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研究課題名（和文）A realistic mechano-sensitive disease model in-vitro for cardiac tissue culture.

研究課題名（英文）A realistic mechano-sensitive disease model in-vitro for cardiac tissue culture.

研究代表者
Ｈｏｅｒｎｉｎｇ　Ｍａｒｃｅｌ（Hoerning, Marcel）

京都大学・高等研究院・客員研究員

研究者番号：２０５８８４９６

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）：我々は、細胞形態学の制御の新たな内部と、新しい力学的に変更可能なヒドロゲルを使用することによって細胞生存率を妨げることなく、筋芽細胞の細胞骨格における規則化を明らかにした。さらに、心臓組織の螺旋波の回転における線欠陥の時空間的動態を可視化する新たな解析法を導入し、線欠陥が螺旋波の安定振動を維持しながら転換、融合、崩壊および安定特異点を形成できることを示した。その上、単一細胞の細胞膜全体の脂質動態を数値化するための新たなマッピング法を紹介した。また、PtdInsP3波動力学が３Ｄ幾何学（すなわちサイズおよび形状）によって直接調節されることを明らかにした。

研究成果の学術的意義や社会的意義
Main goal of this investigation is to understand tissue morphological changes and pattern formation that are associated with cardiac diseases, which is in JAPAN one of the leading causes of death with an annual death rate of approx. 100,000 people (12%).

研究成果の概要（英文）：We revealed novel insides of the regulation of cell morphology and cytoskeletal ordering of myoblasts without interfering cell viability by using novel dynamically changeable hydrogels. Further, we introduced a novel analysis method to visualise spatiotemporal dynamics of line defects in rotating spiral waves of cardiac tissues, showing that line defects can translate, merge, collapse and form stable singularities while maintaining a stable oscillation of the spiral wave. Additionally, we introduced a novel mapping method to quantify lipid dynamics on the entire cell membrane of single cells. We revealed that PtdInsP3 wave dynamics are directly regulated by the 3D geometry (i.e., size and shape).

研究分野：Biology, Physics

キーワード：Cardiac Tissue　Cellmechanics　Patternformation

１．研究開始当初の背景

Muscle cells (myocytes) that mature in an environment that is self-similar, e.g. the rigidity of the extracellular matrix (ECM) and cells matches **($E_{ECM}=E_{cell}$)**, lead to an enhancement in the synchronization dynamics of spontaneous and enforced intracellular Ca^{2+}-signals [Hörning et al. Biophys.J, 2012], morphological changes that support improvement in self-organization, i.e. sarcomere self-organization [Hörning & Entcheva, Springer Series in Mat.Sci., 2015], and even direct stem cell linage specification [Engler et al. Cell, 126, 677, 2006] and self-renewal [Gilbert et al. Science, 2010]. Furthermore, changes in the microenvironment (rigidity) have been shown to regulate morphology [Discher et al. Science, 310, 1139, 2005; Yoshikawa et al, Phys.Chem.B, 2013] and motility [Engler et al, Cell, 126, 677, 2006; Winer et al, Tissue Eng., 15, 147, 2008] of contractile cells, as well as in pancreatic cancer cells [Kaindl et al. PlosONE, 7, e42991, 2012]. Electro spun nanofibrous meshes [Orlova et al. Biomat., 32, 5615, 2011] and three-dimensional scaffolding of elastic polymers [Chung et al. FASEB, 25, 852, 2011] has been used to increase self-organizing tissue assembly, similar as observed *in-vivo*.

２．研究の目的

Investigations on contractile confluent tissues, i.e. heart tissue, has been exclusively explored by using rigidity-fixed non-adjustable hydrogels that only enables the study of developmental processes. By use of stimulus-responsive host-guest hydrogels (here we propose βCDAd-gel) one can overcome this limitation, since it (1) can mimic the natural environment during the development to ensure *in-vivo*-like maturity and tissue growth, similar to conventional hydrogels and (2) can serve as a realistic *in-vitro* disease model to study changes in the non-linear self-organization tissue-signaling dynamics that are associated with severe diseases, such as fetal arrhythmia (spiral waves) and fibrillation (spatiotemporal chaos) in heart. The latter has been investigated *ex-situ* by use of external artificially applied electric disturbances [Isomura, et al. PRE, 2009; Hörning et al. PRE, 2010 / 2012 / 2012, Bitthin et al. PRL 2012].

