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Geometric understanding of local fluctuation distribution of conduction time in lined-up cardiomyocyte network in agarose-microfabrication multi-electrode measurement assay

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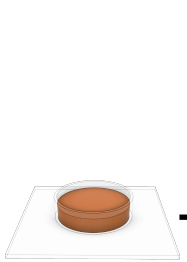
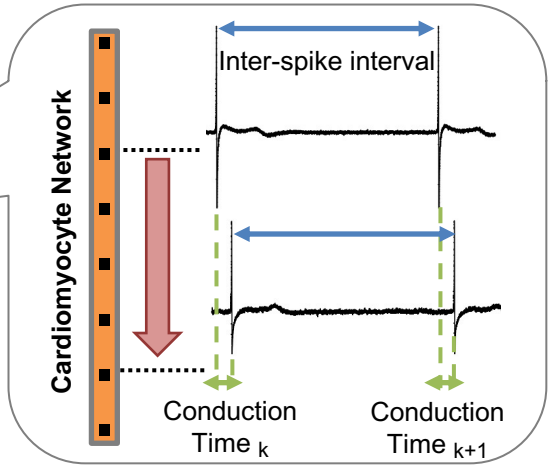
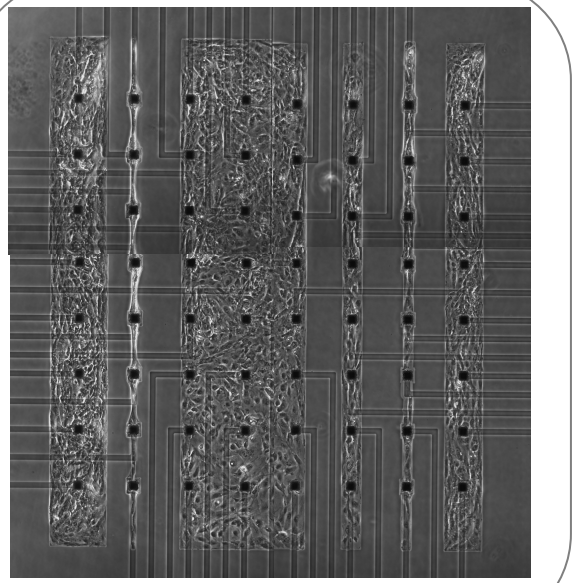


