Conduction Block in Novel Cardiomyocyte Electrical Conduction Line by Photosensitization Reaction

Mariko. Kurotsu, Emiyu. Ogawa, Tsunenori. Arai.
School of Fundamental Science and Technology,
Graduate School of Science and Technology Keio University (Japan)

***Abstract*—We developed a novel cardiomyocyte electrical conduction line. We studied electrical conduction block by extra-cellular photosensitization reaction with this conduction line to study electrical blockade by the photosensitization reaction *in vitro*.**

I. Introduction

We have proposed non-thermal arrhythmia treatment by extra-cellular photosensitization reaction (PR) using talaporfin sodium [1]. The number of atrial fibrillation patients is around 2,200,000 in U.S.A. and this number is increasing along with the aging in the society [2]. Radiofrequency catheter ablation is used as an effective treatment in recent years, but it is still difficult to control the temperature inside a cardiomyocyte tissue to prevent thermal complications. We have proposed myocardial electrical conduction (EC) block by singlet oxygen oxidation produced by the PR. Cardiomyocyte tissue models *in/ex vivo* [3] or mathematical models [4] are used in basic study of EC blocks. However, in order to study therapeutic interaction by the PR these tissue models are difficult to use due to conditioning of the tissue in particular oxygen supply. Moreover, the mathematical models have a restricted performance. Since *in vitro* cell study has been used a basic cell necrosis interaction research in general [5-7], however, *in vitro* EC block studies have not been reported due to the lack of EC line *in vitro*. We developed a novel cardiomyocyte EC line. We studied EC block by extra-cellular PR with this EC line to study EC blockade by the PR *in vitro*.

Resrach supported by Adaptable & Seamless Technology Transfer Program through Target-driven R&D (A-STEP)" of the Japan Science and Technology Agency; AS2415004P.

M. Kurotsu is with The Graduate School of Science and Technology, Keio University, Kanagawa, Japan (corresponding author to provide phone: (+81) 045-563-1151 (ex 42583); fax: (+81) 045-566-1587; e-mail: m.kurotsu@arai.appi.keio.ac.jp).

E. Ogawa is with The Graduate School of Science and Technology, Keio University, Kanagawa, Japan (corresponding author to provide phone: (+81) 045-563-1151 (ex 41079); fax: (+81) 045-566-1587; e-mail: e.ogawa@arai.appi.keio.ac.jp).

T. Arai is with The Department of Applied Physics and Physico-Informatics, Faculty of Science and Technology, Keio University, Kanagawa, Japan (corresponding author to provide phone: (+81) 045-566-1428; fax: (+81) 045- 566-1587; e-mail: tsunearai@appi.keio.ac.jp).

II. Materials and Methods

A. Construction of In Vitro Cardiomyocyte EC Line

Rat cardiomyocytes (Primary Cell Company Ltd., Japan) were grown on 10 mmΦ cover glasses (Dai Nippon Printing Co., Ltd., Japan) in 35 mmΦ dishes. There were cultivated and non-cultivated lines, which widths were 60 and 300 μm, respectively on the cover glass. Non-cultured lines were made from cell gluing check enzyme. The cardiomyocyte lines were only cultured on the cover glass surface without a collagen coat. Cell concentrations for each dish were 4.0×10^5 cells/ml. Culture duration of four days was employed. Environmental condition was 37 ℃ with 5% CO_2. Dulbecco's modified Eagle medium/Nutrient Mixture F-12 (D-MEM/F-12) (Thermo Fisher Scientific Inc., U.S.A.) adding 10% of fetal bovine serum (Thermo Fisher Scientific Inc., U.S.A.) was used as a culture medium. EC block was measured by synchronicity of intracellular Ca^{2+} concentration change using a fluorescent Ca^{2+} indicator, Fluo-4 AM (Thermo Fisher Scientific Inc., U.S.A.). Fluo-4 in the cardiomyocyte cells was excited with an argon laser (800BL; National Laser Co., U.S.A.) at 488 nm in wavelength. Fluo-4 AM fluorescence images were obtained with a confocal laser microscope system (CSU-X1; Yokogawa Electric Company, Japan) with a water immersion lens of magnification of 4. Fluo-4 AM fluorescence was detected at the 500–540 nm band-pass filter with an electron multiplication CCD camera (DU897; Andor Technology, UK) with a frame rate of 300 ms per frame. The obtained fluorescence images were analyzed by the image software, iQ Core (Andor Technology, UK). The average Fluo-4 AM fluorescence intensity inside the cells was used to assess the changes in $[Ca^{2+}]$ in cardiomyocyte cells.

