Influence of microsize groove surface on human osteoblasts behavior and initial adhesion evaluated by cytodetacher

Shih-Ping Yang[1], Tzer-Min Lee[2], Truan-Sheng Lui[1], Ming Che Hsieh[3].
[1]Department of Materials Science and Engineering, National Cheng Kung University, Taiwan
[2]Institute of Oral Medicine, National Cheng Kung University, Taiwan
[3]Institute of Manufacturing Information and Systems, National Cheng Kung University, Taiwan

Statement of Purpose: In orthodontic implant success, surface properties and shapes design are the major factors, especially at bone healing in the early and long-term period. Implant of surface properties can also influence cellular responses from the early stage adhesion and migration to regulate cell physics response such as cell growth, gene expression, secretion of proteinases, differentiation, and even life and death. Titanium and calcium phosphate (Ca-P) has excellent biocompatibility. In our previous study, the cantilever-based technique is used to measure the initial adhesion force of osteoblasts cultured on different kinds of surface-treated disks. This method directly measures the actual strength of attachment between a single cell and its substratum to better assess the cyto-compatibility of biomaterials. Therefore, the aim in this study was to investigate the factors of Ca-P thin films and unidirectional micro-sized structures on the cell response.

Methods: Silicon (Si) wafers with <100> p-type crystal orientation were manufactured patterns by lithography and etching methods. Then, the micropatterns were coated with Ti and then coated with Ca-P by PVD. It was used different instruments (LV-SEM, thin film X-ray and EDX) to investigate the coatings and patterns. In addition, coatings were evaluated with human fetal osteoblasts (hFOB1.19) to investigate cell morphology, cell area, cell elongation, cell proliferation. The cyto-compatibility of specimen is quantitatively evaluated by cyto-detacher, which directly measures the detachment shear force of an individual cell to substrate. Each data was represented the mean $\pm$ standard deviation, and the analysis of one-way variance (ANOVA) was used to evaluate the significance differences. Differences were considered significant at $p \leqq 0.05$.

Results: The results showed that we fabricated two different sizes with 2 and 5 μm (groove-1 and groove-2) unidirectional microgroove patterns coated with 200 nm Ti and Ca-P thin film. The hFOB cells was seeded on the specimens at a density of 5×10^3 cells / cm^2 for 30 minutes, 1, and 3 hours, then fixed with 2.5 % glutaraldehyde at 4 °C, 4 % OsO_4 at 37 °C, 1% tannic acid at 4 °C. The sample was dehydrated with a series of graded ethanol solution and immersed in hexamethyldisilazane for 10 minutes. Finally, after sputter coating with gold, the specimens were observed by SEM. Analysis of SEM observation showed that the cells responded to micropatterns by spreading and elongating morphologies compared to smooth specimens. The hFOB cells behavior, such as cells morphology, area, elongation, and proliferation, can be controlled by the topography of surface in contact with cells. The MTT assay indicated that Ca-P grooved specimens showed the significantly higher value of cells number than control group after 5- and 15-day culture. The groove-1 showed higher cells number than groove-2. In addition, the cyto-compatibility of specimen is quantitatively evaluated by cyto-detacher, which directly measures the detachment shear force of an individual cell to substrate. Among these three specimens, the cells on Ca-P groove-1 have the highest cell adhesion strength (140 nN), followed by the cells on Ca-P grooved-2 specimens (138 nN), Ti grooved- specimens (137 nN) and Ti grooved-2specimens (103 nN). The cells on smooth surface present the lowest cell adhesive strength (40 nN).

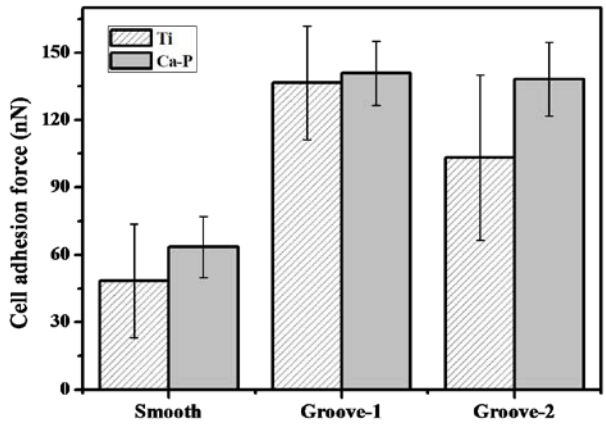

Figure 1. Cell adhesion force by cytodetachor

The improvement in cell adhesion to grooved surface resulted from an enhancement in the elongation of cell shape. It is concluded that the variation of surface morphology will affect the cell shape, adhesion force, and proliferation. The strength of cell adhesion is also considered a reliable measurement for cyto-compatibility. However, only the cantilever-based technique is capable of directly assessing the cell adhesion strength by detaching single cell from the surface of the specimen. The experimental results suggest that the grooved patterns can control the cell shape and thus influence the cell proliferation and cell adhesion force. The cyto-detachment test with nanonewton resolution is a sensitive method to study cell-biomaterials interaction.

Conclusions: (1) Results of the SEM showed that hFOB cells on grooved surface have better spreading and adhesion morphologies than smooth specimens.Ca-P thin film exhibits higher cytocompatibility than Ti substratums. (2) Groove-1 shows the higher value of cell area and elongation index than groove-2. (3) The proliferation data of cells on grooved specimens is significantly higher than smooth specimens. (4) Cells cultured on the grooved specimens have stronger initial adhesion forces.

References:

Wang CC, Hsu YC, Su FC, Lu SC, Lee TM. J Biomed Mater Res 2009;88A:370-387.