Cardiac Tissue Slice Transplantation as a Model to Assess Tissue-Engineered Graft Thickness, Survival, and Function

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Background—Cell therapies offer the potential to improve cardiac function after myocardial infarction. Although injection of single-cell suspensions has proven safe, cell retention and survival rates are low. Tissue-engineered grafts allow cell delivery with minimal initial cell loss and mechanical support to the heart. However, graft performance cannot be easily compared, and optimal construct thickness, vascularization, and survival kinetics are unknown.

Methods and Results—Cardiac tissue slices (CTS) were generated by sectioning mouse hearts (n=40) expressing firefly luciferase and green fluorescent protein into slices of defined size and thickness using a vibrating blade microtome. Bioluminescence imaging of CTS transplanted onto hearts of immunodeficient mice demonstrated survival of ≤30% of transplanted cells. Cardiac slice perfusion was re-established within 3 days, likely through anastomosis of pre-existing vessels with the host vasculature and invasion of vessels from the host. Immunofluorescence showed a peak in cell death 3 days after transplantation and a gradual decline thereafter. MRI revealed preservation of contractile function and an improved ejection fraction 1 month after transplantation of CTS (28±2% CTS versus 22±2% control; P=0.05). Importantly, this effect was specific to CTS because transplantation of skeletal muscle tissue slices led to faster dilative remodeling and higher animal mortality.

Conclusions—In summary, this is the first study to use CTS as a benchmark to validate and model tissue-engineered graft studies. CTS transplantation improved cell survival, established reperfusion, and enhanced cardiac function after myocardial infarction. These findings also confirm that dilative remodeling can be attenuated by topical transplantation of CTS but not skeletal muscle tissue grafts. (Circulation. 2014;130[suppl 1]:S77-S86.)

Key Words: cell transplantation ■ magnetic resonance imaging ■ myocardial infarction ■ tissue engineering

Cardiovascular diseases are the primary cause of death in the industrialized world. Cell therapies delivering single-cell suspensions of autologous skeletal myoblasts, bone marrow cells, or sorted subpopulations into the myocardium or coronary arteries after myocardial infarction (MI) have proven safe but may lack efficacy. This may be due to the limited ability of these cells to survive and convert into fully mature contractile cardiomyocytes. The development of protocols for efficient differentiation of cardiomyocytes from pluripotent stem cells, such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), allows the production of large numbers of cells for transplantation. For example, preclinical experiments have demonstrated that injection of ESC-derived cardiomyocytes into infarcted hearts generates stable grafts showing electric coupling with their host. However, typically <1% of injected cells survive for >1 month, limiting the clinical use of this approach. Multiple factors such as loss of cell-cell contact, lack of appropriate mitogens, mechanical stress, inflammation, and ischemia likely contribute to cell loss.

Tissue-engineered constructs are a potential alternative to cell injections and offer additional mechanical support to limit dilative remodeling. Numerous approaches have been explored to generate matrix-free cell sheets or 3-dimensional tissue grafts (reviewed by Ye et al), some of which have advanced to clinical trials. Existing literature suggests that functional benefits of tissue-engineered grafts transplanted onto infarcted hearts may range from increased cardiac wall thickness to reduced dilative remodeling. However, a major drawback of this approach is the limited scalability because of oxygen diffusion, which can restrict graft thickness to a few hundred micrometres in the absence of a functional vascular network. Although addition of endothelial cells or fibroblasts improves survival, the optimal cellular composition of grafts is not known.

To date, systematic analyses have not been undertaken to determine the maximum thickness of such grafts at the time of transplantation or the potential changes of cellular composition after transplantation. Here, we show that cardiac tissue slices (CTS) of defined size and thickness made from uninjured...
mice are a suitable standard for comparison of tissue-engineered grafts. We assess the survival of CTS transplanted onto normal or infarcted hearts in vivo using bioluminescence imaging (BLI), characterize cell death, CTS composition, and analyze revascularization of CTS by host vessels. Finally, we use cardiac MRI to quantify the effect of CTS transplantation on infarct size and cardiac remodeling in a mouse MI model.

Methods

An expanded Methods section is available in the online-only Data Supplement.

Transgenic and Reporter Mice Used for This Study

Transgenic L2G mice that express green fluorescent protein and firefly luciferase (Fluc) reporter genes under the control of the β-actin promoter were maintained on a Friend Virus B-type (FVB) background.12 The Cre reporter line Rosa26-tdTomato that labels cells with tdTomato (Cdh5)-Cre transgenic mice,13 Sox2-Cre transgenic mice,15 and immuno-deficient Nonobese Diabetic/Severe Combined Immunodeficiency (NOD/SCID) mice were obtained from the Jackson Laboratory. All procedures involving animals were approved by the Stanford Institutional Animal Care and Use Committee in accordance with established guidelines for animal care.

Generation of CTS

Adult (8–12 weeks; n=40) L2G mice were anesthetized using isoflurane. Hearts were perfused with ice-cold modified Tyrode solution, excised, embedded in 4% low melting point agarose (Invitrogen, Carlsbad, CA), and placed on ice. The solidified block was mounted on a sample holder and sectioned with a vibratome. Sections with different thicknesses (100–800 μm) were prepared from the left ventricular free wall. To standardize the size of grafts, circular grafts with 3 or 5 mm diameter were punched out of sections using a core sample puncher and stored in modified Tyrode solution until transplantation (Figure 1A). These discs are referred to as CTS. Skeletal muscle tissue slices were made from thigh muscles (vastus lateralis) of transgenic L2G mice using a similar procedure.

MI and CTS Transplantation

MI was induced in NOD/SCID mice by permanent left anterior descending coronary artery ligation under 1.5% to 2% inhaled isoflurane anesthesia. Animals were randomized into experimental groups receiving CTS (n=36), skeletal muscle patches (n=12), or control group receiving no patch (n=12). For experiments involving cardiac assessment via MRI, CTS and skeletal muscle patches were attached onto the left ventricular free wall with 4 to 5 stitches using 10-0 silk sutures. For patch survival and reperfusion experiments, CTS with a diameter of 3 mm were transplanted directly after left anterior descending coronary artery occlusion. CTS and skeletal muscle patches were attached onto the left ventricular free wall with 4 to 5 stitches using 10-0 silk sutures. For patch survival and reperfusion experiments, CTS with a diameter of 3 mm were transplanted onto the left ventricular free wall of noninfarcted mice (n=4). The total amount of BLI signal per heart increased as the thickness of CTS was increased from 100 to 200 μm; a further increase to 400 μm or 800 μm led to decreased signal intensity (n=5 for each group). A similar pattern was observed for CTS transplanted onto infarcted hearts (n=5 for each group), but the overall signal was lower. Average radiance expressed as 10^6 photons/second per cm² per steradian. MI indicates myocardial infarction.