B. Measurement Capability of EC block by PR

Cardiomyocytes were grown on the 10 mm wide cover glasses in the 35 mmΦ dish for 4 days. EC block by PR was examined using 5 μM Fluo-4 AM dissolved in the culture medium. Cardiomyocytes were exposed to Fluo-4 AM during 20 min at room temperature. The culture medium was then replaced with 3 mL of 20 μg/ml talaporfin sodium dissolved in the culture medium. The reaction was observed under a fluorescence microscope. Figure 1 shows the measurement setup for the measurement of $[Ca^{2+}]$ in rat cardiomyocytes during the PR. Fluo-4 AM was excited with an argon laser at 488 nm and talaporfin sodium was excited at 662 ± 2 nm by a

red diode laser (Optical fuel, Sony, Japan). The laser irradiance was set to 0.14 W/cm^2. Radiant exposure was 40 J/cm^2. Fluo-4 AM fluorescence was obtained with a confocal laser microscope system mounted on a differential interference microscope with a water immersion lens of magnitude of 4. Fluo-4 AM fluorescence was detected through the 500–540 nm band-pass filter with an electron multiplication CCD camera with a frame rate of 300 ms per frame. Observation duration was over 15 mins. The detected images were analyzed by the image software iQ Core.

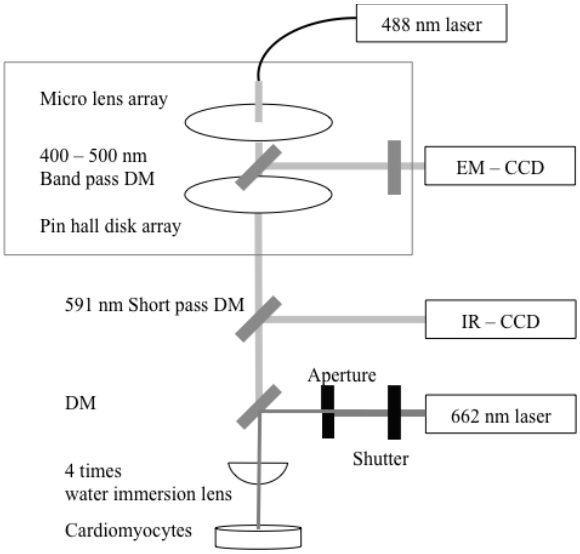

Figure 1. Schematic illustration of the measurement setup for the measurement of [Ca2+] in rat cardiomyocytes during the PR

III. RESULTS

A. Construction of In Vitro Cardiomyocyte EC Line

Figure 2 shows that cultured cardiomyocytes formed in a single line. Figure 3 shows Fluo-4 AM fluorescence image by the confocal microscope with a magnification of 4. Figure 4 shows that example of Fluo-4 AM synchronically intensity change measured at 4 positions in a single line. Fluo-4 AM fluorescence intensity at different positions changed synchronically each other on one lines. Cross correlation function (CCF) on one line was about 0.8 (N=15).

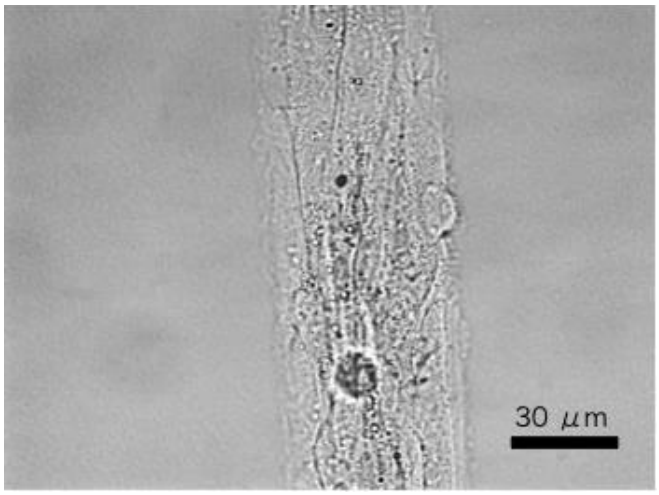

Figure 2. Phase difference image of Cardiomyocyte EC line with a magnification of 4

