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FIGURE LEGENDS

Figure 1. **(A)** Representative image of electrical current over the iPSC-CMs-transplanted heart surface in one cardiac cycle, visualized by 64-channel mapping system at day 2, day 3 and day 7 (n=4 each). Multiple ectopic excitations which were present at day 2 and day 3 were not detected at day 7. **(B)** Frequency of the VPC was calculated by continuous telemetric electrocardiogram monitoring (n=4 each). It appeared slightly greater in the iPSC-CMs-transplanted rats compared to the sham-operated rats, though there was no significant difference.

Figure 2. **(A)** Activation recovery interval (ARI), measured by daily 64-channel mapping studies, was significantly greater in the the iPSC-CMs (iPS) group compared to the other groups from the day 3 onwards (n=4 each). **(B)** Left ventricular ejection fraction (LVEF), measured by daily transthoracic echocardiography, was significantly greater in the iPSC-CMs group compared to the other groups from the day 3 onwards (n=6 each).

Figure 3. Small-angle X-ray scattering analysis of the red-circled area in the iPSC-CMs-transplanted heart at day 14 **(A)** showed appearance of the 1,0 and 1,1 equatorial reflections at the end-systolic phase **(B** and **C)**. In contrast, blue-circled scar tissue of the sham-operated heart **(D)** showed 1,0 reflections only at the end-systolic phase **(E** and **F)**. n=4 each.

Figure 4. Observed 1,0 reflection intensity change, calculated myosin interfilament spacing ($d_{1,0}$) and myosin mass transfer index (equatorial intensity ratio $I_{1,0}/I_{1,1}$) over several consecutive cardiac cycles in an iPSC-CM sheet of a transplant heart. Regular loop like

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relations between myosin mass transfer were evident.

Figure 5. Observed 1,0 reflection intensity change, calculated myosin interfilament spacing ($d_{1,0}$), myosin mass transfer index (equatorial intensity ratio $I_{1,0}/I_{1,1}$) and simultaneously acquired LV pressure acquired over several consecutive cardiac cycles in an iPSC-CM sheet of a transplant heart. Significant 1,0 myosin reflections were only evident for part of the cardiac cycle due to heart movement. When significant actin-myosin reflections were evident the shift in myosin mass towards actin (decrease in intensity ratio) coincided with the rapid increase in LV pressure during systole showing synchronized contraction of the iPSC-CMs in the sheet. Arrows indicate timing of end diastole.

Figure 6. Dsred-labelled transplanted iPSC-CMs expressed clear myosin-positive sarcomeres as shown in the representative confocal micrograph **(A)**. The sarcomere of the transplanted iPSC-CMs consisted of myosin and sarcomeric actin **(B)**. Distribution of the Cx43 did not show the typical intercalated disks in the transplanted iPSC-CMs **(C)**. The scale bar indicates ten micrometers. The iPSC-CMs *in vitro* showed the cardiac myocyte-like sarcomeres with less dense mitochondrial structures (arrow) as shown in the representative electron micrograph **(D)**. *In vivo* transplanted iPSC-CMs showed clear desmosome structure between the cells (arrow heads, **E**). Mitochondria of the transplanted iPSC-CMs *in vivo* gradually showed a dense structure at day 3 **(E)** and then at day 7 **(F)**. n=5 each.

