Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 351 (2006) 209–215
BBRC
www.elsevier.com/locate/ybbrc

Role of the community effect of cardiomyocyte in the entrainment and reestablishment of stable beating rhythms

Kensuke Kojima [a], Tomoyuki Kaneko [b,c], Kenji Yasuda [a,b,*]

[a] *Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, 3-8-1 Komaba, Meguro, Tokyo 153-8902, Japan*
[b] *Department of Biological Information, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, 2-3-10 Kanda-Surugadai, Chiyoda, Tokyo 101-0062, Japan*
[c] *PRESTO, Japan Science and Technology Agency, 4-1-8 Honcho Kawaguchi, Saitama, 332-0012, Japan*

Received 1 October 2006
Available online 20 October 2006

Abstract

To investigate the roles that the community effect and entrainment function of cultured cardiomyocyte play in decreasing beating fluctuation and reestablishing synchronized beating, we developed a single-cell-based two-dimensional network culture assay to measure and compare the dynamics of beating rhythm synchronization of individual cells before and after they form networks. Studying the formation of two-cell networks, we found that their synchronized beating tended to be determined by the cardiomyocyte whose beat rate fluctuated less than that of the other cardiomyocyte. We further found that the strength of this tendency increased with the number of cells in the network. These results indicate that (1) beating fluctuation is one of the important factors influencing the reestablishment of a stable synchronous beating rhythm, (2) the larger networks reduce fluctuation, and (3) the formation of a spatial network can itself stabilize cardiomyocyte beat rates.

Keywords: Cardiomyocyte; Beating fluctuation; Synchronization; Entrainment; Cell network; Single-cell-based culture assay system

The heart is one of the most important organs for toxicology in drug screening, and investigators are trying to make on-chip cell models that are as reliable as tissue/organ models. To make a cell-based heart tissue model on a chip, we need to understand the properties of cultured cardiomyocytes and estimate to what extent the cultured cardiomyocyte network can model heart tissue. The relations between the beat rate and beating fluctuation of cultured cardiomyocytes and the establishment of synchronized beating have therefore been examined extensively. It has been reported, for example, that one beating cell can influence the beat rate of a neighboring cell with which it makes contact and that a group of cultured cardiomyocytes beating synchronously with a rapid rhythm can act as a pacemaker for a contiguous cell sheet [1]. One would thus predict that if a faster-beating single cell comes into contact with a slower-beating one, the faster cell should dominate and cause the latter to take on its rhythm (i.e., act as a pacemaker). While this has been reported [2,3], it has also been reported that the rate at which a synchronized cell pair beats cannot be predicted from the beat rate of the initially faster cell and that the faster cell does not always act as pacemaker [4]. Thus, the mechanisms that underlie the entrainment of cardiomyocytes with different rhythms as a pacemaker group with a common synchronous rhythm in the intact heart are unknown. Some researchers have reported that fluctuation of the beating rhythm of cultured cardiomyocytes decreases as the culture period or the number of aggregated cells increases [5–7]. In the work reported in this paper, we used our on-chip single-cell-based culture assay system [8,9] to investigate the ways in which cardiomyocyte beating fluctuation takes place on the establishment of synchronized beating.

* Corresponding author. Fax: +81 3 5280 8049.
E-mail address: yasuda.bmi@tmd.ac.jp (K. Yasuda).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.10.037

Materials and methods

1064-nm photothermal etching method and cell cultivation system. To culture cells at the single-cell level, we used the newly developed photothermal etching system shown in Fig. 1a. It had a focused laser irradiation module with a 1064-nm infrared laser and had a phase-contrast microscope (IX-70, with a 20× objective lens, Olympus, Tokyo, Japan) with an automated X–Y stage (BIOS 201T, Sigma Koki, Saitama, Japan). Different wavelengths were used for phase-contrast microscopy and micrometer-scale photothermal etching: 520-nm visible light for observing the surface of the agarose chip, and 1064-nm infrared light for melting a portion of the agarose in the observed area. Phase-contrast images were acquired by using a charged-coupled device (CCD) camera (CS230, Olympus). The dichroic mirrors and lens in the system were chosen for their suitability for these two wavelengths simultaneously. Flexible focusing lenses were placed in the path of the infrared laser beam to control the focal positions of the laser in order to correct for their different wavelength-dependent focal lengths between 520 and 1064 nm. Cell preparations on the inverted phase-contrast optical microscope were incubated at 37 °C in a humidified atmosphere of 95% air and 5% CO_2 by using a cell-cultivation system (IN-ONI, Tokai Hit, Shizuoka, Japan).

