Analysis of the electrophysiological properties and arrhythmias in directly contacted skeletal and cardiac muscle cell sheets

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Abstract

Objective: Autologous skeletal muscle cell (SM) transplantation into the in vivo heart sometimes induces serious arrhythmias. The purpose of this study was to investigate the electrophysiology of cardiomyocyte (CM) and SM in direct contact and to study the mechanism underlying the cause of arrhythmia using the recently developed cell sheet engineering technique.

Methods: Primary cultured rat neonatal SM and CM were prepared, and cell sheets were fabricated using temperature-responsive culture dishes. The action potential was recorded by a conventional microelectrode. Intracellular calcium concentration and optical mapping image of the action potential were recorded using Fluo-3 and di-4-ANEPPS, respectively. A video motion-detecting system was used for the detection of arrhythmias.

Results: SM myotubes occasionally displayed automaticity. SM sheets did not display synchronized contraction, but instead groups of myotubes contracted independently. The action potential of SM, induced by artificial pacing, did not expand to the entire sheet but was limited within a restricted, small area around the electrode, and it was unfeasible to generate an electrical connection or propagate an action potential between CM and SM sheets. SM sheets, in which some of the myotubes displayed automaticity, caused fibrillation-like contraction in the co-cultured CM sheets, and this arrhythmia was specifically blocked by the stretch-activated channel blocker GsMTx-4.

Conclusions: These findings show that SM sheets do not contract synchronously or generate functional syncytia with the surrounding CM sheets and that stretch-induced arrhythmias due to spontaneous contraction of SM may occur in the CM sheet.

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1. Introduction

Autologous transplantation with skeletal muscle cells (SM) can improve cardiac function and the long-term post-implantation survival of injected SM has been reported [1–6]. The primary advantages of autologous cell transplantation over organ transplantation are to overcome delays caused by donor shortages and to cease the necessity for immuno-suppressive agents. The main disadvantages of SM transplantation include the high incidence of arrhythmia requiring cardiac defibrillator implantation [2,3,6], and the lack of data showing improved prognosis.

The mechanisms of improved cardiac function are a matter of debate [7,8]. Although isolated cardiomyocytes (CM) and SM establish electrical communication in vitro [9,10], transplanted SM aggregate and become encapsulated by fibrous tissue [11,12], and do not communicate electrically or simultaneously contract with the recipient CM [13]. These findings suggest that the observed improvement of cardiac function in vivo might be caused by an increase in passive wall tension of the infarcted myocardium or by the induction of vascularization to the injected area [7,8]. While it is known that automaticity, re-entry, and triggered activity...
are the common underlying causes of arrhythmias, the precise mechanism of ventricular fibrillation following SM transplantation is unknown. Although clinical trials for SM transplantation have progressed rapidly, investigations into the mechanisms underlying the cause of arrhythmias have been limited due to the lack of a large animal experimental model.

Shimizu et al. reported an in vitro method using cell sheet technology enabling the manufacture of 3-dimensional (3D) tissue grafts that can be readily applied to a transplantable cardiac graft without enzyme digestion [14,15]. Using this process the layered CM sheets maintain extracellular matrix proteins, adhesion molecules, gap junctions, and form electrical connections between layers within a few days.

To investigate the mechanism of ventricular arrhythmias observed in the SM transplantation model, the present study established a novel technique involving the coculture of CM and SM sheets and by observing the induced arrhythmias. The characteristics of spontaneous contraction of SM sheets were investigated using conventional electrodes and by measuring the intracellular Ca2+ concentration ([Ca2+]i). Action potential propagation, electrical connection, and gap junctions between CM and SM sheets were investigated using optical mapping and histological analysis. Results of the present study will help to elucidate the electrical mechanisms involved in SM transplantation and thereby lead to the prevention of associated arrhythmias.

