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論文審査委員	委員長　教　授　吉川　洋史 委　員　准教授　坂田　一郎 委　員　教　授　中林誠一郎 委　員　准教授　前田　公憲 委　員　東京大学特任准教授 寺村　裕治

論文の内容の要旨

The human body is a complex hierarchical structure, consists of genes as a blueprint, macromolecules (including proteins) as a material, cells as a functional unit, organs as a functional segment, and the body as a whole aggregate. Biological phenomena are boldly yet subtly regulated by the ordered intra- and inter-hierarchy interaction. It has been increasingly recognized that the mechanical properties of cells, tissues, and extracellular environments play crucial role in many biological processes. For instance, Engler et al. reported the matrix stiffness-dependent mesenchymal stem cells differentiate into the specific tissue cell types; neurogenic phenotype on soft matrices, myogenic phenotype on stiffer matrices (Engler, A.J. et al, Cell 126, 677-689, 2006). The cells feel mechanical properties of the surroundings and alter their behavior by changing gene expression. These facts highlight the importance of mechanical properties to understand biological phenomena such as development and disease.

The aim of this study is to quantitatively evaluate the mechanics of (multi-)cellular systems to understand the mechanisms of how mechanics are involved and regulating biological processes. This thesis reports two-different, but related topics in which the stiffness and adhesion of cells/tissues were quantitatively evaluated by using advanced optical/force microscopy.

Chapter 2 describes the mechanical property of the organ-like tissues (called as organoids) which can model the disease development and progression. Liver functions are closely correlated with tissue stiffness; once stiffened by cirrhosis, mortality rate is unacceptably high. Non-alcoholic Fatty Liver Disease (NAFLD)

is the most prevailing liver disease (Cotter and Rinella, Gastroenterology 158, 1851-1864, 2020), in which the excess lipid accumulation causes inflammation (Hashimoto et al., J Gastroenterol Hepatol 28, 64-70, 2013). The aggressive phenotype of NAFLD induces the tissue stiffening by collagenous fiber deposition (fibrosis), and eventually develops a cirrhosis; however, effective therapeutics is limited. One possible reason is the lack of the relevant disease model which can mimic the context-dependent pathology of the lipid metabolism disorder, including inflammation and fibrosis-like tissue stiffness change along with the disease progression. Upon the proposition, the study has hypothesized that organoid technology could generate the liver model while possessing non-parenchymal cell population that is crucial to model complex hepatic metabolism disorder including fibrotic tissue stiffness change.

This works showed that the human liver organoids (HLOs) could be successfully generated from human induced pluripotent stem cells (hiPSCs). During the differentiation, it was revealed that retinoic acid treatment can induce the hepatocyte-like, as well as Kupffer-like and stellate-like cells which is responsible for inflammation and fibrosis, respectively. The HLOs stored triglycerides by treated with free fatty acid (FFA). Furthermore, the HLOs showed inflammation- and fibrosis-associated gene expression and cytokine secretion. Importantly, atomic force microscopy (AFM) measurement revealed that the HLOs were stiffened by long-term FFA treatment. Spatial stiffness distribution analysis clearly revealed pathogen-dependent mechanical property pattern, which cannot be distinguishable by conventional force-curve distance analysis. Finally, consistent with clinical studies, obeticholic acid treatment rescued HLOs from fibrotic tissue stiffening. Overall, the study succeeded in establishing a novel liver model that can recapitulate the context-dependent pathology of inflammation and fibrosis. The AFM approach demonstrated the potential applicability of stiffness measurement as a useful clinical readout for high-throughput drug screening.

Chapter 3 describes the development and quantitative evaluation of a cell adhesion model. To generate highly functional tissues *in vitro*, cells must be integrated into tissue with appropriate positions. DNA hybridization is one of the promising approaches that can regulate cell-cell interaction and rationally control the relative cell position. However, the effect of artificially introduced cell-cell contact on the endogenous cell adhesion, such as E-cadherin-mediated binding, is remained unclear. E-cadherin-mediated cell adhesion is vital in the epithelial tissue, in which cells recognize their surroundings and respond through the adhesion structure. Therefore, in this study, the impact of cell surface modification with DNA-PEG-lipids, ssDNA and poly(ethylene glycol)-conjugated phospholipid derivatives (Teramura, Biomaterials 48, 119-128, 2015) on E-cadherin-mediated cell adhesion was evaluated.

For this purpose, cell-cell adhesion model was first designed by seeding living cells onto a planar lipid membrane (PM) displaying E-cadherin and/or DNA-PEG-lipids with well-defined densities. The cell membrane model enabled us to clearly visualize structures of the cell/PM interfaces by fluorescence and interference microscopy, which allows for distinguishing between adhesion sites mediated by E-cadherin and by DNA hybridization. The advanced microscopic technique combined with image analysis revealed

that the cell adhesion can be a two-step process; physical contact mediated by the DNA hybridization within minutes and the subsequent E-cadherin-mediated adhesion in hours. Furthermore, the DNA hybridization can facilitate the E-cadherin-mediated cell adhesion, which may be attributed to positive feedback loop. The self-organization method sometimes requires comprehensive analysis of the cell density/ratio and mechanical/chemical property of the substrate. On the other hand, functional molecules can alter surface energy of the cell assembly by artificially controlling the cell-cell interaction, allowing morphogenesis beyond the spherical structure. The in vitro cell adhesion model established in this study will provide guidelines for the design of functional molecules for use in tissue engineering approach.

