Creation of Engineered Cardiac Tissue In Vitro From Mouse Embryonic Stem Cells

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Background—Embryonic stem (ES) cells can terminally differentiate into all types of somatic cells and are considered a promising source of seed cells for tissue engineering. However, despite recent progress in in vitro differentiation and in vivo transplantation methodologies of ES cells, to date, no one has succeeded in using ES cells in tissue engineering for generation of somatic tissues in vitro for potential transplantation therapy.

Methods and Results—ES-D3 cells were cultured in a slow-turning lateral vessel for mass production of embryoid bodies. The embryoid bodies were then induced to differentiate into cardiomyocytes in a medium supplemented with 1% ascorbic acid. The ES cell–derived cardiomyocytes were then enriched by Percoll gradient centrifugation. The enriched cardiomyocytes were mixed with liquid type I collagen supplemented with Matrigel to construct engineered cardiac tissue (ECT). After in vitro stretching for 7 days, the ECT can beat synchronously and respond to physical and pharmaceutical stimulation. Histological, immunohistochemical, and transmission electron microscopic studies further indicate that the ECTs both structurally and functionally resemble neonatal native cardiac muscle. Markers related to undifferentiated ES cell contamination were not found in reverse transcriptase–polymerase chain reaction analysis of the Percoll-enriched cardiomyocytes. No teratoma formation was observed in the ECTs implanted subcutaneously in nude mice for 4 weeks.

Conclusions—ES cells can be used as a source of seed cells for cardiac tissue engineering. Additional work remains to demonstrate engraftment of the engineered heart tissue in the case of cardiac defects and its functional integrity within the host’s remaining healthy cardiac tissue. (Circulation. 2006;113:2229-2237.)

Key Words: stem cells ■ myocardial infarction ■ myocardium ■ myocytes ■ tissue engineering
improved pharmacological and invasive treatment regimens, the numbers of patients who suffer from heart failure are still increasing (www.americanheart.org/statistics). The majority of these patients at the end stage of disease will die while waiting for a heart transplant, presently the last treatment option, which, unfortunately, is very limited because donor organs are in short supply. Restoration of cardiac function may be achieved by replacing diseased myocardium with a functional cardiac tissue, and therefore cardiac tissue engineering might offer a novel therapeutic modality to patients with malfunctioning myocardium. Additional work remains to demonstrate further if the therapeutic modality to patients with malfunctioning myocardium can provide a therapeutic benefit for replacing diseased myocardium with a functional cardiac tissue, and therefore cardiac tissue engineering might offer a novel therapeutic modality to patients with malfunctioning myocardium.

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Methods

Mouse ES Cells

ES-D3 cells were cultured and maintained as described elsewhere. The cells were dissociated with 200 U/mL collagenase IV (Invitrogen, Carlsbad, Calif) at 37°C for 5 to 10 minutes, and ~7×10⁶ cells in culture medium without leukemia inhibitory factor were transferred into a 250-mL slow-turning lateral vessel (STLV) (Synthecon, Inc, Houston, Tex) for cell expansion and embryoid body (EB) formation. The rotating speed was 15 rpm for the first 12 hours and was then adjusted to 45 rpm. After 3 days in the STLV, 1% ascorbic acid was added into the medium. Two days later, EBs were transferred onto gelatin-coated plates (1 to 3 EBs per square centimeter) and cultured for an additional 7 to 9 days in differentiation medium. (Details of ES cell culture medium and differentiation medium can be found in the online-only Data Supplement.) The cultures were then examined for the presence of beating cells and subjected to analysis of cardiomyocytes by immunostaining of cardiac troponin T (cTNT).

Percoll Enrichment of Cardiomyocytes

Differentiated ES cells containing beating cells were dissociated, resuspended in differentiation medium, and loaded onto a Percoll (Amer sham, Uppsala, Sweden; Pharmacia, Piscataway, NJ) gradient for enrichment of cardiomyocytes as described. In brief, Percoll was diluted in a buffer containing 20 mmol/L HEPES and 150 mmol/L NaCl. The gradient consisted of a 40.5% Percoll layer over a layer of 58.5% Percoll. After centrifugation at 1500g for 30 minutes, cell layers were apparent. Cells at different fractions were collected, washed, resuspended in the differentiation medium, plated into chamber slides, and cultured for an additional 3 days for immunochemical staining. A mixture of fraction IV and V was collected for reverse transcriptase-polymerase chain reaction analysis to define cardiomyocytes or semi-quantitative reverse transcriptase–polymerase chain reaction analysis for detection of the presence of undifferentiated ESCs (for details, see the online-only Data Supplement).