2. Methods

All experimental procedures and protocols were reviewed and approved by the Animal Care and Use committees of Keio University and conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.1. Making myocardial and skeletal muscle cell sheets

Primary cultured CMs were prepared from the ventricles of 1-day-old neonatal Wistar rat (Japan CLEA, Tokyo, Japan) as described previously [16]. The CMs were plated on temperature-responsive culture dishes (provided by Prof. Okano, Tokyo Women’s Medical University) at a cell density of 8 × 10^5/dish as described previously and incubated at 37 °C in a humidified atmosphere with 5% CO₂ [15]. Skeletal myoblasts were obtained from hind limbs of neonate rats (Japan CLEA, Tokyo, Japan) following the method described by Rando and Blau [17]. Using this method 4 × 10^5 cells were obtained, of which 93% were desmin positive, and were harvested on temperature-responsive culture dishes using F-10 medium (Invitrogen, Carlsbad, California, USA) supplemented with 20% FBS (JRH Bioscience, Lenexa, KS, USA), and 2.5 ng/ml bFGF (TECHNE, Minneapolis, MN, USA). Confluence of SM cultures was reached within 4 days providing a final cell number of 5.6 ± 0.6 × 10^6 and was on the center square area of the dishes where poly (N-isopropylacrylamide) (PIPAAm) (temperature-responsive polymer) had been grafted [18]. Culture dishes were then transferred to room temperature (approximately 25 °C) and square CM and SM sheets were obtained. Culture dishes were precoated with laminin (Roche, Mannheim, Germany) as described previously [19], and three culture models were prepared including CM sheets, SM sheets, and a co-culture of overlaid CM and SM sheets. These cell sheets were cultured with supplemented 199/DMEM medium [20] and half of the medium was changed every two days.

In the present study, the day of cell sheet detachment is defined as day 0, and the generation of cell sheets by lowering the temperature is referred to as manipulation. SM and CM sheets were overlaid and co-cultured for 7 days.

2.2. Action potential recording

Using a conventional glass microelectrode, action potentials were recorded from the SM and CM cultures mounted in a thermo-controlled (37 °C) bath on the inverted microscope under different conditions including the addition of acetylcholine, eradication of Ca2+ ions using isomolar Mg2+ ion substitution, adding a Ca2+ channel blocker, and lowering the temperature. Tyrode’s solution [21] was used as the extracellular solution and was changed using the thermo-controlled Y-tube system within 10 ms [19].

2.3. Measurement of transient [Ca2+]i

The spontaneous contraction of myotubes in the SM sheet was monitored under the microscope at day 3 after manipulation. The [Ca2+]i of the myotubes during spontaneous contraction was monitored using the fluorescent calcium indicator Fluo-3 (Molecular Probes, Eugene, OR, USA) as previously described (Fig. 2) [19].

2.4. Electro-optical mapping

The electrical communication between the co-cultured CM and SM sheets at day 3 after manipulation was observed using the membrane voltage indicator, di-4-ANEPPS (Molecular Probes, Eugene, OR, USA) as described previously [22]. The sample was exposed to loading solution containing 10 μM of di-4-ANEPPS at 37 °C for 30 min. Twenty msec of biphasic stimulation generated by the current isolator system (SS-403J, Nihon Kohden, Tokyo, Japan) at the pacing cycle length of 400–600 ms was applied via a pair of Ag–AgCl electrodes (d=200 μm) to each cell sheet. The fluorescent signal from the paced sample was monitored at a wavelength of more than 610 nm with an excitation wavelength of 520 nm. The obtained data was processed according to the original procedure [22].
2.5. Videotape recording and motion detecting analysis

The cultured cells were observed through an inverted-type phase-contrast video microscope (IX70; OLYMPUS, Tokyo, Japan) equipped with a 4× quartz objective lens and a 1× relay lens. The culture dishes were kept at 37 °C using a temperature-controlled chamber. The cell images were introduced into a charged couple device camera (CS220, Olympus, Tokyo, Japan) and recorded by a digital videocassette recorder (wv-DR9, Sony, Tokyo, Japan). Contraction of the overlaid SM and CM sheets was analyzed with the original motion-detecting program (Igor pro 4, Wavemetrics, Inc., Lake Oswego, OR, USA).

