# MATHEMATICAL CORRELATION BETWEEN BIOMATERIAL AND CELLULAR PARAMETERS - CRITICAL REFLECTION OF STATISTICS -

R. Lange[1], F. Lüthen[2], B. Nebe[2], J. Rychly[2], U. Beck[1]

[1]University of Rostock, Dept. of Electrical Engineering and Informatics, Albert-Einstein-Str. 2, 18051 Rostock, Germany
[2]University of Rostock, Department of Internal Medicine, Ernst-Heydemann-Str. 6, 18057 Rostock, Germany

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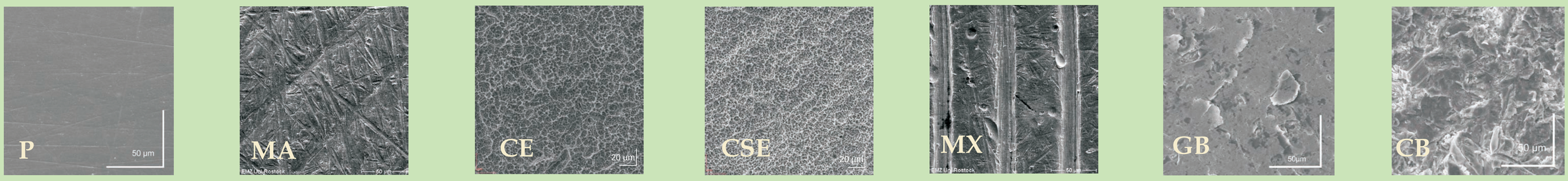
## INTRODUCTION

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
MA - Machining 1
CE - Chemical Etching (solution: 37% HCl; 98% $H_2SO_4$; $H_2O$; 2:1:1)
CSE - Cathodic supported chemical etching (solution: 37% HCl; 98% $H_2SO_4$; $H_2O$; 2:1:1)
MX - Machining 2
GB - Blasting with glass spheres ( 2,7 bar)
CB - Blasting with corundum particles (2,5 bar)




$R_a$=0.07 µm | $R_a$=0.51 µm | $R_a$=1.08 µm | $R_a$=1.23 µm | $R_a$=1.53 µm | $R_a$=2.41 µm | $R_a$=6.56 µm

## MATERIAL CHARACTERIZATION

For the physical characterization of the surface morphology both standardized roughness parameters (ISO 4287, $R_a$, $R_q$, $l_{m0}$, ...) 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 ΔQ) 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: Adhesion (after 10 min), spreading (cell area and shape (relation length/width) after 3h, 24h and 40h) and integrin expression (β1, α2, α3, α5, β3).[6,7]

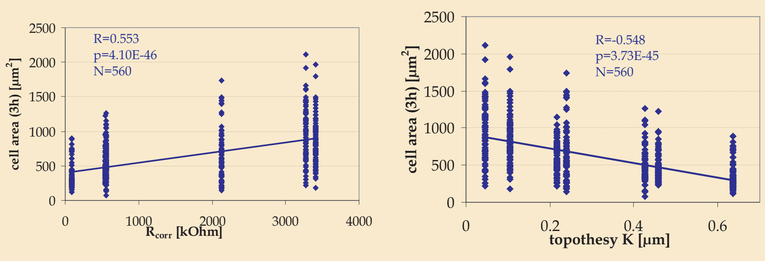
## Averaging only material parameters

cellbiological parameters

material parameters (averaged)

| | Adhesion | Integrin expression | | | | | cell spreading - area | | | spreading - shape L/W | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10 min | beta1 | alpha2 | alpha3 | alpha5 | beta3 | 3h | 24h | 40h | 3h | 24h | 40h |
| Ra [µm] | | | | | | | | | | | | |
| Rq [µm] | | | | | | | | | | | | |
| lm0 [mm] | | | | | | | | | | | | |
| Rz [µm] | | | | | | | | | | | | |
| Rmax [µm] | | | | | | | | | | | | |
| RPc [1/cm] | | | | | | | | | | | | |
| Wt [µm] | | | | | | | | | | | | |
| K [µm] | | | | | | | X-0.548 | -0.471 | -0.491 | | | |
| Dp | | | | | | | | | | | | |
| I corr [nA] | | | | | | | | | | | | |
| R corr [kOhm] | | | | | | | X 0.553 | 0.460 | 0.485 | | | |
| Q [µAs] | | | | | | | | | | | | |
| C6 [µF] | | | | | | | -0.472 | | -0.481 | | | |
| exp C6 | | | | | | | | | | | | |
| exp C2 | | | | | | | | | | | | |
| Df | | | | | | | | | | | | |
| N | 49 | 35 | 35 | 35 | 35 | 35 | 560 | 560 | 560 | 560 | 560 | 560 |

