AN ATTEMPT TO CORRELATE THE CHARACTERISTICS OF MICRO STRUCTURED SURFACES OF TITANIUM IMPLANTS AND BIOLOGICAL PARAMETERS OF ADHESIVED CELLS

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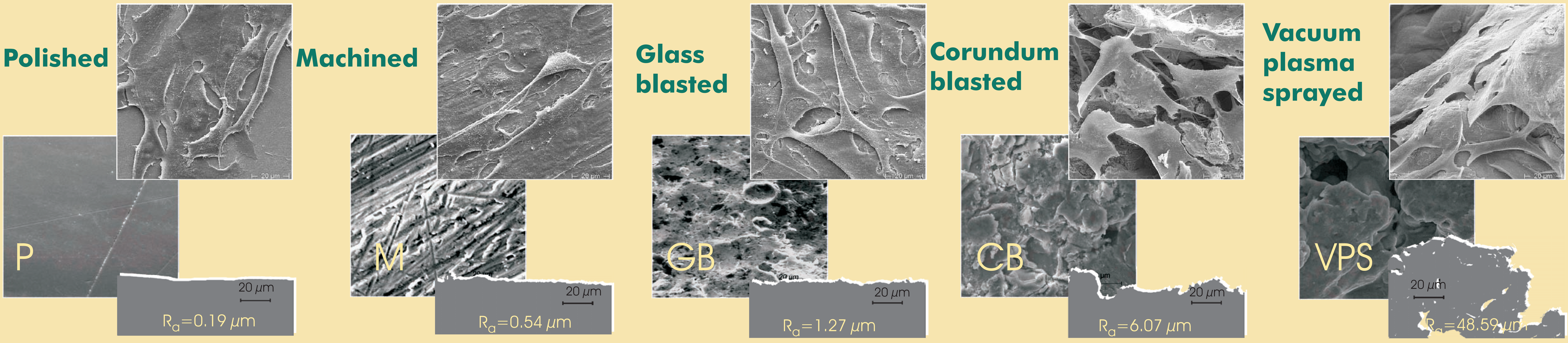
Introduction

One of the premises for biocompatibility studies of implant materials is the determination of morphological characteristics of their modified surfaces. Our investigations here were focussed on the question if the physical and chemical parameters used for the description of the surface of commercially pure titanium with different roughness can be utilised for the prediction of the cellular behaviour of osteoblastic cells.

Material

The surface structure of cp-titanium samples was modified in a range of roughness average R_a from 0.19µm to 48.59µm by polishing (P), machining (M), blasting with glass balls (2.7 bar) (GB), blasting with corundum particles (6 bar) (CB) and vacuum plasma spraying (VPS).
The pictures below show the pure material (in the middle row), MG63 cells on the material after 24h of cultivation (above) and a cross section through the sample (below).



Characteristics of the material surface

Several physical and electrochemical methods like surface profiling (SP), linear sweep voltammetry (LSV), chronoamperometry (CA), electrochemical impedance spectroscopy (EIS) or digital image processing (DIP) [1] were used to obtain parameters like:

- Roughness average R_a (SP)
- Open circuit potential E_{ocp} (LSV)
- Cathodic tafel slope (LSV)
- Corrosion resistance R_{corr} (LSV)
- Corrosion current I_{corr} (LSV)
- Surface increasing (CA)
- Electrochemical double layer capacity C_d (EIS)
- Fractal dimension D_F (LSV, EIS, DIP) [2,3,4]

To check these parameters on linear interdependence the correlation coefficient (Pearson) among each other was calculated. To avoid redundance only parameters that are not strongly dependent from each other were used for correlation with biological parameters.

Correlation between the material characteristics on the one hand and biological parameters on the other hand was done in two ways [9]:

- Spearman's rank ($-1 <= r_s <= 1$) was calculated. This correlation only works on ranked (relative) data, rather than directly on the data itself. The higher the Spearman's coefficient (its modulus, resp.) the stronger the agreement between the correlated data, but there must not be a linear relationship. If $|r_s|$ is greater or equal 0.9 the appropriate field in the correlation matrix is marked green.
- Pearson's correlation coefficient reflects the degree of linear relationship between two variables. Like Spearman's rank it ranges from -1 to +1. The places in the pearson's correlation matrix where $|r_p|$ is greater or equal 0.9 are also marked green.

