Topographie der Biomaterialoberfläche – Einfluss auf Zellarchitektur und Zellfunktion

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[3]Universität Tübingen

Universität Rostock Traditio et Innovatio

Hanau Material's Valley, 24.02.2011

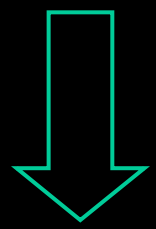
INP Greifswald

Eberhard Karls Universität Tübingen

Einleitung

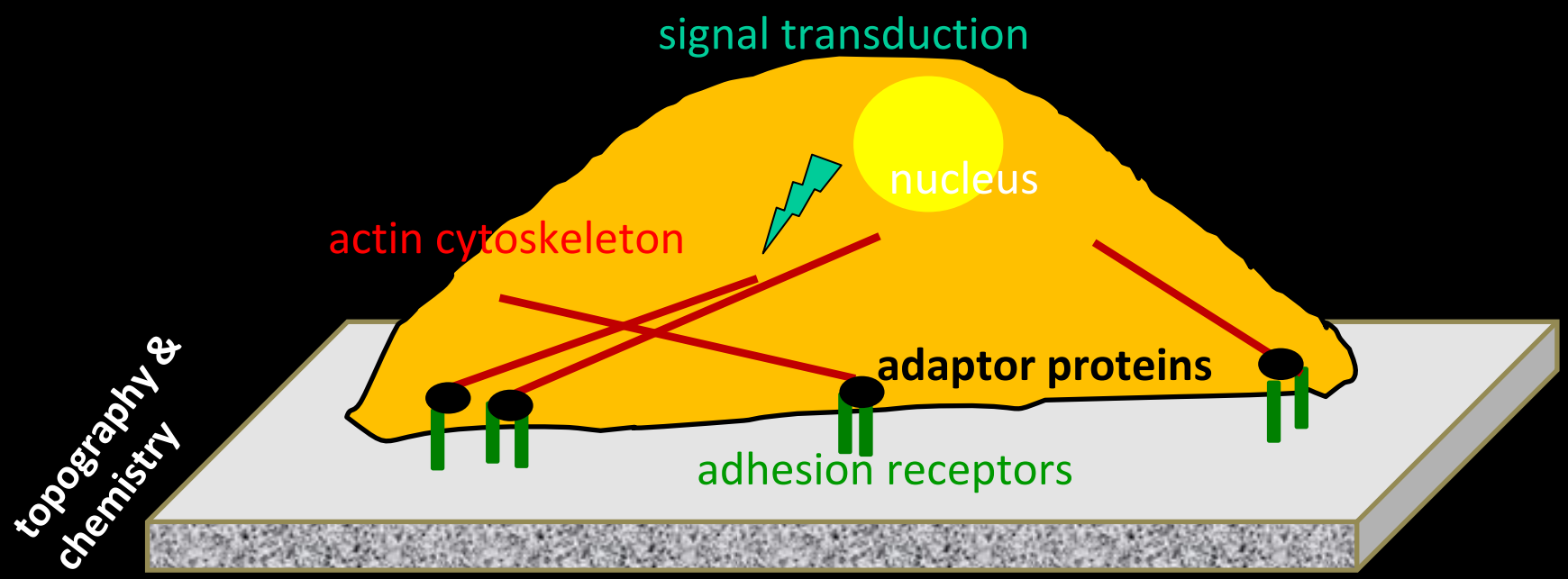
- Interface interactions cells / biomaterial – decisive for the clinical outcome of an implant

- Processes playing a role when cells establish upon a material – initial attachment, cell spreading, proliferation, migration



- Mediated by: extracellular matrix and cellular adhesion structures – e.g. adhesion receptors, actin cytoskeleton, adaptor proteins

Schema - adhärente Zelle auf einer Materialoberfläche



- cell attachment is facilitated by pericellular matrix molecules and by adhesion receptors in focal contacts which include integrins

- adhesion receptors bind extracellulary to surface ligands and are connected intracellularly to adapter proteins (e.g. vinculin, paxillin) that bind to the actin cytoskeleton