We investigated whether co-existence of SM with CM or the spontaneous beating of SM affected the incidence of irregular beating or fibrillation of CM. CM sheets were electrically paced at 100 or 150 beats/min for 60 s, and were monitored by motion detecting analysis. GsMTx-4 is a small peptide found in the venom of the tarantula Grammostola spatulata and specifically blocks cationic stretch activated channels in cardiomyocytes [23–25]. GsMTx-4 (Peptide Institute, Osaka, Japan) was administered at 400 nM to the perfusate when fibrillation-like contraction was observed in the CM sheets. GsMTx-4 was then removed by washing for 30 min, and the CM sheet was re-stimulated for 60 s.

2.6. Immuno-histological analysis

Single or co-cultured cell sheets were fixed with 4% paraformaldehyde (Muto Pure Chemicals, Tokyo, Japan), and embedded into OCT compound (Sakura Finetechnical, Tokyo, Japan). Immunofluorescent staining was performed on the CM sheets at day 3, on the SM sheets at day 1 and day 7, and on the co-cultured CM and SM sheets at day 3 and day 7, using a mouse anti-α-actinin (1:400, Sigma) monoclonal antibody and a rabbit anti-connexin43 (1:400, Sigma) polyclonal antibody at 4 °C overnight. The samples were then incubated with Alexa488-labeled anti-mouse IgG antibody (1:200, Molecular Probes) or TRITC-labeled anti-rabbit IgG antibody (1:200, Dako), respectively, at room temperature for 1 h. The nucleus was stained with 1.0 nM of TOTO-3 (Sigma). Samples were observed with a confocal laser microscope (LSM510, Carl Zeiss International, Jena, Germany).

2.7. Transmission electron microscopy

The co-cultured CM and SM sheets were fixed at day 3 with 2.5% glutaraldehyde for 2 h. The cells were embedded in Epon 812 (Nacalai Tesque, Kyoto, Japan). Sagittally cut ultrathin sections were observed under a JEM-1200EX transmission electron microscope (Nihon Denshi, Tokyo, Japan).

3. Results

3.1. Characteristics of the action potential of skeletal myotube

Spontaneously contracting myotubes were visualized by microscopy in 70% (21/30) of the SM sheets at day 3 after manipulation. The rhythm of spontaneous contraction was different for each myotube within a single culture dish. Fig. 1 shows the recorded action potentials of spontaneously contracting CMs and skeletal myotubes. The duration of the action potential was markedly shorter in SMs than in CMs, consistent with the short refractoriness of SMs (Fig. 1A). Administration of acetylcholine depolarized the membrane potential, and caused arrhythmia-like activity in SMs (Fig. 1B,C). While pacemaker-like potential was observed in SMs, it occurred via a different mechanism than cardiac pacemaker potential as it was unaffected by administration of Ca2+ free Tyrode’s solution or 10 μM GsMTx-4 (Fig. 1D,E). Moreover, the decrease in the rate of the spontaneous rhythm by lowering the temperature did not correspond to the slope of the pacemaker-like potential (Fig. 1F). Therefore, the observed upward slope of SM depolarization was not consistent with a pacemaker-like potential, but a potential caused by recovery immediately after excitation, a common phenomenon of action potentials observed in neuronal and skeletal muscle cells.

3.2. Dissociated contraction of skeletal myotube detected by recording of [Ca2+]i

We previously reported that two CM sheets become electrically bound and contract synchronously [15,21]. However, measurement of [Ca2+]i shows that even a spontaneously contracting SM sheet does not make a single syncytium (Fig. 2) but rather several groups of myotubes contract independently with their own rhythm. Although one myotube (a and c) was observed to have crossed over another myotube (b), each group beat independently and could not be connected. These findings highlighted the difficulty in establishing a synchronously beating SM sheet.