Agarose microchambers. Agarose microchambers for culturing single cells were constructed as shown in parts (b–f) of Fig. 1. First, 50 μl of sol state 2% (w/v) agarose (ISC BioExpress, GenePure LowMelt: melting temperature 65 °C) was placed on the collagen-coated glass slide (collagen-type-1, Nitta gelatin, Osaka, Japan) with a chromium layer (b). Spin coating was then performed at 4000 rpm for 30 s (Spincoater 1H-D7, Mikasa, Tokyo, Japan), forming a 5-μm thick agarose layer on the chip (c). As the 1064-nm laser beam is not absorbed by either water or agarose, it melts only a portion of the agarose on the chromium thin layer because only the chromium layer absorbs the beam. Using this noncontact etching, we can easily make microstructures such as holes and channels within only a few minutes without using any cast molding process. We focused the 1064-nm beam on the agarose layer on the glass slide to melt the agarose at the focal point until the shape of the holes for cells formed. When the focused beam was moved over the chip surface, a portion of agarose layer around the focal spot of laser melted and diffused into the buffer. After the heated spot had been moved, a channel connecting the two adjacent holes was created at the bottom of the agarose layer. Pairs of 30-μm-diameter microchambers connected by 10-μm-wide microchannels were fabricated on the chip (d). A single cell was put into each microchamber manually by using a cell handling pipette (e) and cultured at single-cell level (f).

Cardiomyocytes isolations. Cardiomyocytes were isolated from 13- to 14-day-old mouse embryos (ICR) (Saitama Experimental Animals Supply, Saitama, Japan). A mouse was anesthetized with diethyl ether and embryos were rapidly removed from the mouse. The hearts of the embryos were removed and then washed with phosphate-buffered saline (PBS, 137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, and 1.5 mM KH_2PO_4, pH 7.4) containing 0.9 mM $CaCl_2$ and 0.5 mM $MgCl_2$ to induce heart contraction and remove corpuscles. The hearts were then transferred to PBS without $CaCl_2$, and $MgCl_2$ and the ventricles were separated from the atria, minced into 1-mm^3 pieces with fine scissors, and incubated in PBS containing 0.25% collagenase (Wako, Osaka, Japan) for 30 min at 37 °C to digest the ventricular tissue. After this procedure was repeated twice, the cell suspension was transferred to a cell culture medium (DMEM [Invitrogen Corp, Carlsbad, CA, USA] supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin) at 4 °C. The cells were filtered through a 40-μm nylon mesh and were centrifuged at 180*g* for 5 min at room temperature. After the cell pellet was resuspended in a cell culture medium 100 μl of the suspension (diluted to a final concentration of 1.0×10^5