ECT Construction

Circular ECTs were prepared by a method described previously. In brief, 1.8×10⁶ freshly isolated cardiomyocytes derived from mouse ES cells were mixed with 1.5 mL of liquid collagen type I prepared from rat tails, a basement membrane protein mixture (Matrigel; Becton Dickinson Biosciences, San Jose, Calif), and concentrated serum-containing culture medium (2% DMEM, 20% horse serum, 4% chick embryo extract, 200 U/mL penicillin, and 100 μg/mL streptomycin); pH was neutralized by titration with NaOH. The reconstitution mix was pipetted into circular casting molds and incubated for 30 to 45 minutes at 37°C and 5% CO₂ to allow hardening of the reconstitution mixture. Thereafter, 6 mL serum-containing culture medium (DMEM, 10% horse serum, 2% chick embryo extract, 100 U/mL penicillin, and 100 μg/mL streptomycin) was added to each dish. After 7 days in culture, ECTs were transferred into a modified stretch device and hooked on the working rods for unidirectional cyclic stretch (10%, 2 Hz) for an additional 7 days. Culture medium was changed 12 hours after ECT casting and then every other day while the culture was maintained in casting molds. After transfer into the stretch device, the culture medium was changed daily.

In Vitro Evaluation of ECTs

Five days after culturing in casting molds, the ECTs were strong enough to allow for mechanical stretching. Force measurement and electrophysiology were examined an additional 7 days later (see online-only Data Supplement). Histological sections were prepared, and cardiomyocytes as well as noncardiomyocytes in the ECTs were also examined by confocal laser scanning microscopy or immuno- histochemical staining. The ultrastructure of the ECTs was also examined by transmission electron microscopy.

ECT Implantation

A total of 7 ECTs were implanted subcutaneously into the dorsal skin pocket of 7 nude mice. Four weeks later, the mice were euthanized, and the ECTs were extracted for histological examination. The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Construction of ECT

A total of ~8.25×10⁷ cells containing >46% cTnT-positive cardiomyocytes were procured after step-by-step strategies including STLV-based EB formation, ascorbic acid–enhanced differentiation, and Percoll enrichment (see the online-only Data Supplement). The mixture of these cells with liquid collagen was pipetted into casting mold to form circular ECTs. The circular ECTs condensed after 2 days of culture. Contraction of individual cells was first noted after 24 hours. Synchronous contractions of cell clusters on the surface of the strand started at day 3. Most cell clusters beat unidirectionally along the long axis of the circle. The beating clusters increased in size and contracted independently from each other. The beating rates of the engineered tissue were ~1.2 Hz. Coherent contraction of the complete ECT was achieved 7 days after stretching. Over time, contractions became more regular, and the beating rates could reach 1.3 Hz and even as much as 1.4 Hz at day 10. After 7 days of stretching, the ECT beat regularly and unidirectionally along the direction of stretching (Figure 1a). After removal from the stretching device, the ECT beat continuously at ~1.2 Hz for >10 days (Figure 1b).

Contractile Properties of Circular ECTs

Contractile force and twitch kinetics of the ECTs were investigated in standard organ baths under isometric conditions 6 days after stretching was complete. The ECTs responded to pharmacological interventions (positive inotropic responses to isoprenaline and calcium) in an organotypic manner. At the width of 20% of original length, twitch tension amounted to 0.07±0.004 mN (Figure 2a). The beating rate was 64.2±3.3 per minute. An increase of extracellular calcium enhanced twitch tension from 0.07±0.003 to 0.48±0.012 mN, with a maximal inotropic response at 1.6 mmol/L (Figure 2b, left). β-adrenergic stimulation...
induced an increase of twitch tension from 0.07±0.002 to 0.29±0.032 mN, with a maximal inotropic response at 1 μmol/L isoprenaline (Figure 2b, right). Additionally, isoprenaline increased the beating rate from 65.7±2.8 to 83±3 per minute.

