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The Journal of Heart and Lung Transplantation

http://www.jhltonline.org

An electrically coupled tissue-engineered cardiomyocyte scaffold improves cardiac function in rats with chronic heart failure

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KEYWORDS:
chronic heart failure;
ventricular function;
ventricles;
ejection fraction;
cardiomyocytes;
cell therapy

BACKGROUND: Varying strategies are currently being evaluated to develop tissue-engineered constructs for the treatment of ischemic heart disease. This study examines an angiogenic and biodegradable cardiac construct seeded with neonatal cardiomyocytes for the treatment of chronic heart failure (CHF).

METHODS: We evaluated a neonatal cardiomyocyte (NCM)-seeded 3-dimensional fibroblast construct (3DFC) in vitro for the presence of functional gap junctions and the potential of the NCM-3DFC to restore left ventricular (LV) function in an in vivo rat model of CHF at 3 weeks after permanent left coronary artery ligation.

RESULTS: The NCM-3DFC demonstrated extensive cell-to-cell connectivity after dye injection. At 5 days in culture, the patch contracted spontaneously in a rhythmic and directional fashion at 43 ± 3 beats/min, with a mean displacement of 1.3 ± 0.3 mm and contraction velocity of 0.8 ± 0.2 mm/sec. The seeded patch could be electrically paced at nearly physiologic rates (270 ± 30 beats/min) while maintaining coordinated, directional contractions. Three weeks after implantation, the NCM-3DFC improved LV function by increasing ($p < 0.05$) ejection fraction 26%, cardiac index 33%, dP/dt(+) 25%, dP/dt(–) 23%, and peak developed pressure 30%, while decreasing ($p < 0.05$) LV end diastolic pressure 38% and the time constant of relaxation (Tau) 16%. At 18 weeks after implantation, the NCM-3DFC improved LV function by increasing ($p < 0.05$) ejection fraction 54%, mean arterial pressure 20%, dP/dt(+) 16%, dP/dt(–) 34%, and peak developed pressure 39%.

CONCLUSIONS: This study demonstrates that a multicellular, electromechanically organized cardiomyocyte scaffold, constructed in vitro by seeding NCM onto 3DFC, can improve LV function long-term when implanted in rats with CHF.

J Heart Lung Transplant 2014;33:438–445

Chronic heart failure (CHF) is the leading cause of morbidity and mortality worldwide. Although current medical therapeutics decrease mortality from CHF, they do not reverse the disease process or restore long-term

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1053-2498/$ - see front matter
http://dx.doi.org/10.1016/j.healun.2013.12.004

cardiac function. Recently, a number of novel strategies have been proposed for improving functional and clinical outcomes of patients with CHF such as, cell-based therapies,[1] in vivo tissue reprogramming,[2] and gene therapy.[3] Each of these approaches may carry therapeutic potential, but cell-based therapies offer the least cumbersome approach and are not complicated by in vivo viral or gene administration.

Evaluation of cell-based therapies for CHF has progressed through a number of clinical trials.[4–10] Although questions remain regarding the most effective cell type and dosing strategies, the major limitation to success may be the development of an effective cell-delivery system. Current delivery techniques, for the most part, use direct injection by catheter-based systems that result in limited cellular survival and minimal retention of cells in the target area.[11,12] As a result, new cell-delivery strategies, such as tissue engineered constructs, are being developed that provide structural support facilitating implanted cell survival and integration into the underlying myocardium.[13–15]

Previous studies by our laboratory, and others, have tested a 3-dimensional fibroblast construct (3DFC) consisting of viable human dermal fibroblasts embedded onto a bioabsorbable polymeric Vicryl mesh (Ethicon, Somerville, NJ) that does not elicit an immunologic response.[16–18] Implantation of this 3DFC immediately after myocardial infarction (MI) or in an ischemic CHF model, 3 weeks after permanent occlusion of the left coronary artery, results in formation of a microvascular bed and the consequent increase in myocardial blood flow to the infarcted tissue.[19,20] The embedded fibroblasts are an important component of the bioengineered scaffold because dermal fibroblasts have been demonstrated to play a role in microvascular organization in vitro through paracrine-mediated effects or other factors.[21–23] Yet, implanting the 3DFC alone in CHF did not improve cardiac function.[19,20]

In the present study, we explored the potential of the microvascular bed induced by the 3DFC to support an overlaying population of cardiomyocytes seeded on the 3DFC. We demonstrate that rat neonatal cardiomyocytes (NCM) can be successfully cocultured with human fibroblasts in a biodegradable scaffold and that they form an electromechanically organized syncytium capable of improving the left ventricular (LV) function of a chronically infarcted heart.

