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　４ 年　 ６ 月　 ２ 日現在

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課題番号：１９Ｋ１２８０１

研究課題名（和文）自発進行波による高成熟心筋を用いた新規心筋梗塞治療法の開発

研究課題名（英文）Novel method for treating myocardium infarction by using cardiac tissue ring matured by spontaneous traveling wave

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交付決定額（研究期間全体）：（直接経費）　　3,300,000円

研究成果の概要（和文）：ヒトiPS細胞由来の心筋細胞は梗塞した心臓の修復が期待されますが、その成熟度が治療効果に影響を与える可能性があります。成熟した自己組織化組織リングを作製し、心筋を用いてラット心臓に対する治療効果を評価しました。進行波によってペーシングされる成熟したティッシュシートが準備されました。成熟組織群は、対照群と比較して心筋梗塞のラット心臓に対する治療効果の改善を示しました。さらに、成熟組織の生存率の改善と治療効果の改善は、ミオグロビン、シトクロムCより高い発現に関連している可能性があることがわかりました。3つの論文を発表し、1つの特許を申請し、2021年の米国心臓協会の会議で口頭発表を行いました。

研究成果の学術的意義や社会的意義
This technology could be used to produce matured iPSC-derived cardiac tissue for treating infarcted hearts. We expect that this new technology could be of great usefulness and importance for both researchers and clinical users working on developing regenerative therapy for the human heart.

研究成果の概要（英文）：Cardiomyocytes derived from human iPSCs are expected to repair the infarcted heart, however, their maturation level could affect the therapeutic effect. Supported by the present project, we prepared the matured self-organized tissue rings and evaluate their therapeutic effect on the rat heart with myocardium infarction. The achivements are as following: 1.The matured tissue sheet paced by the traveling wave have been prepared. The cardiac-specific markers have been verified. 2.The mature tissue group demonstrated an improved therapeutic effect on the rat heart with myocardial infarction compared with the control group. In addition, we find the improved survival rate and improved therapeutic effect of matured tissue could be related to the higher expression of myoglobin, cytochrome C, and other maturation related markers.
3. We published 3 papers and applied for 1 patent, we gave an oral presentation at 2021 American Heart Association conference.

研究分野：tissue engineering

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

## １．研究開始当初の背景

The annual number of patients with myocardial infarction in Japan is 80,000, and the annual number of deaths due to heart failure is about 43,000. Fundamental treatments for these severe heart failure are centered on replacement treatments such as ventricular assist devices and heart transplants. However, at present, there are problems such as side effects of artificial hearts and a shortage of donors. Therefore, it is expected to develop a therapeutic method by regenerative medicine using human induced Pluripotency Stem Cell-derived Cardiomyocytes (hiPSC-CMs). Although the human iPS cells (hiPSCs) could be differentiated into the cardiomyocytes (CMs) with high efficiency, when compared with adult CMs they are still immature and poorly organized. This could limit their application in the development of regenerative medicine. In addition, there are few reports on whether matured hiPSC-CMs, compared with their immature counterparts, would have enhanced therapeutic effect on animal model with myocardial infarction. We have previously developed the traveling wave based maturation method (Li et al., Communications Biology, 2020, H29-H31 Wakate B). This method required no external experiment set up or power supply. The traveling wave could spontaneously originate and rapidly pace the hiPSC-CMs and improve the expression of maturation related biomarker, contractile force, and oxygen consumption capability. The traveling wave technique would be an ideal tool for producing matured hiPSC-CMs and investigating whether matured hiPSC-CMs would have enhanced therapeutic effect on heart with myocardial infarction.

## ２．研究の目的

The applicants have previously produced an hiPSC-derived myocardial ring using tissue engineering technology and confirmed that stimulation of spontaneous traveling wave promotes the maturation of cardiomyocytes. In this research project, we aim to develop a new treatment method that dynamically supports the contraction of the heart by inserting this high-maturity organized myocardial ring into the heart of a myocardial infarction model animal. (Figure. 1). The specific target will include: (1) Find optimization conditions that stably and improve the induction rate of spontaneous traveling wave. (2) Elucidate the relationship between tissue maturity and transplantation effect. (3) To elucidate the mechanism of the effect of different maturity on transplantation efficiency and therapeutic effect.