３．研究の方法

We have used different biological systems, ranging from single muscle cells (C2C12 cell line), Neuronal cells and primary culture of cardiomyocyte tissues from neonatal rats to investigate the mechano-sensitive influence of cells/tissues on the extra-cellular matrix. We used to host-guest hydrogels composed of acrylamid and permanently cross-linked host and guest monomers, βCD and Ad, that could be dynamical control the E-Module of the hydrogel by freely diffusing βCD molecules. We developed also a new analysis method to visualize dangerous spatiotemporal alternans in cardiac tissue, as well as time-resolved actin visualization and quantification methods.

４．研究成果

The results of the work we have done can be categorized into two parts, 1. Mechano-sensitive dynamics on hydrogels and 2. Development of dynamic quantification methods. The results of the three main publications can be summarized as:

① We show that PtdInsP3 wave dynamics are directly regulated by the 3D geometry (i.e., size and shape) of the plasma membrane. By introducing an analysis method that extracts the 3D spatiotemporal activities on the entire cell membrane, we show that PtdInsP3 waves self-regulate their dynamics within the confined membrane area. This leads to changes in speed, orientation, and pattern evolution, following the underlying excitability of the signal transduction system. Our findings emphasize the role of the plasma membrane topology in reaction-diffusion-driven biological systems and indicate its importance in other mammalian systems, such as cardiac tissue.
[M. Hörning and T. Shibata, Biophysical Journal, **116**, 2, 372-382, 2019]

② Through *in vitro* experiments of heart tissue observation, we reveal the spatiotemporal dynamics of line defects in rotating spiral waves. We combined a novel signaling over-sampling technique with a multi-dimensional Fourier analysis, showing that line defects can translate, merge, collapse and form stable singularities with even and odd parity while maintaining a stable oscillation of the spiral wave in the tissue.
[M. Hörning et al. Scientific Reports, 7, 7657, 2017]

③ We demonstrated that the Young's modulus of our "host-guest gels", 4–11 kPa, lies in an optimal range not only for static (*ex situ*) but also for dynamic (*in situ*) regulation of cell morphology and cytoskeletal ordering of myoblasts. Compared to other stimulus-responsive materials that can either change the elasticity only in one direction or rely on less biocompatible stimuli such as UV light and temperature change, our supramolecular hydrogel enables to reversibly apply mechanical cues to various cell types *in vitro* without interfering cell viability.
[M. Hörning et al. Scientific Reports, 7, 7660, 2017]

５．主な発表論文等

〔雑誌論文〕（計　5　件）

① **M. Hörning** and T. Shibata, "Three-dimensional cell geometry controls excitable membrane signaling in Dictyostelium cells", Biophysical Journal, **116**, 2, 372-382, 2019 https://doi.org/10.1016/j.bpj.2018.12.012

② H. Umeshima*, K. Nomura, S. Yoshikawa, **M. Hörning**, M. Tanaka, S. Sakuma, F. Arai, M. Kaneko and Mineko Kengaku, "Local traction force in the proximal leading process triggers nuclear translocation during neuronal migration", Neuroscience Research, **142**, 38-48, 2019
https://doi.org/10.1016/j.neures.2018.04.001

③ H. Sakuta, S. Seo, S. Kimura, **M. Hörning**, K. Sadakane , T. Kenmotsu , M. Tanaka and K. Yoshikawa*, ”Optical Fluid Pump: Generation of directional flow via micro-phase segregation/homogenization”, J. of Phys. Chem. Lett., **9**, 5792-5796, 2018 https://doi.org/10.1021/acs.jpclett.8b01876

④ **M. Hörning**, F. Blanchard, A. Isomura and K. Yoshikawa, ”Dynamics of spatiotemporal line defects and chaos control in complex excitable systems.”, Scientific Reports, **7**, 7657, 2017 http://dx.doi.org/10.1038/s41598-017-08011-z

⑤ **M. Hörning**, M. Nakahata, P. Linke, A. Yamamoto, M. Veschgini, S. Kaufmann, Y. Takashima, A. Harada and M. Tanaka, ”Dynamic Mechano-Regulation of Myoblast Cells on Supramolecular Hydrogels Cross-Linked by Reversible Host-Guest Interactions”, Scientific Reports, **7**, 7660, 2017 http://dx.doi.org/10.1038/s41598-017-07934-x

〔学会発表〕（計 8 件）

[invited oral presentation]