3.3. Spontaneously beating SM sheet induces fibrillation in CM sheet

To investigate the mechanism of ventricular fibrillation following SM-transplantation, we examined a co-culture of overlaid SM and CM sheets (Fig. 3A). Of a total of 70 separate SM and CM overlays, spontaneous beating was observed in 20 SM sheets (29%) and no spontaneous beating was observed in 50 SM sheets. The CM sheet was paced with 100–150 bpm for one min. There was no irregular beating in the CM sheets when no spontaneous beating was observed in the corresponding SM sheets (0%,
Fibrillation-like contraction in CM sheets occurred in 4 cases (20%, 4/20) and the corresponding SM sheets displayed spontaneous contraction. When two overlaid CM sheets were cultured, there was no fibrillation-like contraction activity (0/50) observed (data not shown).

Fig. 2. Electrical compartmentation of SM sheets. (A) A light microscopic view of the matured SM (1 × 1 mm). The letters “a” to “e” correspond to the location of the intracellular Ca²⁺ concentration ([Ca²⁺]ᵢ) estimation. (B) The [Ca²⁺]ᵢ was monitored following the addition of Fluo-3. Variations in the [Ca²⁺]ᵢ, signal from “a” through to “e”, in panel A, were traced as the SM spontaneously contracted. Each signal at “a” and “b” displayed its own regular contracting rhythm, whereas signals traced from “c”, “d” and “e” had identical patterns of contraction. (C, D, E) Representative images of fluorescent signals for [Ca²⁺]ᵢ are shown. (C) An increase in [Ca²⁺]ᵢ was observed in the area surrounding area “a” when the other 4 spots were unexcited. (D) An increase in [Ca²⁺]ᵢ was noted only at “b” when “a”, “c”, “d” and “e” were not excited. (E) The areas “c”, “d” and “e”, but not “a” and “b”, were excited. Note that “c”, “d” and “e” belonged to same syncytium, but “a” and “b” belonged to different syncytia. The SM cell sheet consisted of several compartments, and did not excite as a single tissue.

Fig. 1. Electrophysiological properties of skeletal muscle cells. (A) Typical action potentials of the cultured cardiomyocytes (CM) and skeletal muscle cells (SM). SM occasionally displays automaticity as shown in the right panel. (B,C) Acetylcholine (Ach) caused marked depolarization (upper panel) and extrasystolic activity (*) in lower panel) in SM. (D, E) Extracellular Ca²⁺([Ca²⁺]ₒ) was replaced with equimolar levels of Mg²⁺. The automaticity of SM was not affected by the absence of extracellular Ca²⁺([Ca²⁺]ₒ=0) or by verapamil. (F) The cooling of the SM perfusate from 37 °C to 22 °C decreased the rate of the spontaneous rhythm without affecting slope of the pacemaker-like potential.
contraction. With electrical pacing of the CM, the CM sheet began to beat regularly (site “a” in Fig. 3B, and trace in Fig. 3C). Spikes corresponding to dotted lines (in upper and lower traces) indicate contractions caused by the CM sheet and spikes corresponding to straight lines (in lower traces) indicate contractions caused by myotubes in the SM sheet. The cardiomyocytes at site “b” displayed a complex movement caused by a mix of paced CM contractions and spontaneous contractions of myotubes in the underlying SM sheet. The amplitude of the spikes increased when coincidental simultaneous contraction occurred at site “a” and “b” (*).

Fibrillation-like contraction in CMs was observed 28 s after the onset of regular beating (Fig. 3D and E). Fig. 3(E) shows the spontaneous contraction of myotubes immediately after the third contraction of site “a”, a vulnerable period for the CM. Thereafter, fibrillating contraction commenced in the entire CM sheet. This fibrillating contraction activity continued for a few minutes.

3.4. Optical mapping demonstrates electrical disconnection between CM and SM sheets

The induction of fibrillation-like activity in the CM sheet following the spontaneous contraction of myotubes in the SM sheet was possible to be caused by the establishment of an electrical connection between the CM and SM sheets. To further investigate this possibility we examined the optical mapping of overlaid CM and SM sheets. Unlike the pair of CM sheets which exhibited tight electrical connection within a few days [15,21], no electrical communication between the CM and SM sheets (0/50) was observed (Fig. 4). The action potential, generated by electrical stimulation at the end of the CM sheet, propagated only within the CM sheet and did not extend through to the SM sheet (Fig. 4B–E). When the SM sheet was electrically paced, excitation of the SM extended only to a very small area around the pacing electrode, and did not extend to the CM sheet. Movement of the pacing site closer to the junction of the CM sheet did not lead to extension of excitation from the SM sheet to the CM sheet (Fig. 4H–K). Configuration of the action potentials traced from the fluorescent signals are shown in Fig. 4(F) and (L). The duration of the action potentials recorded for the CM sheet were long while those recorded for the SM sheet were short.

