Report for newly appointed faculty startup

1. Name of project leader: Masamichi Imajo
2. Project title: Application of synthetic hydrogel to the control of cellular behavior and functions
3. Report

In multicellular organisms, cellular behavior and functions are strictly regulated by extracellular environment to achieve formation of complex organs and to maintain their homeostasis. Thus, proper regulation of extracellular environment has long been considered a key to successful manipulation of cellular functions in biomedical engineering. Since polymer hydrogel mimics features of extracellular environment in various tissues and can be used for cell culture and implantation, considerable efforts have been devoted to develop hydrogel with specific biological functions.

In ICReDD, I will aim to apply chemically synthesized materials to life science and medical research to deepen our understanding of biological systems and to develop novel therapeutics of incurable diseases. For these purposes, in FY2019, I aimed to develop application of chemically synthesized hydrogel, in collaboration with the Prof. Gong's group, to biological and medical research. This start up fund covered the costs of various cell lines, cell culture reagents, synthesis of hydrogel, molecular cell biology analyses, and PCs for data analysis, and so on. The achievements of this project are described below.

(1) Development of synthetic hydrogel that modulates cellular functions

To establish principles to design synthetic hydrogel with specific biological activities, I first examined what physical parameters of hydrogel have critical effects on cellular behavior. To this end, various kinds of hydrogels with distinct physical and chemical properties were synthesized from different monomers and used as substrates for culturing conventional cell lines, including non-cancerous and cancerous cells (Fig. 1). The results showed that electric charge of hydrogel strongly affects cellular adhesion, survival, migration and proliferation in many cell lines. To strictly control electric charge of

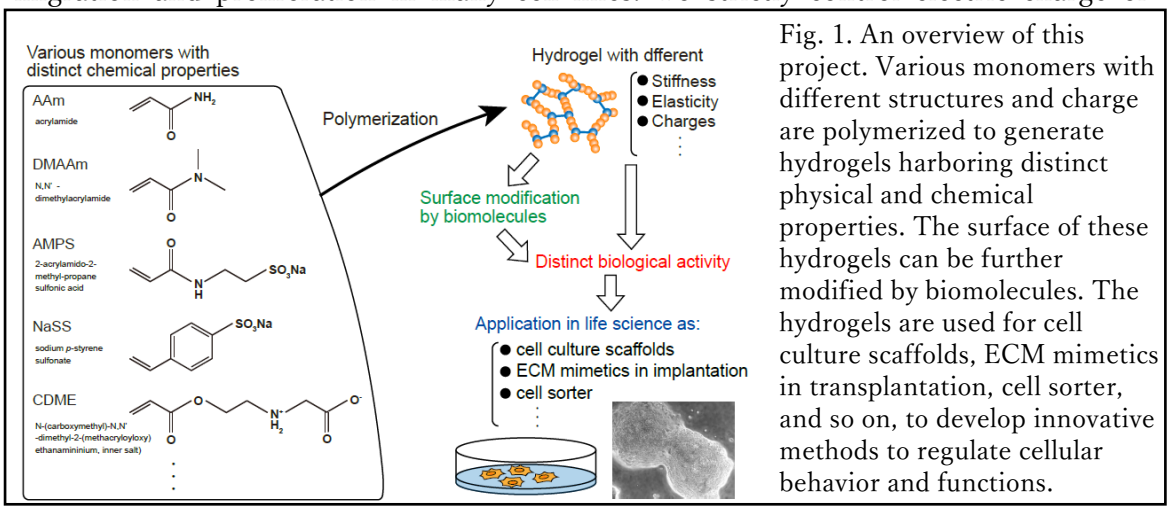

Fig. 1. An overview of this project. Various monomers with different structures and charge are polymerized to generate hydrogels harboring distinct physical and chemical properties. The surface of these hydrogels can be further modified by biomolecules. The hydrogels are used for cell culture scaffolds, ECM mimetics in transplantation, cell sorter, and so on, to develop innovative methods to regulate cellular behavior and functions.

hydrogel, we utilized the hydrogel composed of cationic ((3-acrylamidoproyl)trimethyl-ammonium (APTMA)) and anionic (2-acrylamido-2-methylpropanesulfonate (AMPS)) monomers. Since APTMA-AMPS hydrogel has good biocompatibility and its electric charge could be controlled by changing the ratio of two monomers, we found that APTMA-AMPS hydrogel is an ideal substrate to control cellular functions through changing electric charge of the scaffold matrix. These findings would be a basis for designing hydrogel with specific biological activities in future studies.