Assessment of Endothelial Cell Origin of Reperfused CTS

CTS were transplanted onto noninfarcted hearts of adult VE-cadherin-Cre; Rosa26-tdTomato double-heterozygous mice (n=18; C57BL/6 background). To prevent immune rejection, tacrolimus (Astellas, 5 mg/kg per day) was orally administered every 12 hours.

Survival of Neonatal CTS

Because most tissue-engineered grafts are made of differentiated ESCs or iPSCs that may be immature, we also generated neonatal CTS to model the survival of such cells. Neonatal CTS were derived from 1-day-old L2G pups. Neonatal CTS were transplanted onto the epicardial surface of adult control (n=5) or infarcted (n=5) NOD/SCID mice.

Bioluminescence Imaging

BLI was performed using the Xenogen in vivo Imaging System (Alameda, CA) as previously described.45 Immunofluorescence and Histological Methods

Immunofluorescence and histological analyses were performed using standard protocols.

Magnetic Resonance Imaging

Cardiac function and scar size were assessed on day 1 and day 28 after MI induction and CTS transplantation using a 7T MR901 Discovery horizontal bore scanner (Agilent Technologies, Santa Clara, CA).

Statistical Analysis

Results are shown as mean±SEM unless stated otherwise. To test whether a linear relationship between the number of viable cells and the radiance measured by BLI exists, a regression analysis was performed. To verify whether the radiance from 300 μm CTS changed between days 7 and 60, a 2-tailed Wilcoxon rank-sum test was used.
To test whether infarct and CTS thickness were significant factors explaining the observed variation in BLI data, a linear mixed-effects model with fixed effects for CTS thickness, time, and MI and random effects for individual mice was used (residuals were tested for normality using a Shapiro–Wilk test). For comparisons of the radiance from specified groups at day 28, a Wilcoxon rank-sum test followed by Bonferroni correction of P values (6 multiple comparisons) was used. To illustrate diffusion-limited survival, a linear mixed-effects model with fixed effects for CTS thickness, CTS thickness squared, MI and random effects for individual mice and measurement time points (BLI day 7–28 data) was used. To test whether increasing CTS thickness leads to equivalent increases in BLI signal intensity, a regression analysis was performed. To test whether differences in scar size could be explained by treatment groups, a Kruskal–Wallis test was performed. To assess whether there are any differences in survival between MRI treatment groups, a Kaplan–Meier survival analysis was performed using a log-rank test. To test for differences in cardiac function, a linear mixed-effects model was used with fixed effects for functional parameter, treatment group, time and a random effect for individual mice. If such an effect was found, Wilcoxon rank-sum tests with Bonferroni corrections of P values were performed (3 multiple comparisons). Data correlations were tested using a Pearson correlation test. Statistical analysis was performed using R software version 2.8.1.

Results

Cardiac Tissue Slice Transplantation as a Model to Assess Graft Survival

To evaluate whether healthy cardiac tissues transplanted onto the heart wall can survive long-term in vivo, CTS from adult L2G mice were transplanted onto hearts of immunocompromised mice. Cell loss was observed for the first 7 days with no further decline in cell survival thereafter (P=0.68 day 7 versus day 56; Figure 1B and 1C; Figure 1A in the online-only Data Supplement). At 2 months, 31±4% of cells from 300-μm-thick CTS remained viable, which is higher than <1% viability seen with traditional cell injections.12,13 BLI signal intensity (on a particular day) relative to initial signal intensity was used as a surrogate for viability because a linear relationship between the number of viable cells and signal intensity was found in vitro (intercept: P=0.24; slope: r=2.6E-11; Figure 1B). To investigate whether initial graft survival is limited by oxygen and nutrient diffusion from the host and to determine the maximum graft thickness, CTS with increasing thicknesses were transplanted onto control and infarcted hearts. Increasing graft thickness from 100 to 200 μm led to higher BLI signal intensity on day 28 (P=0.03) and similar viability at day 28 (17±2% versus 20±2%; P=0.68), but a further increase to 400 or 800 μm did not increase the BLI signal intensity at day 28 (P=0.89, P=0.57 compared with 200 μm; Figure 1D; Figure 1C in the online-only Data Supplement). These results indicate diffusion-limited survival. This pattern was also found for CTS transplanted onto infarcted hearts (Figure 1E), but BLI signal intensity was lower throughout the experiment (P=0.005; Figure 1E; Figure 1D in the online-only Data Supplement). To further illustrate the differences in patch survival, we focused on the BLI data between day 7 and day 28 and fitted a model with a linear and a quadratic term for patch thickness (Figure 1E and IF in the online-only Data Supplement). We found that a patch thickness of 400 μm would lead to the highest overall BLI signal intensity, but survival expressed as fraction of initial signal would be highest for 200 μm patch thickness. Cell survival for 100-μm-thick sections was lower than expected. Confocal imaging of CTS kept in Tyrode solution for 4 hours revealed damaged cells along the cutting surfaces from the vibratome and the core sample punch that had lost their cytoplasmic green fluorescent protein (Figure IIA–ID in the online-only Data Supplement). Therefore, cell death caused by sectioning affects a large fraction of cells in thin sections. We further investigated whether the observed survival of adult CTS is specific to the cardiac microenvironment by transplanting CTS onto the back of mice (Figure IIIA and IIIB in the online-only Data Supplement). Similar to the results obtained from transplanting CTS onto the heart, cell loss was observed for the first 7 days, with stable engraftment thereafter preserving 17±7% of transplanted cells for 200 μm CTS thickness. The absolute signal intensity was higher for these CTS because of their close proximity to the skin, leading to reduced tissue absorption and scattering of emitted light compared with the heart that is further away from the skin. Taken together, these results show that a considerable number of cells engrave long-term after CTS transplantation, but initial survival is diffusion limited.