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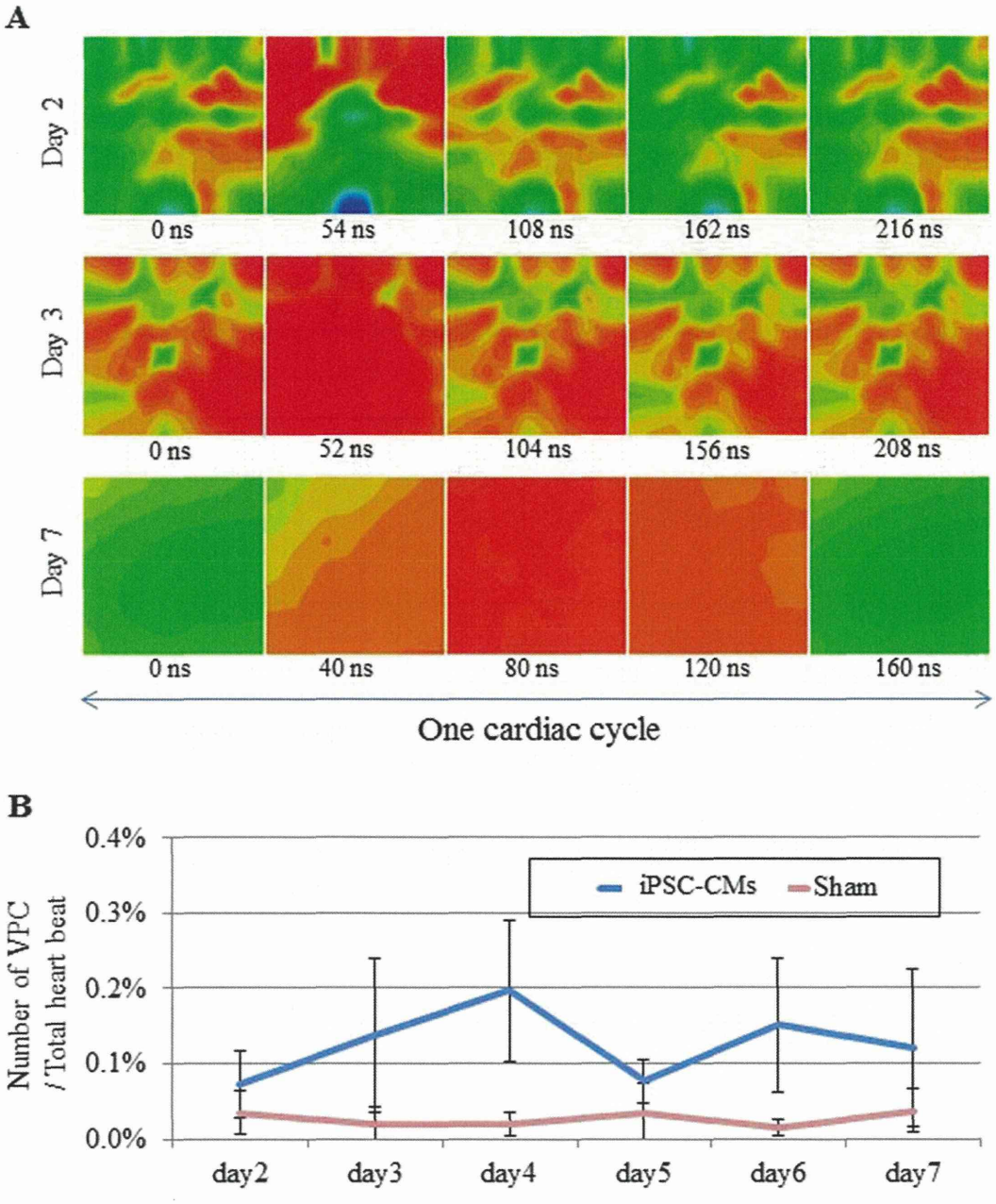