Spearman's rank

	Integrin expression 1	Integrin expression 3	Integrin expression 2	Cell adhesion 5 min	Cell adhesion 10 min	Cell adhesion 15 min	Mineralization	Cell spreading area 3h	Cell spreading area 16h	Cell spreading area 24h	Cell spreading shape 24h	proliferation (cell cycle) 1d	proliferation (cell cycle) 3d	proliferation (cell cycle) 7d	Vinculin dynamics	Vinculin number	Gene expression Alkaline phosphat. 1d	Alkaline phosphat. 3d	Osteocalc. 3d	Osteocalc. 7d	Osteopont. 3d	Osteopont. 7d	Bone sialopr. 3d
roughness average R_a					■	■						■				■							■
open circuit potential E_{OCP}								■			■				■								
cath. tafel slope			■		■								■			■							■
corrosion resistance R_{corr}					■	■						■				■							■
corrosion current I_{corr}					■	■						■				■							■
fractal dimension D_F (LSV)																					■		
fractal dimension D_F (EIS)			■		■								■			■							■
fractal dimension D_F (DIP)			■						■	■													
valid cases	5	5	5	5	5	5	5	4	4	4	4	5	5	5	4	4	5	5	5	5	5	5	5

Pearson's correlation

	Integrin expression 1	Integrin expression 3	Integrin expression 2	Cell adhesion 5 min	Cell adhesion 10 min	Cell adhesion 15 min	Mineralization	Cell spreading area 3h	Cell spreading area 16h	Cell spreading area 24h	Cell spreading shape 24h	proliferation (cell cycle) 1d	proliferation (cell cycle) 3d	proliferation (cell cycle) 7d	Vinculin dynamics	Vinculin number	Gene expression Alkaline phosphat. 1d	Alkaline phosphat. 3d	Osteocalc. 3d	Osteocalc. 7d	Osteopont. 3d	Osteopont. 7d	Bone sialopr. 3d
roughness average R_a				■				■	■			■					■				■		
open circuit potential E_{OCP}											■				■								
cath. tafel slope			■					■	■														
corrosion resistance R_{corr}					X	■		■								■							X
corrosion current I_{corr}				■					■			■					■				■		
fractal dimension D_F (LSV)		■									X												
fractal dimension D_F (EIS)					■	■		■	■							■							■
fractal dimension D_F (DIP)			X						X														
valid cases	5	5	5	5	5	5	5	4	4	4	4	5	5	5	4	4	5	5	5	5	5	5	5

Note: Some biological parameters (cell spreading, GFP-vinculin) couldn't be determined for the very rough modification VPS. That's why in this cases the correlation includes only 4 modifications and thus the correlation coefficient is very uncertain.
Nevertheless few selected correlations where the Pearson' correlation coefficient is near 1 (marked with a cross in the according correlation matrix) are shown in the diagrams left and below.

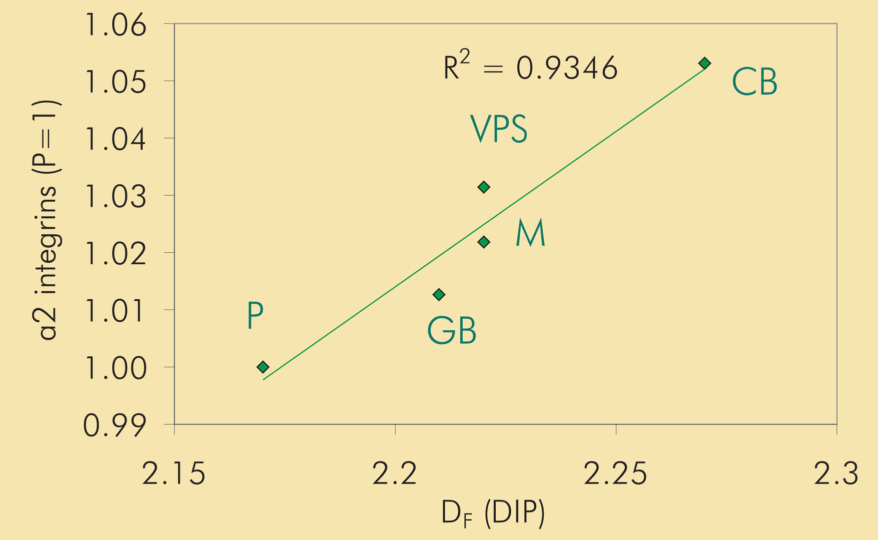
Characteristics of the cell behaviour

Cellular investigations were carried out in human primary osteoblasts (POB) [5] and MG-63 osteoblastic cells. Cells were cultured in DMEM with 10% fetal calf serum (FCS) and 1% gentamycin (Ratiopharm GmbH, Ulm, Germany) at 37°C and in a 5% CO_2 atmosphere. In general, cells were seeded with a density of $3x10^4$ cells/cm^2 onto the titanium materials and into control dishes.
Following methods were applied:

