様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　元 年　 ６ 月　 ５ 日現在

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研究種目：若手研究(B)
研究期間：2017～2018
課題番号：１７Ｋ１４６２４

研究課題名（和文）多能性幹細胞由来心筋永久機関：循環進行波に基づく加速心筋細胞成熟法の開発

研究課題名（英文）Circulating traveling wave enhances the maturation of cardiomyocytes in self-organized tissue

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交付決定額（研究期間全体）：（直接経費）　　3,400,000円

研究成果の概要（和文）：ヒト iPSC 由来心筋細胞は、移植だけではなく、創薬への応用も期待されている。しかし、既存の分化誘導法による得られた心筋細胞はまだ未熟であるため、薬剤評価の応用に十分満たされていない。 申請者は工学的な手法を用いたデバイスを開発することにより、進行波という活動電位が加速に伝播する現象を発見した。また、この現象により、心筋細胞の成熟化を促進することは明らかになった。進行波がないサンプルと比較して、明らかに心機能を上昇したことは示された。さらに、このデバイスはハイスループットシステムにも適用性があり、今後薬剤応答への応用に期待ができる。

研究成果の学術的意義や社会的意義
This technology could be a promising substitution for harmful electrical stimulation method. It can be expected that this new technology, could be of great usefulness and importance for both researchers and industrial/clinical users in the fields of drug screening and heart regeneration.

研究成果の概要（英文）：Cardiomyocytes derived from human iPSCs are expected to be applied not only to transplantation but also to drug discovery. However, since the cardiomyocytes obtained by the existing differentiation induction method are still immature, they are not sufficiently satisfied for the application of drug evaluation. In this research project, we aim to develop an accelerated maturation method of novel pluripotent stem cell-derived cardiomyocytes without using physical stimulation from outside. The applicant discovered the closed loop and the phenomenon that action potential propagates to acceleration by the developed device. The ohenomenon was named travling wave. Furthermore, with the training by travling wave, we aim to develop an accelerated cardiomyocyte maturation method based on the spontaneous and circulating progressive waves safely, simply and stably, that is, the development of a pluripotent stem cell-derived myocardial permanent machine.

研究分野：tissue engineering

キーワード：pluripotent stem cells　cardiomyocytes　maturation　tissue engineering

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９、ＣＫ－１９（共通）

１．研究開始当初の背景

The cardiomyocytes (CMs) derived from human iPS cells (hiPSCs), despite of the high-efficient differentiation, are still immature and poorly organized, which might limit their applications in drug development and regenerative therapy. Rapid electrical stimulation has long been used to pace muscle cells for higher maturation (Nunes et al., Nature methods, 2013). There are several issues existing for electrical stimulation:

(1) The electrical stimulation may possibly cause damage to cells within tissue and thus requires appropriate stimulation protocols.

(2) It is difficult for electrical stimulation to pace the CMs tissue rapidly (>2 Hz) in the long term (e.g., for several weeks), owing to the side effect such like heavy metal poisoning, electrolysis, pH-shift and reactive oxygen species.

(3) The upscaling for electrical stimulation requires complicated external experiment setup and high power consumption.

２．研究の目的

Recently, cardiomyocytes derived from human iPSCs are expected to be applied not only to transplantation but also to drug discovery. However, since the cardiomyocytes obtained by the existing differentiation induction method are still immature, they are not sufficiently satisfied for the application of drug evaluation. Although there are methods for promoting the process of cardiomyocyte maturation by conventional physical methods (for example, electrical stimulation and mechanical training), the cells are damaged and not suitable for long-term training. In this research project, we aim to develop an accelerated maturation method of novel pluripotent stem cell-derived cardiomyocytes without using physical stimulation from outside. The applicant discovered the closed loop and the phenomenon that action potential propagates to acceleration by the developed device. Furthermore, with this phenomenon, we aim to develop an accelerated cardiomyocyte maturation method based on the spontaneous and circulating progressive waves safely, simply and stably, that is, the development of a pluripotent stem cell-derived myocardial permanent machine.

３．研究の方法

This project aims to create a platform for tissue formation and maturation of hiPSCs derived cardiomyocytes. This platform could be a promising substitution for conventional electrical stimulation method. Specifically, the project will be organized in following steps:

To design and optimize the low-attachment substrate for cardiomyocytes to efficiently self-organize into tissue ring and spontaneous originating of high frequency traveling wave.

To characterize the maturation and performance of cardiomyocytes in tissue ring after training by traveling wave.

To develop optimized platform for large-scale production of matured and organized cardiomyocytes.

