Graduate School of Advanced Science and Engineering
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博 士 論 文 概 要
Doctoral Thesis Synopsis

論 文 題 目
Thesis Theme

Establishment of Drug Screening Platform Using Nanopattern Mimicking Diseased Renal Mesangial Matrix

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Experimental models are used for understanding pathogenesis of disease, evaluating the pharmaceutical efficacy and developing therapeutics for the human body. This experimental research can be achieved by means of *in vitro* and *in vivo* models. Thus, the appropriate experimental *in vivo* and *in vitro* models are important for the human disease related studies. *In vivo* animal models address a variety of scientific questions of disease, from basic science to the elucidation of pathological mechanism, and prevention or therapeutic strategies. The use of mice as *in vivo* models for human disease is not only based on the genetic and physiological similarities between the species, but also being able to mimic any human disease or condition by genetic engineering. *In vitro* model has many advantages with comparison to *in vivo* model, such as precise control of chemical and physical environment, decrease in animal numbers, lower expense on animal maintenance and care and shorter time period with higher throughput. Mammalian cell *in vitro* model provides a defined platform for investigation of cell biology, development of tissue engineering, understanding of the pathological underlying mechanisms of diseases and cellular drug response. However, the results obtained from *in vitro* models have often failed to adequately represent the *in vivo* situation, resulting in discrepancy between the *in vitro* and *in vivo* studies. The typical culture system on petri dishes and tissue culture flasks receives completely different environments compared to those in a natural tissue nanoenvironment. These differences induce the alterations in cell morphology, responses and functions, resulting in failing to reflect *in vivo* cell behavior. If the natural nanoenvironment could be mimicked by nanopattern use for *in vitro* model, it could bridge the discrepancy between *in vivo* and *in vitro* models

Inflammatory bowel disease (IBD) is a chronic, remitting and relapsing inflammatory disease of the gastrointestinal tract characterized by inflammation and mucosal tissue damage and is associated with significant morbidity. Ulcerative colitis and Crohn's disease are the two most common forms of IBD. Ulcerative colitis and Crohn's disease differ from each other in physiology but show similar symptoms such as severe diarrhea, rectal bleeding, abdominal pain, fever, and weight loss. Clinical and epidemiological evidence suggests that IBD is a systemic disorder that can affect almost every organ. Renal manifestations and complications in patients with IBD are not rare, and numerous clinical studies have reported that 4–23% of IBD patients experience renal disease such as tubulointerstitial nephritis, nephrolithiasis, and glomerulonephritis, which eventually induce renal disease. The appropriate experimental animal model of IBD-associated renal disease thus has clinical importance for related studies, including pathological mechanisms, prevention and treatment strategies for IBD.

In this dissertation, it was hypothesized that mimicking the natural nanoenvironment by nanopattern could bridge the discrepancy between *in vivo* and *in vitro* models. To examine my hypothesis, both *in vivo* model by dextran sulfate sodium (DSS)-colitis mice model and *in vitro* diseased model by disease-mimic nanopattern were established to induce the cellular responses mimicking the renal disease condition. This dissertation has achieved 1) investigated the glomerular structural change in the experimental mice with DSS-induced colitis, 2) evaluated the effects of disease-mimic nanopattern on mesangial cells (MCs) proliferation and matrix change, and 3) evaluated the effect of TGF-β1 on diseased mimic nanopattern for establishing the drug screening platform. This dissertation consists of five chapters.

Chapter I is the introduction of this dissertation, and it presents the advantages of experimental *in vivo* and *in vitro* models and its importance in the human diseases related studies. The discrepancy between *in vivo* and *in vitro* model due to the difference in the natural nanoenvironment is also provided. In addition, this chapter introduces the solution for reducing the discrepancy between *in vitro* to *in vivo* research. Then, this chapter explains my hypothesis and purpose.