MEA Chip

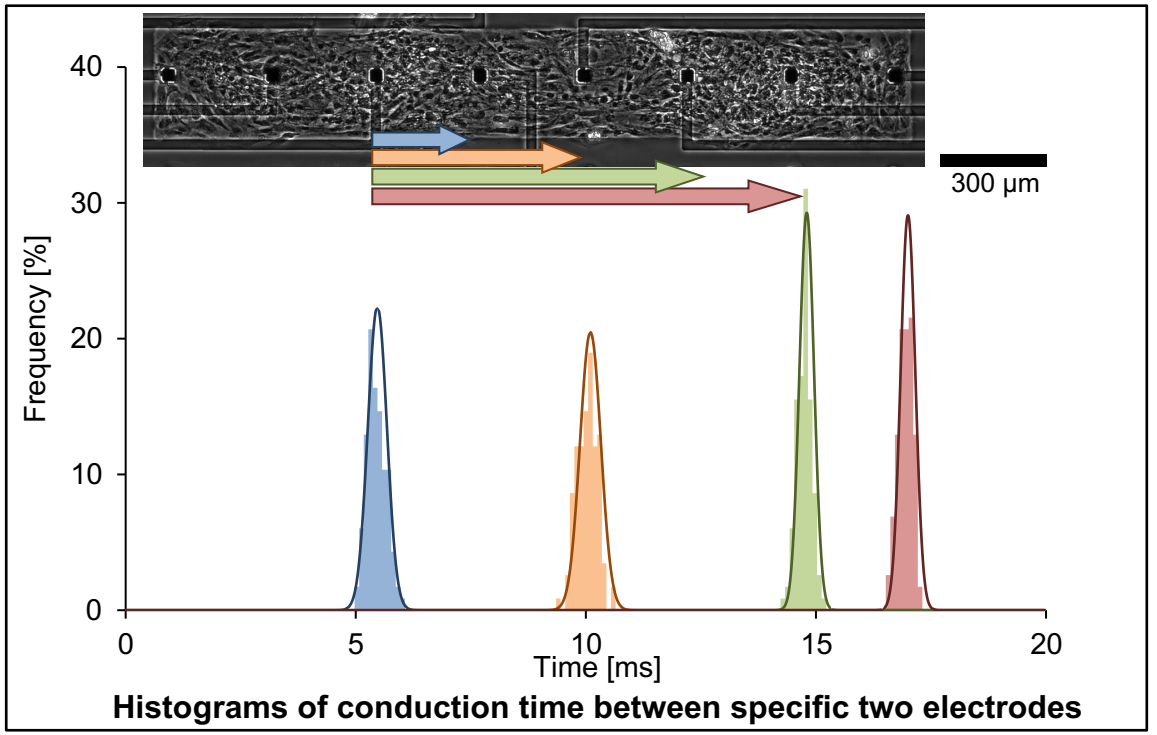
MEA System

Measurement Record and Analysis PC





Cardiomyocyte Networks



Histograms of conduction time between specific two electrodes

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Abstract

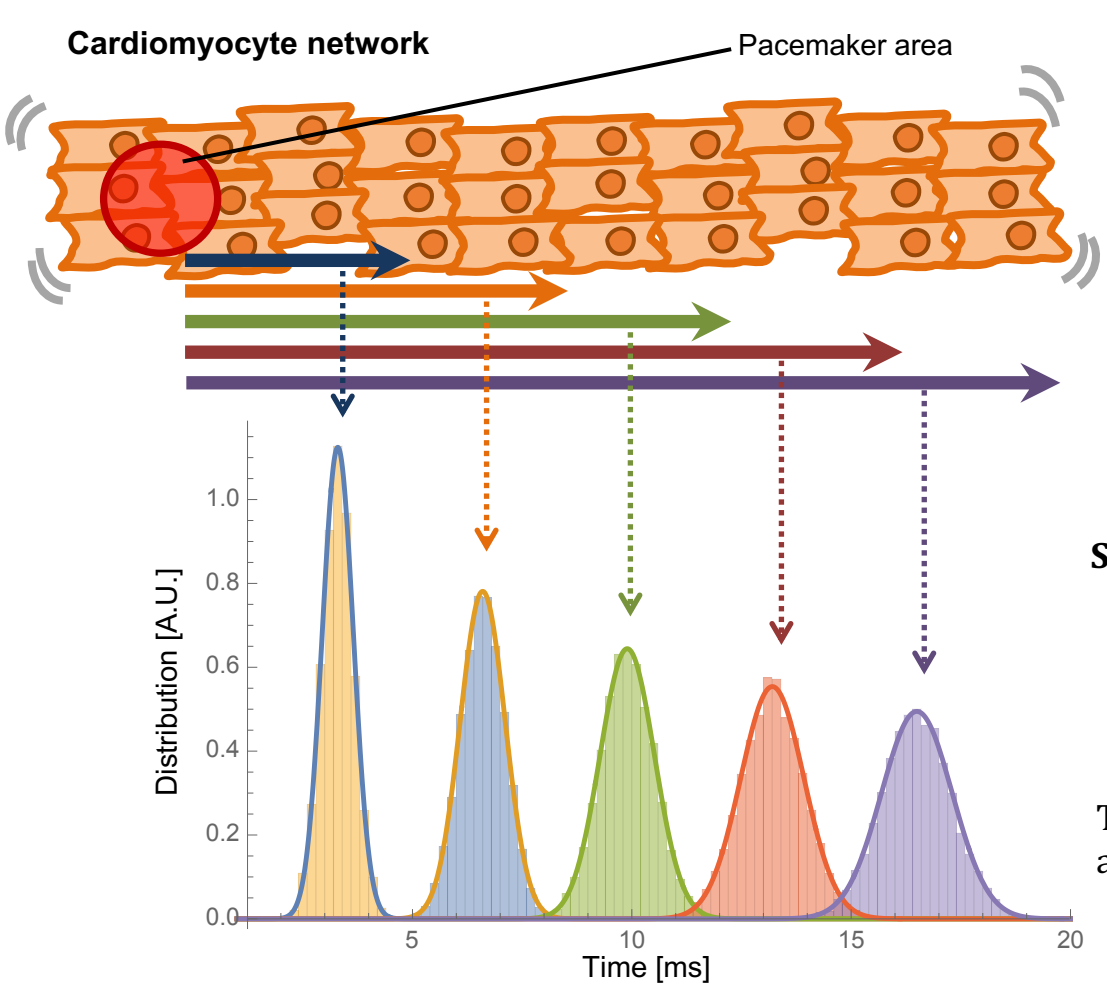
We examined characteristics of the propagation of conduction in width-controlled cardiomyocyte cell networks for understanding the contribution of the geometrical arrangement of cardiomyocytes for their local fluctuation distribution. We tracked a series of extracellular field potentials of linearly lined-up mouse primary cardiomyocytes and human ES cardiomyocytes with 100 kHz sampling intervals of multi-electrodes signal acquisitions and an agarose microfabrication technology. Conduction time between two neighbor microelectrodes showed Gaussian distribution, which indicates this conduction propagation in a unit length was a stochastic firing phenomenon. However, the distributions of conduction time were not expanded but maintained within an identical range of distribution regardless of their propagation distances from 0.3 mm to 1.5 mm, which is against the expected distance-dependent enlarging of the distribution based on the faster firing regulation. In contrast, when Quinidine was applied to the cardiomyocytes, the distributions of conduction time were expanded as propagation distance increased as predicted by the conduction propagation model of faster firing regulation. The results indicate the “faster firing regulation” is not sufficient to explain this conservation of the propagation time distribution in cardiomyocyte networks.

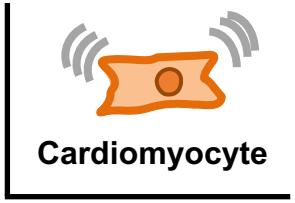
Keywords

on-chip cell network assay, multi microelectrode array, external field potential measurement, conduction distribution, cardiomyocyte network

Introduction

Can explain the excitation conduction by the faster firing regulation?





Simulation of conduction time distribution in the faster firing regulation model

The distribution of conduction time dispersed as the firing signal propagated.

Fig.1. The histogram of conduction time distribution.

Result and Discussion(1)

Agarose Microfabrication and Cell Culture

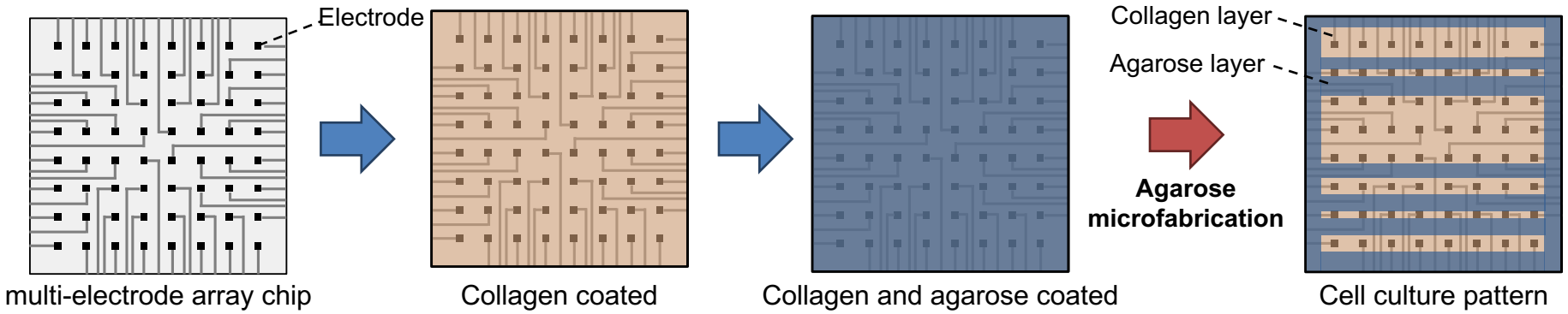


Fig.2. MEA chip microfabrication procedure.

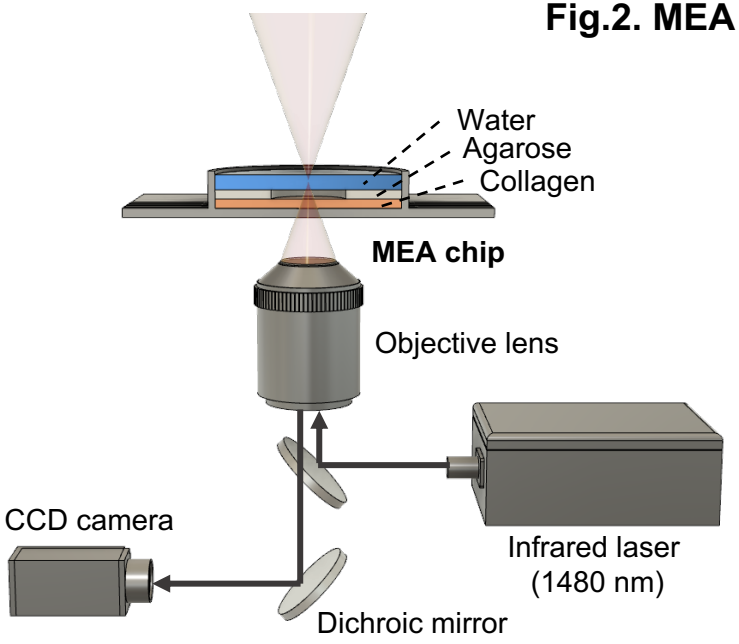


Fig.3. Agarose microfabrication technology.

