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An Electrically Coupled Tissue Engineered Cardiomyocyte Scaffold Improves Cardiac Function in Rats with Chronic Heart Failure

Jordan J. Lancaster, BS^{1,2,3}, Elizabeth Juneman, MD^{1,2}, Sarah A. Arnice, BS^{1,2}, Nicholle M. Johnson, BS^{1,2}, Yexian Qin, PhD⁴, Russell Witte, PhD⁴, Hoang Thai, MD^{1,2}, Robert S. Kellar, PhD⁵, Jose Ek Vitorin, PhD, MD³, Janis Burt, PhD³, Mohamed A. Gaballa, PhD⁶, Joseph J. Bahl, PhD^{1,2}, and Steven Goldman, MD.^{1,2}

¹Cardiology and Medicine, Southern Arizona VA Health Care System, Tucson, Arizona

²Sarver Heart Center, University of Arizona, Tucson, Arizona

³Department of Physiology, University of Arizona, Tucson, Arizona

⁴Medical Imaging, University of Arizona, Tucson, Arizona

⁵Northern Arizona University, Flagstaff, Arizona

⁶Banner Sun Health Research Institute, Sun City, Arizona

Abstract

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 three-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 following 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 near physiological 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 (PDP) 30%, while decreasing ($p < 0.05$) LV end diastolic pressure 38% and the time constant of relaxation (Tau) 16%. Eighteen weeks post 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 PDP 39%.

Correspondence: Jordan Lancaster, BS, Cardiology Section, 1-111C; SAVAHCs, 3601 S. 6th Avenue, PH: (520) 792-1450, ext 6319; FAX: (520) 629-4636, Jordan.Lancaster@va.gov.

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Conclusion—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.

Keywords

Chronic heart failure; ventricular function ventricles; ejection fraction; cardiomyocytes; cell therapy

Background

Chronic heart failure (CHF) is the leading cause of morbidity and mortality worldwide. While current medical therapeutics decrease mortality from CHF, they do not reverse the disease process nor restore long-term 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 re-programming (2), and gene therapy (3). While each of these approaches may carry therapeutic potential, utilization of 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). While 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, employ direct injection via 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) comprised of viable human dermal fibroblasts embedded onto a bioabsorbable polymeric vicryl mesh that does not elicit an immunologic response (16–18). Implantation of this 3DFC immediately after myocardial infarction (MI) or in an ischemic chronic heart failure (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, as dermal fibroblasts have been demonstrated to play a role in microvascular organization *in vitro* through either paracrine mediated effects or other factors (21–223). 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 co-cultured 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, karyology, isoenzymes, and tumorigenicity and are free from viruses, retroviruses, endotoxins and mycoplasma. The 3DFC is provided by Theregen, Inc. (San Francisco, CA) frozen (–75°

$\pm 10^{\circ}\text{C}$) in pieces 5×7.5 cm with an average thickness of 200 micrometers. The 3DFC is thawed in phosphate buffered saline ($34\text{--}37^{\circ}\text{C}$) and handled gently to limit cellular damage. The 3DFC does not generate an immune response (16,17,19,20, Investigators' brochure ITT-101, Theregen).

Cardiomyocyte Isolation, Seeding and Culture

Cardiomyocytes were isolated from 1–2 day old neonatal Sprague Dawley (Harlan, Indianapolis, IN) rat hearts. Briefly, the hearts were excised, atria removed and ventricles minced into 0.5–1 mm portions, and digested in a pancreatin/collagenase solution. Following each enzymatic digest, cardiomyocytes were collected, combined and re-suspended in DMEM with 10% FBS. Lastly, the suspension was differentially plated in Ham's F-12 with 100mg/ml BSA. The 3DFC was thawed, cut into 1.5cm diameter sections and seeded with NCM at densities ranging from 0.6×10^6 to 2.7×10^6 cells/cm² 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% CO₂. Patches were constructed as described for both *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 hrs of seeding. Patches prepared for *in vitro* evaluation were seeded and cultured 1–10 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 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 & Pacing