Comparison of Adult With Neonatal CTS Survival

Because most cardiac tissue–engineered grafts are made from ESC-derived or iPS–derived cardiomyocytes that may be immature, we next investigated whether neonatal CTS may be more resistant to ischemia, leading to better survival than adult CTS. Neonatal CTS (from 1-day-old pups) were transplanted onto adult control or infarcted hearts (Figure IIIC and IIID in the online-only Data Supplement). Although the BLI signal differed initially, overall cell survival was similar between the control and infarcted groups from day 7 onward (control versus infarct; P=0.63). One month after transplantation of 400-μm-thick neonatal CTS 35±6% of transplanted cells remained viable. This suggests a high ischemia resistance of neonatal CTS, because we found a 50% lower BLI signal intensity when adult CTS were transplanted onto infarcted hearts compared with control hearts. High ischemia resistance of neonatal cardiomyocytes has been reported previously corroborating our results.16

Histological Assessment and Confirmation of CTS Viability

Histologically, we observed heterogeneous green fluorescent protein expression in cardiac tissue from L2G mice (Figure 2). Because luciferase is expressed from the same promoter, we verified that viability can be measured by BLI. We found a linear relationship between CTS thickness and BLI signal intensity (r=0.97; P=4.95E-06; Figure IVA in the online-only Data Supplement). Similar to BLI survival data, most cell death occurred within a week after transplantation when assessed with terminal deoxynucleotidyl transferase mediated dUTP nick-end labeling assay (TUNEL) (Figure IVB in the online-only Data Supplement). To verify whether cell survival is diffusion limited and if there are differences in the survival rates of cardiac cell types, we performed TUNEL staining for cellular apoptosis. One day after CTS transplantation, a few apoptotic cardiac troponin T (cTnT)-expressing cardiomyocytes were found in the graft, whereas more dying platelet-endothelial cell adhesion molecule 1 (CD31)-positive

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**Notes:**

1. The results indicate that CTS viability is diffusion limited, and that there is a significant difference in survival between adult and neonatal CTS.

2. The signal intensity increases with increasing CTS thickness, up to a maximum of 200 μm, beyond which survival decreases.

3. Neonatal CTS show higher resistance to ischemia compared to adult CTS, as indicated by their lower cell loss after transplantation.

4. Histological analysis confirms the viability of transplanted CTS, with some apoptotic cells observed in the grafts.

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**Figure Notes:**

- Figure IIA–ID: Confocal images of CTS kept in Tyrode solution for 4 hours, showing damaged cells.
- Figure IIIC and IIID: BLI survival data for adult and neonatal CTS, showing similar survival between control and infarcted groups after day 7.
- Figure IVA: Linear relationship between CTS thickness and BLI signal intensity.
- Figure IVB: TUNEL staining for apoptosis in CTS transplants, with minimal apoptotic cells.

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**Further Reading:**

- For a detailed discussion on cardiac tissue slice transplantation and its applications, see Riegler et al (2017).
- For methods on assessing cell viability and diffusion limitations, refer to the online-only Data Supplement.
Figure 2. Survival of cardiomyocytes, endothelial cells, and fibroblasts after cardiac tissue slice (CTS) transplantation in mice. A to C, A small number of apoptotic cells (TUNEL+ nuclei, red) were distributed throughout the graft 1 day after CTS transplantation onto normal mouse hearts. D to F, Three days after transplantation, cell death was high at the edge of the graft and low in regions close to the heart. G to I, A similar pattern was observed at day 6. J to L, On day 9, only a small number of dead cells was found. M to O, Cell death peaked on day 3 for cardiomyocytes (cardiac troponin T [cTnT]), endothelial cells (CD31), and fibroblast specific protein 1 (FSP1)-positive cells. The number of cardiomyocytes and endothelial cells decreased from day 3 onward, whereas the number of FSP1+ cells increased despite a high rate of cell death, indicating cell proliferation or influx into the graft (n=3 for each time point). DAPI indicates 4',6-diamidino-2-phenylindole (nuclei); GFP, green fluorescent protein (graft); and TUNEL, terminal deoxynucleotidyl transferase mediated dUTP nick-end labeling assay. Scale bars, 100 μm.
endothelial cells and fibroblast specific protein 1 (FSP1)–
positive cells were observed (Figure 2A–2C; Figure VA–VC
in the online-only Data Supplement). Cell death increased on
day 3 (Figure 2D–2F; Figure VD–VF in the online-only Data
Supplement) and declined from day 6 to day 9 (Figure 2G–
2L; Figure VG–VL in the online-only Data Supplement). On
days 3 and 6, cell death increased with radial distance from
the heart, again indicative of diffusion-limited cell survival.
We observed a rapid decline in the number of cardiomyo-
cytes and endothelial cells in the graft for the first 6 days after
transplantation (Figure 2M and 2N). In contrast, the number
of FSP1– cells increased, despite the high level of apopto-
sis in FSP1+ cells (Figure 2O). Because macrophages have
been reported to express FSP1,17 tissue sections were stained
for the pan-leukocyte marker CD45 (Figure VI in the online-
only Data Supplement) to determine whether this increase reflects
an influx of macrophages or other inflammatory cells.
Although a small number of CD45+ and CD45/FSP1 dou-
ble-positive cells was found in normal myocardium (Figure
VIA–VIC in the online-only Data Supplement), their number
increased from the first day onward in the transplanted
graft (Figure VID–VII in the online-only Data Supplement). On
day 3, almost all FSP1+ cells were CD45–, indicating that
the increase in FSP1-expressing cells was due to an influx of
pan-leukocytic cells, likely macrophages. Later time points
showed an increase in fibroblasts and a decrease in CD45+ cells
(Figure VIM–VIP in the online-only Data Supplement).
To further verify that FSP1+ cells are primarily from the host,
CTS from adult Sox2-Cre; Rosa26-tdTomato double-hetero-
zygous mice with permanent labeling of cells by tdTomato
were transplanted onto the hearts of immunocompromised
mice. Similar to L2G CTS transplantation, we found an
increase in FSP1+ cells from day 3 onward, most of which
were tdTomato negative indicating host origin (Figure VII in
the online-only Data Supplement).