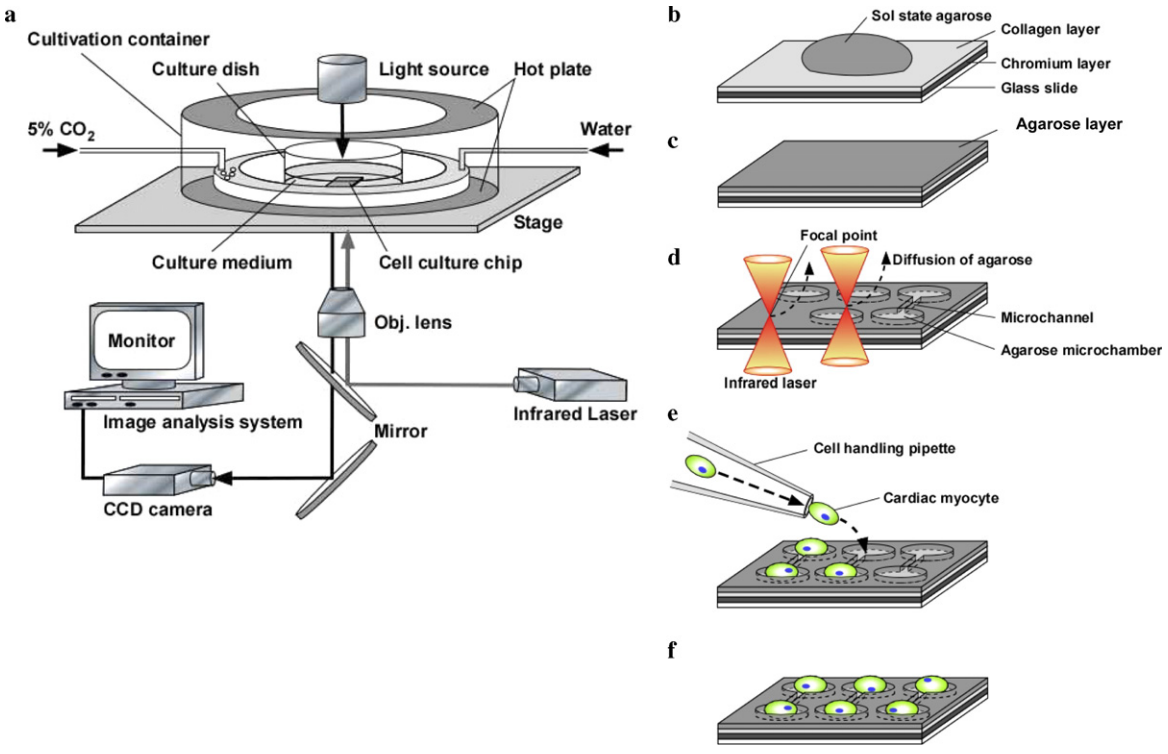

Fig. 1. (a) Schematic drawing of the on-chip single-cell-based Cardiomyocyte cultivation assay. A phase-contrast microscope was used to melt a portion of agarose layer on the chip for the formation of microchamber and to measure the beating rhythm of the cardiomyocytes. The cell preparation on the phase-contrast optical microscope was incubated at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. The spontaneous beating rhythm of cultured cardiomyocytes was evaluated by the image analysis system in which the change of the size (volume cross section) of each cardiomyocyte was estimated and recorded every 1/30 s. (b–f) Process of microchamber formation for the on-chip single-cell-based cardiomyocytes cultures.

cells/ml) was plated on a 35-mm dish, from which the individual cardiomyocytes were picked up and put into microchambers by using a micropipette.

Immunocytochemistry. After the measurements, the preparations were washed with PBS and fixed with 4% paraformaldehyde for 5 min at room temperature. They were then incubated at room temperature for 1 h with blocking buffer (PBS containing 3% skim milk). Connexin 43 was detected by using a rabbit anti-mouse connexin 43 polyclonal antibody (Chemicon, Japan) diluted 1:1000 by PBS containing 1% skim milk and 0.15% Triton X-100. The dishes were incubated with the first antibody for 1 h at room temperature and then washed with PBS three times to remove unbound antibody. The anti-connexin 43 antibody was detected with a donkey anti-rabbit IgG antibody (Alexa fluor 488, Molecular Probes, USA). The polymerized form of actin was detected by using a rhodamine phalloidin (Cytoskeleton, Inc., USA). The dishes were incubated with 100 nM rhodamine phalloidin in PBS for 30 min at room temperature and then washed with PBS three times to remove unbound rhodamine phalloidin. After nuclei were stained for 20 min at room temperature with Hoechst 33342 (1 mg/ml) (CALBIOCHEM, Inc., USA) diluted 1:1000 with PBS, the dishes were washed with PBS three times. The preparations were observed under a fluorescent microscope equipped with a digital CCD camera and a 40× objective.

Image analysis. The spontaneous contraction rhythm of Cardiomyocytes cultured in agarose microchambers was evaluated by a video-image recording method. Images of beating cardiomyocytes were recorded with a CCD camera through the use of a phase-contrast microscope. The temporal change of brightness of the cardiomyocytes image, which depended on the size (volume cross-section) of the cardiomyocytes and thus changed considerably with contraction, was captured every 1/30 s by a personal computer with a video capture board and was recorded.

Results and discussion

Continuous measurement of the change of cardiomyocyte beating rhythm at the single-cell level when cell-to-cell interaction occurs

In the simplest experimental model we used to investigate the dynamics of the reestablishment of synchronized beating by isolated cardiomyocytes, we examined the synchronization process of two cardiomyocytes with initially different rhythms cultured in connected agarose microchambers (Fig. 2a) by observing them and measuring their beating. They initially showed beating rhythms independent of each other (Fig. 2b). When the two cells came into contact physically through the microchannel and interacted with each other (Fig. 2c), their beating rhythms became synchronized (Fig. 2d). Cardiomyocytes cultured in agarose microchambers were subjected to immunocytochemistry for Cx43 and actin. As shown in Fig. 2e, Cx43 and actin were detected normally and labeling for Cx43 was found at the two cardiomyocytes' abutment in the connecting microchannel. These results show that the process of the reestablishment of synchronous beating by cardiomyocytes can be measured at the single-cell level by using our agarose microchamber system and that cardiomyocyte-specific proteins are synthesized normally by cells cultured in agarose microchambers.