Electrophysiological Recordings of ECT
Electrophysiological studies showed both spontaneous beating and active responses to electric or pharmaceutical stimulation. In Tyrode’s solution, the ECTs beat spontaneously and rhythmically, showing a regular ventricular activity, with an amplitude of 0.7±0.1 mV. The beating rate was 72.3±7.6 per minute (Figure 2c). Isoprenaline enhanced the amplitude to a maximal 2.2±0.8 mV at 1 μmol/L (Figure 2d). However, the addition of diltiazem markedly reduced resting tension to below the baseline (Figure 2e).

Histology
Sections of paraffin-embedded ECTs revealed the formation of complexes of multicellular aggregates and longitudinally oriented cell bundles mainly consisting of cardiomyocytes in the circular ECT (Figure 3a, i to iii). The muscle bundles within the ECTs were found mainly in the free lateral edges of the gel with a width ranging from ~60 to 380 mm. In comparison with mature adult rat myocardium, the myocytes in the ECTs had oval or round nuclei, were stained less intensively with eosin, and exhibited less clear cross-striation, indicating a decreased content of myofilaments. However, histological features of myocytes forming ECTs resembled those of myocytes within native differentiated myocardium. Cross-striation was visible although less clear than that in the adult tissues. The overall histological features resembled immature neonatal cardiac tissue (Figure 3a, iv) and were much like those observed in ECTs generated from neonatal rat cardiomyocytes by Zimmermann et al18 and our group19 using the same construction method. These results further illustrate the survival and rearrangement of the ES-derived cells in the ECTs, which is a prerequisite for cardiac tissue engineering. Additionally, blood vessels were observed in the ECTs, which may indicate spontaneous vascularization (Figure 3a, i to iii, arrow).

Cell Type Identification in ECTs
To analyze the overall composition and spatial distribution of cell species within ECTs, vibratome sections were immunolabeled to identify cardiomyocytes (sarcomeric actin). Cardiomyocytes constituted the majority of the phallloidin-tetramethylrhodamine-5-isothiocyanate–positive cellular network with a high degree of sarcomeric organization (Figure 3b). Cell strands formed a network of intensively interconnected cell bundles in most parts of the ECT. Immunohistochemical staining also revealed the presence of fibroblasts (positive for vimentin), neural cells (positive for nestin), and vascular endothelial cells (FVIII) throughout the ECT (Figure 3c).

Ultrastructural Characterization of ECTs
Ultrastructural hallmarks of cardiomyocyte differentiation are Z-band formation, specialized cell-cell junctions, and the reestablishment of an extracellular basement membrane. Most, but not all, of these features were present in the majority of cells. Cardiomyocytes within ECTs displayed a predominant orientation of sarcomeres in registry along the longitudinal cell axis (Figure 4a). These cardiomyocytes were rich in mitochondria and glycogen granulae (Figure 4a to 4e). Sarcomeres were composed of Z bands in most investigated cells; however, I, A, and H bands were not clearly visible. Myofilament bundles were less compacted in comparison with adult cardiomyocytes. In some cells, the myofilaments were short and not well oriented in a specific direction (Figure 4b, 4c), indicating that cardiomyocytes in ECTs exhibit a high, but not terminal, degree of differentiation. Specialized cell-cell junctions responsible for mechanical and electric coupling of cardiomyocytes, such as adherens junctions, desmosomes, and gap junctions, were found throughout the ECTs (Figure 4a to 4e). Transmission electron microscopy provided additional evidence that ECTs are reconstituted from various cell types apart from cardiomyocytes, resembling an organoid cardiac tissue construct. Fibroblasts (Figure 4f) and macrophages (Figure 4g) were observed throughout the ECT.

ECT Implantation Into Nude Mice
A total of 7 ECTs were surgically implanted subcutaneously in the dorsal skin pocket of 7 immunodeficient nude mice. Four weeks later, the implants were extracted to determine the survival and engraftment of the ECTs and, more importantly, to examine for tumorigenesis. No signs of teratoma formation were found with hematoxylin-eosin stain (Figure 5a). The retrieved tissue stained positive for cTnT and cardiac troponin I with striation in immunohistochemical examinations, indicative of the presence of cells with cardiomyocyte phenotype (Figure 5b, 5c, 5d). However, in some regions, individual cells with clear striation stained negative for cTnT (Figure 5c). Histological examination also revealed extensive vascularization throughout the implants (Figure 5a, arrow).