Fig. 3. Fibrillation-like contraction in the CM sheet co-cultured with the SM sheet. (A) Schematic illustration of the observed area where a part of the CM sheet was laid on top of the SM sheet. (B) Light microscopic view of the overlapping area of the SM and CM sheets. The skeletal myotubes contracting spontaneously, were located at “b” under the CM sheet (enclosed by the white arrows). (C) The length of the movement of the cells at the marked points, “a” in the CM sheet and “b” in the overlaid CM and SM sheets, was traced using the video motion analyzer. Complete dissociation of the contracting rhythm between CM and SM was observed after pacing was initiated on the CM sheet. Increased movement of the cell (*) at the “b” was observed following coincidental simultaneous contraction of the SM and CM. (D) Twenty-eight seconds after the onset of pacing, fibrillation-like contraction was observed in the CM. (E) Magnification of the trace at the onset of fibrillation-like contraction. Spontaneous contraction of skeletal myotube occurred immediately after the third contraction of CM (*), and was then followed by fibrillation.
for the SM sheet was short. In summary, this study shows that establishment of an electrical connection between the CM and SM sheets is not feasible, although further analysis may be necessary.

3.5. Immunofluorescent staining of CM and SM sheets

Co-immunostaining reveals that CM sheets express both α-actinin and connexin43 (Fig. 5A and B). The two observed patterns of α-actinin and connexin43 staining in the SM sheet correspond to immature and mature SM. In the SM sheet at day 1 after manipulation, immature SM is revealed by abundant connexin43 and limited α-actinin staining (Fig. 5C and D). In contrast, mature SM is revealed in the SM sheet fixed at day 7, by marked α-actinin staining and the absence of connexin43 staining (Fig. 5E and F). Immature and mature SMs were observed in the overlaid CM and SM sheets at day 3 after manipulation. In the SM sheet layer, the cells that expressed connexin43 did not show α-actinin staining, and conversely, cells that expressed α-actinin did not show connexin43 staining. In contrast, all CMs expressed both proteins. The margin between the CM and SM sheets could be identified in the 3-day-old samples (Fig. 5G). In day 7 samples, SMs formed long myotubes, which strongly expressed α-actinin but not connexin43, while CMs expressed abundant connexin43 (Fig. 5H). These findings indicate that it was not possible to establish an electrical connection between the CM and the mature SM.

3.6. Electron microscopy

Electron microscopy was carried out on co-cultured CM and SM sheets at day 3. At this stage, both CM and immature SM expressed contractile proteins and myofilaments could be observed by transmission electron microscopy, but complete striation was not yet established. The SMs had begun to form myotubes and become multinucleated. (data not shown). The contacts between CM and SM did not show gap junctions or intercalated disks. (Fig. 6A). In contrast, gap junctions and intercalated disks were observed between CMs (Fig. 6B,C). These findings support the immunofluorescence results described above.

