

EFFECT OF GROOVED TITANIUM SUBSTRATUM ON HUMAN OSTEOBLATIC CELL ADHESION

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INTRODUCTION: In this study, we compare 3 different machine-tooled surfaces, with different amplitudes and organizations of roughness. A qualitative kinetic analysis using F-actin labelling reveals the orientation of cells in grooves. A quantitative evaluation of cell adhesion and proliferation is correlated with parameters describing surface roughness at a scale above (macro-roughness) or below the cell size (micro-roughness).

METHODS: Titanium alloy Ti6Al4V bars were machine-tooled to obtain samples presenting organized and regular grooved surfaces with various roughness amplitudes Ra=0.81, 1.21, 3.35 μm (respectively surfaces A, B and H). Primary human osteoblasts were inoculated at 4×10^4 cells/well. For visualization of F-actin cytoskeleton using FITC-phalloidin, cells were fixed after 4 hours, 1, 2, 3 and 6 days. A cell detachment assay was performed after 1, 7, 14, 21 days as previously described¹. A detachment index percentage (DIP) was calculated. The DIP was considered as inversely proportional to the hOB adhesion. Roughness was measured using a tactile profilometer. Profiles were digitised and amplitude (Ra, Rt) and frequency parameters (Order, Delta) were computed. Statistical analysis of the roughness effects on cell proliferation and cell adhesion was performed using SAS[®] software.

RESULTS: Profiles A and B obtain the same quite regular and periodic morphologies. Roughness of machine-tooled H samples presents a more chaotic aspect. Order on H surfaces is lower than on A and B surfaces (27%). Samples H present grooves with an elevated height (Rz =15 μm) and with a large width (PAS =1000 μm). This width is much larger than the cell size. However, in these large grooves, a micro-roughness exists. For this reason, we attempted to analyse the micro-roughness that is at the scale of the cells (<100 μm). For that, we filtered the roughness profiles using the Fourier Transform and retained only those frequencies greater than the inverse of the size of one cell. A new profile was re-created that represented the roughness seen "under the cell". Micro-grooves are deeper for the H samples. The number of micro-peaks is equal for all machine-tooled surfaces and lies around

1100 peaks per inch. However, the microgrooves are rather periodical on samples A and B and unperiodical on samples H. This is confirmed by the Order parameter that is of 23% for surface B, 15% for surface A and less than 10% on surface H (Table 3). We can conclude that the periodicity is higher on B > A > H. Kinetic observation of cell growth on grooved surfaces using F-actin labelling showed the orientation of cells along the grooves at 4h on all surfaces. On H surfaces, cells appeared more elongated than on A and B surfaces. We did not observe visible morphological differences between cells growing on A and B surfaces. Major differences did exist between these surfaces and H surfaces. On H surfaces, SEM observations displayed pictures of cells looking as if they had "fallen into cracks" on the surface. We observed an increase of cell adhesion as a function of time. However, the inter-surface comparison did not demonstrate any significant difference of adhesion between surfaces at each time point. Proliferation was the same on A and B surfaces. Proliferation was higher on H surfaces compared to other surfaces, notably after 21 days of culture.

DISCUSSION & CONCLUSIONS:

We did not observe any significant differences of proliferation and adhesion between A and B surfaces, although these two surfaces were different in their macro-roughness parameters. When micro-roughness was considered, no more differences in roughness appeared between A and B surfaces. On the other hand, the roughness parameters of H surfaces were very different at each scale compared to A and B surfaces. These observations indicate that the cells react more to roughness at their scale (10-100 μm) rather than to roughness at a higher scale (100-1000 μm). This has been previously described² and is coherent with previous results describing various responses of osteoblasts to surfaces presenting grooves of 1 to 10 μm in width³.

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