The influence of the surface topography of Titanium implants on the behaviour of adhesive osteoblastic cells – statistical correlations

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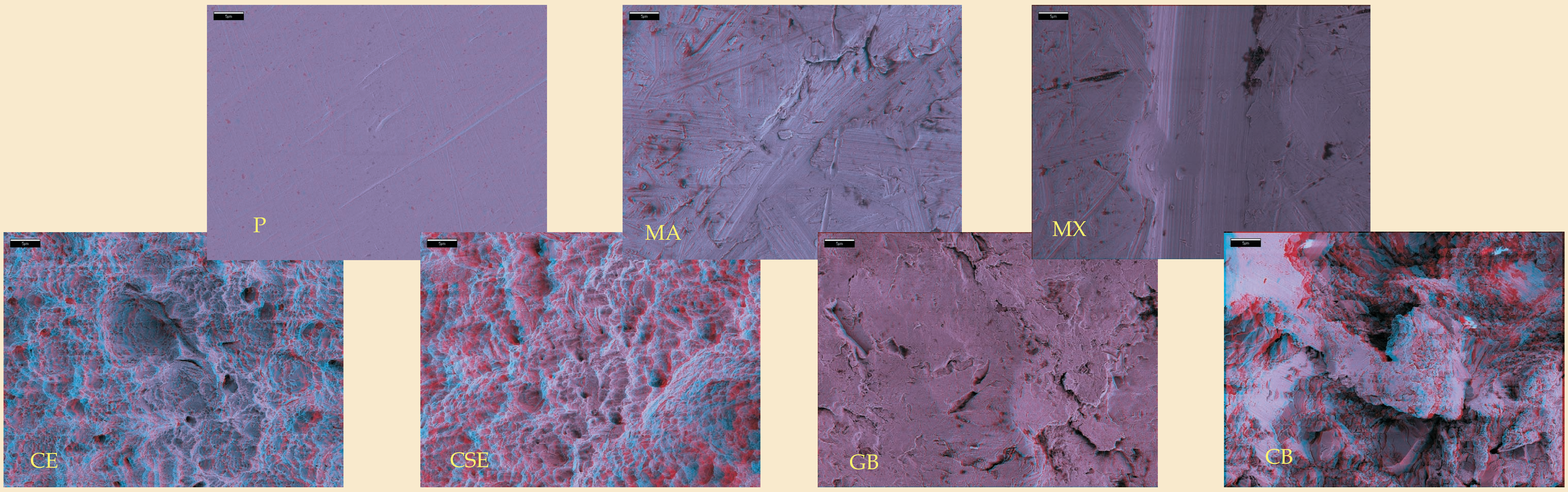
INTRODUCTION

For mathematical modelling of the biomaterial-cell contact it is necessary to find both parameters characterizing physical and chemical properties of the material surface and also such describing the reaction of the adhering cells. Only those material and cell parameters that correlate with each other are applicable to model this contact mathematically. Only few papers are dealing with this special problem [1, 2].
The aim of this paper is to present results of physical/chemical and biological investigations made on differently modified rough titanium implant surfaces in order to find out only the correlating parameters. Furthermore we want to discuss several ways to apply statistical methods to the correlation problem.

MATERIAL PREPARATION

The surface structure of cp-titanium samples was modified in a range of roughness average R_a from 0.07 µm to app. 7 µm by several modification methods:

- P - Polishing (R_a = 0.07 µm)
- MA - Machining 1 (R_a = 0.51 µm)
- CE - Chemical Etching (solution: 37% HCl; 98% H_2SO_4; H_2O; 2:1:1) (R_a = 1.08 µm)
- CSE - Cathodic supported chemical etching (solution: 37% HCl; 98% H_2SO_4; H_2O; 2:1:1) (R_a = 1.23 µm)
- MX - Machining 2 (R_a = 1.53 µm)
- GB - Blasting with glass spheres (2,7 bar) (R_a = 2.41 µm)
- CB - Blasting with corundum particles (2,5 bar) (R_a = 6.56 µm)