Finally, these cross-cutting biomechanical research at various size scales provides the insight into the methodology for functional and complex tissue re-generation, and future application for the disease modeling as well as the mechano-screening approaches.

論文の審査結果の要旨

本学位論文は、力学的な観点から多細胞組織の形成や機能発現について調べた研究について述べられている。第１章に研究背景と目的、第２章と第３章に研究成果、第４章で結論が述べられ、英語で作成されている。以下に学位論文の内容の概略を英語で述べる。

In chapter 1, the scientific background and purpose of this study are summarized. The aim of this study is to quantitatively evaluate the mechanics of (multi-)cellular systems, such as stiffness and adhesion dynamics, to understand the underlying mechanisms of how mechanics are involved in the expression of dynamic biological processes. This thesis reports two-different, but related topics in which the stiffness and adhesion mechanics of cells/tissues were quantitatively evaluated by using advanced optical/force microscopy.

In chapter 2, the correlation between mechanical properties and biological functions of organ-like tissues (called as organoids) was systematically investigated. It is known that liver functions are closely correlated with tissue stiffness; once stiffened by cirrhosis, mortality rate is unacceptably high. Non-alcoholic Fatty Liver Disease (NAFLD) is the most prevailing liver disease in which the excess lipid accumulation causes inflammation. Here, In vitro NAFLD models were established by using human liver organoids (HLOs) induced from human induced pluripotent stem cells (hiPSCs). It was found that retinoic acid treatment during stem cell differentiation can induce the hepatocyte-like, as well as Kupffer-like and stellate-like cells, which are responsible for inflammation and fibrosis, respectively. HLOs that were treated with free fatty acid (FFA) stored triglycerides and showed inflammation- and fibrosis-associated gene expression and cytokine secretion. Importantly, atomic force microscopy (AFM) measurement revealed that the HLOs were stiffened by long-term FFA treatment. Spatial stiffness distribution analysis clearly revealed pathogen-dependent mechanical property patterns. In addition, obeticholic acid treatment rescued HLOs from fibrotic tissue stiffening. Overall, the study succeeded in establishing a novel liver model that can recapitulate the context-dependent pathology of inflammation and fibrosis. We can expect the potential applicability of stiffness measurement as a useful clinical readout for high-throughput drug screening.

In chapter 3, quantitative evaluation of cell adhesion by using advanced optical microscopy was described. To generate highly complex, functional tissues in vitro, cells must be integrated into tissue with appropriate positions. DNA hybridization is one of the promising approaches that can regulate cell-cell interaction and rationally control the relative cell position. However, the effect of artificially introduced cell-cell contact via DNA hybridization on the endogenous cell adhesion, such as E-cadherin-mediated binding, remained unclear. In this study, the impact of cell surface modification with DNA-PEG-lipids, single strand DNA (ssDNA) and poly(ethylene glycol)-conjugated phospholipid derivatives on E-cadherin-mediated cell adhesion was evaluated. In the experiment, a planar and fluid lipid membrane displaying E-cadherin and/or single-strand

DNA with well-defined densities was prepared. Visualization of cells on membranes by fluorescence and interference microscopy revealed that cell adhesion undergoes with a two-step process: artificial adhesion by DNA hybridization within a few minutes followed by biological adhesion via cadherin-cadherin binding within hours. Furthermore, this study revealed that DNA hybridization can substantially facilitate E-cadherin-mediated cell adhesion. The promotive effect is probably due to the enforced binding between E-cadherin molecules in geometrical confinement between two membranes. The in vitro model of cell adhesion can potentially be used to design functional synthetic molecules that can regulate cell adhesion via cell adhesion proteins for tissue engineering.

In conclusions, this cross-cutting biomechanical research at various size scales provides the basic insight into the methodology for functional and complex tissue re-generation, and future application for the disease modeling as well as the mechano-screening of tissue functions.

以上のように申請者は、先端的な光学的・力学的計測法を基盤とし、オルガノド呼ばれる人為的に作製した臓器状組織の力学特性と機能との相関の解明や、化学的アプローチによる細胞組織の秩序構造形成の制御に関する研究で成果を挙げている。これらの研究成果は、組織工学分野において力学的な観点からの新しい計測制御手法を提示するものである。それぞれの研究成果は、再生学や合成化学の研究者との共同成果で生まれたものであり、申請者はその分野横断的な研究の立案・遂行において十分な貢献をしている。これらの研究成果は、再生医学や創薬など様々な基礎研究･産業分野に大きな波及効果をもたらすことが期待できる。

なお第２章の内容は、Cell Metabolism 誌にダブル筆頭著者論文として、Applied Physics Express 誌に共著論文が出版されている。また第３章の内容は、APL Bioengineering 誌に筆頭著者論文として出版し、同誌の Featured Article にも選ばれている。また Scilight 誌において第３章研究に関する特集記事が掲載されている。

以上の理由から本論文は、博士の学位を授与するために質・量とも十分なものであり、合格という結論に至った。