

Preface

Hyper Bio Assembler for 3D Cellular Innovation (Bio Assembler) is a 5-year research project that started in July 2011 with the support of a Grant-in-Aid for Scientific Research on Innovative Areas from the Japanese Ministry of Education, Culture, Sports, Science and Technology. The aim of the Bio Assembler is to build 3D cellular systems that can function in vitro. This is an entirely new research field that has not been previously explored. The Bio Assembler will elucidate the principles of ultra-high-speed measurement, the manipulation techniques, and function expression of cells and tissues.

One of the features of the research project is the close collaboration between robotics experts, who specialize in micro-nanoscale measurement and manipulation, and biological and medical researchers, who construct stem cells and multicellular systems and aim for the medical application of these systems. Another feature is that these experts and researchers work together to create an innovative methodology (the Bio Assembler) that will be able to measure and sort target cells at high speed and construct 3D cellular systems in vitro. By understanding the various characteristics of 3D cellular systems and establishing a technology for constructing them, we hope to make a breakthrough in tissue engineering/biology and establish a new technical area of high-speed micro-nano robotics. Our final goal is to combine the work done in these three research areas in order to create artificial 3D cellular systems with morphologies and functions that can be used in tissue engineering.

We have so far explored this field and obtained new knowledge for creating an artificial 3D cellular system that can be used in regenerative medicine, and established innovative measurement-control technologies based on micro-nano robotics. We now have summarized the results of the project and published our achievements in the innovative field of the Bio Assembler as a book.

The structure of the book reflects the structure of the new research field consisting of three research areas. Part I, “Cell Sort Engineering”, describes the research in the area of measurement and control of cell characteristics, which focuses on measuring the physical properties of cells taken from living organisms at high speed, and sorting the target cells that are useful in constructing cellular systems. Part II, “3D Cellular System Design”, describes the research in the area of “3D cellular system assembly”, which aims at shaping and assembling 3D cellular systems with

complex morphologies. Part III, “Sociocytology”, describes the research in the area of analysis and evaluation of 3D cellular systems, which analyzes and evaluates the growth, differentiation-inducement, morphogenesis controls, and transplantation responses of the constructed 3D cellular systems, and conducts functional elucidation as well as comparison and verification *in vivo*, looking for ways to apply our outcomes to regenerative medicine.

Part I Cell Sort Engineering

Understanding the various properties of cells is crucial. Moreover, ultra-high-speed sorting of active and uncommon cells is important in the construction of three-dimensional cellular systems. This part of our research focuses on methodologies for measuring and sorting cells at high speed. Recently, fluorescent imaging using an optical microscope is being widely used to study the properties of cells. However, cellular mechanical properties are not well investigated. In this part, measurement methods of cellular mechanical properties using high-speed micro-nano robot technologies are shown. In the case of floating cells or objects such as spheroids, microfluidic chips can be used for continuous and sequential measurement. Chapter 1 describes a mechanical characterization method of floating cells which uses an on-chip robot with a microfluidic chip. The mechanical properties of floating cells are measured one by one continuously on the chip. Chapter 2 describes dimensionless evaluation of the deformability of floating cells using a microfluidic chip. Through the dimensional analysis performed on the microfluidic chip, three dimensionless parameters determining stiffness-based deformability are derived, and a new index is introduced based on these parameters. Chapter 3 describes the real-time image processing for active sensing of cells flowing in the microfluidic channel using a high-speed vision system. On the other hand, for the continuous and sequential measurement of the mechanical properties of adherent cells, the atomic force microscope is a more suitable and powerful system. Chapter 4 describes high-throughput measurements of single-cell rheology using atomic force microscopy. As cells have a variety of different properties, sorting techniques are needed for the construction of three-dimensional cellular systems. Many methodologies for sorting of specific cells have been proposed. Chapter 5 describes the application of dielectrophoresis for the discrimination of cells with specific antigens expressed on a cellular membrane. This method is suitable for the high-speed sorting of floating cells. As an example of an application of methodologies for measuring and sorting cells at high speed, Chapter 6 analyzes the physical characteristics of hematopoietic cells using microfluidics-based devices, which ensure efficient detection of rare cell populations in circulating blood. New methodologies for measuring and sorting target cells at high speed are quite important for the Hyper Bio Assembler for 3D Cellular Systems. The methods introduced here will contribute to a better understanding of the various properties of cells and of the synthesis of complex biological tissues in the future.