As you can see in the above correlation matrix it seems that only cell spreading area is significant influenced by material parameters. The highest value for the correlation coefficients R you can find for the correlation between the corrosion resistance $R_{corr}$ and cell spreading area after 3h (R=0.553) and the fractal parameter topothesy K (obtained by surface profiling) and cell spreading area after 3h (R=-0,548). These two correlations are marked with a cross in the correlation matrix above and the corresponding diagrams are shown below. For every surface modification all the values obtained for the corresponding material parameter ($R_{corr}$, K) from single measurements were averaged and pairs of variats were built with the values obtained from the single cellbiological measurements.




Due to the high number of cellbiological experiments (N=560) the statistical reliability is high (p<0.01) but the pearson's coeffizient R is quite low because of the relatively high spread of the biological data up to 30% per material modification.

## STATISTICAL CORRELATION

Correlation between material and biological parameters was made by means of 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. We had to average the data to get pairs of variates per material modification. This was done in different ways:
1. averaging only material parameters per material modification (at the left hand side)
2. averaging only cellbiological parameters per material modification (at the right hand side) and
3. averaging both material and cellbiological parameters per material modification (below)

In the correlation tables you can find the material parameters arranged in the rows and cellbiological parameters in the columns. At the places of this correlation matrices stands the correnspondig **pearson's coefficient R** between the material parameter given by the row and the cellbiological parameter given by the column. Only that places in the correlation matrix where the pearson's coefficient has a statistical significance p<0.01 are coloured marked and only the highest correlation coefficients with p<0.01 are given by their value.

1

2

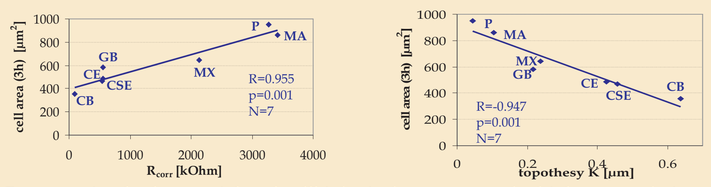
3

## Averaging both material and cellbiological parameters

cellbiological parameters (averaged)

material parameters (averaged)

| N=7 | Adhesion | Integrin expression | | | | | cell spreading - area | | | spreading - shape L/W | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10 min | beta1 | alpha2 | alpha3 | alpha5 | beta3 | 3h | 24h | 40h | 3h | 24h | 40h |
| Ra [µm] | | | | | | | | | | | | |
| Rq [µm] | | | | | | | | | | | | |
| lm0 [mm] | | | | | | | | | | | | |
| Rz [µm] | | | | | | | | | | | | |
| Rmax [µm] | | | | | | | | | | | | |
| RPc [1/cm] | | | | | | | | | | | | |
| Wt [µm] | | | | | | | | | | | | |
| K [µm] | | | | | | | X-0.947 | -0.942 | -0.943 | | | |
| Dp | | | | | | | | | | | | |
| I corr [nA] | | | | | | | | | | | | |
| R corr [kOhm] | | | | | | | X 0.955 | 0.921 | 0.930 | | | |
| Q [µAs] | | | | | | | | | | | | |
| C6 [µF] | | | | | | | | | -0.923 | | | |
| exp C6 | | | | | | | | | | | | |
| exp C2 | | | | | | | | | | | | |
| Df | | | | | | | | | | | | |




By averaging both material and cellbiological parameters the highest correlation coefficients were obtained. But this is due to the fact that measurement variations in material characterization and also in cellbiological characterization are unconsidered. On the other hand the statistical reliability is quite low because of the low number of investigated modifications (N=7).