Discussion
Currently, investigators are focusing on cardiomyocyte proliferation using pluripotent sources, such as skeletal myoblasts, mesenchymal stem cells (MSCs), or ES cells.5–7 Skeletal myoblasts, such as muscle-resident satellite cells, may be propagated
in vitro on a large scale and can survive after injection in vivo.\textsuperscript{20} Thus far, however, research has not demonstrated active contractions of implanted myoblasts and true cell-cell coupling.\textsuperscript{21} In vivo injected myoblasts differentiate into myotubes, not into cardiomyocytes, and remain in isolated myocardium.\textsuperscript{22} Despite concerns about the induction of arrhythmias, clinical trials are being conducted that will eventually clarify whether such treatment is feasible, safe, and beneficial to the patient.\textsuperscript{8} Although there has been improvement in cardiac function after MSC transplantation in animal models, as noted in several reports, transdifferentiation of MSCs into cardiomyocytes is still in dispute, and the conversion efficacy is quite low.\textsuperscript{23–27} This makes MSCs less promising in construction of cardiac tissue patches for repair of large myocardial defects.

We demonstrated the derivation of the ECT through improved techniques for massive production of EBs, cardio-
genic differentiation, and enrichment of cardiomyocytes from differentiated cell mixtures. Most ES cell lines require aggregation of multiple ES cells to efficiently initiate EB formation. Currently, the most robust method for generating most differentiated cell types is through the EB system, in which the ES cells differentiate spontaneously as tissuelike spheroids in suspension culture.\textsuperscript{28–30} Standard methods of EB formation include hanging drop, liquid suspension, and methylcellulose culture.\textsuperscript{31} However, these culture systems have limited production capacity and are not easily amenable to process-control strategies. Gerecht-Nir et al\textsuperscript{31} first introduced rotary cell culture system for culture of human EBs and demonstrated that the STLV system has a 3-fold enhancement in generation of human EBs compared with the static culture by control over the aggregation of differentiating human ES cells, thus enabling scalable cell production for clinical and industrial

Figure 2. Contractile properties and electrophysiology of ECTs. Representative contraction of ECTs was recorded as described by Zimmermann et al.\textsuperscript{18} The spontaneously beating ECTs were immersed in Tyrode's solution supplemented with calcium at 0.4 mmol/L and stretched to the length of maximal force development (a). Calcium was lowered to 0.2 mmol/L and then increased accumulatively to 2.8 mmol/L. After 2 washes with 0.2 mmol/L calcium, isoprenaline was accumulatively added between 0.1 and 1000 nmol/L (b). For electrophysiology, the ECTs beat spontaneously and rhythmically in Tyrode's solution, showing regular ventricular activity (c). Isoprenaline enhanced the amplitude (d). Addition of diltiazem markedly reduced resting tension to below the baseline (e).
Figure 3. Histology of distinct cell types in ECT. Photomicrographs of hematoxylin-eosin–stained paraffin sections from ECTs showed formation of complexes of multicellular aggregates and longitudinally oriented cell bundles (a, i to iii). Vascular vessels were notable in the ECTs (arrow). The histological morphology of the ECTs resembles that of immature neonatal cardiac tissue (a, iv). Immunolabeling indicates formation of cardiac cell bundles with significant striations of sarcomeres in the ECT (actin appears red; α-sarcomeric actin, green; and nuclear, blue) (b). Distributions of fibroblasts (vimentin), vascular endothelial cells (FVIII), and neural cell–like cells (nestin) were also observed as indicated by immunolabeling (green) (c). Bars=30 μm (a), 15 μm (b), or 30 μm (c).
applications. In our study, EBs were initiated and culture expanded in a 250-mL STLV, a vessel that is considerably larger than the 55-mL one used in the report of Gerecht-Nir et al and therefore was more effective for mass production of healthy EBs.

In vitro ES cell differentiation into cardiomyocytes is a unique system that not only allows opportunities to study cardiomyocyte differentiation but also has potential therapeutic applications. ES cells can differentiate spontaneously into beating cardiomyocytes. To improve efficiency for cardiogenic differentiation of
mouse ES cells, we introduced ascorbic acid into our differentiation system as a modification of the one reported by Takahashi et al.\textsuperscript{32} This modification proved more efficient for cardiogenic differentiation of mouse ES cells. It is possible that ascorbic acid may have an effect early in EB formation.\textsuperscript{32} In the present study, a combination of STLV culture and 1% ascorbic acid induction was conducive to mass production of cardiomyocytes from ES cells.