Neonatal cardiomyocyte seeded 3DFC patches were transferred to a thermo regulated 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 ($\sim 7\text{V}$) 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 (NDB-TMA (provided by Dr. Steve Wright, University of Arizona, Tucson, AZ (24)); mol wt 280, net charge 1+, 10 mM), Alexa 350 (mol wt 326, net charge 1–, 10 mM), and Rhodamine Dextran (0.1 mg/ml) (both Molecular Probes - Invitrogen, Carlsbad, CA). Microelectrode tips were created from 1.0 mm filament glass (A–M Systems, Sequim, WA) on a Sutter Instruments puller (Novato, CA), their tips filled by capillary action with the dye mixture and backfilled with 3M KCl; microelectrodes were lowered onto a cell field in the preparation until contact with a cell

surface was achieved and cytoplasm access was gained. The dyes were slowly and continuously injected by a slight capacitance overcompensation of the amplifier (SEC-05LX, NPI electronics, Tamm, Germany). Images were captured at different times during the next 5–20 minutes. Halothane, a known gap junction inhibitor, was added to the superfusate (final concentration ~8mM) to temporarily decrease intercellular dye diffusion and further confirm the transjunctional nature of this phenomenon (25–27).

Multi Electrode Array Electrical Mapping

The NCM-3DFC was electrophysiologically mapped in real-time using a ten-electrode custom designed multi electrode array (AdTech Medical Inc) and 1 kHz low pass filter. Action potentials were collected using a BIOPAC MP150 data acquisition system with MCE100C signal conditioning modules. The MCE module has multiple gain settings from 10–1000 and on-board configurable signal filter. Up to 16 channels of data were acquired simultaneously at 5 kHz sampling rate. Notch filters were configured in the data acquisition system for removing the 60Hz noise and its higher harmonics. Data recordings were collected in 10 seconds intervals. Seeded patches were evaluated *in vitro* at 1–8 days in culture for spontaneous field potential amplitude, duration, conduction velocities, propagation patterns and monophasic action potentials. The NCM-3DFC was maintained at 37°C in standard culture medium.

Coronary artery ligation experimental CHF

All animals in this study received humane care in compliance with the ‘Principles of Laboratory Animal Care’ and the ‘Guide for the Care and Use of Laboratory Animals’. The rat coronary artery ligation model is standard in our laboratory (28–30). In brief, Sprague Dawley rats are anesthetized with ketamine, acepromazine, and undergo a left thoracotomy; the heart is expressed from the thorax and a ligature placed around the proximal left coronary artery. The rats are maintained on standard rat chow, water ad-libitum, and pain medication post operatively. Only rats with EF > 35% following permanent left coronary artery ligation at 3 weeks post MI were enrolled in the study. Three weeks after MI, the chest is re-opened and the NCM-3DFC was implanted with suture on the infarcted ventricle bridging to viable myocardium.

Echocardiography

Closed chest transthoracic echocardiography is performed using a Vingmed, Vivid 7 system echo machine with EchoPac (both GE Ultrasound, Fairfield, CT) programming software with a 10 MHz multiplane transducer with views in the parasternal short axis and long axis, to evaluate the anterior, lateral, antero-lateral, inferior and posterior walls. Systolic displacement of the anterior wall and EF are obtained from 2D and M-mode measurements of myocardial wall thickness and LV dimensions (19,31).

Hemodynamic measurements *in vivo*

Rats are anesthetized with inactin (Sigma) (100 mg/kg intraperitoneal injection), intubated, and placed on a rodent ventilator with a 2F solid state micromanometer tipped catheter with two pressure sensors (Millar Instruments, Inc. Houston, TX) inserted via the femoral artery, with one sensor located in the left ventricle and another in the ascending aorta. The pressure sensor is equilibrated in 37°C saline, LV and aortic pressures/heart rate are recorded, digitized at a rate of 1000 Hz to calculate LV dP/dt and the time constant (Tau) of LV relaxation (19,25–29). Hemodynamics was obtained at the end of study, 6 weeks post MI (3 weeks post implantation of 3DFC) and 21 weeks post MI (18 weeks post implantation).

Statistical Analysis

Data are expressed as mean $\pm$ standard error (SE). The Student t-test is used for single comparison of group versus group analysis of statistical significance ($P < 0.05$).