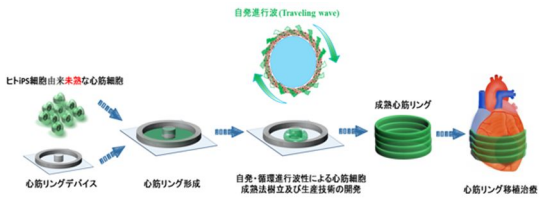


**Figure 1. Formation of myocardial ring tissue with spontaneous traveling wave and application in repairing heart with myocardial infarction.**

## ３．研究の方法

This project aims to create a platform for matured cardiac tissue formation and to use them for repairing heart with myocardial infarction. Specifically, the project will be organized in following steps:

(1) Optimization for stably obtaining matured cardiac tissue. The device design and the culture protocol will be optimized to allow better nutrient diffusion and stable maintaining of traveling wave. The device size will be also improved for larger scale production of the tissue that could be readily used for transplantation.

(2) To elucidate the relationship between tissue maturity and transplantation effect. The matured tissue as well as the control group will be evaluated and used for transplantation on rat heart model with myocardial infarction. The heart function will be monitored for several weeks and the heart will be sectioned and analyzed to compare the therapeutics effect of cardiac tissue with different maturation level.

(3) To elucidate the mechanism of the effect of different maturity on transplantation efficiency and therapeutic effect. The RNA-sequencing, western blot and other biological analysis technologies will be used to compare matured cardiac tissue and the immature cardiac tissue. The mechanism for how traveling wave induces the cardiac maturation and enhance the therapeutic effect will be concluded based on the data analysis.

## 4．研究成果

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

(1) With optimized device design and protocol, the traveling wave could be generated spontaneously and maintained in more than 80% samples in repeated experiments. The traveling wave promoted the maturation of hiPSC-CMs within the tissue, showing improvement in conduction, ultrastructure, energetics as well as contraction (Figure 2). In addition, the mature cardiac tissue demonstrated improved survival post hypoxia culture (Figure 3).

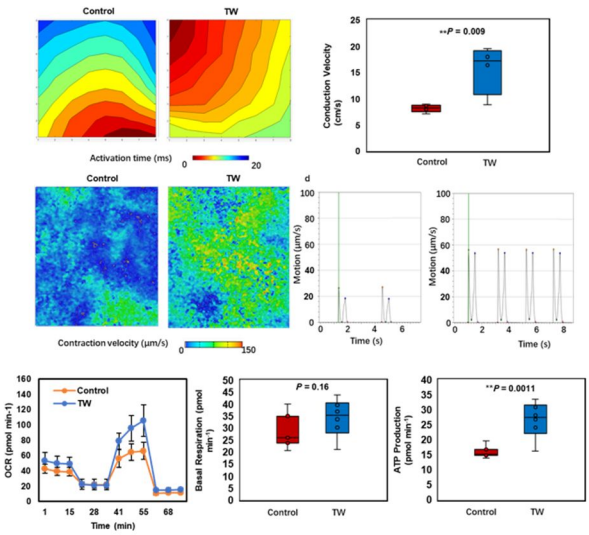


**Figure 2. The traveling wave (TW) enhanced the maturation of hiPSC-CM tissue including conduction velocity of the action potential (Top row), contractility (Middle row) and the mitochondrial function (Bottom row).**

(2) Although the cell differentiation and animal experiments have been affected by the epidemic of Covid-19, we managed to perform part of the preliminary experiments. According to these tests, the traveling wave matured cardiac tissue showed improved retention in the rat heart with myocardial infarction, and the function of the heart exhibited improved left ventricular ejection fraction within 6 week (Figure 4). Right now, more repetitive tests are underway.

