SUMMARY OF Ph.D. DISSERTATION

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Title

Design and control of cell adhesion and construction of a sheet-like cell structure by surface roughness of a particle monolayer

Abstract

Cell adhesion is important for not only formation and maintenance of cell shapes but also regulation of cell functions. In other words, cells change their shapes in response to properties of substrate, resulting expression of cell functions. We often use a culture dish for in vitro cell culture system, however, cells adhering onto a flat culture dish are likely to lose their cellular functions. Therefore, the culture substrate which controls cell adhesions and cellular functions is required in the field of biomaterials and tissue engineering. In this study, we intended to control cell adhesions and cell functions using a particle monolayer. We developed the methods for preparation of a cell sheet by controlling cell adhesion. In addition, we intended to control the shapes of individual cells and a cell sheet by photopatterning of bovine serum albumin (BSA) and by photolithographic technique.

1. Preparation and evaluation of particle monolayer as a culture substrate

Poly (styrene-co-acrylamide) particles (SA particles) were prepared by soap-free emulsion copolymerization. The diameters of SA particles ranged from sub-micrometer to micrometer scale and were monodisperse in size. The monolayer of particles, which were 527 nm (SA527) and 1270 nm (SA1270) in diameter, was prepared by Langmuir-Blodgett deposition. By using field emission scanning electron microscope (FE-SEM) and scanning probe microscope, we showed that particle monolayers consisted of closely-packed particles and had well-ordered indented surfaces. To evaluate the effect of particle monolayer on cell adhesions, cell shapes and cytoskeletons, human umbilical vein endothelial cells (HUVECs) were seeded onto a particle monolayer, incubated in culture medium and observed using optical and fluorescent microscope and FE-SEM. We found that cell-substrate and cell-cell adhesions could be controlled by the surface topology of particle monolayer.

2. Preparation of a cell sheet by using a particle monolayer

To investigate cell detachment from culture substrate, cell monolayer formed on a substrate was pipetted by micropipette. We demonstrated that cells adhering onto an SA527 particle monolayer could be detached as a cell sheet just by pipetting. Prepared cell sheet could reattach onto a culture dish within 20 min, because cell sheet had the layer of extracellular matrix on the bottom side of a cell sheet. This result indicates that a cell sheet prepared by using particle monolayer could be used as a tissue graft. This technique will be a promising method for tissue regeneration.

3. Control of shapes of individual cells and a cell sheet by using cell patterning techniques

At first, we intended to control cell adhesion by patterning photo-reactive BSA onto particle monolayer. It could be observed that cells preferentially adhered onto the region where BSA molecules were not immobilized. Then, we intended to control the shape of a cell sheet by using photolithographic technique. We prepared a patterned particle monolayer by UV patterning of photoresist polymer. Cell monolayer formed on a patterned particle monolayer could be peeled by gentle pipetting, resulting in formation of a patterned cell sheet.