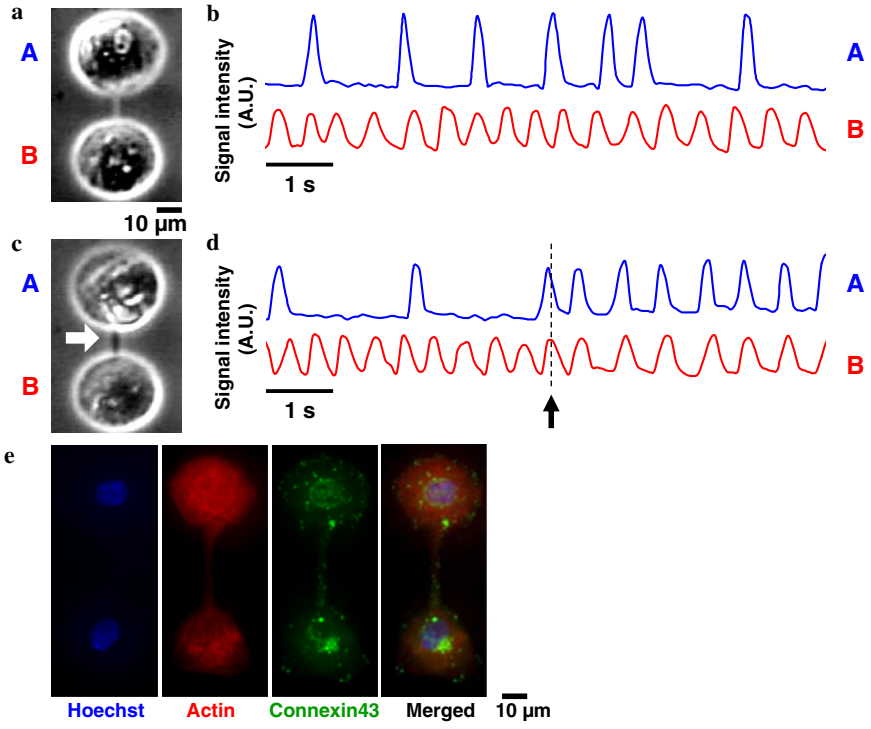

Fig. 2. Interaction of a cardiomyocyte pair cultured in connected microchambers. (a) Micrograph of isolated single cardiomyocytes with different beating rhythms cultured in microchambers A and B. (b) Time course of the beating rhythms of the cells in microchambers A and B before synchronization. (c) Network formation through the microchannel (white arrow). (d) Time course of beating rhythms of the cells in microchambers A and B after synchronization. Dashed line shows the time that synchronization occurred. (e) Immunostaining of two cardiomyocytes cultured in connected microchambers. The cells were stained for Hoechst (blue), actin (red), and connexin43 (green).

Tendency of beat rate and beating fluctuation change before and after the synchronization of two cardiomyocytes with initially different rhythms

We investigated the roles of the beat rate and beat-rate fluctuation of isolated single cardiomyocyte on the reestablishment of synchronous beating by analyzing the changes of beat rate and fluctuation before and after the synchronization of two cardiomyocytes with initially different rhythms. As a result, we found three kinds of synchronization. In one the beating of the two cardiomyocytes synchronized at the faster of the two initial rates (Figs. 3a and b) but with the beating fluctuation of the lower of the two initial rates (Fig. 3c). Another kind of synchronization was one with the beating of the two cells synchronized at the lower of the two initial rates (Figs. 3d and e) and with the fluctuation of the lower of the two initial rates (Fig. 3f). In the third kind of synchronization at neither of the initial rates of the pair (Figs. 3g and h) and with fluctuation of smaller of the initial fluctuations (Fig. 3i). The interbeat intervals of 14 cell pairs before and after synchronization are listed in Table 1. Five of the cell pairs synchronized at the initial rate of the faster cell (Group F), two of the pairs synchronized at the initial rate of the slower cell (Group S), and the other seven pairs synchronized at a rate other than one of the initial rates (Group I). In Table 2 the fluctuation data for the 14 cell pairs whose rate data are listed in Table 1 are grouped according to the change of the fluctuation before and after synchronization. Thirteen pairs synchronized with a fluctuation equal to or less than the initial fluctuation of the slower member of the pair (Group L), and one pair synchronized with a fluctuation larger than that of either of the two initial fluctuations (Group H). These results suggest that the fluctuation of reestablished synchronous beating by isolated cardiomyocytes is influenced more strongly by the fluctuation of the initial fluctuation of the beat rates of the isolated cardiomyocytes than the rate of the reestablished synchronous