Figure 1

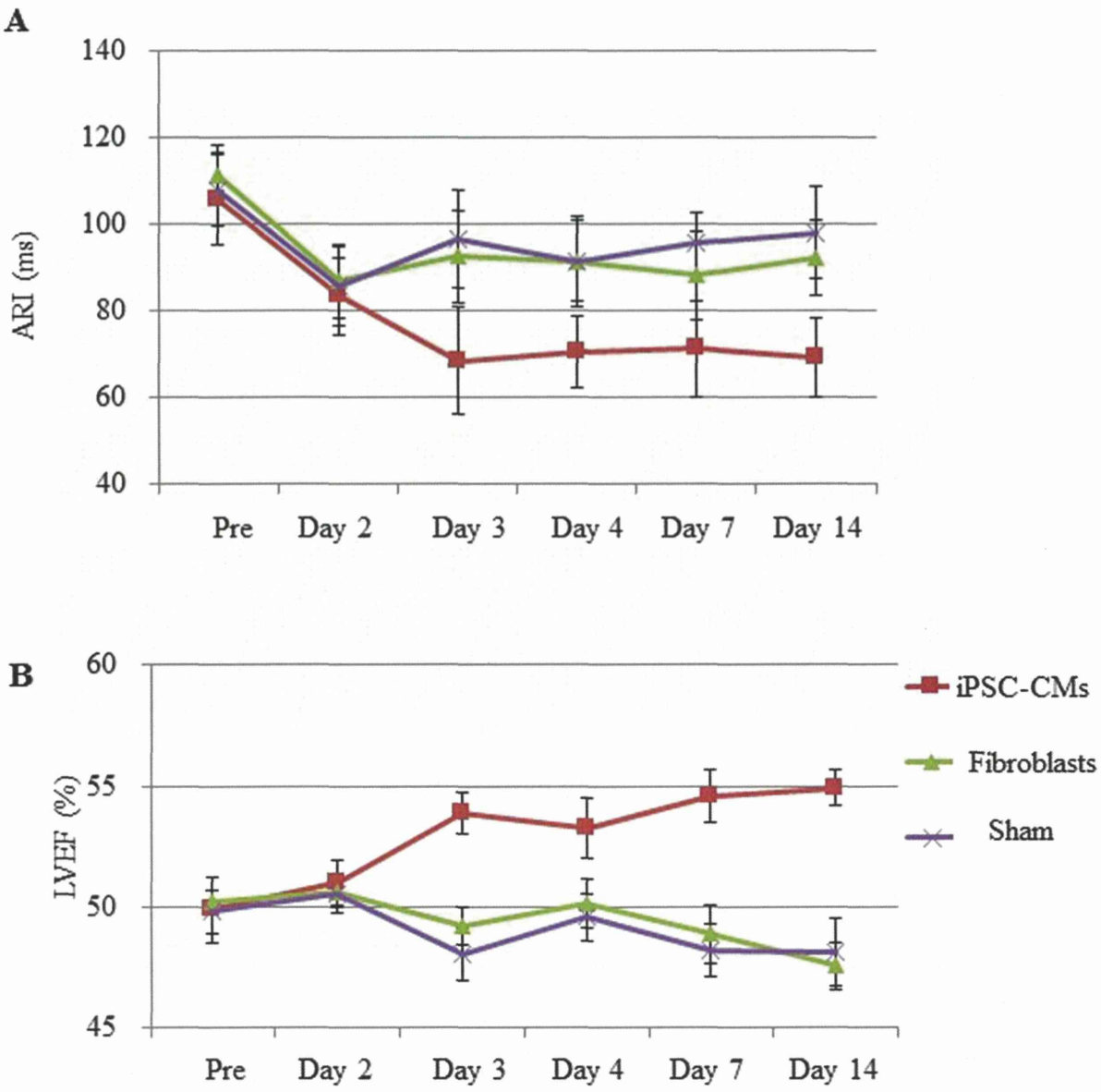

Figure 2

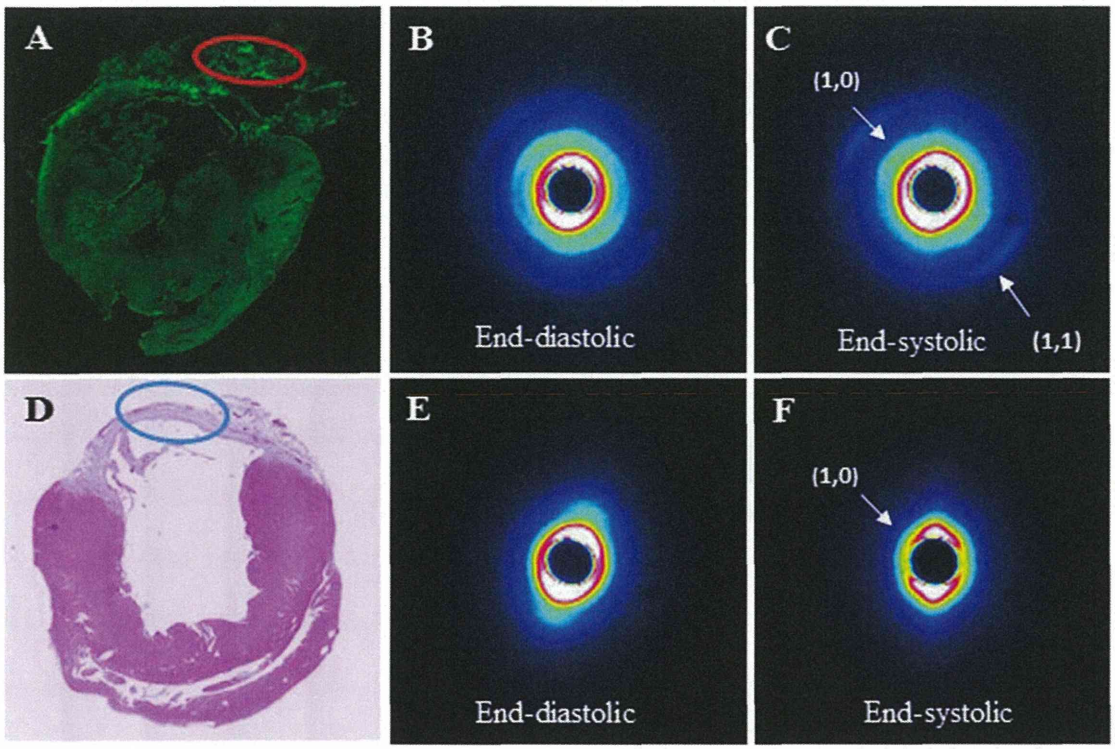