- signals that control cellular activities

Rebl et al. Adv. Biomat. 2010

Fokus

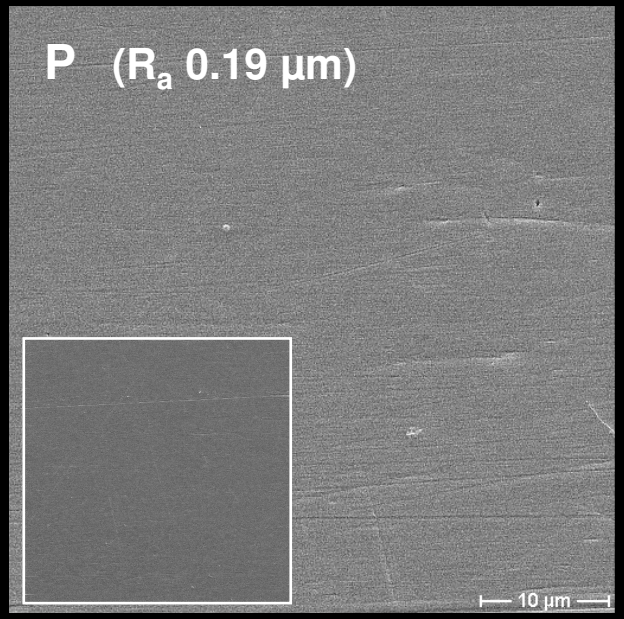
To find out the impact of the material topography
on cell architecture and
on differentiated cell functions

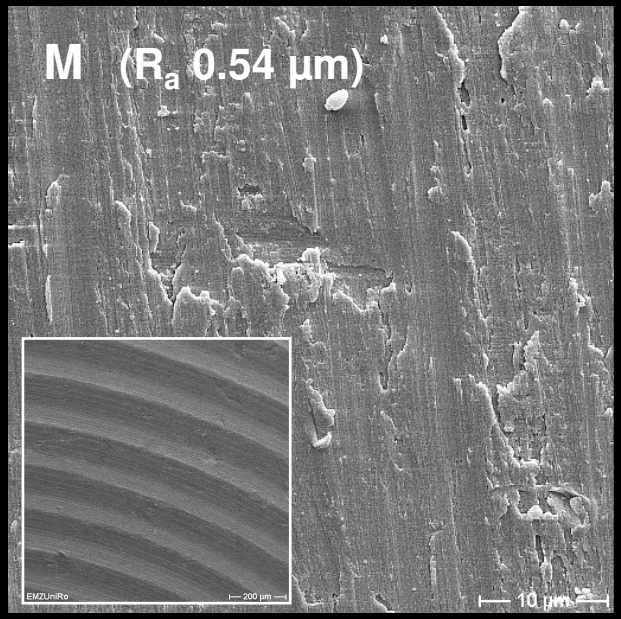
—› stochastic topographical characteristics
—› defined topographical features

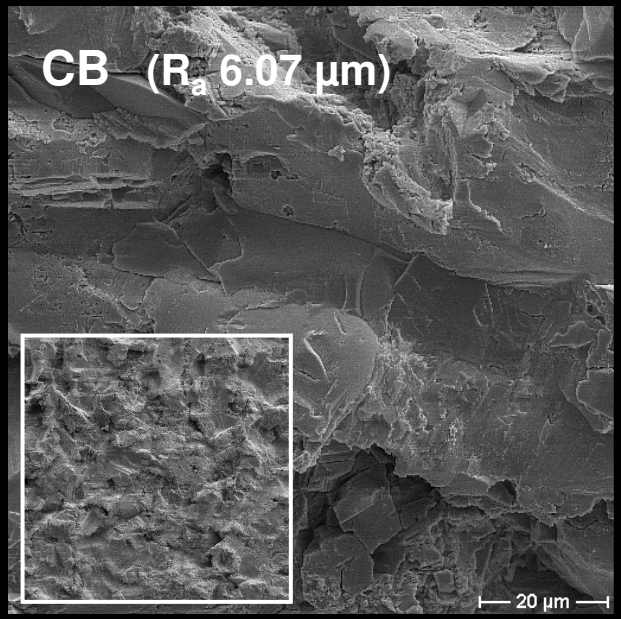
Materials:
>titanium polished, machined, corundum blasted
>cubic pillar arrays, regular geometry