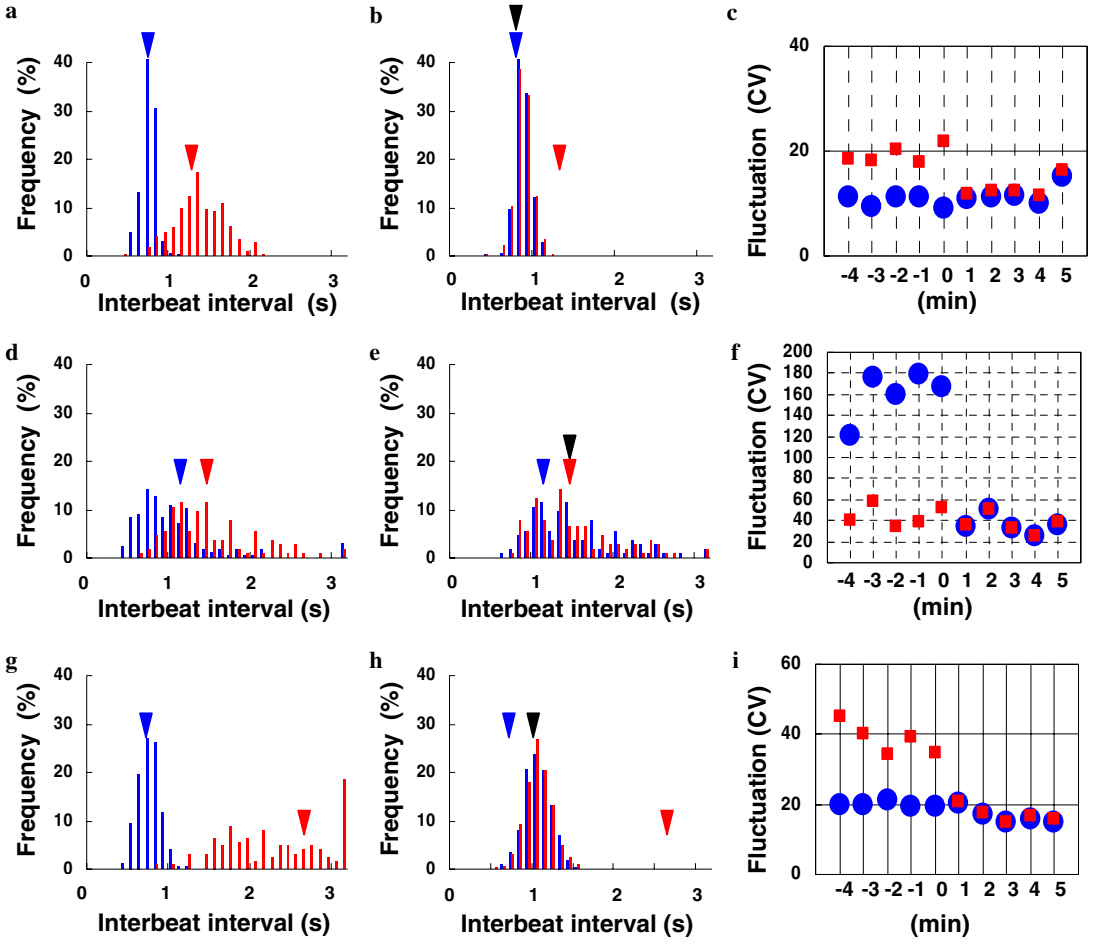

Fig. 3. Distribution of interbeat intervals of two cardiomyocytes and the change of the mean value of beating rhythm fluctuation at intervals of 1 min measured for 5 min before and after synchronization. (a) Distribution of interbeat intervals of two cardiomyocytes before synchronization. The data for the two cardiomyocytes are shown by blue and red bars, and the blue and red triangles show the corresponding mean values. (b) Distribution of interbeat intervals of two cells after synchronization. Blue and red triangle shows the mean values before synchronization, and the black triangle shows the mean value for the two cells after synchronization. (c) The change of the mean value of beating rhythm fluctuation (CV) at intervals of 1 min measured for 5 min before and after synchronization. Blue circles and red squares show the corresponding mean values of beating rhythm fluctuation for 1 min. Results for two other pairs are shown similarly in (d–f) and (g–i), respectively. CV, coefficient of variation (100 × standard deviation/mean beat rate).