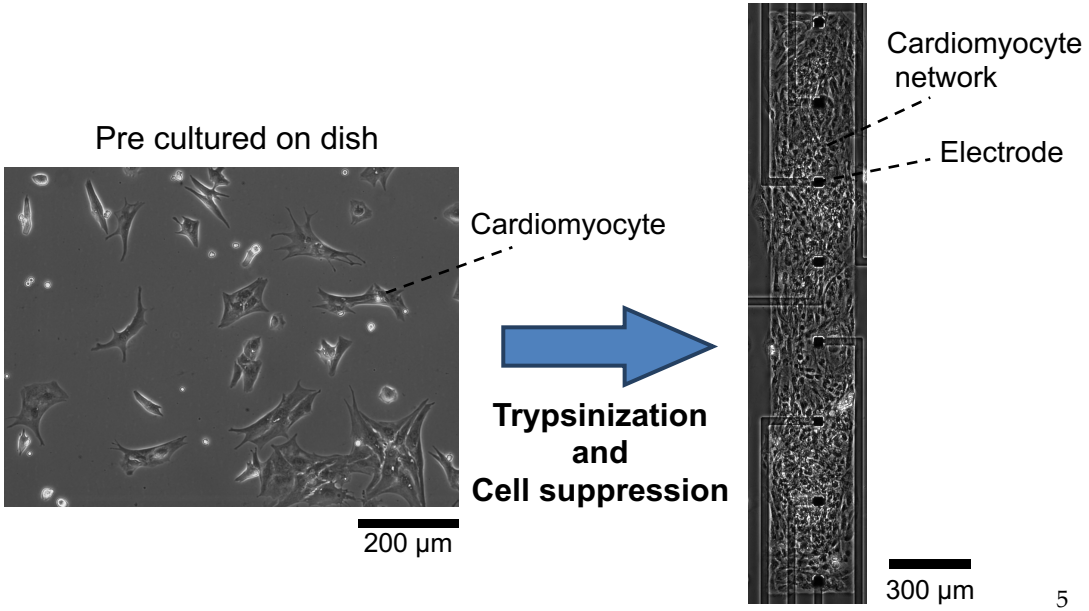


Fig.4. Cultured cardiomyocytes on MEA chip.

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Result and Discussion(2)

Measurement System and Data Analysis

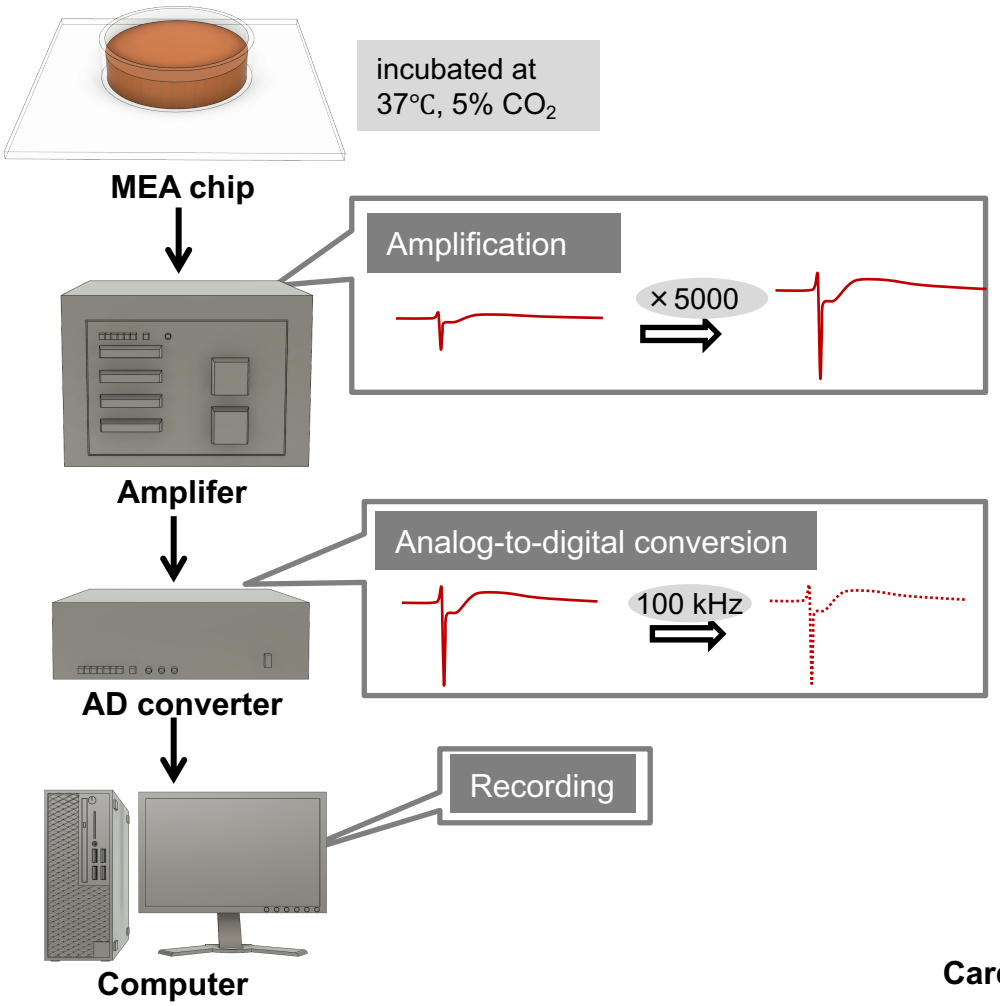

Fig.5. MEA system.