Cells:
>MG-63, human, osteoblast-like

Strukturierte Titanoberfläche - stochastisch







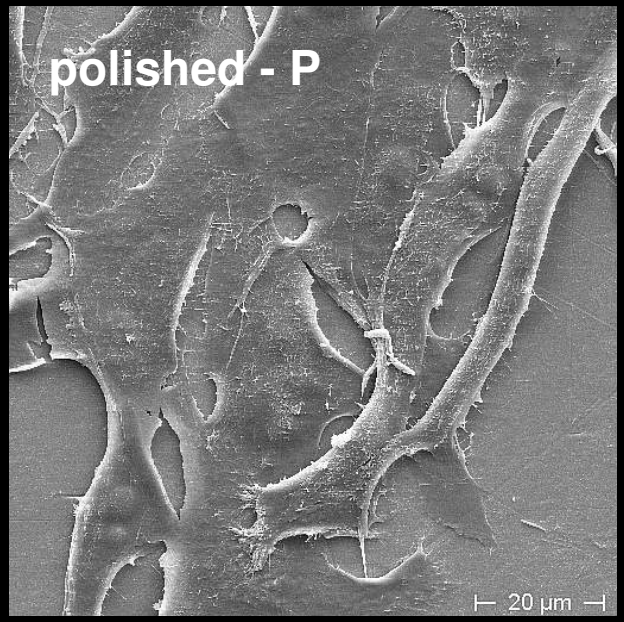
Titanium - P
polished
SiC wet grinding paper