Figure 3

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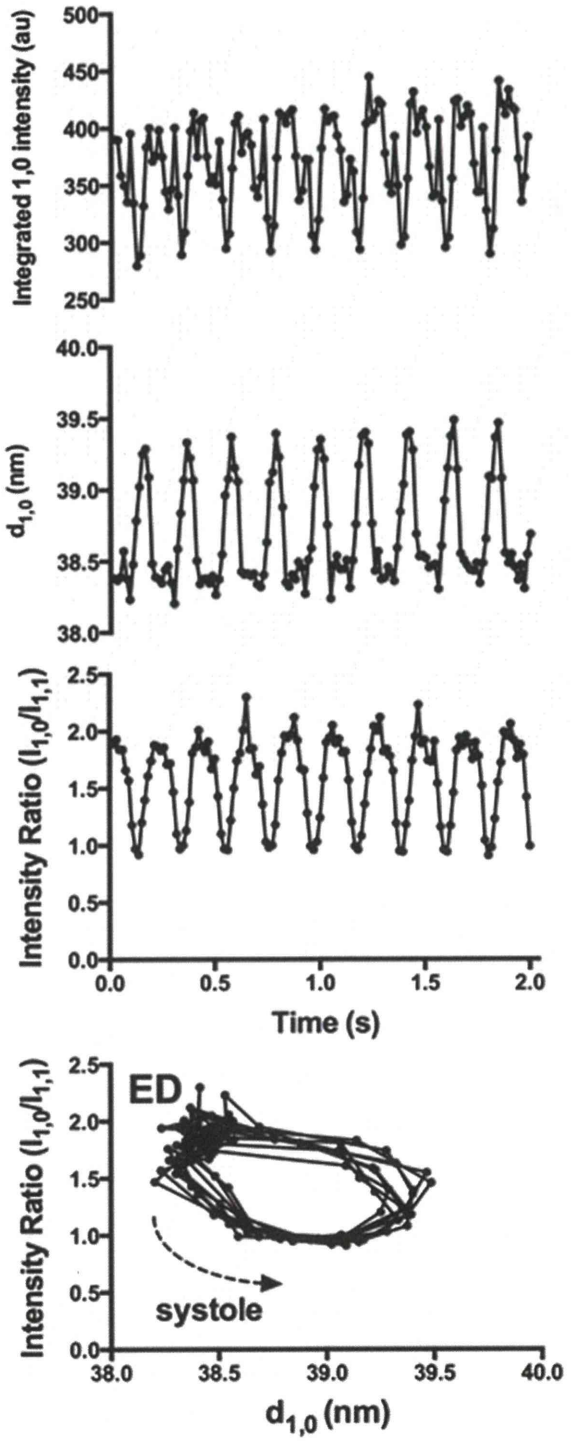