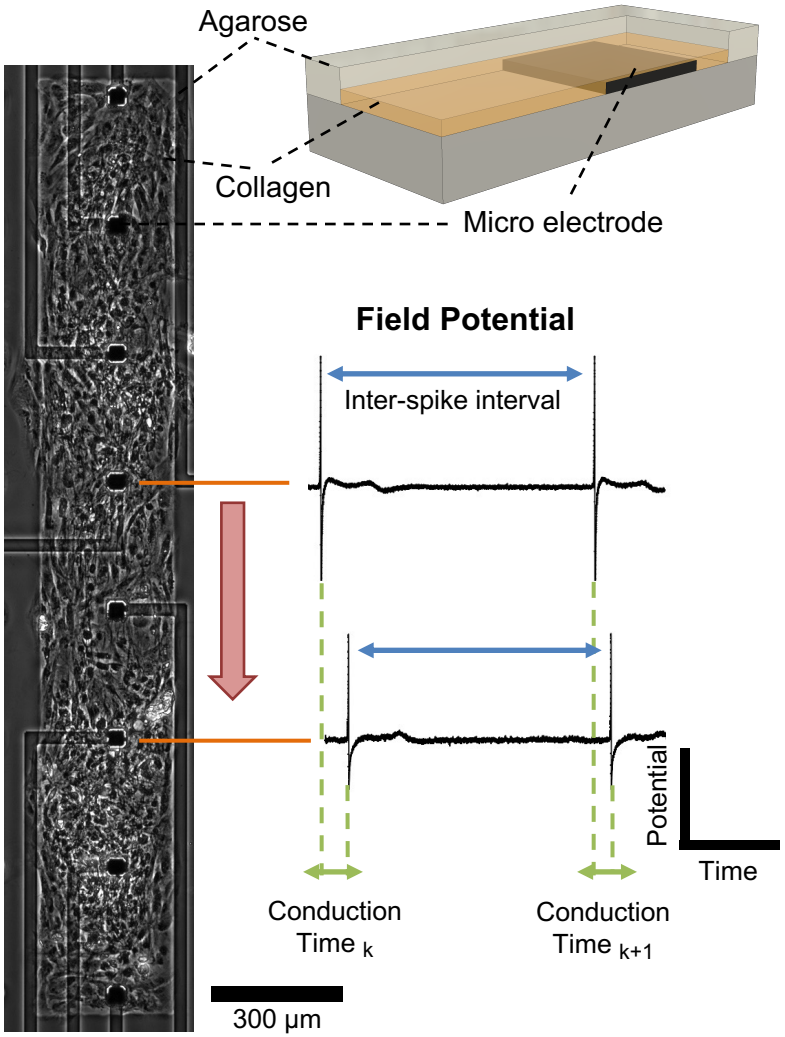

Fig.6. Conduction time measurement and analysis.

Result and Discussion(3)

Distribution of Conduction Time

- Mouse Primary Cardiomyocyte Networks

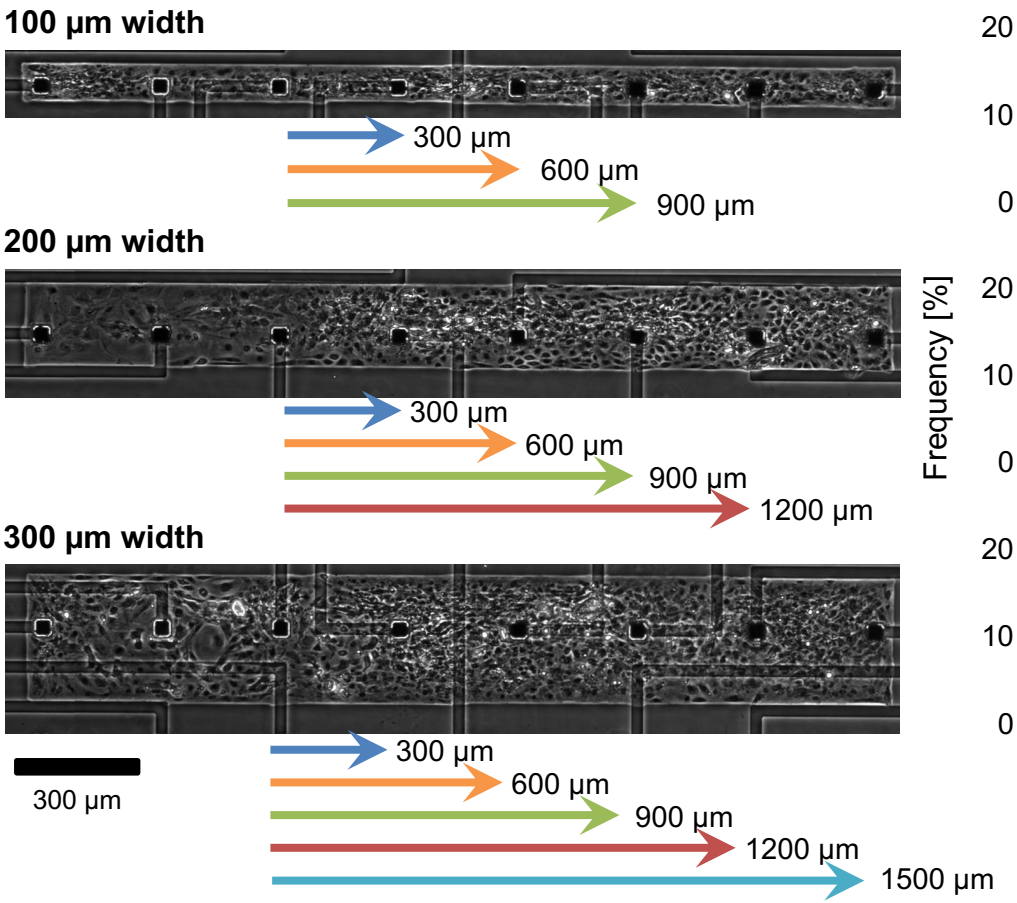

Fig.7. Primary cardiomyocyte networks.