Chapter II presents the aim to investigate the glomerular structural change in the experimental mice with dextran sulfate sodium (DSS)-induced colitis. Firstly, the background of IBD and its effect on other organs due to systemic inflammation are reviewed. In addition, this chapter introduces IBD patients' experience of renal disease, which can lead to the progression of kidney injury and fibrosis, and it eventually could induce kidney failure. Furthermore, damage of glomerular function due to the reduced glomerular filtration rate (GFR) in IBD patients is also mentioned. However, because of lacking funding for glomerular structure change, which is important for the filtration function of kidney. Herein, this chapter focuses on DSS induced renal structural changes, particularly to the glomerular structure. Experimental IBD was induced by administering 3.5% DSS in Slc:ICR strain mice for eight days. Histological changes to colon and glomeruli were examined by periodic acid-Schiff (PAS) and Masson's trichrome staining. Expressions of glomerular collagens were analyzed by immunofluorescent staining and Western blot analysis. Expressions of inflammatory cytokines, interlukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and transforming growth factor beta-1 (TGF-β1) were analyzed by Western blot analysis. The results showed an elevated disease activity index (DAI), colon shortening, massive cellular infiltration and colon damage with inflammatory cell infiltration were also detected in mice after DSS administration, confirming that DSS-colitis mice can be used as an IBD animal model. In addition, DSS-colitis mice showed increased glycoprotein and collagen deposition in glomeruli. Interestingly, significant changes in glomerular collagens, including a decrease in type IV collagen, and an increase in type I collagen were observed. Moreover, higher expression of proinflammatory cytokines, IL-6, TNF-α and TGF-β1 were also detected in renal cortex. These results were not only consistent with the clinical reports in IBD patients but also mention that glomerular structure change is related to glomerular collagen change, suggesting this *in vivo* model could be useful for the study of IBD-associated renal disease.

Chapter III presents that development of an *in vitro* renal disease model to study the influence on mesangial cell behavior. In this chapter, three different fibril-forming nanopatterns mimicking the diseased mesangial matrix (MM) were designed for disease-mimic. In addition, one network-forming nanopattern mimicking the normal MM was designed for normal-mimic, and an unpatterned flat control was also used. Normal mouse mesangial cell line SV40MES13 (MES13) was used to evaluate cell morphology, cell proliferation, extracellular matrix (ECM) synthesis change and expressions of ECM synthesis related proteins after 24 h or 48 h of seeding on TiO_2 nanopatterns. The staining of F-actin filaments by rhodamine-conjugated phalloidin revealed F80/200 nanopattern induced MES13 cell to present fusiform and elongated morphology. The altered morphology of MCs could affect cell functions such as cell adhesion, proliferation and MM component secretion. The results of cell proliferation determined by 5-ethynyl-2′-deoxyuridine (EdU) proliferation assays showed a significant increase in cells cultured on disease-mimic nanopatterns, including F80/80, F80/200 and F200/80. In addition, the highest proliferation was showed in cells cultured on the F80/200 nanopattern. Aberrant MC proliferation in mesangium is commonly observed in patients with glomerular disease. Increased type I collagen and fibronectin expressions were detected in cells cultured on the F80/80, F200/80 and particularly the F80/200 nanopattern. Moreover, higher TGF-β1 expression was detected in the cell leading edge when grown on the F80/200 nanopattern compared to the other nanopatterns, revealing that the F80/200 nanopattern-induced ECM component change could be mediated by TGF-β1. The results indicated that disease-mimic nanopattern induced cell morphological change, proliferation and matrix change. In contrast, the normal-mimic nanopattern actually resulted in cells displaying normal proliferation and the production of normal MM components. These results were consistent with the *in vivo* animal model, which I established previously. These results bring the conclusion that the disease-mimic nanopattern induced disease-like cell behavior and normal-mimic nanopattern induced normal-like cell behavior.