Table 1
Interbeat intervals (s) of Cardiomyocyte pairs before and after synchronization

Group F			Group S			Group I		
I_F	I_S	I_{Syncro}	I_F	I_S	$I_{synchro}$	I_F	I_S	$I_{synchro}$
0.64	1.23	0.76	1.10	1.40	1.40	0.64	2.7	0.94
0.93	1.01	0.83	0.59	0.63	0.62	0.84	1.77	1.10
0.74	1.13	0.82				0.56	1.21	0.89
0.87	1.43	0.86				0.71	0.92	0.81
0.94	2.18	0.89				0.56	1.21	0.90
						0.43	3.10	1.00
						0.53	1.06	0.74

I_F, I_S, and $I_{synchro}$ are respectively the interbeat intervals of the cell with the faster beat rate, the cell with the slower beat rate and the pair after synchronization.

Table 2
Beating fluctuation (CV) of Cardiomyocyte pairs before and after synchronization

Group L				Group H			
f_H	f_L	$f_{synchro}$		f_H	f_L	$f_{synchro}$	
25.1	12.3	12.3		88.7	26.7	78.1	Group I
29.0	17.9	18.1					
20.8	16.4	8.92	Group F				
117	19.7	18.9					
164	14.7	13.2					
16.4	11.6	10.7	Group S				
149	41.2	41.7					
42.9	20.1	17.3					
19.7	11.7	10.9					
29.3	17.9	18.7	Group I				
46.5	19.7	15.9					
20.5	17.5	12.9					
22.8	21.7	11.8					

f_H, f_L, and $f_{synchro}$ are, respectively, the fluctuations of the cell with the higher beating fluctuation, the cell with the lower beating fluctuation and the pair after synchronization.

beating is influenced by the initial beat rates of the isolated cardiomyocytes. It might therefore be thought that a cardiomyocyte whose beat rate fluctuates less than that of another cardiomyocyte entrains the beating rhythm of that cardiomyocyte, but we observed one pair of cells in which this did not happen (Group H). This indicates that the influence of a single cell is still not strong enough to account for the process of entrainment in heart tissue.

Beating rhythm synchronization caused by the interaction between a cell population and a single cell

We previously showed that fluctuation of the rate at which a community of cardiomyocyte beat decreased with increasing community size and that a population of only nine cells beat stably and synchronously with a fluctuation of about 10% [10]. In the present work, we explored the relation between entrainment and community size by examining the synchronization process of a cardiomyocyte network formed by the interaction of single cardiomyocytes cultured in a 3×3 grid of agarose microchambers with connecting microchannels. After nine isolated cells were cultured in the nine-chamber agarose microcultivation chip for 24 h, we started to measure the synchronization process continuously and found that when a three-cell network came into contact with another cell and formed a four-cell network (Fig. 4a), the 4th cell synchronized at the initial rate of the 1st cell in the three-cell network (Figs. 4b and c) and with a fluctuation close to the initial fluctuation of the 1st cell (Fig. 4d). When a 5th cell came into contact with the four-cell network and formed a five-cell network (Fig. 4e), it synchronized at a rate equal to the initial rate of the 1st cell in the four-cell network (Figs. 4f and g) and with a fluctuation equal to that of the four-cell network (Fig. 4h). Thereafter a three-cell network formed by the connection of 6th, 7th, and 8th cells came into contact with the five-cell network consisting of the 1st–5th cells and thereby formed an eight-cell network. When this eight-cell network interacted with the 9th cell (Fig. 4i), the 9th cell synchronized at the rate of the eight-cell network (Figs. 4j and k) and with a fluctuation equal to that of the eight-cell network (Fig. 4k). These results suggest that the beating rhythm of a single cardiomyocyte tends to be entrained to the

