Elucidating organ engineering toward regenerative medicine

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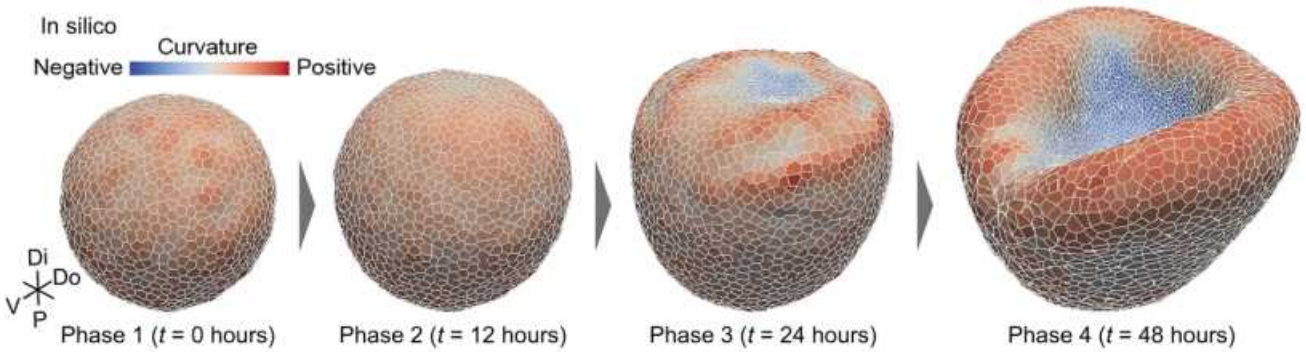

Figure 1. Simulation results of optic cup formation in Okuda et al.[1]

Many incurable diseases still remain today, such as Alzheimer's disease, dementia, Parkinson's disease, lumbar spondylosis, heart failure. Most of those diseases are caused by cellular aging, a problem that is becoming worse as life expectancy increases. Furthermore, when organs are severely damaged or dysfunctional, they cannot be cured by current pharmaceutical treatments. To solve this major problem, organ transplant is a prime candidate. However, donor-based transplantation has challenges such as immune rejection or lack of donors. Therefore, regenerative medicine using stem cells is expected to be a promising solution.

We, living things, started from a single cell, and this cell proliferated and differentiated appropriately to form the brain, eyes, heart, liver, and other parts of our complex body. Utilizing this ability, embryonic stem cells (ES cells) have been engineered from the internal cell mass in blastocyst, a structure formed in the early stages of mammalian development[2,3]. Research on these ES cells has advanced our understanding of developmental processes and organ morphogenesis. In addition, Yamanaka's group established a reprogramming technique to revert differentiated somatic cells into undifferentiated induced pluripotent stem cells (iPS cells)[4], enabling ethical acquisition of stem cells and greatly advancing regenerative medicine.

However, there are still many challenges to realize in regenerative medicine. In order to perform regenerative medicine, it is necessary to be able to engineer the exact organ adapted to the patient. Yet, so far, the creation of complete organs such as the brain, heart, and liver from ES and iPS cells has not been achieved. Artificial organ-like structure that develop from those stem cells and recapitulate organ-specific functions is

called “organ-oid”, organoid (although the detailed definitions vary from paper to paper[5]). Organoids exhibit many interesting features of developmental processes, but are not as mature as real organs in both morphology and function. To overcome this, many researchers are trying to improve culture protocols and sometimes using genetic approaches.

My personal opinion is that more mechanical interpretation is needed to understand and improve the morphological development of organoids. Elucidating which cells proliferate or differentiate and what signals are used during development is a major goal for organoid researchers. One important aspect of this signaling is mechanotransduction, which is the process of sensing and responding to stress from the surrounding environment. For example, mesenchymal stem cells, which have the ability to differentiate into nerves, muscles, and bones, have been shown to change their cell type in response to the stiffness of the surrounding material[6]. However, there is no universal method for non-invasively measuring the stress applied between cells, so it is necessary to devise conditions and select an appropriate measurement method depending on the situation. Furthermore, the instruments and analyses are highly specialized and difficult for non-specialists to use. While it is very difficult to clarify the mechanical properties of organoids, such research is also required to understand the organoid system.

With this in mind, beautiful experiments using organoids have been conducted by Eiraku’s group. He has been focusing on the mechanical properties of complex deformation and engineered the first organoid of an optic cup, which is the origin of a retina in Sasai’s Group[7]. In this study, the optic cup was assumed to be axisymmetric, and a two-dimensional deformation model was designed using the vertex model. In the vertex model, the cells are approximated as polygons, and the time evolution of the vertices that define cell boundaries is determined by appropriately constructed free energy[8]. Later, Okuda et al. in his group revealed more detailed properties[1]. They designed the three-dimensional vertex model, in which cells are modeled as polyhedrons and time-developed by the free energy determined by volume, height and total surface area of each cell and perimeter, area, and curvature of apical and basal surface (**Fig. 1**). The parameters of the model were determined from the natural values measured in several experiments. Furthermore, they extended the model by considering the increase and decrease in cell number, i.e., cell division and apoptosis (programmed cell death). They succeeded in the recapitulation of the three-dimensional deformation process of the optic cup formation by computer simulation. Also, various phenotypes were obtained by simulating with different

parameters, and these phenotypes are consistent with experiments using activators/inhibitors that activate/inhibit the function of specific enzymes. Additionally, they predicted intercellular stresses from simulations and validated the estimated stresses with a combination of several experiments, including incisions with micro-tweezers, activation/inhibition of myosin activity involved in force generation, and monitoring of calcium transients, which are known to cause cell contraction. They used physical modeling, computer simulations, and biological experiments to discover the cell's deformation rules and feedback system for mechanosensing. The precise mechanism of force generation by myosin revealed in this study could be applied to a wide variety of morphological deformations of cell populations.

These mechanistic studies will allow us to evaluate protocols by observing cell interactions, which will contribute to protocol refinement. In addition, model-based simulations may provide optimal cell ratios and situations at a lower cost than experiments. However, these studies alone are not sufficient. Of course, genetic and biochemical studies on organoid development are both necessary and very important. Moreover, understanding the biological phenomena of animals that are close to humans will provide the basis for our knowledge and ideas for analyzing cell behavior. In addition to technical issues, solutions to legal and distribution issues are also necessary for the realization of regenerative medicine.

There are great expectations for regenerative medicine, which has the potential to eliminate intractable diseases. Realizing this technology presents many challenges in various fields. In particular, the study of mechanical properties requires a lot of experiments, while analysis and calculations may be too complicated for non-specialists. However, thanks to several experiments, the engineering of organoids by cell assembly is becoming clearer in terms of physical modeling, computer simulation, and biological experiments. In the future, human research, which has developed over a wide range of fields, will be integrated to elucidate the engineering of organs, and this will be mimicked or utilized to design protocols and realize regenerative medicine.

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