Part II 3D Cellular System Design

High-speed robotic construction is crucial to achieve three-dimensional cellular systems in vitro. Part II of our research, “3D Cellular System Design”, looks into ways of achieving the construction of 3D cellular systems that function as tissue, and proposes innovative construction technologies for 3D cellular systems based on micro-nano robotics, microfluidics, and MEMS, starting from the construction of simple 3D cellular systems and ultimately aiming to build tissue with a high oxygen requirement. Significant progress has been made by working together with front-line researchers toward this goal.

This part covers each step of the construction methodologies, from 1D and 2D structures, up to large-scale, complicated 3D tissues. In Chapter 8, we discuss typical expansion methods from 1D to 2D, which provide high-throughput cell assembly featuring heterogeneous hydrogels produced by using microfluidic devices. Single cells are encapsulated at high speed in hydrogel materials, which function as unit structures in constructing further large tissues. Expansion methods from 2D to 3D are shown in Chaps. 9, 10, and 14. Chapter 9 describes an on-chip fabrication technique based on the manipulation and self-assembly of 3D cellular structures. Chapter 10 describes fabrication of 3D cellular tissue utilizing MEMS technologies based on the folding method and a micro magnetic plate. In Chapter 14, we propose a device for rapid transfer of living cell sheets, named “cell scooper”. The cell part assembly and the introduction of vascular-like structures are the most crucial technologies for building large tissues. Chapter 7 introduces cell manipulation and cellular part assembly using micro hand systems and other robotic devices. Chapter 12 proposes an approach for engineering 3D tissue with inner tubular structures, based on electrochemical cell detachment. This approach can be applied to fabricate vascularized bone, liver, and other tissues, by integrating parenchymal cells and other biomaterials. Furthermore, in Chapter 11, we show photo fabrication techniques that enable the construction of tissues using hydrogel. Quantitative evaluation of cell–hydrogel adhesion by advanced optical techniques is shown in Chapter 13.

For the “Hyper Bio Assembler for 3D Cellular Systems”, the technology of constructing large tissue-like structures with target cells is very important. The new methodologies introduced in this part will contribute to the realization of 3D cellular systems in vitro, and provide a better understanding of artificial tissues.

Part III Sociocytology

In life sciences, advanced technologies including rapid DNA sequencing, flow cytometry, confocal laser scanning microscopy, and transgenic animals have made remarkable progresses. We expect the “Hyper Bio Assembler” project to provide deep insights into the underlying mechanisms of living beings, as well as diseases, and the basis for preventive, diagnostic, and therapeutic strategies, including regenerative medicine.

In Part III of our study, we brought together several papers describing exciting topics for research in the life sciences from the point of view of sociocytology. Although tissues, organs, and all the multicellular living beings are composed of various types of cells and the extracellular matrix, in traditional, conventional biology based on reductionism, purified and isolated cells were subjected to investigation, and soluble factors have been believed to be the major factor to play a role in regulating cell functions. Recently the importance of interactions between various types of cells and solid extramatrix have been given much attention. We would like to call this novel approach, “sociocytology”, as we believe that by introducing micro nanorobotics tools into this new field, the revolutionary understandings of cells and biology will be realized. In Chap. 15, the surrounding microenvironments modulating the growth and morphology of 3D tissues on hydrogels with different mechanical stiffness are discussed. Chapter 16 introduces calcium phosphate-based scaffold materials used for regenerating hard tissues in bone defects. A microfabricated culture device for assembling the spheroids of chondrocytes in a 3D cellular construct is also described in this chapter. Chapter 17 presents an in vivo imaging method, which sheds light on the organogenesis of transplanted liver buds, derived from induced pluripotent stem (iPS) cells. Chapter 21 describes the in vitro analysis of the formation and mineralization of bone tissue, derived from bone marrow stromal cells (BMSCs). Chapter 22 describes cell sheet-based bio-assembler technologies used for creating multicellular, functional 3D tissues. With the aid of advanced robotics and biomechanics, these technologies should solve problems that former technologies were not able to solve. A bionic simulator-on-a-chip using organ explants of embryonic chick heart is presented in Chap. 18. In particular, mechano-regulation of cell and tissue functions and properties are quantitatively described here. Also we present “old” unanswered questions that can be re-examined with these increasingly powerful methods. In Chap. 19, an in vitro 3D imaging system for the tempo-spatial dynamics of cellular mechanics which uses fluorescent microscopy and digital image analysis is presented. Chapter 20 describes the in vitro imaging of cellular migration and invasion on 3D matrices for evaluating tumor invasion.

Through shedding light on sociocytology, major discoveries may be made in the future. We believe the Hyper Bio Assembler can provide the ideal tools for the rapid dissemination and discussion of all aspects of sociocytology, including the interactions among various cell types and between cells and the extracellular matrix.

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