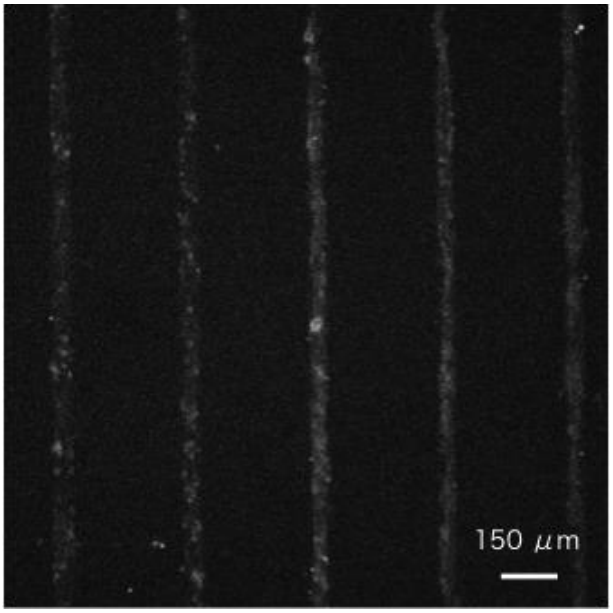

Figure 3. Fluo-4 AM fluorescence image by the confocal microscope with a magnification of 4

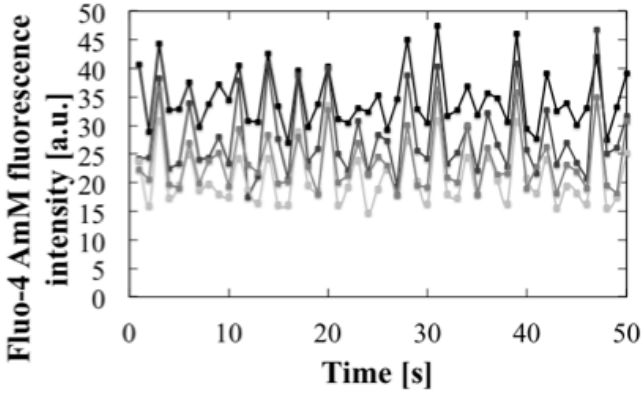

Figure 4. Example of Fluo-4 AM synchronically intensity change measured at 4 positions in a single line.

B. Measurement Capability of EC block by PR

Irradiation zone was set to slant to observe response differences by distances from a irradiation zone. We observed Fluo-4 AM fluorescence intensity change by PR. The measurement zone was 40×340 μm, there might be about 2 ×5 cells in the zone. Figure 5 shows that Fluo-4 AM fluorescence intensity increased in the irradiation zone after 10 J/cm^2. Then, high fluorescence intensity zone was spread to up and down lesion with radiant exposure increasing. Table 1 summarized CCFs between the irradiation zone and non-irradiation zone on certain EC line by 48 correlations with PR in duration. In this analysis, outlying observations were identified and excluded by the equation 1 described below (x_1: sample value, μ : average, σ : standard deviation).

$$\tau = \frac{x_1 - \mu}{\sigma} \quad (1)$$

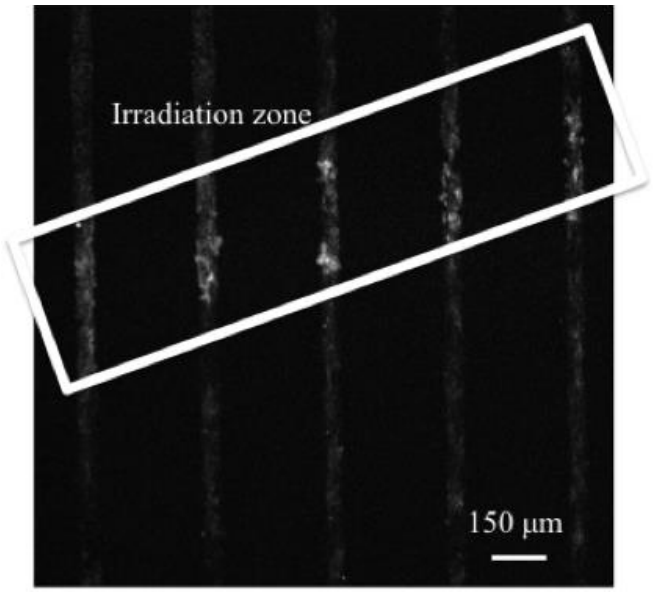

Figure 5. Example Fluo-4 AM fluorescence image after 10 J/cm^2 PR

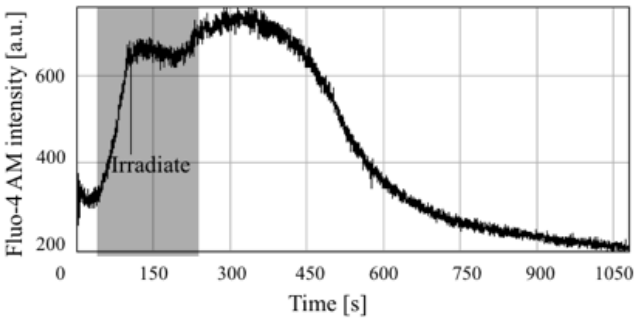

Figure 6. Typical time history of Fluo-4 AM fluorescence intensity in irradiation zone