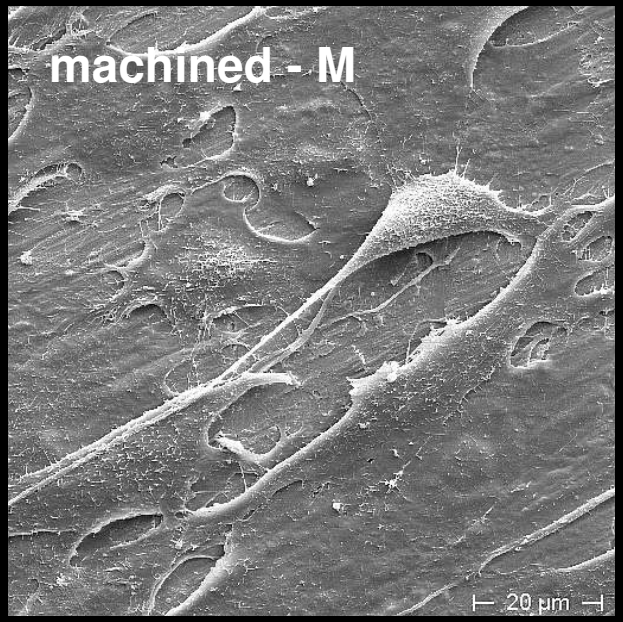
Titanium - M
machined

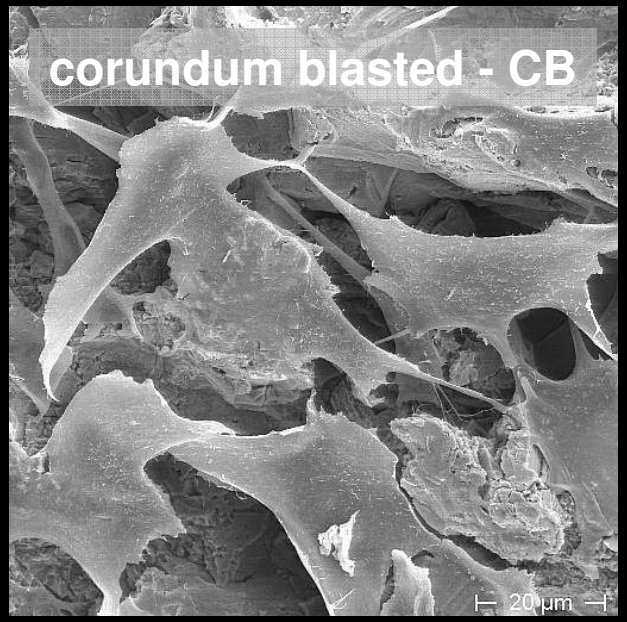
Titanium - CB
corundum blasted

Lüthen et al. Biomaterials 2005

Morphologie der Osteblasten: REM

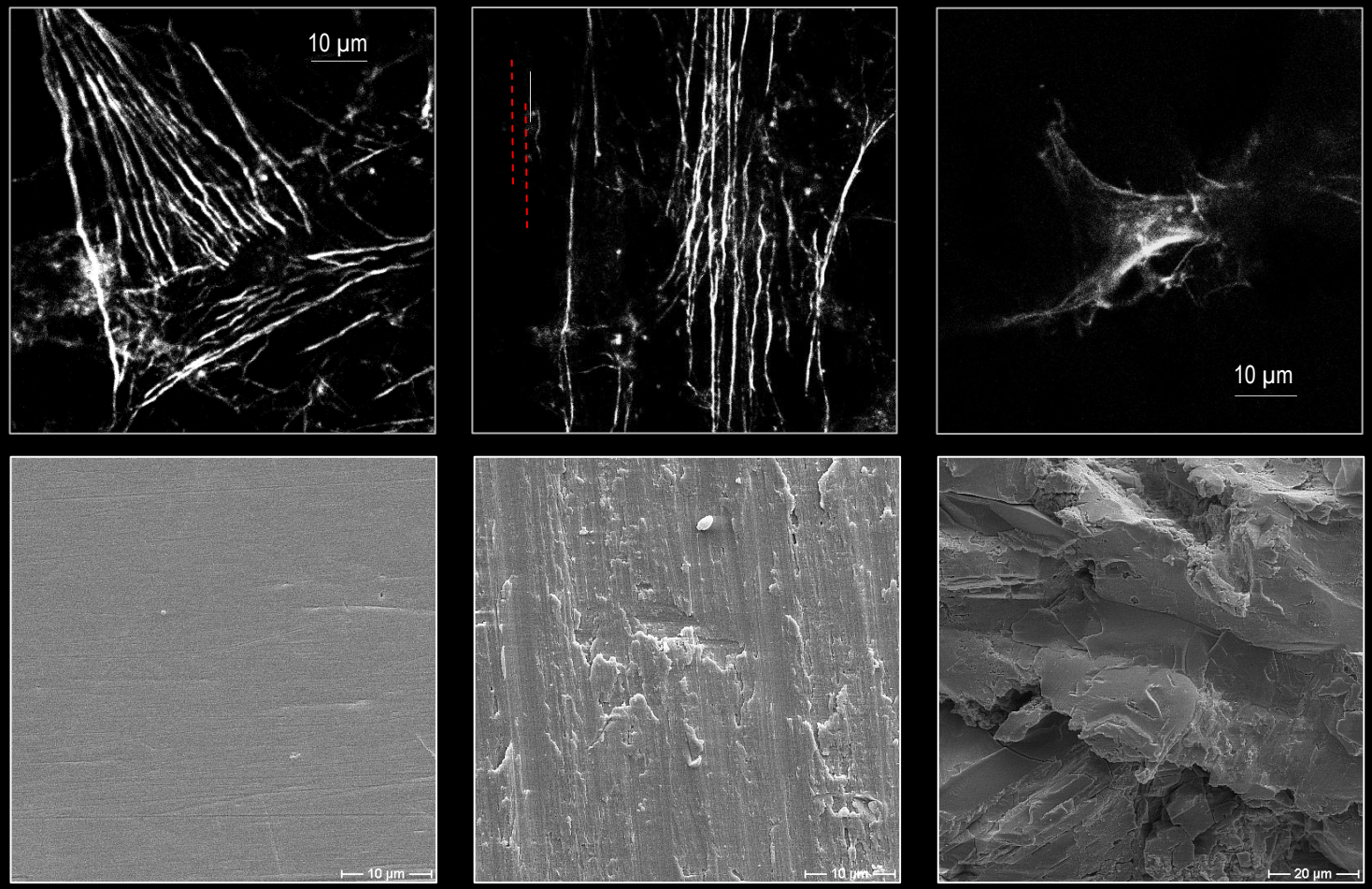






Lüthen et al. Biomaterials 2005

Formation von Aktin in Osteoblasten – ausgerichtet auf maschiniertem Ti, fragmentiert auf Ti CB



