論文の内容の要旨

論文題目　Effects of Mechanical Stimulations on Cultured Cells in the Presence of Immobilized Biological Macromolecules
(固定化生体高分子存在下における培養細胞に対する機械刺激の効果)

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Cells sense external biochemical signals and applied physical forces, and act in response to these inputs. Development of molecular biology has revealed various biological phenomena induced by various biological molecules and provided various medical applications. On the other hand, recent progress of mechanobiology has also increased our understanding how mechanical inputs regulate cell behaviors and how the deregulation might induce the disease. The physical stimuli include shear stress, stretch force, compression, interstitial flow, osmotic pressure, tension, and the physical properties of contacting substrate and some of them are employed for medical therapeutics. Considering these situations, the combination of biological signals and physical inputs is expected to develop a new field of medical science and therapeutics. However, the combination of these two stimuli has not been investigated so much. Therefore, this dissertation focused on the combination for mechanobiological progress and medical application.

This dissertation is composed of five chapters involving Chapter I of introduction with references and Chapter V of conclusion and perspectives in addition to Chapters II-IV summarizing the research achievements.

Chapter I describes the background of mechanobiology and the objectives of this study. First, short history of cell biology including the basic structure and cytoskeleton. Secondly skeletal system is described including healing stages and bone remodeling. Thirdly the background of mechanobiology studies including mechanical sensing system, and the impact on physiology and pathology is described. Fourthly biomaterials science is summarized from the points of biological signaling, stiffness, topographical cues, and combination with mechanobiology. The mechanobiology on the bio-signal immobilized materials is provided. Finally, as the objectives of this thesis, how the bio-signal molecules were immobilized on materials which are available for physical stimuli, and the objective of each chapter is described. For immobilization of bio-signal molecules, photo-immobilization and mussel-inspired methods were employed on silicone elastomer and polystyrene surfaces. As the bio-signal molecules gelatin and insulin-like growth factor were used as a cell adhesion protein and a cell growth protein, respectively. For physical stimuli, mechanical stretching and ultrasound were applied.

In Chapter II, as a study of mechanobiology, to apply the physical stretching on cells silicone elastomers (SE) was employed as a substrate material, because it is flexible, stretchable and non-toxic. SE has linear elastic behavior, meaning that their bulk properties are not altered by compression, shear,

hydrostatic and tension forces. However, it is a bioinert biomaterial to prevent any kind of interaction with biological tissue. To allow cellular adhesion, immobilization of adhesion promoters such as gelatin, collagen, fibronectin, etc. is usually required. Previously in order to immobilize bio-signal molecules, lithography and etching procedure were utilized on SE through the several step methods. Additionally, soft lithography requiring the fabrication of a stamp, which is used to transfer biological macromolecules onto the substrate by direct contact printing was also reported on SE. However, since these immobilization linkages were fundamentally due to physical adsorption (van der Waals forces), they were not stable. Therefore, this study used photo-immobilization method which enables covalent immobilization to SE. The surface of SE was modified with photo-reactive gelatin bearing azidophenyl groups.

Two types of photo-reactive gelatin were prepared: one by coupling with azidoaniline and another by coupling with azidobenzoic acid. The silicone surface was hydrolyzed by oxygen plasma and then gelatin was micropatterned on the surface using a photomask. The surface wettability was tuned by these treatments. The thickness of the gelatin layer was measured by a reflective confocal laser microscope, and it was regulated by the amount of loaded gelatin. From the cell adhesion test, cell adhesion rate was significantly enhanced by immobilization of gelatin on the surface, and the enhancement was dependent on the type of modified gelatin. Surface coated by azidoaniline-coupled gelatin showed higher cell adhesion activity than aziobenzoic acid-coupled one due to its enough positive charge. The stripe-pattern immobilization regulated the shape of cells adhered to silicone and high aspect elongation of the cell was observed. Although substrate which was homogeneously coated by gelatin showed the same tendency of fibroblasts (perpendicular orientation) against stretching stress as the non-immobilized surface, the micropatterned gelatin surface resisted such deformation by stretching stress. Microscopic observation showed that cytoskeleton fiber formed, oriented, and resisted the shape change by mechanical stress, although some reorganization of the cell cytoskeleton was observed. This study suggests that cytoskeleton fiber formation and orientation are important for the response to mechanical stress.

In Chapter III, the cellular response in the form of shape and actin cytoskeleton rearrangement towards cyclic mechanical strain instead of static mechanical strain was investigated on the gelatin surface either with or without micropatterns. Two types of cell line, fibroblast and osteoblast, were selected because they were known as a stretch-responsive cell. Sinusoidal strains were applied in pathological range at short interval of 4 hours. After cyclic strain, on the immobilized gelatin surface without patterns, both cells behaved with different tendency. Fibroblasts showed shorter and spherical phenotypes and accumulation of actin-monomers, while osteoblasts remained their cellular shape and perpendicular alignment of actin-polymers to the strain direction. Both cells showed a similar behavior after mechanical strain on the narrow micro-patterned gelatin surface. Fibroblasts and osteoblasts demonstrated the morphological shrink (became spherical and isotropic) and the actin cytoskeleton

degradation. The narrow stripe-pattern adhesive gelatin by strongly rearranging the actin cytoskeleton controlled the cellular shape and orientation and the unique cell-type specific response became united towards dynamic strain. The importance of patterns against physical stress was demonstrated.

In Chapter IV, as another external force, ultrasound in the form of low intensity pulsed ultrasound (LIPUS) was applied to the osteoblast cells in cooperativity with immobilized growth factors. For the immobilization of the growth factors, insulin like growth factor (IGF) was designed with mussel-inspired peptide, which allows binding on different kind of surfaces. The differentiation of osteoblast on IGF-immobilized substrate was analyzed with and without LIPUS. As a result, a combination of LIPUS and immobilized-IGF showed higher differentiation with upregulated cytoskeleton proteins, compared with each separately applied LIPUS or immobilized-IGF. This study shows the important sight of the cooperative and additive effect of LIPUS with IGF-immobilized surface on the differentiation of osteoblast.

Chapter V summarizes the conclusions of each chapter and describes the future outlook of the combinatorial uses of physical stimulation (stretch/ultrasound) and biochemical stimulation (adhesive pattern/growth factor). The combination will contribute a new insight of medical sciences and various applications through the control cell shape, orientation, actin dynamics and differentiation.