Chapter IV presents that evaluation of physical and chemical cues on disease-mimic nanopattern as a drug screening platform. For drug discovery process, the screening of libraries containing hundreds of thousands of compounds requires screening by the disease-mimic platform. Thus, disease-mimic model is necessary. This chapter aimed to evaluate the effect of TGF-β1 on diseased mimic nanopattern for establishing the drug screening platform. Development of a new drug screening platform using disease-mimic condition including chemical cue and physical cue was evaluated by diseased matrix components synthesis. Model drugs were used to determine whether the disease-mimic condition could be controlled. Normal mouse mesangial cell line MES13 was used to investigate type I collagen synthesis change after treated by 1 ng/mL TGF-β1 on each nanopattern for 48 h. Dexamethasone (DEX, a steroid anti-inflammatotory drug, 10 nM) and A83-01 (TGF- β1 inhibitor, 1μM) were used as modelling drugs. Results showed slightly increased type I collagen expression was detected in cells cultured on disease-mimic nanopattern (F80/200) without TGF-β1, whereas no obvious type I collagen expression in cell cultured on normal-mimic nanopattern and flat control. After treating with TGF-β1, significantly increased type I collagen was detected in cells cultured on disease-mimic nanopattern. In contrast, slightly increased type I collagen were detected in cells cultured on normal-mimic and flat-control. However, significantly decreased type I collagen was detected in cells cultured on disease-mimic nanopattern after co-treating with TGF-β1+A83-01 and TGF-β1+DEX, even lower than disease-mimic nanopattern induced type I collagen expression (without TGF-β1). An appropriate biomarker is also an important part for screening platform. Reactive oxygen species (ROS) plays an important role in the modulation of inflammation-related factor production of cellular oxidative metabolism in kidney injury. ROS production was investigated in cells after treating with 1 ng/mL TGF-β1 on each nanopattern for 12 h by staining with CellROX fluorogenic probe. Results showed slightly increased ROS production was detected in cells cultured on disease-mimic nanopattern without TGF-β1, whereas no obvious ROS induction in cell cultured on normal-mimic nanopattern and flat control. After treating with TGF-β1, significantly increased ROS production was detected in cells cultured on disease-mimic nanopattern. In contrast, slightly increased ROS production was detected in cells cultured on normal-mimic and flat-control. In addition, significantly decreased ROS production was detected in cells cultured on disease-mimic nanopattern after co-treating with TGF-β1+ A83-01 and TGF-β1+DEX, even lower than disease-mimic nanopattern induced ROS production (without TGF-β1). This chapter indicates that TGF-β1 (chemical cue) and disease mimic nanopattern (physical cue) enhanced type I collagen expression and ROS production in cell. Model drugs, A83-01 and DEX, decreased type I collagen expression and ROS production in cells by physical and chemical cues. Therefore, this *in vitro* model could be used as a drug screening platform and ROS could be the biomarker for this disease-mimic model.

Chapter V describes the achievement, discussion, conclusions and future perspectives of this dissertation. Based on the results that 1) *In vivo* DSS-colitis mice model showed consistent results with clinical reports of IBD patients, and 2) *In vitro* model using disease-mimic nanopattern showed consistent results with DSS-colitis mice model, these findings could support my hypothesis that "Mimicking the natural nanoenvironment by nanopattern bridges the discrepancy between *in vivo* and *in vitro* models". In addition, this *in vitro* culture using disease-mimic physical and chemical cues could be used as a drug screening platform. Finally, this work might be applied to other organ-specific disease-mimic systems after the adjustment of the parameters according to organ-specific nanostructure and to establish the organ-specific *in vitro* fibrosis model.