Results

Cardiomyocyte Seeding

Cardiomyocyte seeding densities were evaluated between 0.6 to 2.7×10^6 cells/cm². Lower density cardiomyocyte preparations (0.6 to 1.2×10^6 cells/cm²) displayed spontaneous, non-synchronized contractions after 48 hours in tissue culture. At 72 hours these contractions began synchronizing and by 84 hours cell contractions were fully synchronized. Higher density cardiomyocyte preparations (1.8 to 2.7×10^6 cells/cm²) displayed synchronized and spontaneous contractions of the entire scaffold after 48 hours in tissue culture (Video 1). Contractions increased in robustness from 48 hours to 5 days. At day 5, scaffolds seeded with 2.7×10^6 cells/cm² contracted in a consistent rhythmic and directional fashion. Neonatal cardiomyocytes plated in 35 mm² plates (control) displayed contractions at 97 ± 4 beats/min while NCM-3DFC was recorded at 43 ± 3 beats/min (Sup. Fig. 1&2) with a mean displacement of 1.3 ± 0.3 mm (Sup. Fig. 3) and a contraction velocity of 0.8 ± 0.2 mm/sec (Sup. Fig. 4) ($N=10$).

In Vitro Cellular Composition

In vitro quantification of the cellular composition of the NCM-3DFC show a fibroblast nuclear density ($N=5$) of 931 ± 72 and 974 ± 95 nuclei/mm² and a cardiomyocyte nuclear density ($N=4$) of 5808 ± 527 and 4923 ± 287 nuclei/mm² at 2 and 8 days in culture, respectively. Thus, a 5:1 ratio of myocyte to fibroblast nuclear density and no cell loss between two and eight days in culture.

Field Stimulation

External field stimulation was applied to the NCM-3DFC at varying culture time points (2–6 days) in temperature controlled (37° C) wells. The NCM-3DFC contracted synchronously (Video 2) when paced for 20-second intervals at physiological rates up to 270 ± 30 beats/min (32).

Cell-to-Cell Communication

Dye injections into the fibroblasts of the 3DFC alone resulted in no detectable transjunctional diffusion (Fig. 1A). In contrast, dye diffusion readily occurred from the injected myocytes toward neighboring cells on the NCM-3DFC (Video 3, Fig. 1B). Furthermore, as can be expected from the gradual organization of cell bundles, junctional connectivity increased from day 2 through 6 days (data not shown). When the NCM-3DFCs were exposed to halothane, dye was retained in the donor cell, reaching higher concentrations but failing to diffuse to the neighboring cells in spite of the obvious intercellular gradient (Fig. 1C).

Electrical Mapping

The spontaneous electrical activation of the NCM-3DFC was mapped by electrically recording constructs at varying times in tissue culture. The NCM-3DFC yields max and min transverse conduction voltage amplitude of $42 \mu\text{V}$ and $-75 \mu\text{V}$ respectively. A consistent sequence of transverse activation was seen beat-to-beat (Video 4, Fig. 2).

Hemodynamics and Echocardiography

At 3 weeks after implantation the NCM-3DFC increased ($p<0.05$) EF from 31 ± 2 to $39\pm 1\%$ (Fig. 3), cardiac index from 0.46 ± 0.05 to 0.61 ± 0.06 mL/(g min), $dP/dt(+)$ from 4651 ± 250.4 to 5806.2 ± 192.1 mmHg/sec, $dP/dt(-)$ from 2852.9 ± 147.7 to 3516.9 ± 229.5 mmHg/sec, and peak developed pressure (PDP) from 112.2 ± 7.6 to 146.4 ± 5.4 mmHg (Table 1), while decreasing ($p<0.05$) LV end diastolic pressure from 24 ± 2 to 15 ± 3 mmHg and Tau from 24.9 ± 1.2 to 20.9 ± 1.1 msec (Table 1). At 18 weeks post implantation, the NCM-3DFC improved LV function by increasing ($p<0.05$) EF from $21.8\%\pm 2.8$ to $33.6\%\pm 4.1$ (Fig. 3), MAP from 84.3 ± 1.7 to 101 ± 2.9 mmHg, $dP/dt(+)$ from 4047.7 ± 216.6 to 4713.0 ± 118.4 mmHg/sec, $dP/dt(-)$ from 2172.3 ± 130.2 to 2915.3 ± 218.6 mmHg/sec and PDP from 98.3 ± 9 to 137.3 ± 5 mmHg (Table 2). Importantly, the rats maintained normal sinus rhythm throughout the entire study and the echocardiography revealed the previously infarcted area contracted in sync with the rests of the heart with no arrhythmias and no evidence of dyssynchrony.