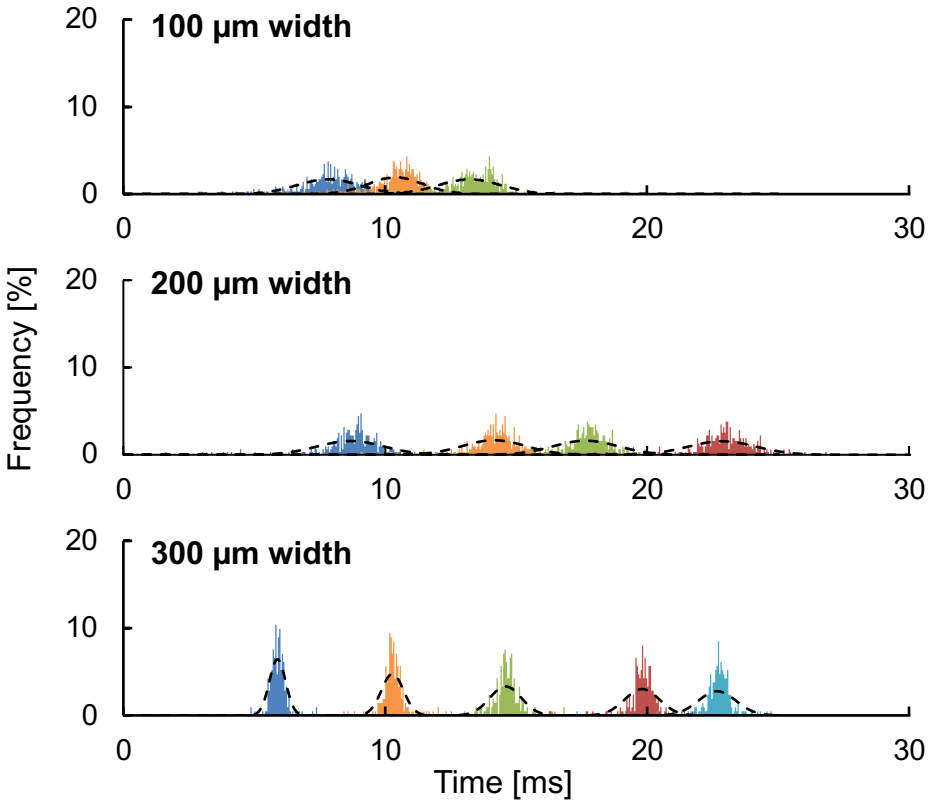

Fig.8. Histograms of conduction time between specific two electrodes

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Result and Discussion (4)

Human ES Cardiomyocyte Networks

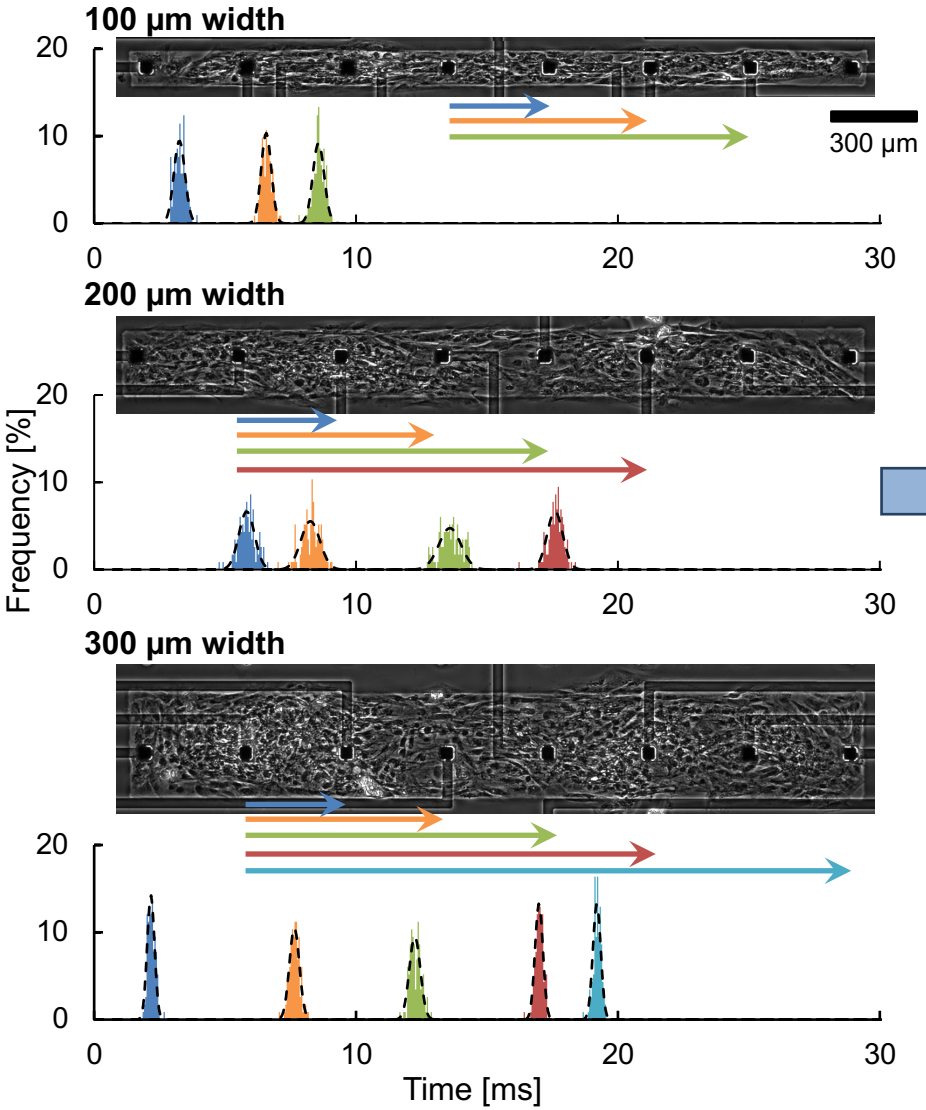


Fig.9. Histograms of conduction time.

