Cytocompatible and multilayered phospholipid polymer hydrogel for regulation of cellular behavior

その他のタイトル	細胞挙動を調節するための細胞適合性リン脂質ポリマーハイドロゲル多層膜
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博士論文（要約）

Cytocompatible and multilayered phospholipid polymer hydrogel for regulation of cellular behavior

（細胞挙動を調節するための細胞適合性リン脂質ポリマー

ハイドロゲル多層膜）

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The primary objective of this dissertation is to create a defined Three-dimensional (3D) cell culture model that provided a multilayered hydrogel platform for the spatial patterning of cells, temporal control over soluble molecule diffusion within 3D model, and the ability to monitor cellular behavior throughout the culture period. Figure 1 shows overview of this thesis.

Chapter 1 Motivation and design of the thesis

3D *in vitro* models span the gap between 2D cell cultures and whole-animal systems. These models can replicate some features of the *in vivo* architecture and allow control of the degree of homotypic and heterotypic cell–cell interactions, enabling more accurate quantitative studies. However, the application of these microfabrication technologies to elucidate the underlying mechanism is still a challenge.

As shown in Figure 2, the main reason for the slow progress in this area is the complexity of the *in vivo* microenvironment. For example, for more detailed information on indirect cell–cell interactions, they should be separated from other parameters and studied following the "cue–signal–response" rules in a simplified environment. Another probable reason is that most microfabrication technologies are complex and require significant preparation and instrumentation not commonly available in biology laboratories.

To solve these issues, the experimental design should incorporate following aspects:

a) Suitable biomaterials for 3D cell culture.

b) Easy availability, low cost and simple manipulation.

c) Ability of patterning of multiple types of cells within 3D scaffolds with spatially localized gradients of soluble molecules.

d) Control the diffusion of these soluble biomolecules to provide temporal administration of cellular stimuli.

e) Systems to monitor cellular behavior within these environments throughout the experiment without sacrificing cells.