TABLE I. AVERAGE CCF BETWEEN THE IRRADIATION ZONE AND NON-IRRADIATION ZONE ON CERTAIN EC LINE BY 48 CORRERATIONS WITH PR IN DURATION

Time [min]	*PR*	*control*
0 (Control)	0.76±0.20	0.81±0.14
0-1 (Irradiation duration)	0.59±0.19	0.81±0.083
15-16 (12 mins after the PR end)	0.15±0.056	0.78±0.11

IV. DISCUSSION

A. Construction of In Vitro Cardiomyocyte EC Line

The synchronized pulsations of some cardiomyocytes on single line may indicate that there were gap junctions between each cardiomyocyte. Because they couldn't pulsation synchronically without ion transmit [5]. It is well known that Ca^{2+} concentration changes dynamically with pulsation. Because extracellular Ca^{2+} concentration is 10000 times bigger than that of intra-cardiomyocytes. So we could observe the cardiomyocyte EC by Ca^{2+} using Fluo-4 AM. Therefore, we might construct *in vitro* cardiomyocyte EC line.

B. Measurement Capability of EC block by PR

CCFs between irradiation and non-irradiation zone significantly decreased by the PR. Significant differences of CCF between irradiation and non-irradiation zone were under 0.005, 3×10^{-6}, 6×10^{-16}. This result meant that cardiomyocytes on single line didn't pulse synchronically by PR. And this may indicate that EC was blocked by PR. Therefore this contracted *in vitro* cardiomyocyte line can be used to study EC block by PR.

V. CONCLUSION

Cardiomyocytes formed in a single line. Fluo-4 AM fluorescence intensity at different positions changed synchronically each other on one lines. CCF on one line was about 0.8 (N=15). The synchronized pulsations of some cardiomyocytes on single line may indicate that there were gap junctions between each cardiomyocytes. We observed Fluo-4 AM fluorescence intensity change on single lines by the PR. CCFs between irradiation and non-irradiation zones significantly decreased by PR. These results indicate EC was blocked by PR. Therefore this contracted *in vitro* cardiomyocyte line can be used to study EC change by PR.

ACKNOWLEDGMENT

This study was supported by Adaptable & Seamless Technology Transfer Program through Target-driven R&D (A-STEP)" of the Japan Science and Technology Agency; AS2415004P

REFERENCES

[1] T. Kimura, S. Takatsuki, S. Miyoshi, K. Fukumoto, M. Takahashi, E. Ogawa, A. Ito, T. Arai, S. Ogawa and K. Fukuda, "Nonthermal Cardiac

Catheter Ablation Using Photodynamic Therapy", *Circ. Arrhythm. Electrophysiol.,* 2013, vol. 6, pp. 1025-1031.

[2] Véronique, L. R, Alan S. G., Donald, M. L. J., Robert, J. A., Jarett, D. B., Todd, M. B., Mercedes, R. C., Shifan, D., Giovanni, S., Earl, S. F., Caroline, S. F., Heather, J. F., Cathleen, G., Kurt, J. G., Susan, M. H., John, A. H., Michael, H., Virginia, J. H., Brett, M. K., Steven, J. K., Daniel, T. L., Judith, H. L., Lynda, D. L., Diane, M. M., Gregory, M. M., Ariane, M., David, B. M., Mary, M. M., James, B. M., Claudia, S. M., Dariush, M., Michael, E. M., Graham, N., Nina, P. P., Wayne, D. R., Paul, D. S., Randall, S. S., Tanya, N. T., Melanie, B. T., Nathan, D. W., Judith, W. R., "Heart Disease and Stroke Statistics 2011 Update: A Report From the American Heart Association," *Cir*, 2010, vol. 123, pp. 18-209.

[3] H. Wakimoto, C. T. Maguire, P. Kovoor, P. E. Hammer, J. Gehrmann, J. K. Triedmana and C. I. Berula, "Induction of atrial tachycardia and fibrillation in the mouse heart", *Cardiovasc. Res*., 2001, vol. 50, pp. 463–473.

[4] C. H. Luo and Y. Rudy, "A dynamic model of the cardiac ventricular action potential. I. Simulations of ionic currents and concentration changes", *Circ. Res*., 1994, vol. 74, pp. 1071-1096.

[5] J. M. Burt and D. C. Spray, "Single-channel events and gating behavior of the cardiac gap junction channel", *Proc. Natl. Acad*., 1988, Vol. 85, pp. 3431-3434.