MATERIAL CHARACTERIZATION

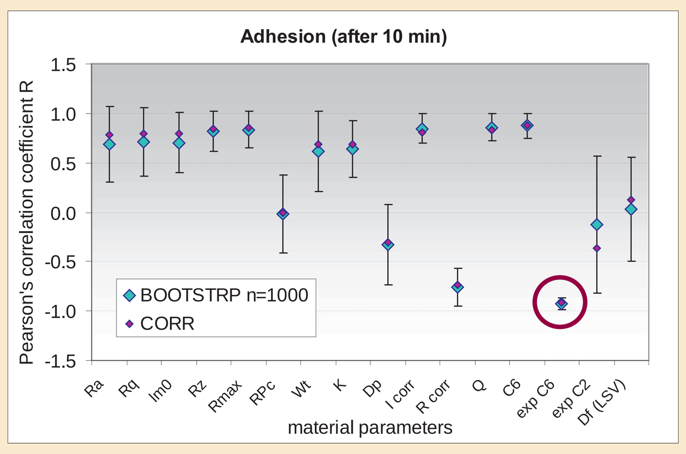
The above shown SEM-pictures of the material modifications are stereo image pairs. Looking at this pictures trough the special red/blue glasses gives a good 3D-impression of the material surface.
For the physical characterization of the surface morphology both standardized roughness parameters (DIN EN ISO 4287, R_a, R_q, I_{m0}, ...) and additional parameters like fractal dimension D_p and topothesy K were calculated from the surface profile [3,4]. Additional electrochemical parameters were determined by methods of Linear Sweep Voltammetry (corrosion current I_{corr}, corrosion resistance R_{corr}), Chronoamperometry (electrical displacement flux Q) and Electrochemical Impedance Spectroscopy (capacities C and exponents of the CPE). The fractal dimension D_f was determined with an electrochemical experiment, too.[5]

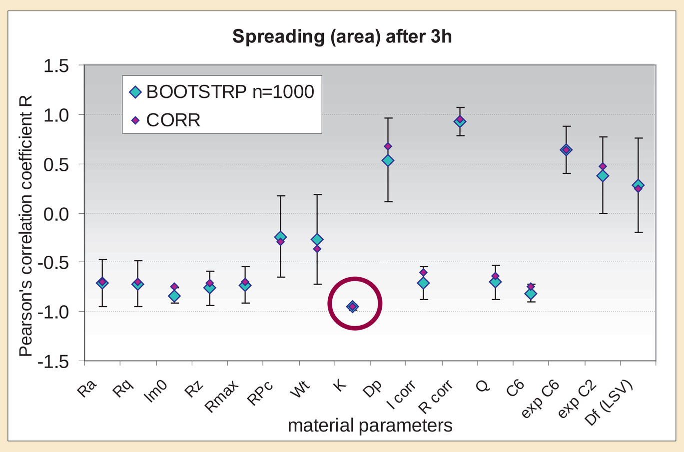
CELLBIOLOGICAL CHARACTERIZATION

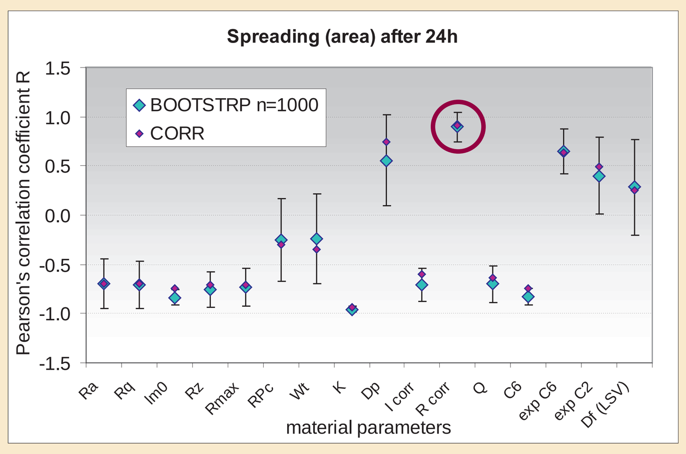
Cellular investigations were carried out with MG-63 osteoblastic cells. Cells were cultured in DMEM with 10% fetal calf serum (FCS) and 1% gentamycin (Ratiopharm GmbH, Ulm, Germany) at 37°C and in a 5% CO_2 atmosphere. In general, cells were seeded with a density of $3x10^4$ cells/cm^2 onto the titanium materials and into control dishes. Following cellular parameters were investigated to evaluate the correlation to physical/chemical properties of the titanium material:
- Cell Adhesion (after 10 min),
- Cell spreading (cell area and shape (relation length/width) after 3 h, 24 h and 40 h),
- Integrin expression (2, 3, 5, 1, 3) and
- Length of the 1-integrin contacts [6,7].