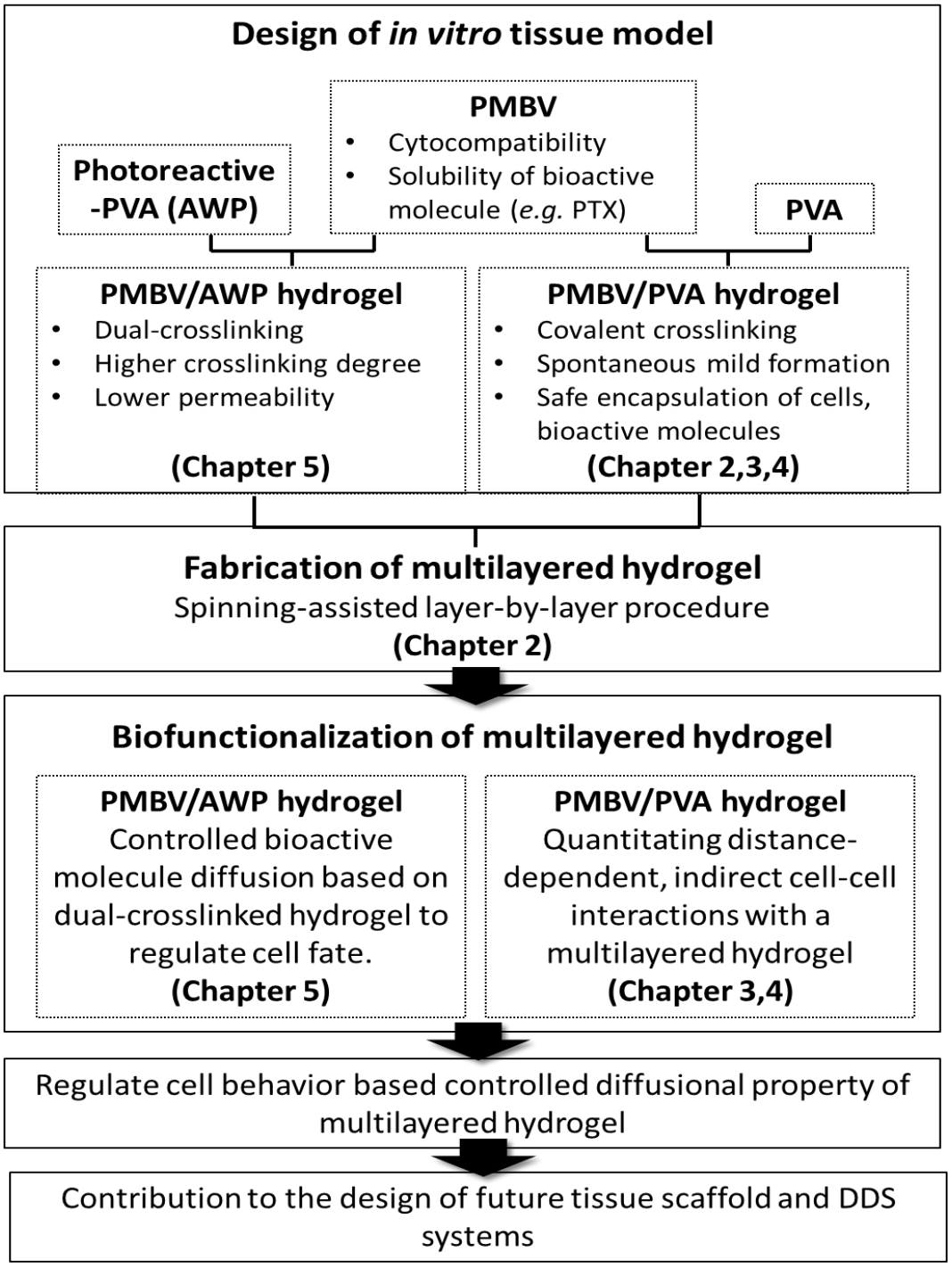

Figure 1 Overview of this thesis.

