medium, collagen (type I, 3 mg/mL; Invitrogen) and Geltrex (Invitrogen), was mixed with 5×10^6 SMs, cast into custom-made molds and incubated for 3 days at 37°C in 5% CO_2. These molds direct the mechanical strain exerted by polymerization along the axis between the fixed polyester meshes thus generating passive mechanical tension (preconditioned ETCs [P-ETCs]). As a control for the preconditioning effects, the cell matrix mixture was poured directly onto the cell culture dish (nonpreconditioned ETCs [NP-ETCs]; Figure 1). After 3 days, the ETCs were transferred to differentiation medium, consisting of DMEM, 2% horse serum (PAA Laboratories, Cölbe, Germany), 0.5 μg/mL Fungizone, 1% penicillin/streptomycin, and incubated for 10 days with medium changed daily.

ETC Transplantation

Animals received humane care in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. The local governmental authorities of the State of North Rhine-Westphalia (LANUV) approved all experiments.

Female C57BL/6 mice (6-8 weeks old) were operated on as previously described.[22] Briefly, after induction of anesthesia (initial, 5% for maintenance, 1.5% isoflurane (Deltaselect, Pfullingen, Germany) in an equal mixture of N_2O and O_2), the heart was exposed through a left lateral thoracotomy and the middle part of a fully differentiated, labeled ETC (approximately 3 mm × 3 mm) was transplanted onto the left ventricle, and held in position using 4 prolene sutures (8/0; Ethicon, Norderstedt, Germany; Figure 1).

Histochemistry

Mice were killed, hearts were removed, rinsed, and perfused with phosphate-buffered saline (PBS; Invitrogen). Cryosections of the hearts (10 μm) were stained with Masson's trichrome method following the manufacturer's instructions (Sigma-Aldrich). For immunofluorescence staining, cryosections were fixed and permeabilized with 4% paraformaldehyde, 0.25% Triton X-100, 0.5 M NH_4Cl (all Sigma-Aldrich) in PBS and blocked with 5% bovine serum albumin (Invitrogen) in PBS. The primary and secondary antibodies were diluted in PBS with 1% bovine serum albumin.

Primary antibodies were antidesmin (GeneTex, Irvine, Calif), anticonnexin 43 (Sigma-Aldrich), antidystrophin and anticardiac troponin I (both Santa Cruz Biotechnology, Santa Cruz, Calif) at concentrations recommended by the manufacturer and detected with species-specific Alexafluor-488– or Alexafluor-568–conjugated secondary antibodies (Invitrogen). Nuclei were stained using 4′,6-diamidino-2-phenylindole (DAPI, 1:1000; Invitrogen). Fluorescence microscopy was performed using an Eclipse Ti-U microscope with NIS Elements BR v3.10 software (Nikon, Düsseldorf, Germany).

Y-Chromosomal PCR

Genomic DNA was prepared from whole hearts using the DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany). Polymerase chain reactions