① **M. Hörning,** ”Spatiotemporal dynamics of line defects in cardiac tissue.”, 3rd International Conference on Biomedical Technology, Hannover, Germany, 11/2017

② **M. Hörning,** ”Interaction of cardiac tissue morphology and dynamics in mechano-sensitive environments during development.”, 11. Workshop des Projekthauses NanoBioMater, Stuttgart, University of Stuttgart, Fraunhofer IGB, Germany, 04/2017

[oral presentation]

③ **M. Hörning**, and T. Shibata, ”Three-dimensional membrane confinement and geometry dictate excitable signaling dynamics in Dictyostelium cells.”, DPG-Spring meeting of the German Physical Society, Regensburg, Germany, 04/2019

④ **M. Hörning**, and T. Shibata,”The three-dimensional geometrical confinement of membranes mediates the excitable signaling dynamics in Dictyostelium cells.”, EMBO - EMBL Symposium: Biological Oscillators: Design, Mechanism, Function, EMBL Advanced Training Centre, Heidelberg, Germany, 06/2018

[poster presenation]

⑤ I. Findeisen, A. Anton, **M. Hörning** and S. Nussberger, "Watching single TOM protein translocation channels at work", Symposium Nano-BW 2018 des Kompetenznetzes ?Funktionelle Nanostrukturen? Bad Herrenalb, Germany, 09/2018

⑥ **M. Hörning**, F. Blanchard, A. Isomura and K. Yoshikawa, "Spatiotemporal dynamics of line defects in cardiac tissue.", EMBO/EMBL Symposium : Biological Oscillators: Design, Mechanism, Function, EMBL Heidelberg, Germany, 06/2018

⑦ **M. Hörning**, Emilia Entcheva, Satoru Kidoaki, Kenichi Yoshikawa , "Self-regulation of the mechanotransduction dynamics in developing cardiac tissues", EMBO/EMBL Symposium Mechanical Forces in Biology, EMBL Heidelberg, Germany, 07/2017

⑧ **M. Hörning**, M. Nakahata, P. Linke, A. Yamamoto, M. Veshgini Y. Takashima, A. Harada and M. Tanaka, "Quantification of Dynamic Mechano-Response of Myoblast using Stimulus Responsive Matrix", 54th Annual Meeting of the Biophysical Society of Japan, Tsukuba, Japan, 11/2016

〔図書〕（計 0 件）

None.

〔産業財産権〕
○出願状況（計 2 件）

名称：原田明、高島義徳、中畑雅樹、田中求、ホールニング マルセル
発明者：原田明、高島義徳、中畑雅樹、田中求、ホールニング マルセル
権利者：原田明、高島義徳、中畑雅樹、田中求、ホールニング マルセル
種類：培地用高分子ゲル、培地、細胞の培養方法及びキット
番号：2016-148725
出願年：2016
国内外の別：domestic

名称：A. Harada, Y. Takashima, M. Nakahata, M. Tanaka, and M. Hörning
発明者：A. Harada, Y. Takashima, M. Nakahata, M. Tanaka, and M. Hörning
権利者：A. Harada, Y. Takashima, M. Nakahata, M. Tanaka, and M. Hörning
種類："Polymer gel for　medium, medium, and method and kit for culturing cells"
番号： PCT/JP2017/026832
出願年： 2017
国内外の別： international

○取得状況（計 0 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

None.

６．研究組織

(1)研究分担者
研究分担者氏名：
ローマ字氏名：
所属研究機関名：
部局名：

職名：
研究者番号（8 桁）：

(2)研究協力者
研究協力者氏名：柴田達夫
ローマ字氏名：Tatuso Shibata

研究協力者氏名：原田明
ローマ字氏名：Akira Harada

研究協力者氏名：高島義徳
ローマ字氏名：Yoshinori Takashima

研究協力者氏名：中畑雅樹
ローマ字氏名：Masaki Nakahata

研究協力者氏名：田中求
ローマ字氏名：Motomu Tanaka

研究協力者氏名：梅嶋 宏樹
ローマ字氏名：Hiroki Umeshima

研究協力者氏名：見学 美根子
ローマ字氏名：Mineko Kengaku

研究協力者氏名：磯村 彰宏
ローマ字氏名：Akihiro Isomura

研究協力者氏名：Francois Blanchard
ローマ字氏名：Francois Blanchard

研究協力者氏名：吉川 研一
ローマ字氏名：Kenichi Yoshikawa