Cardiomyocytes regenerate non-human primate hearts.


Study Hypothesis

The human heart has a limited capacity for regeneration after injury. Cardiac cell therapy aims to repair the injured heart by replacing dead and damaged cells with de novo cardiomyocytes. Human pluripotent stem cells (from embryonic or induced pluripotent lineages) represent an ideal source of cardiomyocytes and have been extensively studied in this context. Several preclinical studies in small animal models have shown that these stem cell–derived cardiomyocytes can electrically couple with the host myocardium and improve cardiac function.1 However, it is unclear whether promising findings in small animal models can be reproduced in large animal models, which have a heart size and heart rate closer to that of a human. In this study, Chong et al2 examine whether exogenously delivered human embryonic stem cell–derived cardiomyocytes (hESC-CMs) will engraft and electrically integrate with the host myocardium in a nonhuman primate model of myocardial infarction (MI).

How Was the Hypothesis Tested?

To determine whether cardiomyocytes could be transplanted on a large scale, the authors conducted preliminary experiments in a mouse model of MI to test whether cryopreserved hESC-CMs could engraft to damaged mouse myocardium as efficiently as noncryopreserved hESC-CMs. Subsequently, large numbers of hESC-CMs were generated from human embryonic stem cell lines and cryopreserved. Specifically, the authors used embryonic stem cell lines that had been genetically modified to express the fluorescent calcium indicator, green fluorescent protein/calmodulin/MB fusion protein 3. This enabled them to monitor calcium fluxes (a marker for electric activation) in hESC-CMs and provided an elegant system to examine electric coupling between the hESC-CMs and the host myocardium. The purity of hESC-CM preparations was assessed with flow cytometry.

Next, MI was induced in 7 pigtail macaques by inflating a balloon catheter in the left anterior descending artery to create ischemia (90 minutes), followed by reperfusion. Two weeks later, macaques were treated with hESC-CMs (1×10⁹ cells), which were injected directly into the infarct region and surrounding border zones, or with a sham injection containing no cells (vehicle only). Immunosuppression commenced 5 days before cell delivery. Two macaques were euthanized 14 days after treatment (1 cell-treated, 1 sham-treated), 3 macaques were euthanized 28 days after treatment (2 cell-treated, 1 sham-treated), and 1 cell-treated macaque was euthanized 84 days after treatment. One macaque developed complications after MI and was excluded from the study. All macaques underwent full necropsy.

For each heart, the authors analyzed infarct size, graft size, and graft composition (including myofibril content, sarcomere alignment, cardiomyocyte size, and host–graft interactions) using histology and immunohistochemistry techniques. Microcomputed tomography was used to generate 3-dimensional images of the coronary vasculature to assess the growth of host blood vessels into the graft. All hearts underwent ex vivo fluorescent imaging using a modified Langendorff perfusion system to test electromechanical coupling between engrafted hESC-CMs and the host myocardium (made possible by GCaMP3 fluorescence). The influence of hESC-CM grafts on left ventricular function was investigated with echocardiography, which was performed before MI, before cell delivery and before death. The potential adverse consequences of hESC-CM grafts were explored by analyzing ECGs collected via telemetry throughout the experiment.

Principal Findings

The authors’ initial experiments showed that cryopreservation of hESC-CMs had no adverse impact on hESC-CM graft size in a mouse MI model, indicating that hESC-CMs could be cryopreserved with good viability for transplantation on a large scale. They then went on to generate hESC-CMs from embryonic stem cell lines stably expressing GCaMP3. Flow cytometry confirmed high yields of cardiomyocytes, showing that the majority of cells (73% ±12) were positive for cardiac troponin T. hESC-CMs beat spontaneously in vitro, fluorescing with each contractile cycle.
Next, they used an established model of ischemia/reperfusion to generate MIs in all macaques. After treatment, all cell-treated hearts showed extensive remuscularization of the infarcted region. The hESC-CM graft size ranged from 0.7% to 5.3% of the left ventricle and made up 40% of the infarct volume, on average. By 14 days post-treatment, there were frequent connections between host and graft cells, including the formation of new intercalated disks. By 84 days post-treatment, blood vessels from the host coronary network had extended into the graft. hESC-CMs also appeared to mature over time, with marked increases in myofibril content, sarcomere alignment, and cardiomyocyte size observed in the graft collected at 84 days compared with the graft collected at 14 days post-treatment.

When examined in a modified Langendorff perfusion system, all cell-treated hearts showed regular epicardial fluorescent calcium transients, indicating electric activation of the hESC-CM grafts. Furthermore, fluorescent pulses were perfectly synchronized with host ECG QRS complexes during spontaneous depolarization and atrial pacing, providing strong evidence for electromechanical coupling between engrafted hESC-CMs and the host myocardium. Conversely, echocardiography showed inconsistent effects of cell treatment on left ventricular function, with no statistically significant improvements observed.

All cell-treated macaques experienced nonfatal arrhythmias after treatment, including premature ventricular contractions and ventricular tachycardia. No arrhythmias were detected in these animals before cell delivery. In contrast, all sham-treated macaques maintained normal sinus rhythm and heart rate throughout the experiment. There was no evidence of teratoma or other tumors, and no human cells were detected outside the heart, in any of the cell-treated macaques.

**Implications**

In this study, Chong et al take an important step toward translation of cardiomyocyte transplantation into clinical therapy. Most significantly, they demonstrate that hESC-CM transplantation may be feasible on a clinical scale. They also show that exogenous delivery of hESC-CMs to the injured heart of nonhuman primates leads to significant remuscularization of the myocardium, electric integration between the graft and host myocardium, and perfusion of the graft by host blood vessels. Importantly, this latter finding suggests that hESC-CM grafts may be viable long-term.

Despite these encouraging findings, the study highlights several challenges that need to be overcome if this emerging technology is to be developed for human clinical trials. Most concerning was the development of arrhythmias, which were observed in all cell-treated macaques. This finding was in stark contrast with the authors’ previous studies in mice, rats, and guinea pigs, suggesting that larger hearts with slower basal heart rates may be inherently more susceptible to arrhythmia after cardiomyocyte transplantation. Given that adult human hearts are >5× larger and beat at a slower rate than adult macaque hearts, much additional work will be needed to understand the causes of arrhythmia in this setting. In addition, it remains uncertain whether cardiomyocyte transplantation confers a beneficial effect on cardiac function in large animal models, let alone in those with larger infarcts or established heart failure. Nevertheless, by demonstrating that large-scale hESC-CM delivery is possible and that it leads to significant remuscularization of the nonhuman primate heart, Chong et al have overcome a major hurdle to clinical translation. This work provides a compelling rationale for further translational work on cardiomyocyte transplantation as a therapy for heart failure.

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**Disclosures**

None.
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Anna P. Pilbrow

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