Actin

Titanium topography

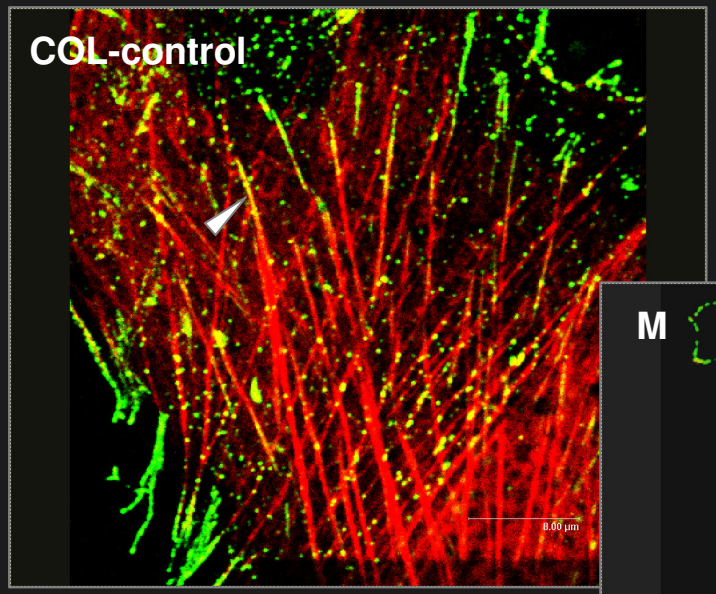
polished

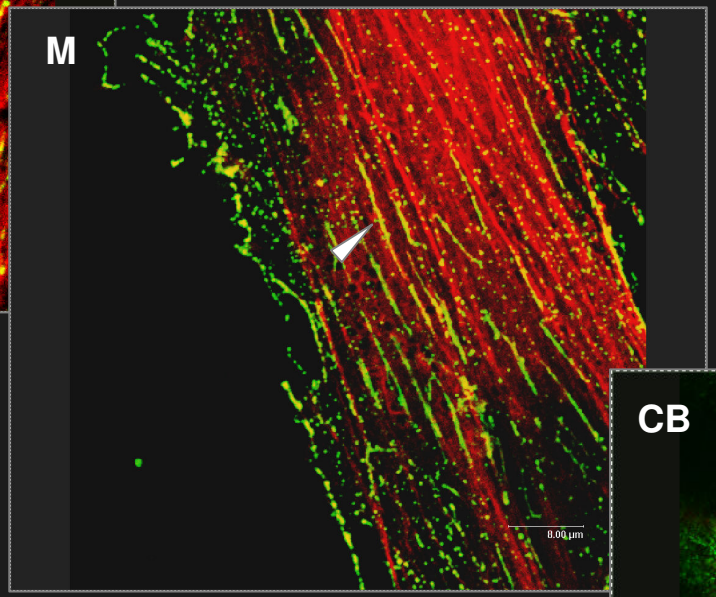
machined

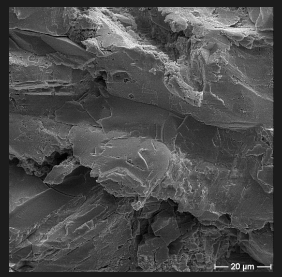
corundum blasted (CB)

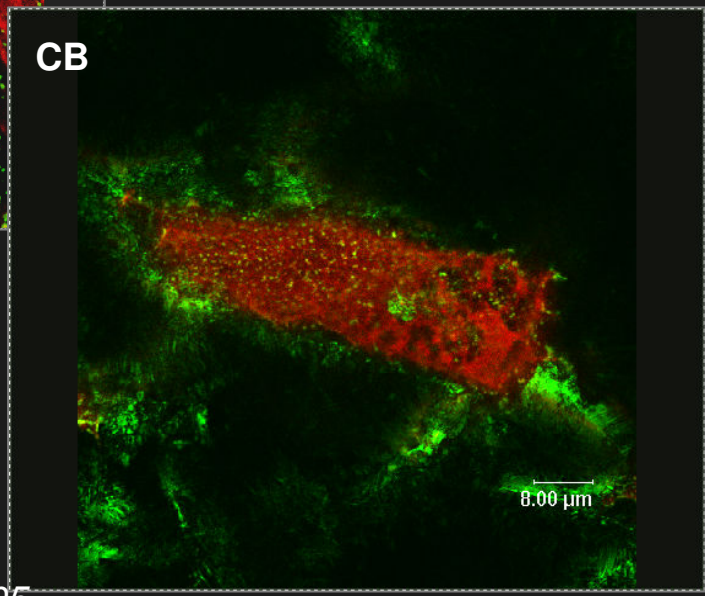
Lüthen et al. Biomaterials 2005

Ko-Lokalisation *(Pfeile)* von Integrinrezeptoren mit Aktin - beeinträchtigt durch Topographie des Ti CB