早稲田大学　博士（工学）　学位申請　研究業績書

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Academic papers	1. ○**Chia-Jung Chang**, Rin Minei, Takeshi Sato and Akiyoshi Taniguchi. “The Influence of a Nanopatterned Scaffold that Mimics Abnormal Renal Mesangial Matrix on Mesangial Cell Behavior”. Int J Mol Sci. **2019** 20(21). 2. ○**Chia-Jung Chang**, Pi-Chao Wang, Tzou-Chi Huang and Akiyoshi Taniguchi. “Change in Renal Glomerular Collagens and Glomerular Filtration Barrier-Related Proteins in a Dextran Sulfate Sodium-Induced Colitis Mouse Model”. Int J Mol Sci. **2019** 20(6). 3. Hso-Chi Chaung, Chin-Dong Chang, Pi-Hang Chen, **Chia-Jung Chang**, Shyh-Hwa Liu and Chih-Cheng Chen. “Docosahexaenoic acid and phosphatidylserine improves the antioxidant activities in vitro and in vivo and cognitive functions of the developing brain”. Food Chemistry **2013**, 138: 342–347.
Conference paper	**Chia-Jung Chang**, Chih-Cheng Chen, Chin-Dong Chang and Hso-Chi Chaung. “The Anti-oxident Effects of Docosahexaenoic Acid and Arachidonic Acid on Neuronal Cells and Astrogliocytes”. International Conference on Bioscience, Biochemistry and Bioinformatics (ICBBB) 2011. IPCBEE 5:39-43 (2011).
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	Dublin, Ireland. 6. **Chia-Jung Chang**, Chih-Cheng Chen, Chin-Dong Chang and Hso-Chi Chaung. “The Anti-oxident Effects of Docosahexaenoic Acid and Arachidonic Acid on Neuronal Cells and Astrogliocytes”. International Conference on Bioscience, Biochemistry and Bioinformatics (ICBBB) **2011**. Sentosa, Singapore. Poster Presentations: 1. **Chia-Jung Chang** and Akiyoshi Taniguchi. “Influence of Mesangial Cell Behavior on Disease-mimic Mesangial Matrix”. 41th Japanese Society for Biomaterials meeting, **2019**. Tsukuba, Japan. 2. **Chia-Jung Chang** and Akiyoshi Taniguchi. “Novel Use of a Dextran Sulfate Sodium-induced Colitis Mouse Model in the Study of IBD-associated Renal Disease”. 30th Annual Conference of the European Society for Biomaterials ESB **2019**. Dresden, Germany. 3. **Chia-Jung Chang** and Akiyoshi Taniguchi. “The Effects of Nanopatterned Scaffold Mimicking the Abnormal Renal Mesangial Matrix on Mesangial Cell Behavior”. 30th Annual Conference of the European Society for Biomaterials ESB **2019**. Dresden, Germany. 4. **Chia-Jung Chang** and Akiyoshi Taniguchi. “Effects of Artificial Nanotopographical Surfaces on Renal Mesangial Cell Behavior”. MANA International Symposium **2019**. Tsukuba, Japan. 5. **Chia-Jung Chang** and Akiyoshi Taniguchi. “Establishment of Disease Model Using Nano-patterned Scaffold”. 40th Japanese Society for Biomaterials meeting, **2018**. Tsukuba, Japan. 6. **Chia-Jung Chang**, Tzou-Chi Huang and Pi-Chao Wang. “Influence of DSS-induced colitis on kidney injury and ECM change of mice”. Proc Physiol Soc 37 (2016), p345-346. **2016**. Dublin, Ireland. 7. **Chia-Jung Chang**, Tzou-Chi Huang and Pi-Chao Wang. “Influence of DSS-induced colitis on kidney injury and ECM change of mice”. The 48th Annual Meeting of The Japanese Society for Matrix Biology and Medicine, p137 (2016). **2016**. Nagasaki, Japan. 8. **Chia-Jung Chang**, Tzou-Chi Huang and Pi-Chao Wang. “Effects of DSS-induced colitis on kidney injury and ECM accumulation of mice”. Tsukuba Students Research Exchange Workshop, p16 (2016). **2016**. Ibaraki, Japan. 9. **Chia-Jung Chang**, Chih-Cheng Chen, Chin-Dong Chang and Hso-Chi Chaung. “Effects of Docosahexaenoic Acid and Phosphatidylserine on Neurexin II Gene Expression and Neuronal Apoptosis”. 2009 Joint Spring Conference of the Chinese Society of Veterinary Science and

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	the Taiwan Association of Veterinary Science and Animal Husbandry (CSVS/TAVSAH), p55 (2009). Taiwan.
Patent	Hso-Chi Chaung, Chih-Cheng Chen, **Chia-Jung Chang**. “Bioactive Liposomes and Method for Making the Same”. R.O.C. Patent I378937. Taiwan.