４．研究成果

The targets in proposed plan have been well fulfilled specified as following:

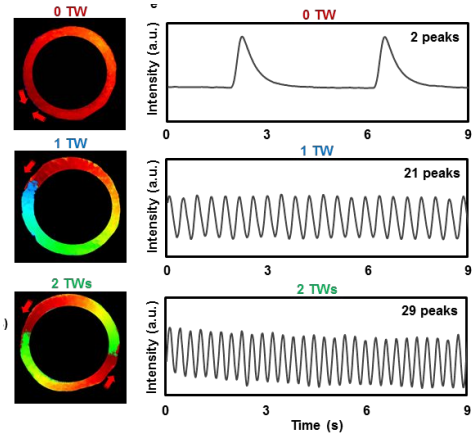

図 1. Traveling wave promoted rapid beating of cells in tissue ring.

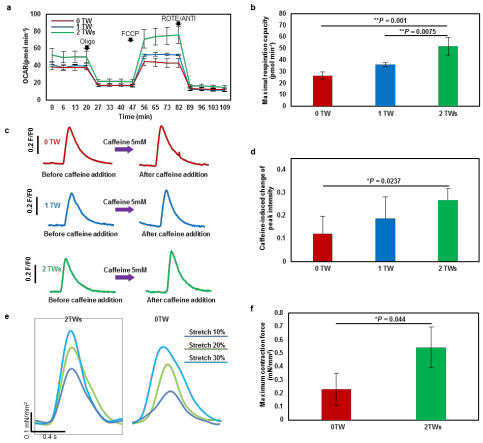

図 2. TWs improve Ca^{2+}-handling properties.

(1) A novel substrates have been designed and optimized to create cardiac tissue ring with spontaneous traveling wave. The traveling wave could be generated spontaneously in more than 80% samples in repeated experiments. (**図 1**)

(2) The cardiomyocytes were trained by traveling wave demonstrated improved mitochondrial function and calcium handling properties (**図** 2),

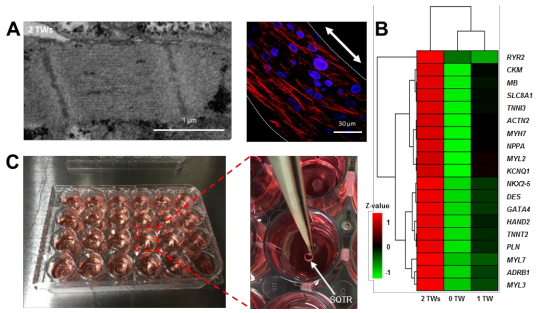

図 3. (A) The matured cardiomyocytes in the ring. (B) The cardiac maturation related gene expression of cardiomyocytes in the tissue ring. (C) Large scale production of tissue ring.

(3) We also found that the matured sarcomere structure and up-regulated gene expression after cardiomyocytes were trained by traveling wave (**図** 3A, B). Forthermore, a system compatible to cell culture dish have been created for up scaled production of ring. (**図** 3C)

(4) As for the publication, based on the research work, one patent has been submitted. Two papers have been published. And one manuscript has been sent out for review in *Science Advances*.

5．主な発表論文等

〔雑誌論文〕（計　2　件）

① Yu, L.†; **Li, J.**†; , Minami, I.; Qu X.; Miyagawa S.; Fujimoto, N.; Hasegawa K.; Chen, Y.; Sawa, Y.; Kotera, H.; Liu, L., Clonal Isolation of Human Pluripotent Stem Cells on Nanofibrous Substrates Reveals an Advanced Sub-clone for Cardiomyocyte Differentiation. ***Advanced Healthcare Materials,* 2019, in Press**. (†Co-first author) (査読あり)

② Yu, L.; **Li, J.:** Hong, J. Takashima, Y.; Fujimoto, N.; Nakajima, M.; Dong, X.; Dang, Y.; Yang, W.; Minami, I.; Okita, K.; Luo, C.; Tang, F.; Chen, Y.; Tang, C.; Kotera, H.; Liu, L., Low Cell-Matrix Adhesion Reveals Two Novel Subtypes of Human Pluripotent Stem Cells. ***Stem Cell Reports*, 2018,** 11, 142-156. (査読あり)

〔学会発表〕（計　2　件）

① 劉莉、李俊君、南一成など、『組織工学技術による新規心筋成熟法の開発』 第18回日本再生医療学会(口頭発表)，Mar. 2019, Kobe, Japan

② Li, J.; Minami, I.; Yu, L; Chen, Y.; Nakatsuji, N.; Kotera, H.; Liu, L., Generating functional anisotropic and 3D tissue-like construct using induced pluripotent stem cell-derived cardiomyocytes. Biomedical Engineering Society Annual Meeting, Oct. 2017, Phoenix, USA.

〔図書〕（計　　件）

〔産業財産権〕
○出願状況（計　1　件）

名称：Ring-like cardiac tissue construct
発明者：Liu, L.; Li, J.;
権利者：Kyoto University
種類：特許
番号：JP2017-047027
出願年月日：2017.12
国内外の別：国内

○取得状況（計　　件）
名称：
発明者：
権利者：
種類：
番号：
取得年：
国内外の別：

〔その他〕
ホームページ等

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