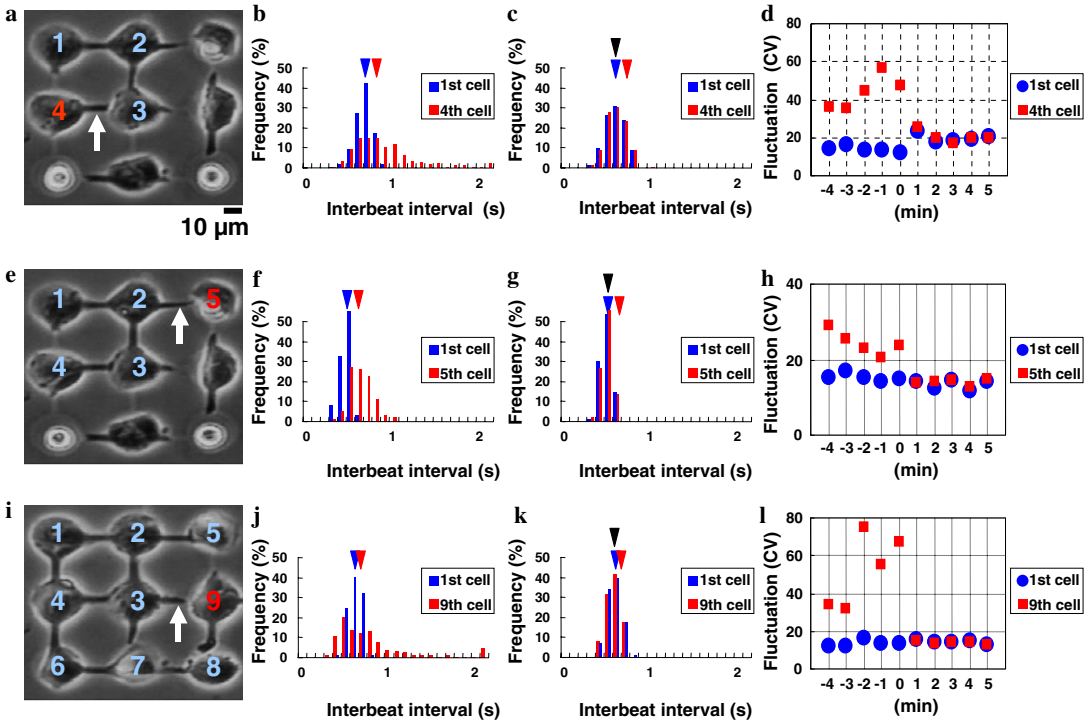

Fig. 4. Distribution of interbeat intervals and the change of the mean value of beating rhythm fluctuation at intervals of 1 min for 5 min before and after synchronization by the interaction between cardiomyocytes groups and single cardiomyocytes. (a) Micrograph of nine isolated single cardiomyocytes cultured in agarose microchambers. The tree-cell network formed by the 1st, 2nd, and 3rd cells came into contact with the 4th cell. The white arrow indicates the interaction point. (b,c) Distributions of the interbeat intervals of (blue) the 1st cell in the three-cell network and (red) the 4th cell for 5 min before and after synchronization. The blue and red triangles in (b,c), respectively, show the mean interbeat intervals of the 1st cell in the three-cell network before synchronization and the 4th cell before synchronization. The black triangle in (c) indicates the mean interbeat interval after synchronization. (d) The change of mean fluctuation of beating rhythm at intervals of 1 min for 5 min before and after synchronization. Blue circles and red squares, respectively, show the mean fluctuations of the 1st cell in the three-cell network and the 4th cell. (e) The four-cell network that was formed by the 1st, 2nd, 3rd, and 4th cells came into contact with the 5th cell at the point indicated by white arrow. (f,g) Distributions of the interbeat intervals of (blue) the 1st cell in the four-cell network and (red) the 5th cell for 5 min before and after synchronization, respectively. Blue and red triangles, respectively, show the mean interbeat intervals of the 1st and 5th cells. The black triangle shows the mean interbeat interval after synchronization. (h) The change of mean fluctuation of beating rhythm at intervals of 1 min for 5 min before and after synchronization. Blue circle and red square, respectively, show the mean fluctuations of the 1st cell in the four-cell network and the 5th cell. (i) The eight-cell network formed by the 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, and 8th cells came into contact with the 9th cell at the point indicated by the white arrow. (j,k) Distribution of interbeat intervals of (blue) the 1st cell in the eight-cell network and (red) the 9th cell for 5 min before and after synchronization. Blue and red triangles, respectively, indicate the mean interbeat intervals of the 1st and 9th cells. The black triangle shows the mean interbeat interval after synchronization. (l) The change of mean fluctuation of beating rhythm at intervals of 1 min for 5 min before and after synchronization. Blue circles and red squares, respectively, show the mean fluctuations of the 1st cell in the eight-cell network and the 9th cell.

rhythm of the cell network and the strength of this tendency increases with the size of the network. Therefore it is thought that the fluctuation of the rate at which a network of cardiomyocytes beats decreases as the size of the network increases.