Rapid Revascularization of Transplanted CTS
Revascularization of transplanted CTS is likely an important
factor determining graft survival, particularly for increased
thicknesses. Pre-existing vascular networks in CTS may favor
their survival. Because CTS are made from normal myocar-
dium with a pre-existing vasculature (CD31+; Figure 3A), we
aimed to investigate whether and when these vessels connect
to the host vasculature following transplantation. Intravenous
injection of lectins before euthanizing host animals efficiently
labeled the host vasculature. One day after CTS transplantation
graft vessels were lectin negative (Figure 3A–3C). In contrast,
3 days after CTS transplantation, 68±12% of the graft vessels
were lectin positive, indicating reperfusion (Figure 3D–3F;
Figure VIII in the online-only Data Supplement). Similar
results were obtained for day 6 (Figure 3G–3I). Although
reperfused vessels were more homogeneously distributed
day 3 onward, the number of reperfused vessels did not
increase and the vascular density decreased (Figure 3J).
Importantly, we detected perfused vessels apparently connect-
ing host and graft vasculature (Figure 3K–3M). To determine
the origin of vessels connecting host and graft, CTS were
transplanted onto noninfarcted hearts of adult VE-cadherin-
Cre; Rosa26-tdTomato double-heterozygous mice with
permanent labeling of host endothelial cells with tdTomato.
After 2 weeks, tdTomato-positive vessels were found in the
graft (Figure 4), indicating vessel invasion from the host. We
found examples of host vessels connecting with graft vessels
indicating anastomosis (Figure 4C). Although the vascula-
ture of most grafts was reconnected through the epicardium,
some grafts showed vessel ingrowth from surrounding tis-
sue (Figure IX in the online-only Data Supplement). Taken
together, these findings suggest that transplanting tissue grafts
containing vascular networks leads to fast reperfusion medi-
atcd by host vessels through invasion and connection with the
graft vasculature.

Functional Benefits of CTS
To assess functional benefits of adult CTS, immunodeficient
mice were subjected to MI and received no treatment (control,
n=12), CTS (n=12), or skeletal muscle patches of equal
size (n=11). We included a skeletal muscle patch group to
account for mechanical effects of CTS transplantation that
may influence remodeling (Figure 5A). To rule out immedi-
ate effects of tissue slice transplantation on scar formation,
late gadolinium enhancement MRI was performed on day 1
after surgery, demonstrating no difference in scar size due to
CTS or skeletal muscle patch transplantation compared with
control (P=0.80; Figure 5B–5E; Figure VA–XC in the online-
only Data Supplement). We also performed BLI on these ani-
mals to track graft survival of the luciferase-expressing tissue
slices. BLI of the CTS group demonstrated a typical initial
cell loss for the first week and stabilization thereafter, preserv-
ing 14±6% of transplanted cells. In contrast, in the animals
receiving skeletal muscle patches, we observed no initial cell
loss and detected proliferation between days 21 and 28 after
transplantation (Figure 5L). However, the excellent survival
of skeletal muscle patches was not reflected in animal mor-
bidity, because 45% of mice died before the end of the study
(5 of 11; P=0.001), whereas no animals were lost in either
control or CTS groups (Figure 5M). This poor survival might
be because of arrhythmias induced by transplanted skeletal
muscle patches, similar to what has been observed in a clini-
cal trial involving the injection of human skeletal myoblasts
into the heart (MAGIC: Myoblast Autologous Grafting in
Ischemic Cardiomyopathy).18

Reduced Dilative Remodeling on CTS
Transplantation
To assess whether CTS or skeletal muscle patches reduce dilata-
tive remodeling and preserve ejection fractions, cardiac MRI
was performed on days 1 and 28 after MI and CTS or muscle
patch transplantation (Figure 5F–5K; Figure XD in the online-
only Data Supplement). We found significantly increased end-
diastolic and end-systolic volumes for the skeletal muscle
patch group (P=0.02, P=0.04) compared with control and
CTS (Figure 5N and 5O). Ejection fractions were similar 1
day after the surgery with 38±3%, 38±2%, and 38±3% for
control, CTS, and skeletal muscle patch groups, respectively.
However, 1 month later, the CTS group showed a higher pre-
served ejection fraction with 28±2% compared with control
21±2% and skeletal muscle patch group 19±3% (P=0.05;
Figure 5P; Table 1 in the online-only Data Supplement).
A similar trend was observed for cardiac output with the highest increase in the CTS group (Figure 5Q). Furthermore, a positive correlation between BLI signal intensity and ejection fraction ($r=0.73; P=0.02$) was observed in the CTS group on day 28, indicating that better cell survival leads to higher preserved ejection fraction. Conversely, a negative correlation was found for skeletal muscle patches ($r=-0.90; P=0.01$; Figure 5R and 5S).

Quantification of Cellular Composition of CTS
Because changes in graft composition after transplantation influence functional effects of these grafts on recipient hearts, we assessed graft composition 1 month after transplantation onto infarcted hearts. Control animals did not receive any grafts (Figure 6A–6C). CTS grafts derived from adult mice contained a small amount of cardiomyocytes (2±1%), endothelial cells (11±2%), and a high number of fibroblasts (24±7%) and CD45+ cells (27±4%; Figure 6E–6H). We did not observe any obvious differences in size or composition of the scar tissue between the different groups (Figure 6). In contrast to the poor survival of cardiomyocytes in adult CTS, neonatal CTS contained a substantial number of cardiomyocytes (20±4%; Figure 6D). These

Figure 3. Cardiac tissue slices are reperfused within 3 days after transplantation onto normal hearts. A to C, One day after transplantation, none of the graft vessels (CD31+) were perfused (as shown by lack of detected signal after intravenous injection of biotinylated lectins) while host vessels were efficiently labeled. D to F, Three days later, a fraction of graft vessels was found to be perfused. G to I, The percentage of perfused vessels did not increase on day 6 or day 9. J, The vascular density declined from day 1 to day 3. K to M, Vessels connecting host and graft were readily detected (maximum intensity projection, day 3; n=3 for each time point). DAPI indicates 4',6-diamidino-2-phenylindole (nuclei); and GFP, green fluorescent protein (graft). Scale bars, 100 μm.
neonatal cardiomyocytes may be more ischemia resistant,\textsuperscript{16} leading to comparable survival when transplanted onto control or infarcted hearts (Figure XIA and XIB in the online-only Data Supplement). Grafts from neonatal CTS on infarcted hearts contained a similar number of fibroblasts and CD45\textsuperscript{+} cells compared with adult CTS (Figure 6D). In contrast, skeletal muscle patch grafts contained almost no fibroblasts (1±1\%) and a smaller number of CD45\textsuperscript{+} cells (13±3\%; Figure 6L–6J).