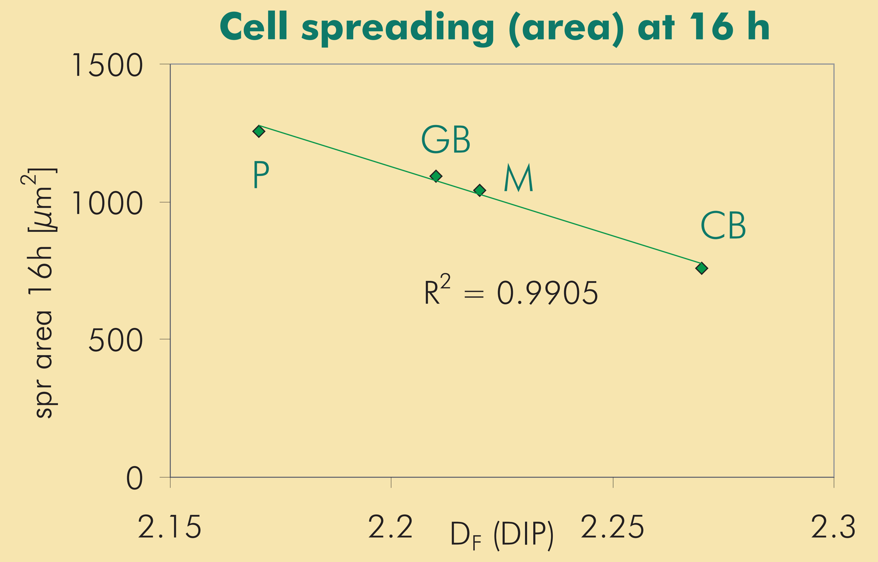
- Cell adhesion (at 5, 10, 15 min)
- Integrin expression (1, 3, 2, 5, 3) [6]
- Mineralization
- Length of 1 integrin contacts (POB, Mg63 cells) [7]
- Cell spreading: area (at 3,16,24,40 h) [6]
- Cell spreading: shape (ratio length to width at 3, 16, 24, 40 h)
- Proliferation (cell cycle at 1, 3, 7d)
- Vinculin contacts in living cells (number, length, dynamics) [8]
- Fibronectin expression
- Gene expression (alkaline phosphatase, osteocalcine, osteopontine, bone sialoprotein at 1,3,7d)

Most of the results are relative values referred to the polished surface, that was set equal one or 100%.
Like physical parameters for the description of the material these biological parameters were correlated among each other (Pearson correlation) to find out those that are redundant and thus to minimize the total number of parameters.

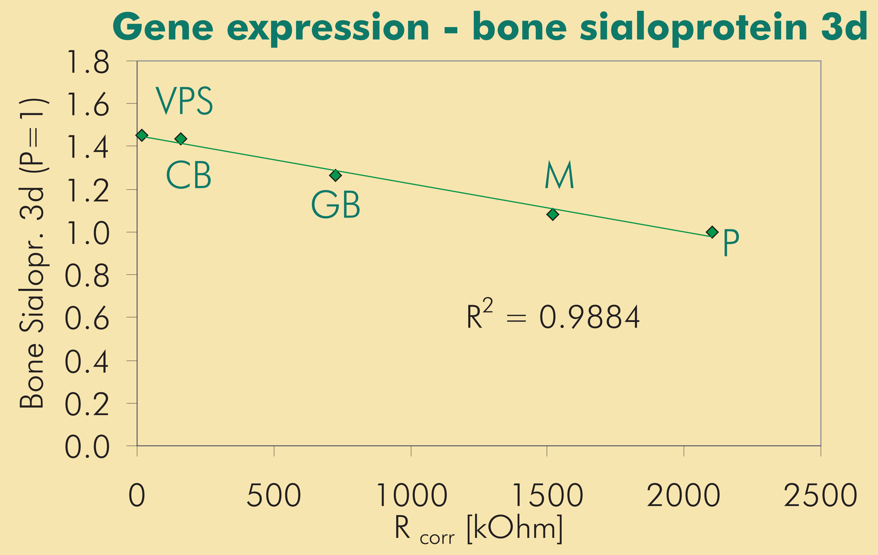
Integrin expression 2 (P=1)