LV Cross Sections

As shown in Figure 4, implantation of the NCM-3DFC is well tolerated and within three weeks of implantation the synthetic vicryl components have degraded and are not visible. As shown by echocardiography and hemodynamics, implantation of the NCM-3DFC results in improvements of cardiac function. This may be in part due to the effective integration of the transplanted myocytes or, alternatively, and enhanced survival/migration/function of endogenous myocardium under the implant. In either case, an increased presence of myocytes in or around the infarcted area was observed (Fig. 4).

Discussion

Previous work from our laboratory has evaluated the use of the 3DFC alone for the treatment of acute MI (19) and CHF (20). While the 3DFC alone improved LV function if implanted at the time of an MI, it did not improve LV function when implanted 3 weeks after coronary ligation. This suggests that once LV remodeling is established the implantation of fibroblasts, which alone can release cytokines and increase myocardial blood flow, is not sufficient to reverse maladaptive remodeling and improve LV function. The goal of the present study was to evaluate the angiogenic 3DFC as a platform for cardiomyocyte seeding and as a cell delivery system for cardiac repair and restoration of LV cardiac contractility in a setting of CHF.

We have shown that NCM can be seeded and co-cultured onto the 3DFC resulting in NCM-3DFC patches that contract spontaneously in a rhythmic, synchronous manner in culture (Video 1). This suggests that the seeded myocytes achieve structural organization and formation of gap junctions allowing electromechanical synchrony. We confirmed the existence of functional gap junctions by injecting junctional permeant dye into contractile cardiomyocytes, and documenting its cell-to-cell diffusion (Video 3, Fig. 1B). To further confirm the transjunctional nature of this diffusion, gap junctions were transiently inhibited by halothane administration (25–27), effectively blocking dye passage (Fig. 1C). Quantitative evaluation of cellular composition showed no loss of cells during tissue culture between two and eight days and a nuclear density ratio of 5:1 (NCM to fibroblasts). To determine response to external electrical signaling the NCM-3DFC were subject to electrical field stimulation. Patches maintained rhythmic and synchronized contractions while displaying increased rates of contraction, suggesting the potentially ability to track in sinus rhythm *in vivo* (Video 2). Furthermore, the NCM-3DFC was electrically mapped using a MEA system showing consistent beat-to-beat electrical activation and demonstrating cellular coupling (Video 4, Fig. 2). It is important to highlight that the NCM-3DFC patches were

implanted into rats within 18hrs of construction, allowing for cellular adhesion of the seeded NCM onto the 3DFC, but prior to the onset of spontaneous contractions and subsequent cellular organization; potentially allowing the underlying native myocardium to dictate organization *in vivo*. When the construct was implanted in rats with CHF, the rats maintained sinus rhythm with coordinated contractions and no arrhythmias were observed. Echocardiographic data showed no LV dyssynchrony, supporting the observation that the patch does not alert local LV electrical-mechanical function (Fig. 4).

When implanted into rats with CHF and EFs below 35%, the NCM-3DFC improved ($p<0.05$) LV function 3 weeks after implantation by increasing EF, CI, $dP/dt(+)$, $dP/dt(-)$ and PDP while lowering EDP and LV relaxation (Table 1). In addition, long term improvements were also observed 18 weeks after implantation by increasing EF, MAP, $dP/dt(+)$, $dP/dt(-)$ and PDP (Table 2 and Fig. 3). While the mechanism of action has not been fully elucidated, histologic cross sections at 3 and 18 weeks post implantations show an increase in viable myocardium underneath the patch (Fig. 4). This population of cells may be in part from the transplanted NCMs, endogenous cardiomyocyte migration or enhancement of the native viable myocardium. It is likely that one component of this beneficial effect is due to a paracrine mediated response generated from the transplanted cells. Our previous work has shown that once implanted, the dermal fibroblasts embedded in the 3DFC secrete angiogenic cytokines establishing microvascular support and increased blood flow (20). The angiogenic cytokines may be supplementing and facilitating the survival of the transplanted cardiomyocytes.