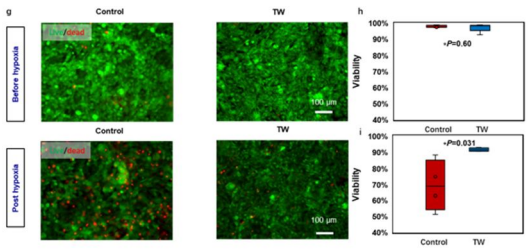


**Figure 3. The traveling wave (TW) matured hiPSC-CMs demonstrated improved survival post hypoxia culture.**

(3) By using RNA-sequencing, we found that the integrin related pathway underlies the traveling wave induced maturation of hiPSC-CMs (Figure 5). RNA-sequencing data indicated that most of the alpha and a beta subunit of integrin in TW group showed upregulation compared with the Control group. In addition, a number of downstream genes were also upregulated such as ECM related and pro-maturation signaling pathways related genes.

（4) As for the publications, based on the research work, one patent has been submitted. three papers have been published. We have given two oral and two poster presentations in the international conferences.

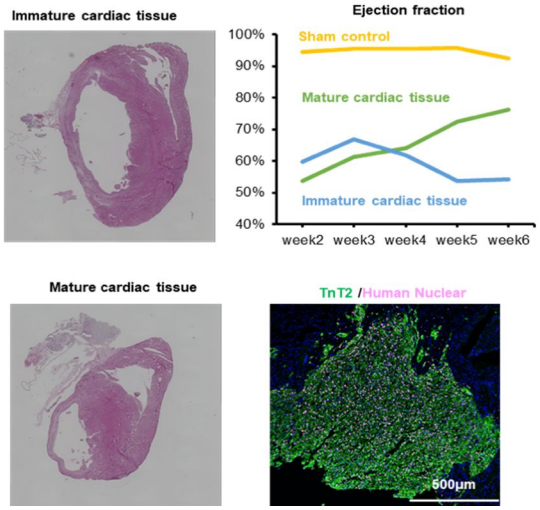


**Figure 4. The mature cardiac tissue demonstrated large retention in the rat heart and improved the function of heart within 6 weeks after occurrence of myocardial infarction.**

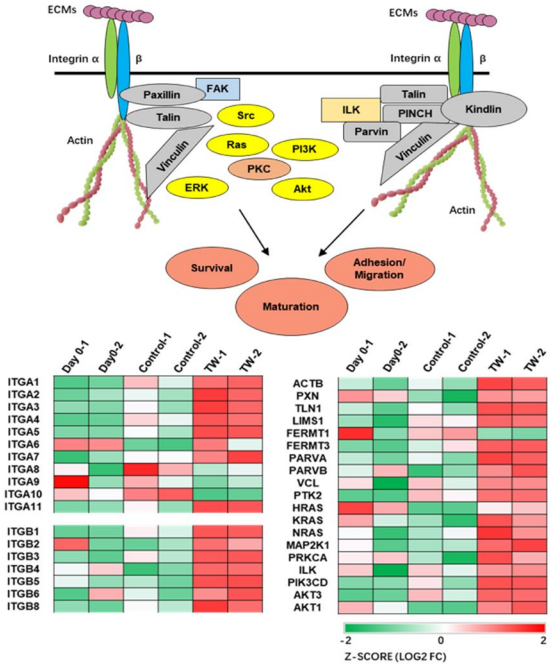


**Figure 5. Integrin pathway for traveling wave induced hiPSC cardiac maturation. (Top) Schematic representation of an integrin pathway that could lead to the cell survival, adhesion/migration, and growth/maturation. (Bottom) Heatmaps showing expression of Integrin and extracellular matrix related genes.**

５．主な発表論文等

〔雑誌論文〕　計3件（うち査読付論文　2件／うち国際共著　0件／うちオープンアクセス　2件）

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| １．著者名<br>Li Junjun、Hua Ying、Miyagawa Shigeru、Zhang Jingbo、Li Lingjun、Liu Li、Sawa Yoshiki | ４．巻<br>21 |
| ２．論文標題<br>hiPSC-Derived Cardiac Tissue for Disease Modeling and Drug Discovery | ５．発行年<br>2020年 |
| ３．雑誌名<br>International Journal of Molecular Sciences | ６．最初と最後の頁<br>8893～8893 |
| 掲載論文のDOI（デジタルオブジェクト識別子）<br>10.3390/ijms21238893 | 査読の有無<br>有 |
| オープンアクセス<br>オープンアクセスとしている（また、その予定である） | 国際共著<br>－ |