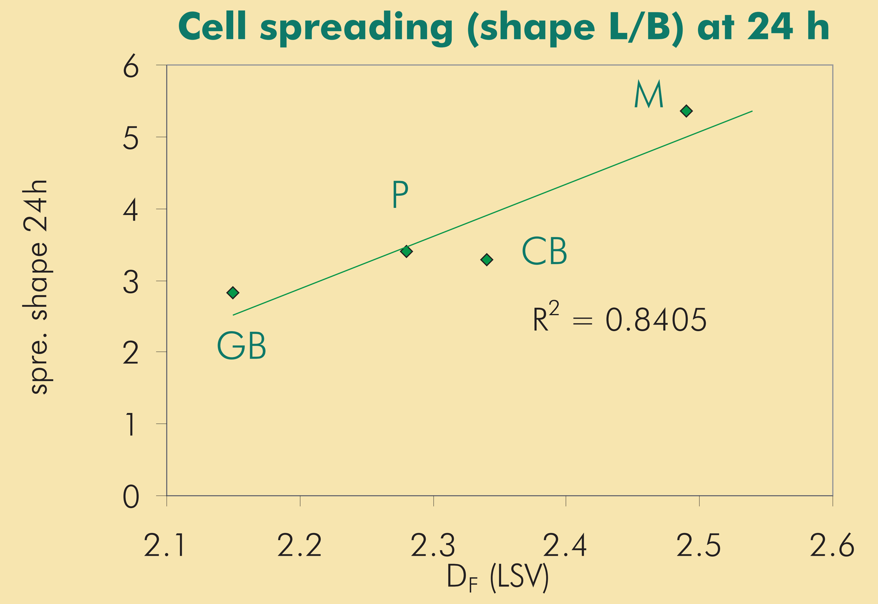
Cell spreading (area) at 16 h



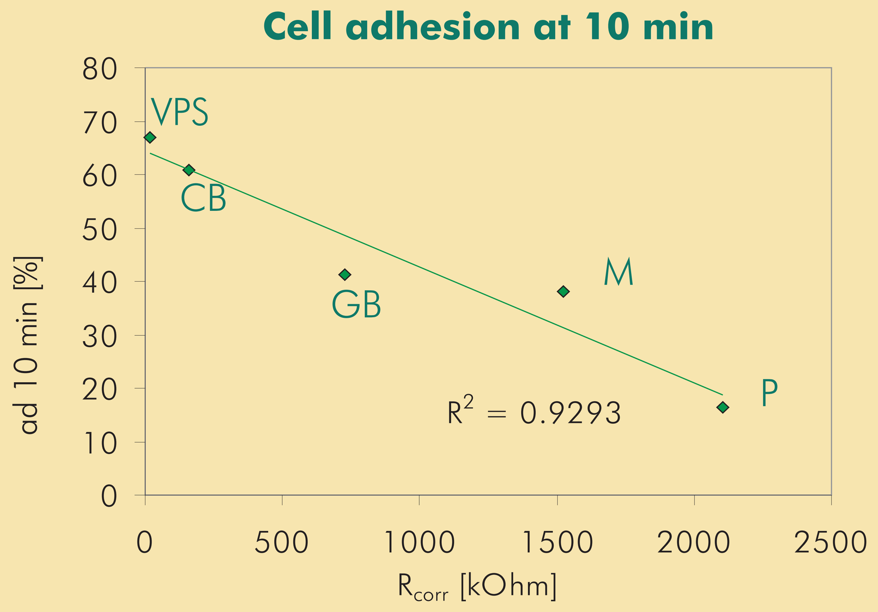
Gene expression - bone sialoprotein 3d



Cell spreading (shape L/B) at 24 h



Cell adhesion at 10 min



Summary

As can be seen in the correlation tables above and the diagrams on the left there is a good correlation between material parameters, especially fractal dimension D_F on the one hand and cell spreading parameters on the other hand. There can be observed a good correlation between the expression of 2 integrins and the fractal dimension D_F obtained from digital image processing (DIP), too. Likewise the corrosion resistance R_{corr} seems to have greater influence on the cell behaviour than other material parameters like roughness average R_a. So we can find a very good correlation of R_{corr} with the gene expression of bone sialoprotein and also with cell adhesion parameters.

Because of the small number of modifications the significance of our calculations is not very high. To confirm our assumptions we are going to broaden our spectrum of surface modifications to nine to fill in the gaps and thus to get a higher significance of correlation.

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