Purification of ES-derived cardiomyocytes is important for use of these cells in tissue engineering or implantation. This is because there is a mixture of noncardiac cell types in the cells dissociated from EBs. Current strategies for purification of cardiomyocytes rely mainly on genetic selection, by transfection of either cardiac-specific promoters\textsuperscript{30} or green fluorescent protein.\textsuperscript{33–35} However, although proven highly effective, the genetic modifications may carry risks for clinical applications. Xu et al.\textsuperscript{6} first successfully used density gradient centrifugation in Percoll to enrich cardiomyocytes from the disassociated human EB cell mixtures. This physical method is simple and comparatively effective and, most importantly, elicits no genetic alterations of the cells of interest. In our previous works, this method also proved successful in enriching cardiomyocytes from a mouse ES cell line in comparison with neonatal rat cardiomyocytes.\textsuperscript{17} This study provides further evidence for the effectiveness of this strategy. Without any genetic alterations, the cardiomyocytes enriched from this system are more advantageous in tissue engineering applications.

Several important structural features of ECT, similar to neonatal mouse cardiac cells, were visible on electron microscopy (Figure 4). The ECTs not only contained a cardiomyocyte network but represented true organoids because of the presence of noncardiac cell types. Our ES cell–derived ECTs exhibited cardiac tissue–like contractile function and electrophysiology, similar to those tissue-engineered cardiac muscles from the progenitor ventricular cardiomyocytes from the neonatal fetus.\textsuperscript{8} Contraction occurred synchronously, which gives functional substantiation to the morphological observation of complete electric coupling. Furthermore, the constructs responded to pharmacological stimulation (calcium and isoprenaline) in a native cardiac muscle–like manner and showed a force response to classic inotropic interventions, such as isoprenaline and diltiazem. The main functional difference between ECTs and adult ventricular tissue is that the spontaneous contractile activity in ECTs is characteristic of immature myocardium.

Although previous reports have demonstrated the possibility of differentiating mouse ES cells into cardiomyocytes\textsuperscript{5–7} and successfully constructing tissue-engineered cardiac tissue grafts with the use of neonatal muscle cells,\textsuperscript{18,19,36–40} this is the first report of in vitro ECT constructs from ES cell–derived cardiomyocytes. The ECTs resembled natural cardiac tissue both structurally and functionally, although the cells were at a less mature developmental level. We think that this may be due to the following factors: (1) The development of contractile properties of the ECT is related to the unidirectional and phasic stretch stimulations; (2) the total cell number for each ECT in our study was $1.8 \times 10^7$, which is larger than that in the study of Zimmermann et al.\textsuperscript{18} (1 to $10^7$); and (3) the supplement of Matrigel in the construct is also beneficial to extracellular matrix secretion, ECT development, and contractile property improvement.\textsuperscript{11}

Although it has been reported that ES cells lose their tumorigenic potential when induced to differentiate immediately after removal of leukemia inhibitory factor from the medium,\textsuperscript{41} it must be noted that not simply the transplantation of mouse\textsuperscript{42} and human ES cells\textsuperscript{43} but also the transplantation of ES-derived “differentiated” cell populations would result in the outgrowth of teratomas.\textsuperscript{44,45} The protocols for in vitro differentiation of mouse ES cells and culture of ECTs, as described in this report, may therefore prove insufficient to eliminate the cancer risk. However, in our examination of the presence of undifferentiated cells in both of the Percoll-enriched cell clusters, the genes associated with tumorigenesis, including CD30 and Oct-4, were not detected by reverse transcriptase–polymerase chain reaction. Carcinogenesis was also not observed in the ECTs after subcutaneous implantation in all 7 nude mice for 4 weeks. Instead, cell types other than cardiomyocytes were found in both of the Percoll-enriched cell clusters and ECTs. These results, together with the results of Xu et al.\textsuperscript{6} in human ES cells, indicate that cardiomyocytes, together with some noncardiomyocytes, enriched from mouse ES cells by our protocol are differentiated and will be unlikely to give rise to tumors, at least in the short term. Long-term and more extensive investigations remain for future studies to ensure the safety of potential ECT transplantation therapy. However, interestingly, in some regions, in addition to cTnT- and cardiac troponin I–positive cardiomyocytes in the implanted ECTs, individual cells with clear striation were found to have stained completely negative for cTnT despite repetitive staining experimentally (Figure 5c, white arrow). We hypothesize that this puzzling phenomenon may occur for the following reasons. The phenotypic instability of immature cardiomyocytes derived from ES cells may account in part for this phenomenon. Additionally, the local microenvironment may play an important role in the
phenotypic stability of cells differentiated from ES cells. Although this subcutaneous microenvironment provides necessary nutrition for the implants, it is not suitable for the phenotypic maintenance and maturation of cardiomyocytes within the ECTs because of lack of mechanical stimulation and biological clues. Further examinations will be necessary to reveal the influence and effective factors of the subcutaneous microenvironment on the phenotype stability of differentiated cardiomyocytes derived from ES cells.

