

# 博士論文（要約）

## **Microstructural Analysis of Bone Matrix Surface and Effects of Topography on Osteoclast and Osteoblast Differentiation**

(骨マトリックス表面の微細構造解析および  
破骨細胞と骨芽細胞の分化におけるトポグ  
ラフィーの効果)

丁 憲煜

Bone grafts have been used to maintain bone structure and to help bone regeneration for bone defects. Tissue engineering, which implants bone tissue regenerated *in vitro*, has attracted attention in recent years. The key elements of tissue engineering (cells, scaffolds, and signals) have been actively studied. Particularly, these elements are attempted to reproduce *in vivo* environment and conditions for achieving tissue regeneration *in vitro*. Regarding bone regeneration, we need to use and reproduce bone-related cells, matrix, and growth factors in bone remodeling.

This study began with the idea that bone loss due to age would result in changes in bone structure, which would affect the activity of bone-related cells because of microtopographical changes. We attempted to assess changes in microtopographical structure on the surface of the bone and utilize them to control bone differentiation for the application of bone regeneration. Therefore, the purpose of this dissertation is to analyze the microstructural changes of the bone surface directly that might affect cellular behaviors and to analyze the differentiation of osteoclasts and osteoblasts by microtopographical differences through *in vitro* experiments.

In Study 1, we assessed the microstructural characteristics of the inner surface of the cortical bone from SD rat in four different models by scanning electron microscope. Our results showed that the surface microtopography and roughness differ depending on the bone model by the canaliculi holes of osteocyte and the bone fiber arrangement. Thus, it could be helpful for the basic understanding of the bone microstructure and the changes in the bone surface by the various conditions. Further, combining with the additional technique to analyze the bone surface or other elements, it is expected that this study would be used as an index in the diagnosis of bone disease. In addition, if the surface microtopography of each bone model is mimicked and used as a topographical element for *in vitro* cell culture, more meaningful applications will be possible for cell differentiation and behavior control in tissue engineering.

In Study 2, we examined the effect of the micro-topographic surface on the promotion of osteoclast differentiation of bone marrow-derived precursor cells. First, we fabricated micro-groove (thickness;  $2\ \mu\text{m}$ , depth;  $1\ \mu\text{m}$ ) patterned substrates of PDMS which have different spacings of each groove 1 to  $10\ \mu\text{m}$ , and coated with vitronectin for cell adhesion and osteoclast induction. The differentiated osteoclasts were observed on each substrate, and at spacing  $1\ \mu\text{m}$  the most osteoclasts were differentiated compared to other substrates, confirmed

by TRAP staining and gene expression level. Also, on observation of the podosome on the substrates, the ratio of podosome stages was different at  $1\mu\text{m}$  that the clusters were relatively less, indicating that the formation of podosome was dependent on the curvature of the grooves. Moreover, as shown in the results of myosin II inhibition using blebbistatin and siRNA, the topographic effects were disappeared. Therefore, it can be concluded that the  $1\mu\text{m}$ -spacing groove pattern affected osteoclast differentiation, leading to stabilization of podosome and promotion of differentiation by lessening myosin II. These findings would be useful for understanding the differentiation process of osteoclasts on the topographic surface and controlling cell differentiation in regenerative medicine.

In Study 3, the same PDMS cell culture substrates with microgroove patterns as in Study 2 was used to induce osteogenic differentiation of MSCs extracted from the bone marrow of SD rats. Picro-Sirius red staining confirmed collagen deposition on all substrates and ALP activity, an early marker of osteogenic differentiation, was measured to assess the progress of differentiation. As a result, the differentiation was delayed according to the microgroove spacing (1 and  $5\mu\text{m}$ ) compared to the flat surface. Moreover, cell adhesion and cytoskeleton of MSC on the substrates were observed by immunofluorescence staining, and it showed that the tendency of cell area and FA area in each substrate were similar to each other. In other words, surface topography affects cell spreading, and cell adhesion, and thus can be interpreted as affecting bone differentiation. Through this study, it is expected that the technique to control bone regeneration by surface microtopography will be one more step closer.

In summary, we assessed the surface topography of bone and elucidated the effect of surface topography on osteoclast and osteoblast differentiation for bone regeneration with tissue engineering approaches. Although it is well known that the bone structure becomes thinner and the bone density decreases as the aging and osteoporosis, the microstructural changes of the bone surface were not evaluated. Bone surface observation and analysis through four different models revealed microtopographical differences due to canaliculi and bone fiber arrangement. It is expected that future studies will be applied to the early diagnosis of bone disease by observing microstructural changes. Also, it is expected that these microtopographical changes can be applied to the surface processing of the scaffold and applied to cellular activities to contribute to bone regeneration and better development of bone implants. The tests in the

differentiation of osteoclasts and osteoblasts using surface topography of well-designed microgroove patterns *in vitro* were also associated with topographical changes in the bone surface as shown in Study 1. By applying the topographical changes to the micropatterns, we elucidated that they affect the osteoclast and osteoblast differentiation according to the spacing of microgrooves. The surface properties of an implant in dental and orthopedic are not only important for proper fixation force into the bone, but also for achieving bone regeneration after implantation.

Further, if the tissue regeneration rate can be controlled by surface topography on the part of the implant according to the zone of the bone tissue, the more efficient implant system can be constructed. Therefore, the experiment of cell differentiation by the substrate with surface topography (microgrooves in different spacing) can be meaningful. However, our results have limitations because there are many biomaterials and surface processing methods currently available in clinical practice, which is different from what we used and might show the various aspects of results from ours. Therefore, it is expected that various applications to the surface structure of bone graft materials will be possible based on the mechanism of osteoclast and osteoblast differentiation by surface topography.