Methods

The 3DFC

The 3DFC is a cryopreserved bioabsorbable scaffold populated with human neonatal fibroblasts.[16,17] The fibroblasts have been tested for cell morphology, karology, isoenzymes, and tumorigenicity and are free from viruses, retroviruses, endotoxins, and mycoplasma. The 3DFC was provided by Theregen Inc (San Francisco, CA) frozen (–75° ± 10°C) in 5 × 7.5 cm pieces with an average thickness of 200 μm. The 3DFC is thawed in phosphate buffered saline (34°C–37°C) and handled gently to limit cellular damage. The 3DFC does not generate an immune response[16–20] (investigators' brochure ITT-101, Theregen).

Cardiomyocyte isolation, seeding, and culture

Cardiomyocytes were isolated from 1- to 2-day-old neonatal Sprague-Dawley (Harlan, Indianapolis, IN) rat hearts. Briefly, the hearts were excised, the atria were removed, and the ventricles were minced into 0.5- to 1-mm portions and digested in a pancreatin/collagenase solution. After each enzymatic digest, cardiomyocytes were collected, combined, and resuspended in Dulbecco Modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS). Lastly, the suspension was differentially plated in Ham's F-12 with 100 mg/ml bovine serum albumin.

The 3DFC was thawed, cut into 1.5-cm diameter sections, and seeded with NCM at densities ranging from 0.6×10^6 to 2.7×10^6 cells/cm^2 using methods developed in our laboratory. The NCM and NCM-3DFC were cultured in 10% FBS in DMEM-LG (Gibco-Invitrogen, Carlsbad, CA), maintained at 37°C and 5% CO_2. Patches were constructed as described for in vitro and in vivo evaluation. The NCM-3DFC patches for in vivo evaluation were seeded, cultured, and implanted onto the rat heart 3 weeks after left coronary artery ligation within 18 hours of seeding. Patches prepared for in vitro evaluation were seeded and cultured 1 to 10 days.

Quantitative measurements of cardiomyocytes and fibroblasts

Serial stained tissue samples were reacted with specific markers for cardiomyocytes (troponin) and fibroblasts (vimentin). Immunohistochemically reacted glass slides were digitally scanned (whole-slide scanning) using an Aperio scanner (Aperio, Vista, CA), with subsequent FACTS (Feature Analysis on Consecutive Tissue Sections). Whole-slide scanning allowed for an objective digital quantitative analysis of the entire tissue sections to ensure against investigator bias. The parameters for a generic image analysis algorithm for quantifying cells were independently adjusted for counting cardiomyocytes and fibroblasts. This was used for the objective analysis. The algorithm-based FACTS process performs image-to-image registration on prepared tissue sections on each glass slide. The FACTS process was used to align the serial sections to ensure that corresponding areas were analyzed in each stained slide to provide a fair sample comparison.

Field stimulation and pacing

NCM-seeded 3DFC patches were transferred to a thermoregulated culture well (Bipolar Temperature Controller; Medical Systems, Greenvale, NY) containing culture medium maintained at 37°C. The NCM-3DFC were paced (S44; Grass Instruments, Quincy, MA) by field stimulation (~7 V) through 99.7% pure silver wires placed into the culture well on opposing sides of the NCM-3DFC. The pacing rate varied from 60 to 270 ± 30 beats/min for 10 seconds.

Cell-to-cell communication

Functional gap junction formation was examined in 3DFC, NCM-3DFC, and halothane-treated NCM-3DFC patches at 6 days. Three dyes were injected simultaneously: [2-(4-nitro-2,1,3-benzoxadiol-7-yl)aminoethyl]trimethylammonium (NBD-TMA [provided by