One of the clinical hurdles of tissue-engineering scaffolds is the method of delivery. In this study, the delivery of a patch/scaffold onto the infarcted myocardium required an open thoractomy. This method of delivery is suitable for those patients who undergo open thoracotomies for placement of ventricular assist devices or coronary bypass grafting. With the advancement of thoracic robotic-assisted surgery, the patch/scaffold could be placed minimally invasively, through a small working port. This surgical approach has been shown to lead to shorter ventilation times and hospitalizations when compared to an open thoracotomy approach.

We believe that there is future potential for cell-based therapy for heart failure. For the type of delivery system outlined here to advance into clinical trials for CHF, work will have to be done developing a patch with a clinically relevant cell type. In addition, further investigation is needed into understanding the mechanism(s) of action of cell-based therapy such as fate of transplanted cells, defining paracrine factors stimulated by different cell types, and potential minimally invasive approaches to delivery patches to the heart.

Conclusion

This study demonstrates that a multicellular, electromechanically organized, cardiomyocyte scaffold can be engineered *in vitro* by seeding and co-culturing NCM onto 3DFC. When implanted in a rat model of CHF the NCM-3DFC improves LV function both short and long term when evaluated using echocardiography and hemodynamics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Time elapsed images, 40x magnification, of A) 3DFC, B) NCM-3DFC, and C) NCM-3DFC treated with halothane, at day 6 of culture. In all images, dye-filled electrode is at the left, and its tip allows the location of the injected cell (donor). From this cell, dye may diffuse across gap junctions to other (recipient) cells. The dye NBD easily occupies the cytoplasmic space, but does not penetrate the nucleus; thus, individual recipient cells can be located by the image of NBD fluorescence surrounding a central, less fluorescent area. A) No detectable intercellular dye transfer occurs from the injected fibroblast to neighboring cells. B) The dye injected into a single neonatal cardiomyocyte spreads through established gap junctions toward neighboring cells. C) After halothane treatment, a transient decrease on the speed of transjunctional dye diffusion was observed. In this particular image, the reflection of the highly concentrated dye in the injected cell is perceived, but only one recipient cell (adjacent to the injected one) can be clearly identified after 5 minutes.

Figure 2.

Electrical activation mapping was performed on the NCM-3DFC in tissue culture 5 days after co-culturing using a custom designed multi-electrode array (MEA) with 18 recording sites spaced $500\mu\text{m}$ apart (A). Recordings were performed from 10 electrodes; each recording site was numbered sequentially as channel 1–10 (B). The electrical activation of the patch shows consistent beat-to-beat activation as shown in 7 sec interval displaying the peak transverse conduction voltage for each individual channel (C). The amplitude is shown with all channels superimposed in a beat-to-beat sequence (D) and during a single activation (E). The amplitude was recorded as 0.03 to 0.42 and -0.13 to -0.75 mV (D & E).

Figure 3.

Echocardiography evaluation of ejection fraction in rats with chronic heart failure 6, 10, 14 and 18 weeks patch implantation. Implantation of the NCM-3DFC improves ($p < 0.05$) ejection fraction as compared to controls. Data are mean $\pm$ SE. α and β denote statistical significance ($p < 0.05$) CHF vs. NCM+3DFC and Sham vs. CHF respectively. CHF = chronic heart failure, NCM = neonatal cardiomyocytes, 3DFC = 3 dimensional fibroblast construct. Sham, N=8; CHF, N= 20-2; NCM-3DFC, N=26-3.

Figure 4.

Trichrome-stained LV cross sections of 6wk CHF control receiving an infarct but no treatment (A–C), CHF + NCM-3DFC 6 weeks after coronary artery ligation (3 weeks after implant) (D–F) and CHF + NCM-3DFC 21 weeks after coronary artery ligation (18 weeks after implant) (G–I). Hearts were excised; right ventricles removed and cut in 5μm transverse sections along the midpoint of the ventricle. Healthy myocardium is represented as red-purple, collagen/scar as blue, and red blood cells as small red dots. Asterisk (A, D, and G) and box insets (B, E, H) represents area of corresponding higher magnification. Plus signs (+) in panel D and G highlight the location of the NCM-3DFC implant. Implantation of a cardiomyocyte patch results in increased LV wall thickness (D&E and G&H vs. A&B) and preservation and/or generation of myocardium (D–F and G–I). The synthetic vicryl components of the patch are degradable and not detectable after three weeks *in vivo*. Remnants of the suture used for implantation of the NCM-3DFC can be seen as the bright red clusters (E&F).