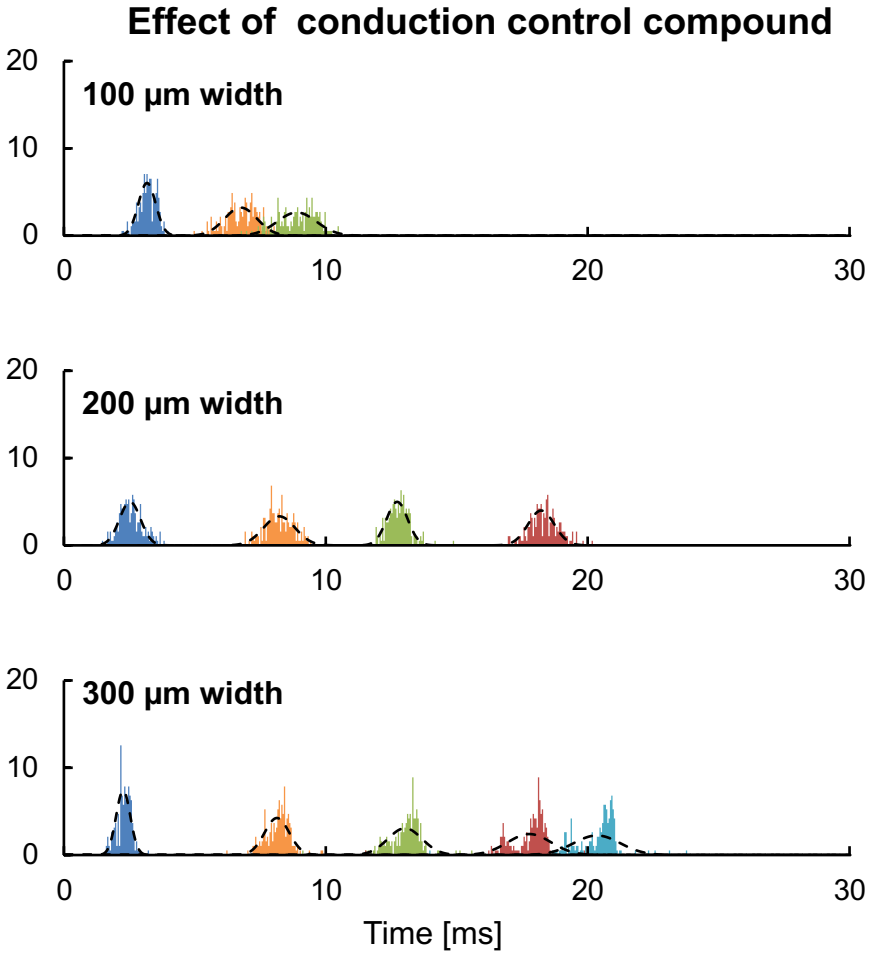
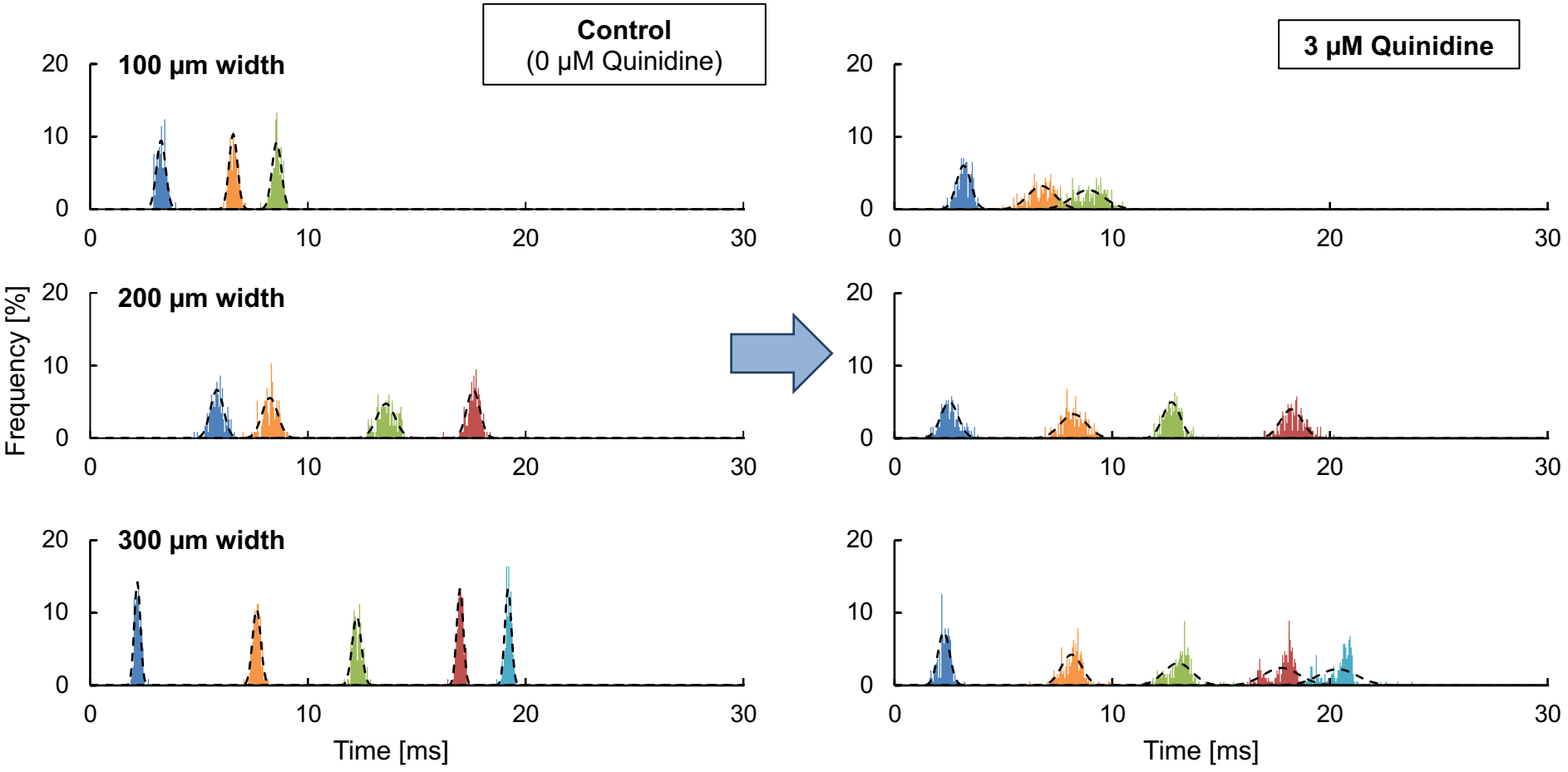


Fig.10. Histograms of conduction time when 3 μM Quinidine was applied.

Result and Discussion (4)

Human ES Cardiomyocyte Networks



The distributions of conduction time maintained within an identical range of distribution regardless of their propagation distances.

The distributions of conduction time were expanded as propagation distance increased.

Result and Discussion (5)