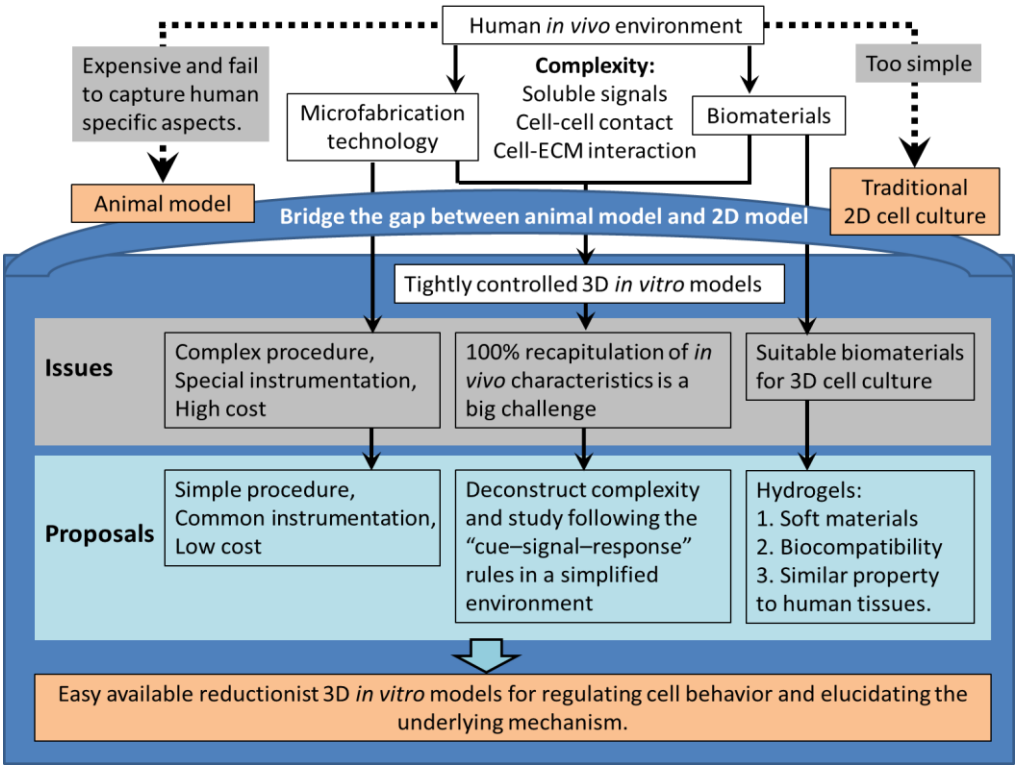

Figure 2 Motivation of this research to develop an easy available reductionist 3D *in vitro* model for better understanding *in vivo* environment.

According to these aspects of experimental design, the following contributions have been done for this project:

Chapter 2 Fabricate of PMBV/PVA multilayer hydrogel

The contribution of this chapter is developing an easily applicable and generic approach to construct PMBV/PVA multilayer hydrogels by using spinning-assisted layer-by-layer (LbL) procedures. The LbL assembly is based on the chemical reaction between PMBV and PVA in aqueous medium. The thickness of the multilayered hydrogel is regulated by number of gel layers. This approach is compatible with existing laboratory techniques and instrumentation. All of multilayered hydrogels were built on commonly available 35 mm cell culture dish. A commercial centrifuge was transformed into spin-coater.

Chapter 3 Fabrication of precise spatial cellular multilayered phospholipid polymer hydrogel

The contribution of this chapter is development of the multilayered hydrogels that allowed the design of cell patterning gels by building 2 cell-laden layers separated by PMBV/PVA multilayer hydrogel. These multilayered hydrogels that mimicked stratified structure of human

tissue were stable in cell culture medium and suitable for cell incorporation due to tens of micrometer scale thickness. The cells remained alive during the spinning process and maintained their metabolic activity for at least 24 h.

Chapter 4 Quantitating distance-dependent, indirect cell-cell interactions with PMBV/PVA multilayer hydrogel

The contribution of this chapter is making use of finely tuned structure and property of PMBV/PVA hydrogels to build up a customized reductionist 3D *in vitro* model for exploring the relationship between diffusion distance and indirect cell–cell interactions (soluble factor interactions), things that have seldom been investigated experimentally. Due to the presence of MPC units in hydrogels, a key feature of this hydrogel system is that it permits the diffusion of soluble factors and decouples soluble signals from other signals (cell-cell contact and cell-ECM interaction). In a case study, I have used this 3D model for patterning tumor cells and stromal cells and quantitatively modulating soluble cell–cell signaling. Cell behavior was evaluated by combination of FUCCI fluorescent reporter technology and microscopy. Then I observed clear differences in the dynamics of cell cycle progression in HeLa-Fucci cells depending on the distance separating them from co-cultured cells.

Chapter 5 Regulation of cells encapsulated in a multilayered hydrogel containing a bioactive reagent

The contribution this chapter is control of cell behavior based on diffusion of bioactive reagent that was layer-specific loaded within multilayered hydrogels. This 3D model was sandwich constructed, where cell-containing gel layer was separated from PTX reservoir layer by a spacing of dual-crosslinked PMBV/AWP hydrogel for controlled PTX diffusion. It was also shown that the permeability of dual-crosslinked hydrogel was so low that it worked as a diffusion-controlling barrier and barrier effects could be finely controlled by varying thickness of diffusion-controlling barrier and PTX concentration. I found that PTX induced weaker apoptotic effects of HeLa cells as barrier thickness increased and PTX concentration decreased.

Finally, these achievements will aid in the elucidation of how cells behave within spatially defined microenvironments similar to physiological tissue. It is clear that a great deal of work remains to fully understand the underlying mechanism. However, I believe that this work provides initial insights that may prove useful in guiding the design of future 3D cell culture systems.