(2) Elucidation of molecular mechanisms that mediate cellular responses to electric charge of the scaffold matrix

The above findings that electric charge of hydrogel significantly affects cellular behaviors have suggested that cells are equipped with some mechanisms to sense and respond to electric charge of the surrounding materials. To reveal such mechanisms, I conducted large-scale integrated analysis of transcriptomic and proteomic changes caused by alteration of electric charge of the hydrogel scaffold. I identified more than 1,000 transcripts and 3,000 proteins whose abundance is regulated by electric charge of the scaffold matrix. Notably, in several cancer cell lines, expression of genes comprising a stem cell signature was increased by a certain range of electric charge, suggesting that stemness of cancer cells is regulated by electric charge of the scaffold matrix. These results, for the fist time, identified genes and proteins that respond to electric charge of the scaffold matrix, which should pave the way for future elucidation of cellular mechanisms for sensing and responding to electric charge of the surrounding environment.

(3) Application of synthetic hydrogel that modulates functions of pluripotent stem cells

As described above, electric charge of hydrogel affects expression of genes comprising a stem cell signature. Thus, the effects of electric charge of hydrogel on pluripotent stem cells, such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), were investigated in collaboration with a postdoctoral researcher, Dr. Akira Hirota, in the Prof. Tanaka group. We found that a certain range of electric charge promotes, whereas opposite electric charge suppresses, differentiation of mouse ESCs. Importantly, on the hydrogel with certain electric charge that suppresses the differentiation, ESCs could maintain their naive pluripotent state even in the presence of humoral factors inducing the transition to the primed pluripotency. These results indicate that functions of pluripotent stem cells can be controlled by modulating electric charge of the scaffold matrix. In future studies, we will perform detailed analyses of the effects of electric charge on the functions of human iPSCs and the underlying molecular mechanisms, thereby aiming to develop novel methods that contribute to advancing the regenerative medicine.

(4) Establishment of a novel culture method for intestinal stem cells by using synthetic hydrogel

Tissues exposed to environmental assault require constant renewal driven by tissue-resident stem cells. In these tissues, defects in stem cell functions have been implicated in many diseases, and transplantation of stem cells cultured and expanded *in vitro* has been considered a promising strategy to promote tissue repair and to induce disease remission. An example of such tissues is the intestinal epithelium, in which chronic inflammation (inflammatory bowel disease (IBD)) has been shown to impair the epithelial barrier function and transplantation of intestinal stem cells (ISCs) could be a novel therapeutic approach to treat IBD (Fig. 2). However, the current methodology for culturing ISCs includes complicated procedures and is not suited to expand ISCs sufficiently for in vivo transplantation, which hampers clinical implementation of the ISC transplantation therapy of IBD. In this project, I aimed to develop a novel innovative method that enables rapid and convenient culture of ISCs by using chemically synthesized hydrogel. By modulating physical properties of hydrogel and formulation of culture media, I found condition under which mouse ISCs can adhere to the hydrogel, maintain their undifferentiated state, and proliferate vigorously. Thus, although further optimization of the culture condition and validation of ISC functions are needed, I have successfully developed a prototypic method for culturing ISCs. Since this novel culture method has clear advantages over the conventional culture method, I decided to apply for the AMED grant to further optimize the culture condition, to adjust the method to the human ISC culture, and to establish application of cultured ISCs to the regeneration therapy for IBD. Thus, the achievements of this grant have provided a basis for the next important project that will be supported by external funding in future.

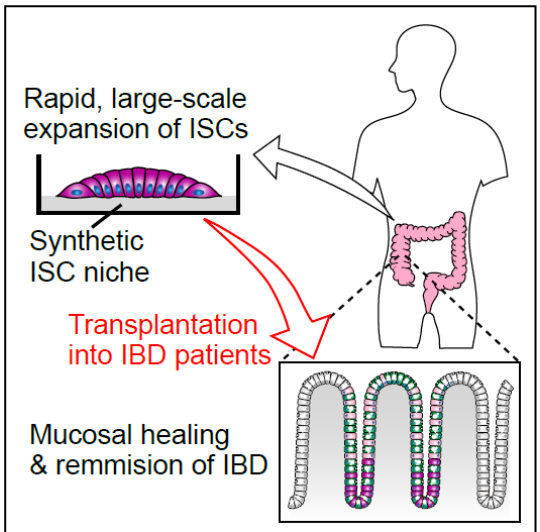

Fig. 2. A graphical abstract of a concept of stem cell transplantation therapy for IBD.

4. Research achievement

[Publication]

Masamichi Imajo. Analysis of Retinoic Acid Receptor Signaling in Colorectal Cancer, Methods Mol. Biol., Retinoid and Rexinoid Signaling, 85-93, 2019

[Presentation]

Masamichi Imajo, Yu Muta, Michiyuki Matsuda. Alterations of ERK activity dynamics underlying tumor-specific traits in the intestinal epithelium. The 38th Sapporo International Cancer Symposium, 2019