Distribution of Conduction Time

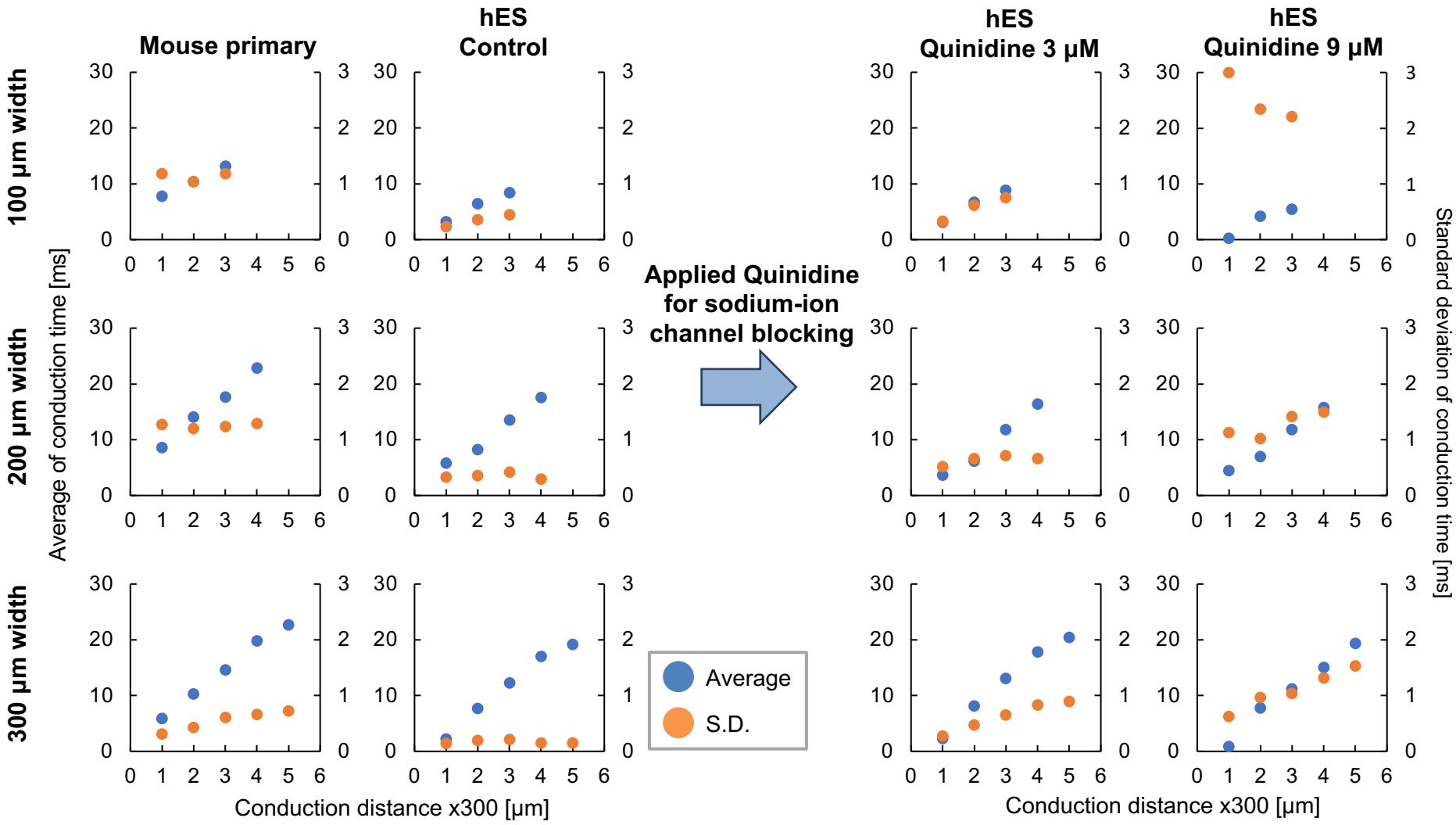


Fig11. Comparison of fluctuations of conduction time in cardiomyocyte networks.

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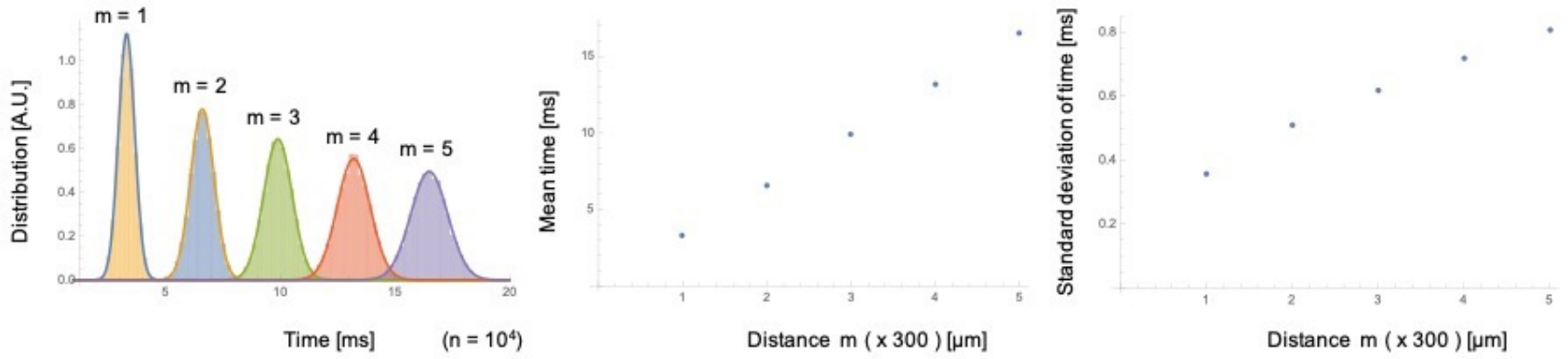
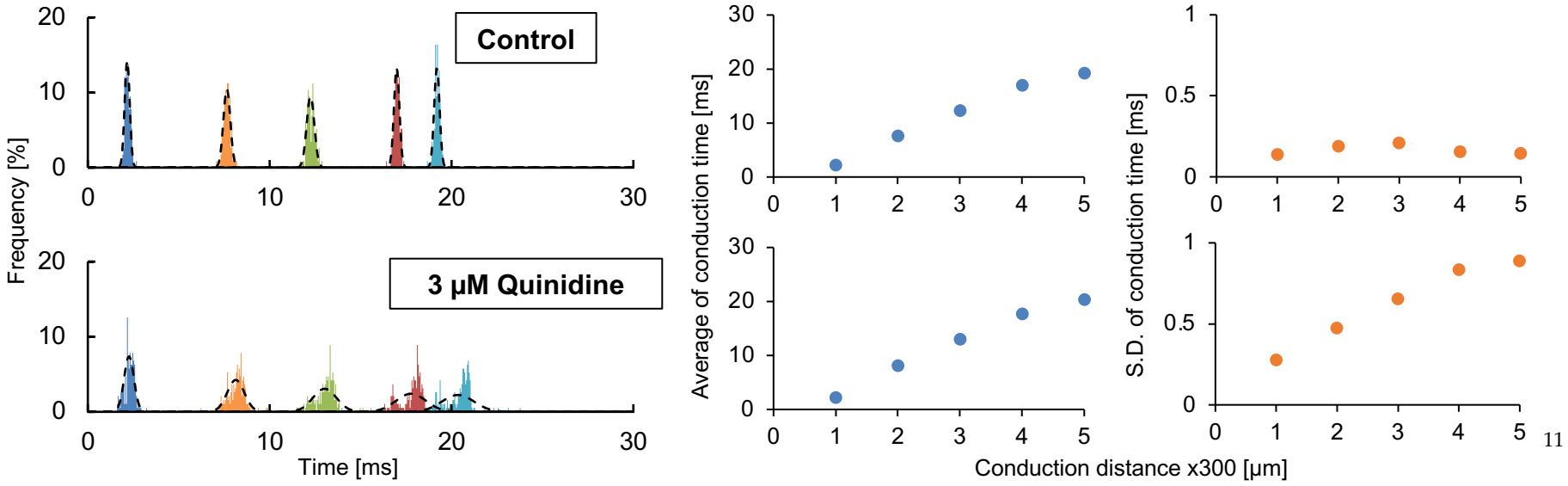
Result and Discussion(6)

Can explain the excitation conduction by the faster firing regulation?