Table 1
Three week endpoint hemodynamics for rats treated with NCM-3DFC

Hemodynamic values three weeks after NCM-3DFC implantation in rats with CHF. Data are mean $\pm$ SE. α , $p < 0.05$ CHF vs. NCM+3DFC and β , $p < 0.05$ Sham vs. CHF. Implantation of the NCM-3DFC improved ($p < 0.05$) EDP, CI, dP/dt, Tau and PDP as compared to untreated CHF rats. Sham, N = 7–20; CHF, N = 6–12; NCM-3DFC, N = 7–13. MAP, mean arterial pressure; SYS, systolic pressure; EDP, end diastolic pressure; CI, cardiac index; PDP, peak developed pressure; CHF, chronic heart failure; NCM+3DFC, neonatal cardiomyocyte 3 dimensional fibroblast construct.

	MAP	SYS	EDP	CI	dP/dt (+)	dP/dt (-)	Tau	PDP
	mmHg	mmHg	mmHg	(ml/(minXg))	mmHg/s	mmHg/s	ms	mmHg
Sham	129 $\pm$ 4	128 $\pm$ 4	5 $\pm$ 1	0.52 $\pm$ 0.04	7146 $\pm$ 285	6368 $\pm$ 468	15 $\pm$ 1	171 $\pm$ 5
CHF	103 $\pm$ 4 β	124 $\pm$ 5	27 $\pm$ 2 β	0.45 $\pm$ 0.05 β	4651 $\pm$ 250 β	2853 $\pm$ 148 β	25 $\pm$ 1 β	112 $\pm$ 8 β
NCM+3DFC	100 $\pm$ 5	126 $\pm$ 4	15 $\pm$ 3 α	0.61 $\pm$ 0.06 α	5806 $\pm$ 192 α	3517 $\pm$ 230 α	21 $\pm$ 1 α	146 $\pm$ 5 α

Table 2
Eighteen week endpoint hemodynamics for rats treated with NCM-3DFC

Hemodynamic values 18 weeks after NCM-3DFC implantation in rats with CHF. Data are mean $\pm$ SE. α , $p < 0.05$ CHF vs. NCM+3DFC and β , $p < 0.05$ Sham vs. CHF. Implantation of the NCM-3DFC improved ($p < 0.05$) MAP, dP/dt, and PDP as compared to untreated CHF rats. Sham, N= 8; CHF, N=3–6, NCM+3DFC, N=2–3. MAP, mean arterial pressure; SYS, systolic pressure; EDP, end diastolic pressure; CI, cardiac index; PDP, peak developed pressure; CHF, chronic heart failure; NCM+3DFC, neonatal cardiomyocyte 3 dimensional fibroblast construct.

	MAP	SYS	EDP	CI	dP/dt (+)	dP/dt (-)	Tau	PDP
	mmHg	mmHg	mmHg	(ml/(minXg))	mmHg/s	mmHg/s	ms	mmHg
Sham	119 $\pm$ 2	119 $\pm$ 2	8 $\pm$ 1	0.35 $\pm$ 0.03	7355 $\pm$ 400	6103 $\pm$ 287	16 $\pm$ 1	178 $\pm$ 6
CHF	84 $\pm$ 2 β	105 $\pm$ 7 β	28 $\pm$ 3 β	0.32 $\pm$ 0.03	4048 $\pm$ 217 β	2172 $\pm$ 130 β	29 $\pm$ 1 β	98 $\pm$ 9 β
NCM+3DFC	101 $\pm$ 3 α	111 $\pm$ 6	17 $\pm$ 7	0.33 $\pm$ 0.06	4713 $\pm$ 118 α	2915 $\pm$ 219 α	25 $\pm$ 2	137 $\pm$ 5 α