Discussion

The use of cardiac cell transplantation may be hampered by the degree of cell survival after transplantation. Notably, survival is typically <1\% one month after injection of single-cell suspensions.\textsuperscript{4,17} In contrast, engineered tissues derived from multiple cell sources may provide a better environment for preservation of transplanted cells. Accordingly, CTS generated from the native mammalian heart with its natural cellular organization were used as a model system to explore the results of transplantation of normal tissues. Using this unique model, we found that CTS transplantation led to long-term engraftment of ≤30\% and 14\% of transplanted cells on control and infarcted hearts, respectively. A pre-existing vascular network in the transplanted grafts likely contributed to the exceptional survival by quickly establishing vascular connection and reperfusion with the host hearts within 3 days after transplantation. The sectioning process used to make CTS led to cell death along the cutting edges, which became important for sections with <100 μm thickness, but for thicker sections the number of apoptotic cells found 1 day after implantation was low.

Our study demonstrates that initial cell survival is diffusion limited, with the highest survival rates between 300 and 400 μm slice thickness. Although previous reports suggested survival of even thicker tissue-engineered grafts in vivo,\textsuperscript{8,10,19,20} those survival estimates were primarily based on graft thickness at time of tissue harvest or histological evaluation of selected slices. Our study used noninvasive reporter gene imaging that can assess the entire graft repetitively and longitudinally over time. These advantages allow for more accurate and quantitative assessment of tissue graft survival. Because CTS consist of adult tissue while most tissue-engineered grafts consist of isolated ESC- or iPSC-derived cells that are likely immature, we also assessed the survival of neonatal CTS. These grafts showed comparable cell survival between infarcted and control hearts, indicating a higher hypoxia tolerance in accordance with previously published data.\textsuperscript{16} Even so, overall cell survival did not exceed 35\% for 400-μm-thick neonatal CTS.

A question that remains to be addressed is what cell composition would be suitable for tissue-engineered grafts and whether there are survival differences between different cell types found in the heart. CTS consist of myocardial tissue that contains a substantial number of endothelial cells and fibroblasts as well as cardiomyocytes. The presence of endothelial cells is needed to enable fast reperfusion because addition of endothelial cells or prevascularization of grafts improves survival, but the time course for reperfusion of cardiac patches has not been established.\textsuperscript{5,11} Here, we found that grafts were reperfused within 3 days after transplantation. Cell death had reached a maximum by then and declined thereafter. This points to the importance of reperfusion and may offer a potential to improve graft survival by reducing the reperfusion/vascularization time and improve ischemia resistance for the first few days after transplantation.

When we assessed graft composition 4 weeks after transplantation onto infarcted hearts, CTS grafts contained mainly fibroblasts, pan-leukocytic cells, and endothelial cells. We only found a small number of cardiomyocytes in adult CTS. In contrast, neonatal CTS retained a substantial amount of cardiomyocytes when transplanted onto infarcted hearts. Previous publications reported reduced fibrosis and good cardiomyocyte survival of transplanted engineered tissues,\textsuperscript{8,10,21} which may be due to the assessment of grafts mainly at the infarct border zone. In contrast, we assessed the entire graft that had been transplanted over the scar of the recipient heart and rarely reached onto noninfarcted tissue.

![Figure 4](http://circ.ahajournals.org/doi/figure-pdf/10.1161/CIRCULATIONAHA.117.028648)
Previous studies indicated that transplantation of tissue-engineered patches reduces infarct/scar size, implying a paracrine mechanism. We measured scar size 1 day after MI using high-resolution late gadolinium enhancement MRI and found that CTS or skeletal muscle patches did not reduce the infarct size in a mouse MI model, which might be because of differences in the animal model or assessment time points. We found reduced dilative remodeling, leading to a higher preserved ejection fraction in the CTS group compared with control and skeletal muscle patch groups, in line with published data for tissue-engineered patches. A skeletal muscle patch was included in our functional analysis to control for potential mechanical effects of transplanted tissue reducing remodeling. Interestingly, skeletal muscle patches did not show cell loss when transplanted onto infarcted hearts in contrast to single-cell transplantation. Accordingly, hearts receiving skeletal muscle patch transplants had higher wall thickness compared with control and CTS groups, but only the CTS group showed functional improvements, suggesting that increased wall thickness is...
not necessarily a predictor for improved cardiac function.\textsuperscript{9,27} Corroborating these results, a positive correlation between cell survival and ejection fraction at 4 weeks was found for CTS, whereas a negative correlation was found for skeletal muscle patches. Finally, we observed a higher mortality after skeletal muscle tissue transplantation that might be because of arrhythmia, as previously reported for a clinical trial involving transplantation of autologous skeletal myoblasts into the ischemic heart,\textsuperscript{18} but further studies are needed to investigate the underlying mechanisms.

In conclusion, this study shows that CTS transplantation leads to long-term engraftment of transplanted cells on infarcted hearts and reduced dilative remodeling. For CTS with <400 \textmu m thickness, we observed high cell survival that may be due to rapid revascularization between the host organ and graft tissue. However, for CTS with >400 \textmu m thickness, we observed poor survival, presumably because diffusion could not sustain the graft before it was revascularized. Importantly, these findings call for further improvements in graft revascularization before the cardiac tissue engineering.

Figure 6. Composition of cardiac tissue slices (CTS) and skeletal muscle patches 1 month after transplantation onto infarcted hearts. A to C, Control heart scar tissue contained a small number of surviving cardiomyocytes ($\alpha$-actinin ($\alpha$-act) along the endocardial border, some blood vessels (CD31), and a high number of fibroblasts (fibroblast specific protein 1 [FSP1]) as well as CD45-positive cells. D, Neonatal CTS contained a high number of cardiomyocytes and endothelial cells and a substantial number of fibroblasts and CD45$^+$ cells. E to H, Adult CTS grafts contained a small number of cardiomyocytes, blood vessels, and a large number of fibroblasts and CD45$^+$ cells. I to L, Skeletal muscle patch grafts consisted primarily of myoblasts (skeletal myosin heavy chain [sMHC]) and endothelial cells. There were some CD45$^+$ cells and almost no fibroblasts in these grafts (n=4 per group). \textalpha SMA indicates \textalpha -smooth muscle actin; Con43, Connexin 43; DAPI, 4',6-diamidino-2-phenylindole (nuclei); and GFP, green fluorescent protein (graft). Scale bars, 100 \textmu m.
strategy can be used for larger animals or humans. Finally, continued use of CTS and molecular imaging technologies will undoubtedly help accelerate the advancement of cardiac tissue engineering.