Furthermore, vascularization of the construct remains a major challenge for future studies of myocardial tissue engineering. It is commonly known that a clinically significant mass of myocardial construct will die within an hour without blood perfusion, whereas the process of angiogenesis and vasculogenesis from the surrounding tissue will take days to develop. More importantly, when implanted, these vessels in the construct need to be connected to a feeding vessel rapidly to prevent tissue necrosis. In our study, liquid collagen was used as a scaffold. In the ECTs, the gelled collagen still allows for mass transfer and medium diffusion through the construct, and this was further improved during the mechanical stretching. Additionally, the presence of vascular endothelial cells and fibroblasts in the construct may give rise to vasculature formation as indicated in Figure 5, similar to recent findings by Levenberg et al.46 It is noteworthy that embryonic fibroblasts can become smooth muscle actin–positive cells induced by surrounding vascular endothelial cells, which promote the stabilization of the vessel structures over time.46 These factors may be the major, if not the only, reason that we could generate ECTs with a thickness of up to 800 μm. However, to engineer a cardiac tissue patch for repair of large myocardial defects, it must be noted that the present thickness is still immature, resembling the neonatal stage, and thus further improvement of tissue engineering strategies, such as local delivery of growth factors, prevascularization of the implanted scaffold before cell seeding,47,48 and incorporation of endothelial cells into the bioengineered tissues49,50 is needed to investigate the potential medical application of ECT transplantation therapy.

In summary, our results strongly suggest that ES cells can be used as a source of seed cells for tissue engineering, especially engineering of those tissues or organs that are based on cell types difficult to obtain from other sources, including cardiac tissue. Further studies will be necessary to demonstrate engraftment of the engineered heart tissue in the case of cardiac defects and its functional integrity within the host’s remaining healthy cardiac tissue. Long-term observations will help in ruling out the possibility of tumorigenesis. It is important to note that ES cells can be culture-expanded in bioreactors and differentiated into cardiomyocytes with significant efficacy. More importantly, the differentiated cardiomyocytes can be purified and enriched to a high purity by simple density-gradient centrifugation. The ES-derived cardiomyocytes demonstrated structural and functional capacity for cardiogenesis, both in vitro and in vivo.

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Disclosures

None.

References


**CLINICAL PERSPECTIVE**

A goal of cardiac tissue engineering is production of pieces of cardiac tissue that can repair damage from a myocardial infarction or other bouts of ischemia. Research performed by these authors at the Beijing Institute of Basic Medical Sciences and the University of Connecticut demonstrates the feasibility of inducing development and growth of cardiomyocytes from murine embryonic stem (ES) cells. Using a mechanical stretching device on this tissue, the researchers grew the ES cell–derived cardiomyocytes into a ring structure. In this study the authors report on the mass production of embryoid bodies in a bioreactor, followed by induction of development into cardiomyocytes under ascorbic acid treatment, followed by enrichment through a Percoll gradient. These cells were mixed with liquid collagen, generating a cardiac tissue ring structure. The engineered ring was subjected to mechanical stretching, producing a formed piece of cardiac tissue. This tissue piece had the unique characteristics of all cardiac tissue; the cells were contractile in unison and responded to mechanical and pharmaceutical stimulation. One concern about engineered tissue from ES cells is its propensity to induce teratoma formation after implantation into an animal. In this study’s preliminary tests, implanting the tissue subcutaneously in nude mice did not produce teratomas up to 4 weeks after implantation. Despite the great potential for engineering medically useful cardiac tissue from ES cells, significant research remains to be done on transplantation procedures and host tissue integration and vascularization.
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