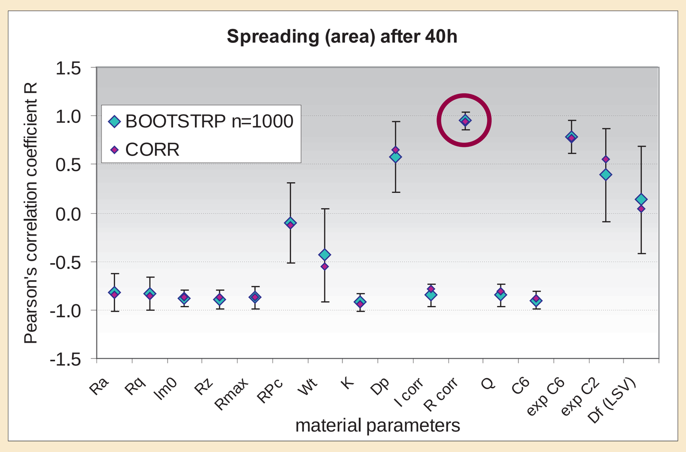
CORRELATION

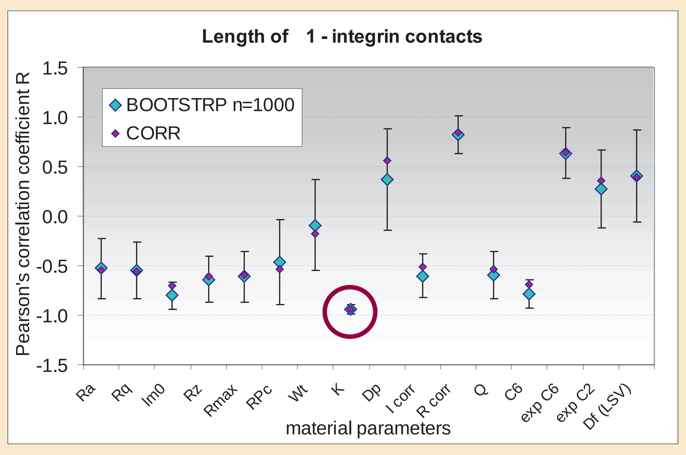
Correlation between material and biological parameters was made using the statistical program SPSS presuming a **linear dependence**. Because of the specific measurements of material and cellular parameters we couldn't build pairs of variates from single measurements. That's why we had to average the data to get pairs of variates per material modification. So we got N=7 supporting points (one for every modification) to get the pearson's correlation coefficient R for every combination between material and cell biological parameters. The results for R are shown in the below diagrams for some selected cellbiological parameters.
Because N=7 supporting points are quite few points for a statistical correlation analysis we looked for a possibility to increase statistical accuracy and we found that resampling of data by using the Bootstrap method can be a way out [8]. Bootstrap analysis was made by means of the statistics toolbox of the software MATLAB. For n=1000 resampling procedures we got the mean and standard deviation of the pearson's correlation coefficient for every combination between material and cellbiological parameters. This results are also shown in the below diagrams in comparison to the results of the conventional method described above. For those cases where the absolute value of pearson`s correlation coefficient is high and there is also a good congruence between the results of the conventional and bootstrap method, i.e. the standard deviation got by the bootstrap method is low (these cases are marked with a red circle in the diagram), the respective correlation diagram is shown below.