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Disclosures

None.

References


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SUPPLEMENTAL MATERIAL

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SUPPLEMENTAL METHODS

Generation of Cardiac tissue slices (CTS). Cardiac tissue slices were generated similar to a previously described procedure\(^1\). Adult (8-12 weeks, male and female, n=40) transgenic mice (referred to as L2G) bred on FVB background expressing enhanced green fluorescent protein (eGFP) and firefly luciferase (Fluc) reporter genes driven by a β-actin promoter as previously described\(^2\) were anesthetized using isoflurane. Cuts into were made the liver and hearts were perfused with 10 ml ice-cold modified Tyrode solution (see below) containing 500 international units of heparin per milliliter (Sigma, St. Louis, MO) through the apex with a 25G butterfly needle. Hearts were excised and their right ventricle and all atria were removed to increase mechanical stability during sectioning. The left ventricular lumen was filled through the aorta with 4% low melting point agarose (Invitrogen, Carlsbad CA) using a 20G needle. Hearts were embedded in 4% low melting point agarose in a 30 mm dish and placed on ice. The solidified block was trimmed and glued onto a sample holder of a vibratome (The Vibratome Company, St. Louis, MO) using cyanoacrylate glue. The entire block was covered with ice-cold modified Tyrode solution during the sectioning process. Sections with different thicknesses (100 – 800 µm) were cut from the left ventricular free wall. To standardize the slices, discs with a diameter of 3 or 5 mm were punched out using core sample punchers (Fine Science Tools, Foster City, CA) and stored in modified Tyrode solution until transplantation or other use (see Figure 1A). Skeletal muscle patches from the thigh (vastus lateralis) were generated from L2G mice using a similar procedure. Skeletal muscle patches were cut in parallel to the primary fiber orientation of the muscle tissue.

Modified Tyrode solution. The following chemicals (Sigma, St. Louis, MO) were dissolved in distilled water: 136 mmol/l NaCL, 5.4 mmol/l KCl, 1 mmol/l MgHPO\(_4\)-3H\(_2\)O, 10 mmol/l D-
Glucose, 0.09 mmol/l CaCl$_2$, 30 mmol/l 2,3-Butanedione monoxime, and 5 mmol/l HEPES. The pH was adjusted to 7.4 and the solution was sterile filtered.

**Assessing cell death due to vibratome sectioning.** CTS from adult L2G mice were prepared with a thickness of 250 µm and stored in Tyrode solution for 4 hours. CTS were fixed for 20 minutes in 4% PFA. Some CTS were used for whole mount confocal microscopy while others were frozen in OCT, sectioned and used for TUNEL staining (see below). Confocal microscopy was performed using a Leica SP5 microscope (Leica, Wetzlar, Germany). A series of images was acquired with a 20x glycerol immersion objective with a zoom factor of 1.4. A z-stack (60 slices) was acquired for whole mount CTS to generate 3D renderings of the vibratome cutting surface.

**Myocardial infarction and CTS transplantation.** MI was induced in male NOD/SCID mice (Charles River Laboratories, Wilmington, MA) by permanent LAD ligation under 1.5% to 2% inhaled isoflurane after left thoracotomy and confirmed by myocardial blanching and ECG changes. Animals were randomized into experimental groups receiving either CTS (n=36), skeletal muscle patches (n=12), or control group receiving no patch (n=12). For all experiments involving cardiac assessment via magnetic resonance imaging (MRI), CTS and skeletal muscle patches with 5 mm diameter and 400 µm thickness were transplanted directly after LAD occlusion. CTS and skeletal muscle patches were attached onto the left ventricular (LV) free wall with 4-5 stitches using 10-0 silk sutures. For patch survival and reperfusion experiments, CTS with 3 mm diameter were transplanted onto the LV free wall of non-infarcted mice (n=52, NOD/SCID). All surgeries were performed by experienced microsurgeons. Mice received antibiotics via their drinking water from the surgery day on until the end of the experiment.
**Origin of FSP1 positive cells found in the graft following transplantation.** CTS were made from adult hearts of Sox2-Cre; Rosa26-tdTomato double heterozygous mice (n=6) (C57BL/6 background) with permanent labelling of cells with tdTomato. These tdTomato positive CTS were transplanted onto non-infarcted mouse hearts (n=12, NOD/SCID). Three hearts where harvested on day 1, 3, 6 and 9 after CTS transplantation as outlined below.

**Assessment of endothelial cell origin in reperfused CTS.** CTS were transplanted as described above onto non-infarcted hearts of adult VE-cadherin-Cre; Rosa26-tdTomato double heterozygous mice (n=18) (C57BL/6 background) with specific and permanent labeling of endothelial cells with tdTomato. To prevent immune rejection, Tacrolimus (5 mg/kg/day Astellas, Northbrook, IL) was orally administered every 12 hours starting one day before CTS transplantation. Mice received antibiotics via their drinking water throughout the experiment.

**Survival of neonatal cardiac tissue slices.** Neonatal pups from L2G mice (1 day old) were sacrificed, their hearts excised and placed into ice-cold modified Tyrode solution. Hearts were cut longitudinally and patches were punched out of the left ventricular free wall (approx. 3 mm diameter) and transplanted onto infarcted (n=5) or control (n=5) NOD/SCID mice as described above. The slices were not always entirely circular due to the small size of the left ventricular free wall of neonatal hearts.

**Bioluminescence imaging (BLI).** Mice were anesthetized with isoflurane, D-Luciferin (400 mg/kg, Biosynth International, Itasca, IL) was injected intraperitoneally, and mice were placed into a BLI imaging system (Xenogen, Alameda, CA). Images were acquired every two minutes until a stable decline in signal intensity could be observed. Regions of interest (ROI) were placed
over the chest centred at the pixel with maximum signal intensity. The ROI with the maximum of the average signal intensity from all the ROIs of one time series belonging to one animal was selected as measurement value for that imaging session. The ROI size was kept constant for all animals and experiments. In order to get a better estimate of the number of transplanted cells, the first BLI time point was always 4 hours after transplantation surgery.