Fig. 4. Electrical disconnection between SM and CM sheets. (A) A representative light microscopic view of the co-cultured CM and SM sheets at day 3 after manipulation. White dotted lines indicate the boundary between cell types, and the yellow dotted line indicates the observation area in experiment H to L. (B, H) Schematic illustration of the arrangement of the two sheets. The Yellow symbol denotes the pacing electrode. (C, D) Optical mapping of the action potential was recorded using di-4-ANEPPS. The edge of the CM sheet was paced, and sequential optical images of action potentials after 35 ms (C) and 119 ms (D) were shown. Propagation of the action potentials was blocked at the center of the junction. (E) Activation map of action potentials presented isochronally (the interval between each isochronal line denotes 7 ms). The red line with an arrowhead shows a trace of the excitation wave front. (F) The recorded electrical potentials of the points, "p" to "t" in Panel E. Wide duration of these action potentials indicated that the signals originated from CMs. (G) The sequence of impulse propagation along the excitation wave front. (I, J, K) Optical images of the yellow dotted box area in Panel A after pacing the surface of the SM sheet at 21 (I), 42.5 (J) and 52.5 (K) ms after pacing. The pacing electrode was located just above the excitation wave limit after CM pacing. (L) A typical narrow action potential was recorded at the point "*", suggesting that this optical image was obtained from SM not CM.
3.7. Suppression of fibrillation-like contraction of the CM sheet by GsMTx-4

The above findings show that, despite the absence of an electrical connection, the spontaneous beating of the SM sheet induces serious arrhythmic activity in the CM sheet. The frequency of the fibrillation-like contraction and the incidence of spontaneous myotube beating in SM sheets are summarized in Fig. 7(A). These suggest that the passive mechanical stretch caused by the spontaneous contraction of myotubes in the SM sheet may activate the stretch-activated ion channels (SACs) in the CM and induce fibrillation-like contraction in the CM sheet. To investigate this possibility, we administered GsMTx-4, a specific blocker for SACs, to the culture media. We found that fibrillation-like contraction in CM sheets was inhibited and that the spontaneous contraction of myotubes in the SM sheets was unaffected. This finding was further supported by the re-induction of fibrillation-like contraction activity following the removal of GsMTx-4 by washing (Fig. 7B).

4. Discussion

4.1. Necessity of the evaluation of electrical and mechanical junction of cardiac and skeletal muscle cells and the appropriateness of the use of cell sheet engineering

A number of experimental studies and clinical trials report that transplantation of skeletal myoblasts into the
infarcted area improves cardiac function, but also causes lethal arrhythmias in humans [1–6]. Due to the difference in electrophysiological properties between skeletal myoblasts and cardiomyocytes and the unnatural existence of SM in the heart, in vitro electrophysiological studies are necessary to analyze the electrical connection, contraction pattern, and arrhythmogenicity of the transplanted skeletal muscle cells in vivo. The recently developed cell sheet engineering technique provides a new method to examine these interactions that have remained largely undetermined due to the difficulty in generating experimental models. Cell sheet engineering enables flat cardiac and skeletal muscle tissue to be generated in any desired shape and to be cocultured by simply overlaid; thus, it is suitable for cell transplantation experiments. In our experimental models, in which partially overlaid cell sheets were analyzed by optical mapping system, detailed observation of the action potential propagation and the effect of mechanical stretch on arrhythmia between two cell types could be examined.

The present study demonstrated that (1) SM occasionally displayed spontaneous contraction, (2) SM sheets did not show synchronized contraction, but each myotube contracted according to the original rhythm, (3) electrical stimulation of the SM sheet did not extend to the entire sheet but was restricted to a small area around the electrode, (4) it was not feasible to generate an electrical connection or action potential propagation between CM and SM sheets, (5) the SM sheet, in which some of the myotubes contracted spontaneously, caused fibrillation-like contraction in the cocultured CM sheets, and (6) the fibrillation-like contraction observed in CM sheets was blocked by GsMTx-4, suggesting the involvement of stretch-activated ion channels in this arrhythmia.

Fig. 6. Transmission electron micrographs of the 3-day-old co-cultured CM and SM sheets. (A) Low magnification overview of a representative junction between CM and SM (arrows). No intercalated disks or gap junctions are observed. Nu, nucleus. (B) Well-differentiated myofilaments (Mf) and abundant mitochondria (Mt) in the CM are shown. Gap junctions (GJ) are observed between CMs. (C) Intercalated disk (ICD) is observed between CMs (arrow heads).