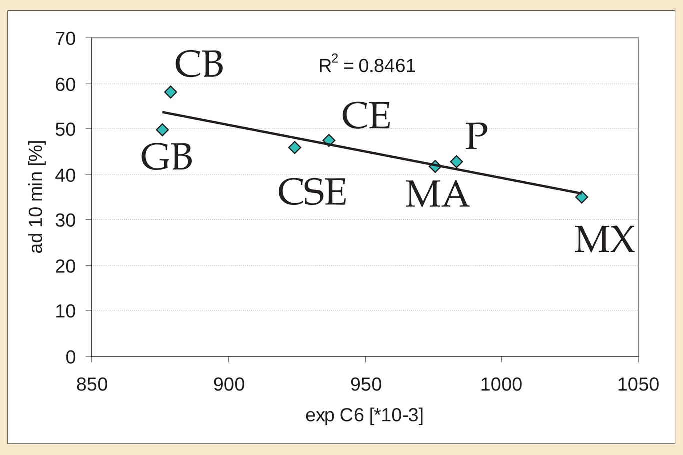


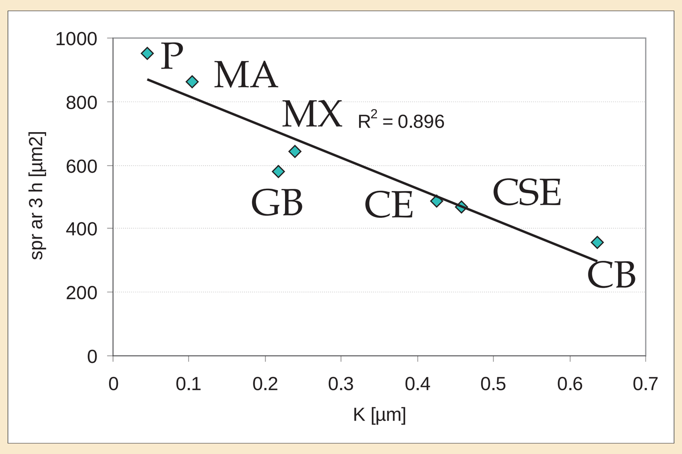


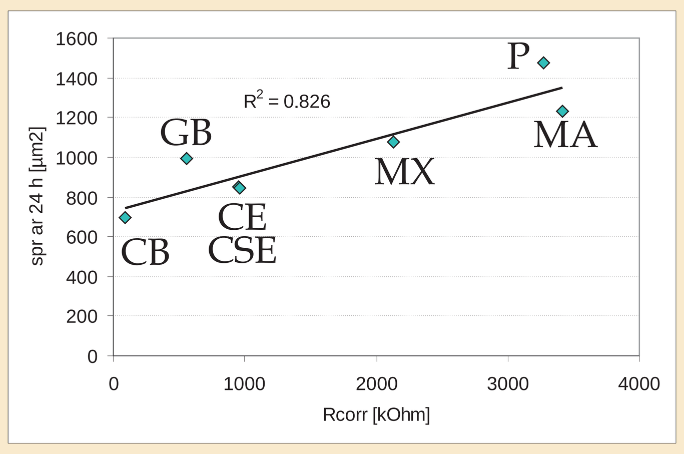


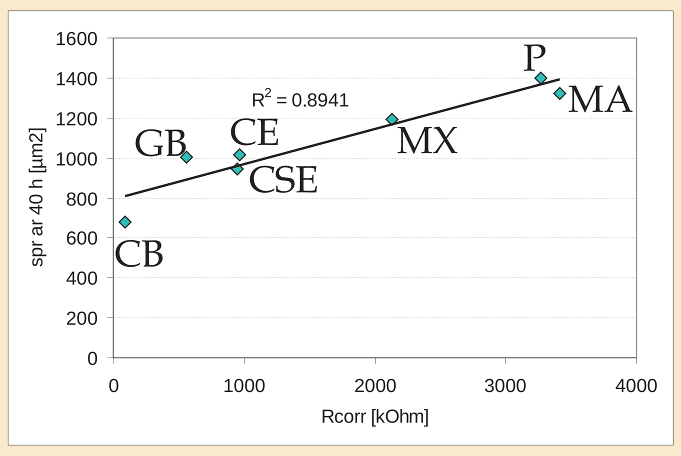


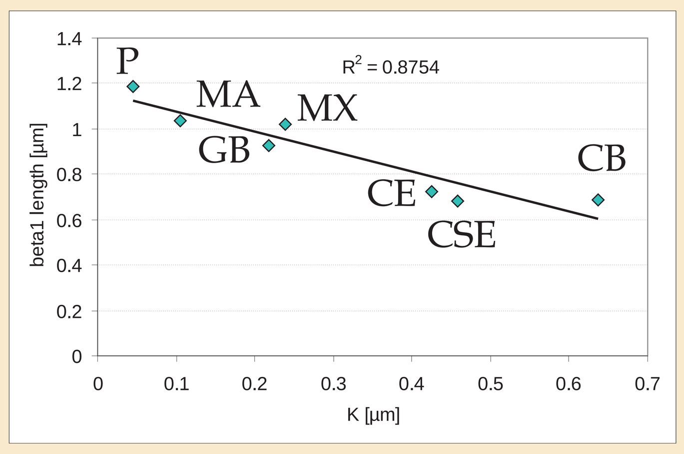












SUMMARY

Only few ones of all investigated parameters both on material and on cellular side were applicable for correlation. For example we found in our studies that fractal structure parameter topothesy K and the corrosion resistance R_{corr} have influence on the spreading behaviour (cell area) of the osteoblastic cells and on length of 1 integrin contacts. On the other hand it seems that there is no appreciable influence of material parameters on the integrin expression (not shown) and spreading behaviour (cell shape, not shown) and only few influence on cell adhesion.
In our investigations Bootstrap method, complementing conventional statistical methods, proved to be a good instrument for increasing statistical significance of the correlation results, especially if only few data are available.

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