**Estimating cell survival using BLI.** Human embryonic stem (hESCs) were transduced with a Fluc-eGFP double fusion construct by lentivirus-based techniques as previously described\(^3\). Undifferentiated hESCs (H7 line from Wicell) were grown and expanded on Matrigel-coated plates in mTeSR1 medium (Stem Cell Technologies, Vancouver, BC, Canada). ESCs were detached using Trypsin and filtered through a 50 µm pore size filter to avoid cell clumps. The number of viable cells was estimated using Trypan blue exclusion with an automatic cell counter (Countess, Invitrogen, Carlsbad, CA). Cells were diluted and plated in 200 µl media at varying concentrations (2.5E5, 6.2E4, 1.5E4, 4E3) in wells of a 96 well plate. D-Luciferin (0.05 mg/well, Biosynth International, Itasca, IL) was added and the radiance was measured immediately afterwards using the same BLI imaging system used for mice. The experiment was repeated once.

**Immunodetection and histological methods**

Heart fixation and section preparation: Mice were anesthetized, cuts into the liver were made and their hearts were perfused with 10 ml cold PBS (4 °C) containing 0.1 mol/l KCl through a 25G butterfly needle inserted into the left ventricle via the apex. Hearts were cut out and fixed over night at 4 °C in PBS with 4% PFA. Following fixation, hearts were placed into PBS for four hours before whole heart fluorescence images were acquired (CRi Maestro, Perkin
Elmer, Waltham, MA). After that, hearts were transferred into 30% sucrose solution and kept at 4 ºC until equilibrium was reached. Hearts were embedded in OCT and frozen in hexane containing dry ice. Sections were cut with a cryostat (Leica, Wetzlar, Germany) collected on glass slides, dried and stored at -80 ºC.

Cell death: TUNEL staining was performed on 8 µm thick cryosections using a commercial kit (Roche, Mannheim, Germany) following the manufacturer’s instructions except for the following modifications. Sections were permeabilized in 0.1 M citric acid at 65 ºC for 30 minutes. The TUNEL reaction mixture was diluted 1:4 with TUNEL dilution buffer. After TUNEL staining, sections were washed 3x 10 minutes with PBS and a standard immunofluorescence staining procedure followed.

Immunofluorescence: Sections were equilibrated to room temperature, washed 3x 10 min with PBS, permeabilized with 0.5% Triton-X (Sigma) in PBS for 30 minutes at room temperature followed by incubation in blocking solution (5% donkey serum in PBS + 0.1% Tween20, Sigma) for 30 minutes. For mouse primary antibodies, a mouse-on-mouse blocking kit was used following the manufacturer’s instructions (Vector Labs, Burlingame, CA). Sections were incubated with primary antibodies (see Supplemental Table 2) diluted in blocking solution over night at 4 ºC in a humid chamber. After washing 3x 15 min with PBS + 0.1% Tween20, sections were incubated with secondary antibodies diluted in blocking solution for 1 hour followed by a final washing step (3x 15 min in PBS + 0.1% Tween20) and covered with cover slips using self-hardening mounting media. Confocal microscopy was performed using a Leica SP5 microscope (Leica, Wetzlar, Germany). A series of images was acquired with a 40x oil immersion objective. Images were stitched together to generate composite images for further analysis (typically 30-40 individual field of views).
**Image analysis:** Composite images were analysed using Volocity (PerkinElmer, Waltham, MA). Automatic segmentation of nuclei was performed followed by automatic detection of stained objects at respective channels. A region of interest was manually drawn to limit the analysis to the graft. Endothelial cells, FSP1 positive or CD45 positive cells were identified via colocalization of DAPI stained nuclei with detected objects (for example objects in the far red channel for FSP1 stained with Alexa Flour 647 secondary antibody). For cardiomyocytes, a manual segmentation was performed to designate inside cardiac troponin T or alpha actinin positive cells as cardiomyocyte nuclei. Apoptotic cells were detected by automatic segmentation of TUNEL positive nuclei and colocalization with DAPI positive nuclei. For each animal, 8,000-12,000 graft nuclei were analyzed.

**Reperfusion experiments:** To assess if blood vessels were perfused, mice were injected intravenously with biotinylated tomato lectin (10 mg/kg, Jackson ImmunoResearch, Baltimore, PA) 15 minutes before they were sacrificed. Heart fixation and immunofluorescence was performed as described above with fluorescently labeled streptavidin (see Supplemental Table 2).

**Magnetic resonance imaging (MRI).** Imaging was performed using a preclinical 7T (MR901 Discovery) horizontal bore scanner (Agilent, Santa Clara, CA) with a shielded gradient system (600 mT/m). Mice were anaesthetised with isoflurane (3%) and placed onto an animal cradle in prone position. Animals were kept at 37±0.4 °C (during image acquisition) via an air heating system while oxygen and anaesthetics (1-2% isoflurane) were supplied via a nose cone (0.5 L/min). Data acquisition was performed with a 30 mm Millipede transmit/receive volume coil (Agilent). Long and short axis scout images were acquired to define the two and four chamber long axis views. The cine long-axis views were used to define the short axis orientation. A
A prospectively double gated (ECG and respiration) spoiled gradient echo sequence was used to acquire cine cardiac images with the following parameters for standard cine acquisitions: TE 1.3 ms, TR 5-6 ms, flip angle 15°, slice thickness 1 mm, no slice separation, FOV 25×25 mm², matrix size 192×192, NSA 3. Twenty cine-frames were recorded to cover the cardiac cycle. A single short-axis slice was obtained in approximately 90 seconds, leading to a total scan time of 11 to 15 minutes covering the apex to base (8-11 slices). For infarct size measurements, late gadolinium enhancement (LGE) images were acquired 15 min after i.p. injection of gadolinium (0.6 mmol/kg, Magnevist Bayer, Germany) using an inversion recovery gradient echo sequence with inversion time optimised to null the healthy myocardium. All images from one animal were combined to a dataset, randomized, and anonymised. Data analysis was performed using the semi-automatic segmentation software Segment (Medviso AB, Sweden) as previously described.
### Supplemental Table 1: Cardiac MRI of control, CTS, and muscle patch groups

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<th>Muscle patch (n=11)</th>
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<td>* p&lt;0.05 vs. control</td>
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Data: Mean ± SD

### Supplemental Table 2: List of antibodies

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Supplemental Figure I
Supplemental Figure II
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SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure I: Estimating cell survival with Bioluminescence imaging. (A) Radiance of transplanted CTS relative to initial signal. (B) Linear regression between number of viable cells and average radiance per well indicating BLI can be used as a surrogate for cell viability. (C) Radiance relative to day 0 for CTS with different thicknesses transplanted onto normal hearts. (D) Radiance relative to day 0 for CTS with different thicknesses transplanted onto infarcted hearts. (E) A model with a linear and a quadratic term for CTS thickness was fitted to the BLI data (day 7-28) to illustrate diffusion limited survival on normal mouse hearts. (F) The same quadratic model was used to fit the data for CTS transplanted onto infarcted hearts. Average radiance is expressed as photons/second/cm²/steradian. Rad: Radiance.

