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論 文 要 旨

THESIS SUMMARY

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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

The dissertation, entitled "Cell behaviors in response to protein-immobilized surfaces and mechanical forces", aims to investigate the synergistic effect of biochemical stimuli from protein-immobilized surfaces and mechanical stimulation on cell behaviors, such as unicellular morphogenesis and osteogenic differentiation.

In Chapter I, the complex biochemical and physical microenvironments around cells are reviewed. The biomaterial scaffolds employing cell adhesion molecules, growth factors, and their micropatterning are discussed. Subsequently, physical forces applied to control cell behaviors are introduced. Finally, current applications of mechanical forces and biomaterials are summarized. The research aims and outline in this thesis are described.

In Chapter II, the morphology change of cells on micropatterns by cyclic stretch was studied. It's found that cyclic stretch can cause shape shrinkage of highly elongated cells, while elongation of cells with low initial extension level on gelatin micropatterns. Here, fibroblasts geometrically confined by photo-immobilized gelatin micropatterns on a silicone elastomer were subjected to cyclic stretch for 20 hours. The surface of the elastomer was plasma-treated, coated with the prepared phenyl azide-modified gelatin, and photo-irradiated with or without a photomask with strip micropatterns of different widths. Micropatterns of various widths successfully induced cell alignment and polarization along the gelatin hills, as seen by a markedly smaller cell area and longer cells compared to the non-pattern surface. 10-μm pattern with a broader space for cell adhesion resulted in larger and longer cells compared to the 2-μm pattern. The mechanical force did not affect cell growth but significantly altered the cellular morphology on both non-patterned and micropatterned surfaces. It was found that cells on non-pattern surfaces showed a perpendicular re-orientation against stretch directions, inducing increasing cell length and decreasing cell area under the stretch. The width of strip micropatterns provided a different extent of contact guidance for fibroblasts. Cells with a high extension on 10-μm pattern would contract under parallel cyclic stretch, while cells with a low extension on 2-μm pattern kept elongating along the micropattern after stretch. The vertical stretch against the strip micropattern induced an increase in cell area and length more than the parallel stretch in both 10-μm and 2-μm patterns. Based on the results, it's hypothesized that cyclic stretch causes opposite shape changes according to the initial extension level. The hypothesis was verified by the decreasing cell shape of highly extended pre-osteoblast cells after cyclic stretch on 10-μm patterns. These results addressed an unresolved question regarding how aligned cells modulate their shapes in response to external cyclic stretch and may guide biomaterial and biophysical design for tissue engineering in the future.

In Chapter III, the synergistic enhancement of osteogenic differentiation by immobilized bone morphogenetic protein-2 (BMP-2) and ultrasound stimulation is described. The adhesive recombinant BMP-2 homodimer containing peptides composed of 3,4-dihydroxyphenylalanine (DOPA) and lysine was successfully prepared (DOPA-BMP-2) by using *Escherichia coli* expression and enzymatic modification. The amount of DOPA-BMP-2 bound on the tissue culturing plate increased with increasing feeding concentration and the resulting surface turned more hydrophilic. DOPA-BMP-2 uniformly formed multi-molecular layers on the surfaces according to atomic force microscopy results and layer calculation, considering the size of the hydrodynamic size of the BMP-2 homodimer. When a pre-osteoblast cell was cultured on the surface bound with or without DOPA-BMP-2, the bound DOPA-BMP-2 significantly enhanced cellular expression of alkaline phosphatase (ALP), which is one of the markers showing the early stages of osteogenic differentiation. The enhancement level by bound DOPA-BMP-2 was more than that by soluble one. Furthermore, calcium deposition, a late stage of osteogenesis, was first induced after only 7 days on the immobilized surface, but it was not observed in cells cultured with soluble form until 14 days. Low-intensity pulsed ultrasound (LIPUS) was employed to study the combined effect with the bound BMP-2. It was discovered that ALP activity of the cell was modestly increased when LIPUS was applied with bound BMP-2 in the first 3 days, although only LIPUS did not affect the cell differentiation. When LIPUS and bound BMP-2 were combined in the final 4 days of a seven-day culture, a synergistic improvement in calcium deposition was observed. To understand how ultrasound and bound BMP-2 worked in concert to promote cell differentiation, the focal adhesion and cytoskeleton dynamics, involved in mechano-transduction as a crucial component of cells, were studied by

immunostaining, fluorescent microscopy observation, and following ImageJ analysis. Interestingly, the number of focal adhesion (FA) formation was significantly increased by combined treatment of bound BMP-2 and LIPUS compared to BMP-2-only and LIPUS-only groups. This result suggested that ultrasound and bound BMP-2 together could enhance the interplay of integrin/FA and BMP receptors to increase FA formation, resulting in downstream differentiation.

In Chapter IV, the results and discussion of this dissertation are summarized. This dissertation will contribute to our understanding of how cells react to biochemical and biophysical microenvironments.