Simulation Result



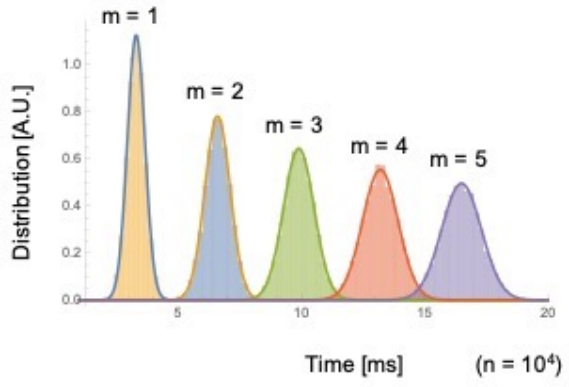
Experimental Result of 300 μm width hES cardiomyocyte network

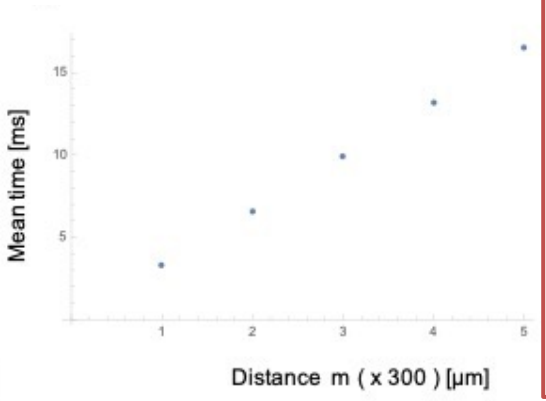


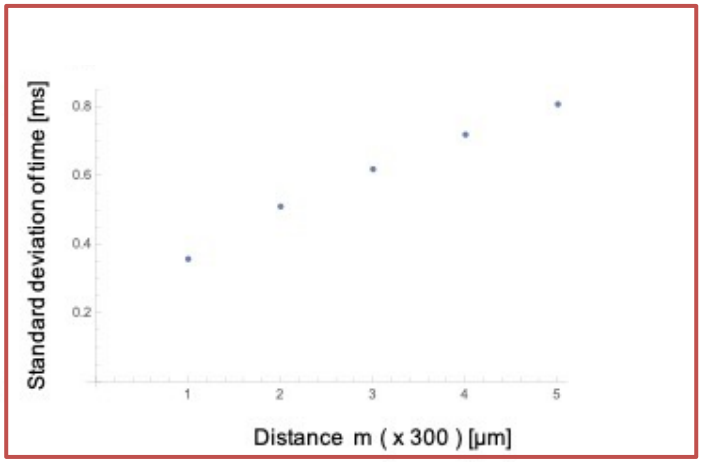
Result and Discussion(6)

Can explain the excitation conduction by the faster firing regulation?

Simulation Result







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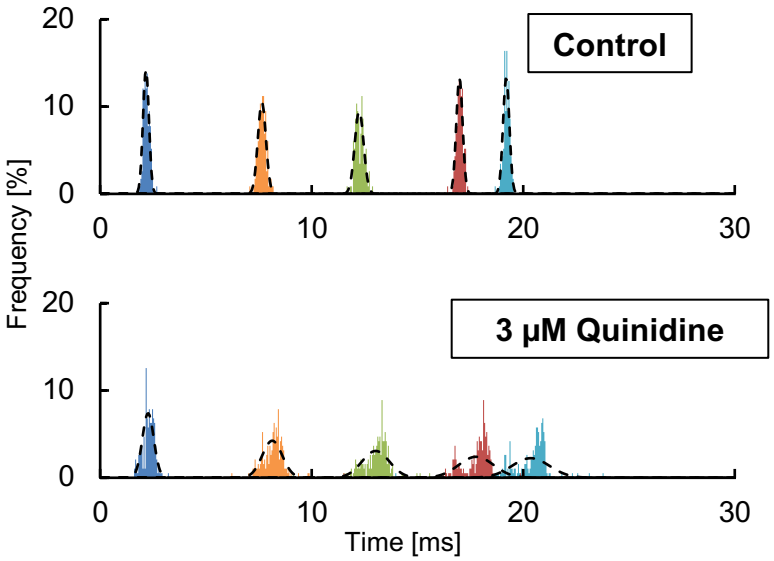

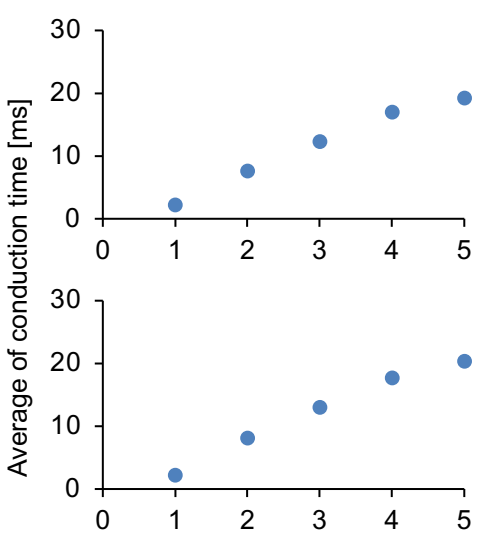

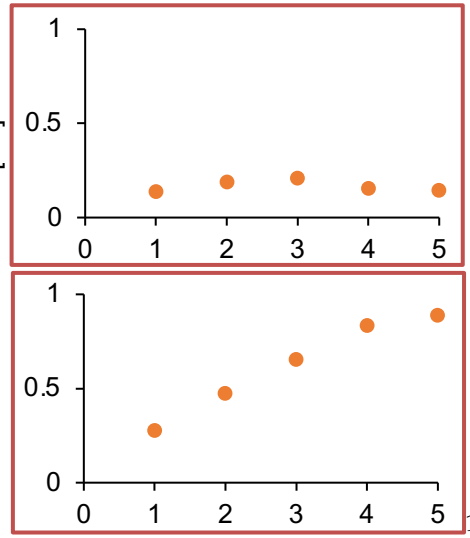

Conclusions

（1）We succeed in constructing measurement assay of excitation conduction time in width-controlled linearly lined-up cardiomyocyte network on the multi electrode array chip.

（2）We observed the distributions of conduction time maintained their range of distribution without any expansion regardless of its propagation distances from 0.3 mm up to 1.5 mm, which is against the conventional conduction connection rule, “faster firing regulation” .

（3）We also observed the distributions of conduction time were expanded as propagation distance increased when Quinidine was applied, which was followed to “the faster firing regulation.”

Above（2）and（3）suggest the existence of some unknown cooperative conduction propagation regulation in cardiomyocyte conduction, which is disappeared by the sodium channel blocking.

In detail, please visit our publication in this issue
Sakamoto, Kazufumi, et al. *Micromachines* 11.12 (2020): 1105.

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Acknowledgments

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