Possible interpretation of the role of the fluctuation of cardiomyocyte beat rates

Our agarose microchamber system lets us regulate the cell type and community size of cultured cells at the single-cell level. This could not be done when using the conventional cell cultivation method, so the prolific growth of cardiac fibroblasts made it hard to culture only cardiomyocytes and investigate the properties of a single cell in a group of cells. By using single-cell-based cultivation, we were able to investigate ways in which the fluctuation of the rates at which cardiomyocytes affect the reestablishment of synchronized beating. The results of the present experiments can be summarized as follows:

1. When two isolated, independently beating cardiomyocytes came into contact, they tended to beat synchronously at a rate that fluctuated no more than that of the cell whose beat rate fluctuated less than that of the other cell.
2. When initially isolated cardiomyocytes formed a network, it tended to entrain to their rhythm the beating rhythm of single cells whose beating rhythm fluctuated more than that of the network.
3. The entrainment activity of cell networks increased with their size.

They might indicate that unstable isolated cardiomyocytes reestablish a cell network that beats stably and

synchronously beating. A novel finding of this study is that a cardiomyocyte network containing only a few cells acquires to form a stable rhythm. And moreover, once the cell or the cell network acquired the stable beating, the additional attachment of an unstable cell can synchronize to the stable cell or cell network with following their stable beating intervals. This phenomenon also suggests that the factor of stability is very important to determine the fate of the beating frequency of the network after the connection of unstable cells. No previous paper has reported mechanisms that underlie the process of entrainment of cells with different rhythms into the common synchronous rhythm of the intact heart. The molecular mechanism by which the increase in the number of cardiomyocytes results in a decrease in fluctuation and causes entrainment has not yet been clarified, but it has been reported that there is a relation between synchronized, stable beating rhythms and increased expression of such proteins as ion-channel proteins and connexin 43 [11]. The formation of a network might therefore increase the expression levels or activities of proteins that contribute to the beating of cardiomyocytes. In conclusion, by using a single-cell-based assay system, we found evidence that the fluctuation of the rhythms of cultured cardiomyocytes is the most important factor affecting the reestablishment of stable synchronized beating and that entrainment is caused by cell networks whose beat rate does not fluctuate excessively. On-chip cardiomyocyte networks that have only a few cells may thus model heart tissue accurately enough to be useful in drug screening.

Acknowledgments

Financial support, in part from a Grant-in-Aid for JSPS Fellows, Grants-in-Aid for Science Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and the Japan Science and Technology Agency (JST), is gratefully acknowledged.

References

[1] I. Harary, B. Farley, In vitro studies on single beating rat heart cells. II. Intercellular communication, Exp. Cell Res. 29 (1963) 466–474.
[2] K. Goshima, Y. Tonomura, Synchronized beating of embryonic mouse myocardial cells mediated by cells in monolayer culture, Exp. Cell Res. 56 (1969) 387–392.
[3] G.E. Mark, F.F. Strasser, Pacemaker activity and mitosis in cultures of newborn rat heart ventricle cells, Exp. Cell Res. 44 (1966) 217–233.
[4] R.L. DeHaan, R. Hirakow, Synchronization of pulsation rates in isolated cardiac myocytes, Exp. Cell Res. 70 (1972) 214–220.
[5] Y. Yamauchi, A. Harada, K. Kawahara, Changes in the fluctuation of interbeat intervals in spontaneously beating cultured cardiac myocytes: experimental and modeling studies, Biol. Cybern. 86 (2002) 147–154.
[6] J.R. Clay, R.L. DeHaan, Fluctuations in interbeat interval in rhythmic heart-cell clusters, Biophys. J. 28 (1979) 377–389.
[7] T. Okamoto, T. Takahashi, T. Kishi, E. Kurimoto, K. Goshima, Beating of myocardial cells and its regulation: fetal cardiomyocytes 35 (1990) 1657–1664.
[8] H. Moriguchi, Y. Wakamoto, Y. Sugio, K. Takahash, I. Inoue, Y. Yasuda, An agar-microchamber cell-cultivation system: flexible change of microchamber shapes during cultivation by photo-thermal etching, Lab Chip 2 (2002) 125–132.
[9] K. Kojima, H. Moriguchi, A. Hattori, T. Kaneko, K. Yasuda, Two-dimensional network formation of cardiac myocytes in agar microculture chip with 1480 nm infrared laser photo-thermal etching, Lab Chip 3 (2003) 292–296.
[10] K. Kojima, T. Kaneko, K. Yasuda, Stability of beating frequency in cardiac myocytes by their community effect measured by agarose microchamber chip, J. Nanobiotechnol. 3 (2005) 4.
[11] M. Oyamada, H. Kimura, Y. Oyamada, A. Miyamoto, H. Ohshika, M. Mori, The expression, phosphorylation, and localization of connexin 43 and gap-junctional intercellular communication during the establishment of a synchronized contraction of cultured neonatal rat cardiac myocytes, Exp. Cell Res. 212 (1994) 351–358.