Figure 4

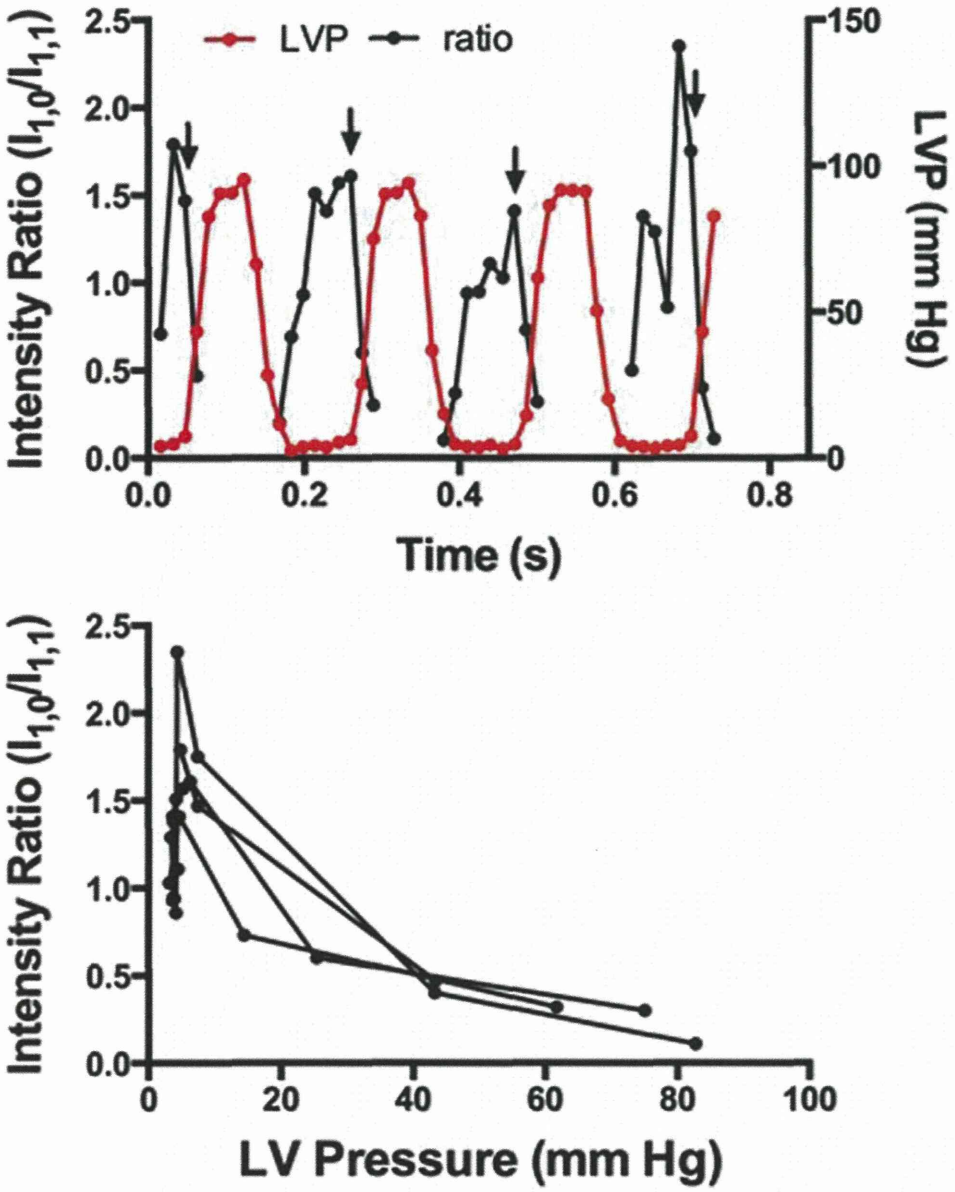

Figure 5

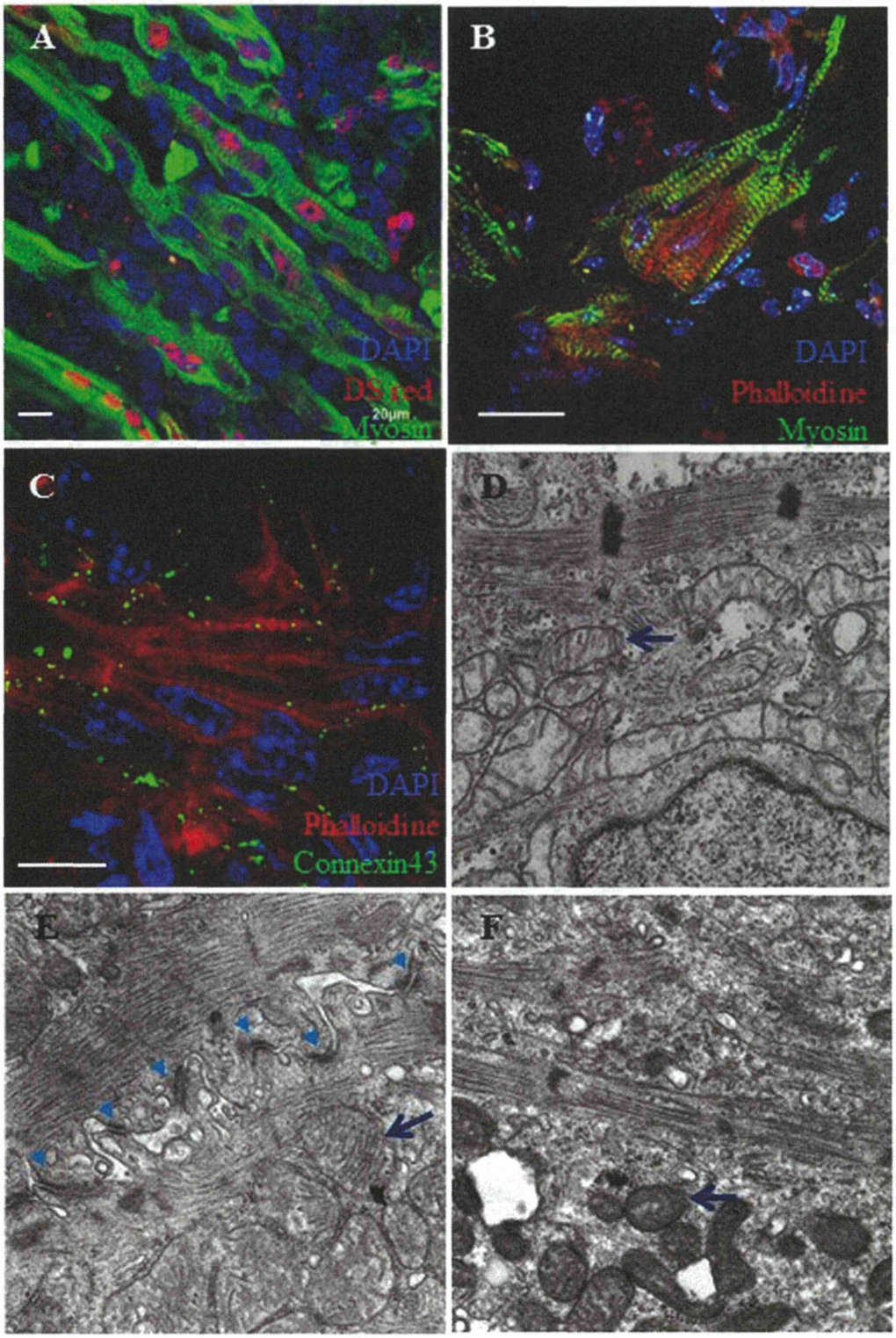

Figure 6

Cell-sheet Therapy With Omentopexy Promotes Arteriogenesis and Improves Coronary Circulation Physiology in Failing Heart

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Cell-sheet transplantation induces angiogenesis for chronic myocardial infarction (MI), though insufficient capillary maturation and paucity of arteriogenesis may limit its therapeutic effects. Omentum has been used clinically to promote revascularization and healing of ischemic tissues. We hypothesized that cell-sheet transplantation covered with an omentum-flap would effectively establish mature blood vessels and improve coronary microcirculation physiology, enhancing the therapeutic effects of cell-sheet therapy. Rats were divided into four groups after coronary ligation; skeletal myoblast cell-sheet plus omentum-flap (combined), cell-sheet only, omentum-flap only, and sham operation. At 4 weeks after the treatment, the combined group showed attenuated cardiac hypertrophy and fibrosis, and a greater amount of functionally ($CD31^+$/lectin$^+$) and structurally ($CD31^+$/α-SMA^+) mature blood vessels, along with myocardial upregulation of relevant genes. Synchrotron-based microangiography revealed that the combined procedure increased vascularization