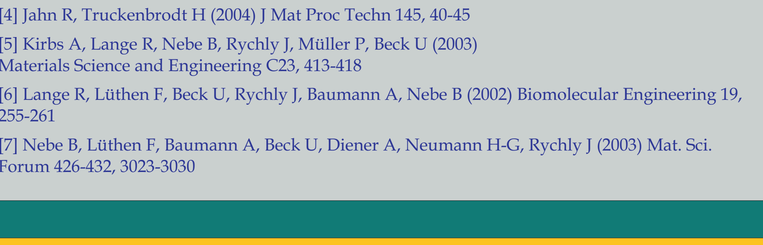
## Averaging only cellbiological parameters

cellbiological parameters (averaged)

material parameters

| | Adhesion | Integrin expression | | | | | cell spreading - area | | | spreading - shape L/W | | | N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10 min | beta1 | alpha2 | alpha3 | alpha5 | beta3 | 3h | 24h | 40h | 3h | 24h | 40h | |
| Ra [µm] | | | | | | | | | | | | | 21 |
| Rq [µm] | | | | | | | | | | | | | 21 |
| lm0 [mm] | | | | | | | | | | | | | 21 |
| Rz [µm] | | | | | | | | | | | | | 21 |
| Rmax [µm] | 0.857 | | | | | | | | | | | | 21 |
| RPc [1/cm] | | | | | | | | | | | | | 21 |
| Wt [µm] | | | | | | | | | | | | | 21 |
| K [µm] | | | | | | | X-0.941 | -0.937 | -0.938 | | | | 21 |
| Dp | | | | | | | | | | | | | 21 |
| I corr [nA] | | | | | | | | | | | | | 35 |
| R corr [kOhm] | | | | | | | X 0.888 | 0.856 | 0.865 | | | | 35 |
| Q [µAs] | | | | | | | | | | | | | 35 |
| C6 [µF] | 0.860 | | | | | | | | -0.913 | | | | 51 |
| exp C6 | | | | | | | | | | | | | 51 |
| exp C2 | | | | | | | | | | | | | 51 |
| Df (LSV) | | | | | | | | | | | | | 25 |

By averaging the cellbiological data the high variations of these measurements remain unconsidered and thus the correlation coeffizient is only influenced by the comparatively lower variations of the material data. That' why the value for R is quite higher. At the same time the case number N and consequently the statistical reliability decreases. This fact is also demonstrated in the correlation diagrams below for the examples cell area (3h) = f($R_{corr}$) and cell area (3h)=f(K).




In the left diagram the influence of the material parameter $R_{corr}$ on the spreading bevaiour (cell area) of the osteoblastic cells is presented. With increasing corrosion resistance the cell spreading area also increases. The corrosion resistance is a material parameter that is determined by electrochemical methods and characterizes the electrochemically active surface area in such manner that a high $R_{corr}$ points to an even surface with low average roughness $R_a$ and a low $R_{corr}$ to a very rough surface with high $R_a$. This would mean that cells can better spread on material surfaces that are even than on rough surfaces.
The interpretation of the right diagram is more difficult because the physical meaning of the fractal parameter topothesy K is not yet so clear like that of the corrosion resistance.

## 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 of the osteoblastic cells. On the other hand it seems that there is no appreciable influence of material parameters on the integrin expression and only few influence on cell adhesion.
In that cases we found a correlation the correlation coefficient and its statistical significance heavily depend on the method of averaging the available data to get pairs of variats per material modification. So critical error discussion of the results is necessary. But this is a general problem of statistical handling of data, especially if only few data are available. May be that new unconventional methods like bootstrap method can show a way out of this dilemma [1].

## REFERENCES

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Regina Lange
University of Rostock, Department of Computer Science and Electrical Engineering
Albert-Einstein-Str. 2, D-18051 Rostock
+49 381 498 7236 Fax +49 381 498 7202
E-Mail: regina.lange@uni-rostock.de