4.2. Automaticity, contraction pattern, and excitation conduction of skeletal myotubes

Murry reported that transplanted skeletal myoblasts can survive and establish new muscle tissue when grafted into injured rat hearts, and that this muscle could contract when stimulated electrically [11]. Hagege reported that a 72-year-old man, who had autologous cultured myoblasts from his vastus lateralis injected to an area of transmural inferior myocardial infarction in non-reperfused scar tissues, showed improvement in symptoms and ventricular ejection fraction and well developed skeletal myotubes [5]. Leobon reported the differentiation of grafted myoblasts into mature, peculiar hyperexcitable myotubes that had a contractile activity independent of neighboring cardiomyocytes [13]. Reinicke reported that CMs and myotubes could make gap junctions in vitro and showed that, although undifferentiated rat skeletal myoblasts expressed N-cadherin and connexin43, both proteins were markedly down regulated after differentiation into myotubes [9]. Although CM sheets can connect electrically in vitro as previously reported [15], the present study demonstrated that CM sheets did not electrically connect to SM sheets. The study also demonstrated that some of the skeletal myotubes had automaticity, but contracted independently and not synchronously as a single tissue. In addition, electrical stimulation-induced excitation did not extend within SM sheets. It has also been reported that most of the grafted SM is separated from the host myocardium by scar tissue [11,12,26]. Taken together, most grafted SM is electrically isolated from the surround-
ing myocardium, and does not contract synchronously. It is possible that the electrically isolated large mass in the ventricular wall is a potential substrate for recurrent arrhythmia. A large animal model is required for further investigations in this area.

4.3. Automaticity and arrhythmogenecity of SMs in CMs

Automaticity was observed in 29% of the SM when co-cultured with CM. If CM and SM were electrically connected it may provide a substrate for recurrent arrhythmias. Reinicke reported that when neonatal or adult cardiomyocytes were co-cultured with skeletal muscle, approximately 10% of the skeletal myotubes contracted in synchrony with adjacent cardiomyocytes, and that cardiomyocytes can form electromechanical junctions with some skeletal myotubes in co-culture and induce their synchronous contraction via gap junctions [9]. They speculated that, if similar junctions could be induced in vivo, they might be sufficient to make skeletal muscle grafts beat synchronously with the host myocardium. Since early stage skeletal myotubes have low levels of connexin43, they have the potential of connecting electrically to CM. Recently Rubart reported that the most transplanted skeletal myoblasts were functionally isolated from the host myocardium. Few of them were, however, exited synchronously with adjacent host cardiomyocytes and it might be resulted from fusion events between donor myoblasts and host cardiomyocytes [27]. But the present study demonstrates that mature SM cell sheets do not make electrical connections or gap junctions with CM sheets. These findings suggest that if an electrical connection between SM and CM exists it would be temporal. Moreover, if an electrical connection is constructed between SM and CM, the possibility of arrhythmias due to SM automaticity must be considered, since the duration of the action potential of SM is significantly shorter than that of CM.

4.4. Possibility of stretch-induced arrhythmia due to the contraction of SMs

The present study demonstrated an interesting tachycardia-like contraction in CM sheets when co-cultured with SM sheets displaying automaticity. The rate of induced arrhythmia was faster than the automaticity of the SM, and was blocked by the specific stretch-activated ion channel blocker GsMTx-4. Since GsMTx-4 did not affect the automaticity of SM, mechanical stretch caused by contracting SM might be a critical underlying cause of arrhythmia. Since this irregular contraction was analyzed by motion-detecting program in this model, the detailed mechanism of arrhythmogenecity remains unclear. But it is possible that the induced arrhythmia resembles the catheter-induced ventricular tachycardia from left ventriculography. Therefore, stretch-induced arrhythmia is a potential cause of ventricular fibrillation after SM transplantation when automaticity is displayed in the transplanted area.

In conclusion, since SM transplantation is a clinically applicable therapy for patients with severe heart failure, the precise analysis of the underlying electrophysiology and arrhythmogenecity is critical before the widespread application of SM transplantation.
use of this method. Although evaluation of cell-to-cell relationships is insufficient as a model for cell transplantation, cell sheet engineering is a suitable model for the analysis of the relationship between cardiac and skeletal muscle tissues.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.cardiores.2005.03.014.

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