in resistance arterial vessels with better dilatory responses to endothelium-dependent agents. Serial ^{13}N-ammonia PET showed better global coronary flow reserve in the combined group, mainly attributed to improvement in the basal left ventricle. Consequently, the combined group had sustained improvements in cardiac function parameters and better functional capacity. Cell-sheet transplantation with an omentum-flap better promoted arteriogenesis and improved coronary microcirculation physiology in ischemic myocardium, leading to potent functional recovery in the failing heart.

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INTRODUCTION

Heart failure following myocardial infarction (MI) is a major cause of death and disability worldwide. Despite advances in drug and device therapy, recovery of cardiac function and prevention of transition to heart failure in MI patients remain unsatisfactory, indicating the need for development of novel therapeutic alternatives.[1] Myocardial regenerative therapy with cell-sheet transplantation has been shown to induce angiogenesis via paracrine effects in a chronic MI model.[2,3] However, the proangiogenic effect of the stand-alone cell-sheet treatment may be insufficient to fully relief ischemia in the chronic MI heart that involves a large territory of the left ventricle (LV), since the coronary inflow of the ischemic/infarct myocardium is dependent upon collateral arteries from other territories.[4,5] In addition, microvascular dysfunction is present in critical chronic MI heart across a wide range of the peripheral coronary tree.[6] This highlights the need for a comprehensive understanding of the mechanism of angiogenesis induced by a cell-sheet therapy in ischemic hearts.

For successful therapeutic neovascularization of ischemic tissues, it is essential to induce robust angiogenic responses (angiogenesis), and establish functionally and structurally mature arterial vascular networks (arteriogenesis) that show long-term stability and control perfusion.[5] Establishment of mature vessels is a complex process that requires several angiogenic factors to stimulate vessel sprouting and remodeling (endothelial tubulogenesis accompanied with a pericyte recruitment) of the primitive vascular network. Endothelial vasodilator function of coronary microvessels (resistance arterial vessels) is also an important determinant of myocardial perfusion in response to increased myocardial oxygen demand, playing a critical role in neovascular therapies.[6–8] The attenuated therapeutic effects observed in the previous clinical trials were caused by multiple factors including

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