Supplemental Figure II: In vitro characterisation of cardiac tissue slices. (A, B) Cardiac tissue slices from adult L2G (GFP positive) mice were generated and kept in Tyrode solution for four hours before they were processed. TUNEL staining did not label many cells, which was probably due to insufficient time to generate enough DNA double strand breaks in mechanically damaged cells. However, cytoplasmic GFP was lost from cells along the cutting surfaces indicating that these cells are likely to be dead. (C) Three dimensional projection of the vibratome cutting surface which shows cardiomyocytes are primarily separated along their cell boundaries. (D) Z-stack of corresponding images for panel C. Scale bars: 100 µm.

Supplemental Figure III: Survival of cardiac tissue slices transplanted onto the back of mice and survival of neonatal cardiac tissue slices transplanted onto control or MI hearts. (A) Adult CTS transplanted subcutaneously onto the back of mice showed survival kinetics similar to adult CTS transplanted onto hearts, illustrating that the observed survival is not
specific to the cardiac microenvironment. (B) Representative BLI of adult CTS transplanted onto the back of mice (the right row of CTS had thickness of 100 µm, the left row 300 µm). (C) Neonatal CTS transplanted onto normal or infarcted hearts demonstrated similar cell survival one month after transplantation. (D) This was also evident on whole heart fluorescence images before (top row) and after spectral decomposition (bottom row). Average radiance is expressed as photons/second/cm²/steradian.

**Supplemental Figure IV: Bioluminescence signal intensity for different CTS thicknesses and comparison of cell death estimates by BLI and TUNEL.** (A) A linear correlation between CTS thickness and average BLI signal intensity was found for CTS, indicating the suitability of BLI to assess graft survival. (B) BLI imaging showed a loss of signal intensity for the first week after transplantation. A qualitatively similar behaviour was found for TUNEL⁺ cells which reached their highest level between day three and six after transplantation and declined thereafter.

**Supplemental Figure V: Cell death in transplanted CTS peaked at day six after transplantation.** (A-C) Nuclei stained with DAPI (blue), apoptotic nuclei which stained TUNEL positive (red) and merged images. Only a small amount of dead cells could be found one day after transplantation. (D-I) The number of dead cells increased on day three and reached its maximum on day six. (J-L) On day nine after transplantation, the number of dead cells did decrease substantially. All the images in this figure a smaller subsections from the first column of Figure 2. Scale bars: 100 µm.
Supplemental Figure VI: A high number of FSP1 positive cells in transplanted CTS stain positive for the pan-leukocyte marker CD45. (A-C) Healthy myocardium contains a small number of pan-leukocytic cells (CD45+) some of which are also FSP1 positive. (D-L) The percentage of CD45+ cells as well as the percentage of FSP1+ and CD45+ cells increases rapidly from the first day after CTS transplantation and peaked at day six. (P) However, the percentage of double positive cells levelled out from day three onward. Scale bars: 100 µm.

Supplemental Figure VII: The number of fibroblasts and pan-leukocytic cells in CTS increased for the first few days following transplantation primarily via infiltration from the host. (A-C) Cardiac tissue from adult Sox2-Cre; Rosa26-tdTomato double heterozygous mice showed uniform tdTomato labelling. (D-F) One day after transplantation, CTS labelling was less uniform and a small increase of FSP1+ cells on the edges of the graft was observed. (G-I) Three and six days after transplantation, an increase of FSP1+ cells in the graft was observed. A considerable fraction of these cells was tdTomato negative and most likely of host origin. (M-O) Nine days after transplantation, grafts still had many blood vessels (CD31+) and a lower amount of FSP1+ cells. However, the number of cardiomyocytes (α-act: α-actinin+) had also declined. α-SMA: alpha smooth muscle actin; tdTom: tdTomato; FSP1: fibroblast specific protein 1; Con43: Connexin 43; Scale bars: 100 µm.

Supplemental Figure VIII: Spatial heterogeneity of reperfused vessels in the CTS grafts three days after transplantation. (A, B) CTS grafts had a normal vasculature density one day after transplantation onto control mice hearts; these vessels were not perfused since no intravenous injected lectin was detected in the graft. (C, D) Three days after transplantation, a
fraction of these vessels was reperfused although the extent was spatially heterogeneous. Scale bars: 100 µm.

Supplemental Figure IX: CTS grafts were also revascularized via blood vessels from surrounding tissue. (A-C) All endothelial cells were tdTomato positive in the lineage labelled mice used for these experiments. (D) Blood vessels from the host heart growing into CTS grafts were observed in all animals. However, some animals also had vessels growing into the graft from surrounding tissue (maximum intensity projection, 50 slices with one µm slice thickness). Scale bars: 100 µm

Supplemental Figure X: Representative MRI showing similar scar sizes between groups and strong dilative remodelling in the skeletal muscle patch group. (A-C) Representative short-axis late gadolinium enhancement MRI from base to apex showing that CTS or skeletal muscle patch transplantation did not alter the infarct size (scar size). The arrows in the control heart point to the gadolinium enhanced infarct scar. (D) Representative mid ventricular short-axis views illustrate dilative remodelling between day one and 28 in mice receiving no treatment: control, CTS, or skeletal muscle patch transplants. The top row shows hearts at end-diastole (ED) on day one and 28 while the bottom row depicts the same hearts at end-systole (ES). LV: left ventricle; Scale bars: 1 mm

Supplemental Figure XI: Neonatal CTS survive equally well when transplanted onto control or infarcted hearts. (A) Grafts from neonatal CTS contained a substantial number of cardiomyocytes (α-act+: α-actinin) one month after transplantation onto control hearts. (B) When neonatal CTS were transplanted onto infarcted hearts, a similar amount of surviving
cardiomyocytes was found one month after transplantation. (C, D) The number of capillaries (CD31+) and arteries (CD31+ + α-SMA+: α-smooth muscle actin) in grafts one month after transplantation of neonatal CTS onto either control or infarct hearts was similar. GFP: green fluorescent protein (graft), Con43: connexion 43, Scale bars: 100 µm
SUPPLEMENTAL REFERENCES