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| １．著者名<br>Li Junjun、Zhang Lu、Yu Leqian、Minami Itsunari、Miyagawa Shigeru、H?rning Marcel、Dong Ji、Qiao Jing、Qu Xiang、Hua Ying、Fujimoto Nanae、Shiba Yuji、Zhao Yang、Tang Fuchou、Chen Yong、Sawa Yoshiki、Tang Chao、Liu Li | ４．巻<br>3 |
| ２．論文標題<br>Circulating re-entrant waves promote maturation of hiPSC-derived cardiomyocytes in self-organized tissue ring | ５．発行年<br>2020年 |
| ３．雑誌名<br>Communications Biology | ６．最初と最後の頁<br>- |
| 掲載論文のDOI（デジタルオブジェクト識別子）<br>10.1038/s42003-020-0853-0 | 査読の有無<br>有 |
| オープンアクセス<br>オープンアクセスとしている（また、その予定である） | 国際共著<br>－ |

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| １．著者名<br>Suzuki Kota、Miyagawa Shigeru、Liu Li、Kawamura Takuji、Li Junjun、Qu Xiang、Harada Akima、Toda Koichi、Yoshioka Daisuke、Kainuma Satoshi、Kawamura Ai、Sawa Yoshiki | ４．巻<br>40 |
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| ３．雑誌名<br>The Journal of Heart and Lung Transplantation | ６．最初と最後の頁<br>767～777 |
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| オープンアクセス<br>オープンアクセスではない、又はオープンアクセスが困難 | 国際共著<br>－ |

〔学会発表〕　計4件（うち招待講演　0件／うち国際学会　4件）

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| １．発表者名<br>Li, J., Zhang, L., Minami, I., Yu, L., Miyagawa, S., Horning M., Qiao, J., Qu, X., Hua, Y., Chen, Y., Sawa, Y., and Liu, L. |
| ２．発表標題<br>Circulating traveling waves enhance the maturation of hiPSC-derived cardiomyocytes in self-organized tissue ring |
| ３．学会等名<br>The 8th Meeting of the International Federation for Artificial Organs（国際学会） |
| ４．発表年<br>2019年 |

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| １．発表者名<br>Li, J., Zhang, L., Minami, I., Yu, L., Miyagawa, S., Horning M., Qiao, J., Qu, X., Hua, Y., Chen, Y., Sawa, Y., and Liu, L. |
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| ３．学会等名<br>CiRA 2019 International Symposium（国際学会） |
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| １．発表者名<br>Li, J., Liu, L., Miyagawa, S., Kawamura, T., Matsuura, R., Hua, Y., Qu, X., Sougawa, N., Sawa, Y., |
| ２．発表標題<br>Human iPSC derived cardiac tissue matured by spontaneous traveling waves for improved drug assessment. |
| ３．学会等名<br>American Heart Association Scientific Session（国際学会） |
| ４．発表年<br>2021年 |

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| ４．発表年<br>2020年 |

〔図書〕　計0件

〔出願〕　計1件

| 産業財産権の名称 | 発明者 | 権利者 |
|---|---|---|
| 心筋細胞層の製造方法、心筋細胞層、およびその利用 | 劉莉、澤芳樹、宮川繁、李俊君、武田真季、三好隼人 | 同左 |
| 産業財産権の種類、番号 | 出願年 | 国内・外国の別 |
| 特許、2021-176040 | 2021年 | 国内 |

〔取得〕　計0件

〔その他〕

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６．研究組織

| | 氏名<br>（ローマ字氏名）<br>（研究者番号） | 所属研究機関・部局・職<br>（機関番号） | 備考 |
|---|---|---|---|
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７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

| 共同研究相手国 | 相手方研究機関 |
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