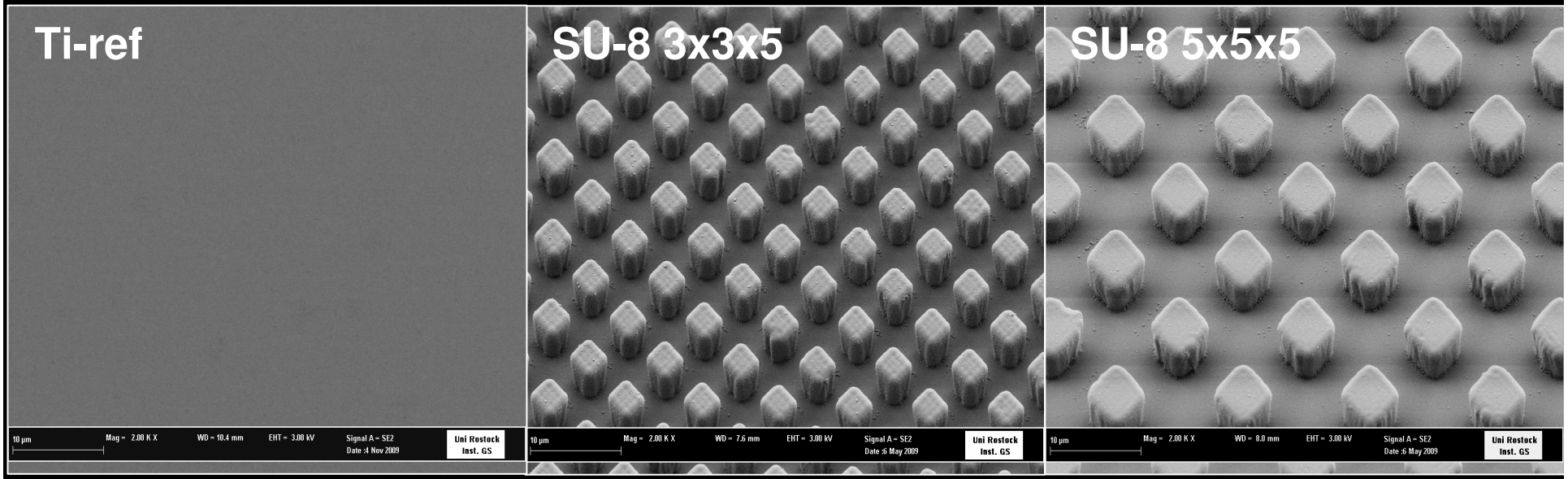






Lüthen et al. Biomaterials 2005

Strukturierte Titanoberfläche - definiert

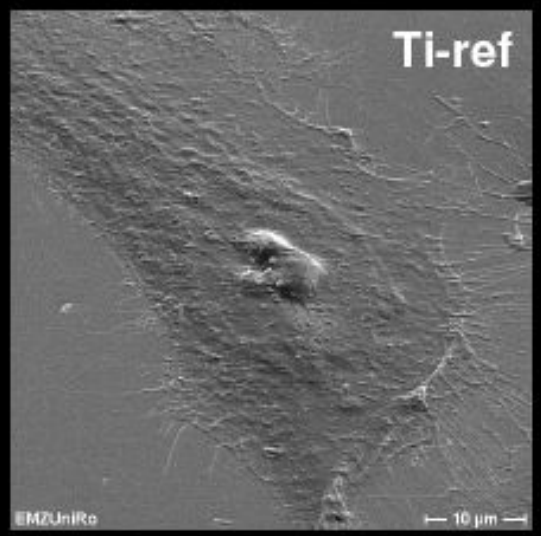


Cubic pillar structures 3x3x5, 5x5x5 µm
(Prof. D. Kern, University of Tuebingen)

Process:
Silicon-wafer
+ negative photoresist SU-8
+ photolithographic process for structuring
+ sputter-coated with 100 nm titanium

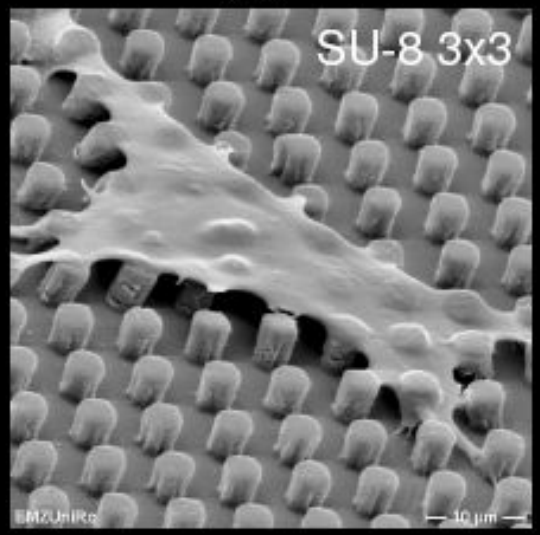
Matschegewski et al. Biomaterials 2010

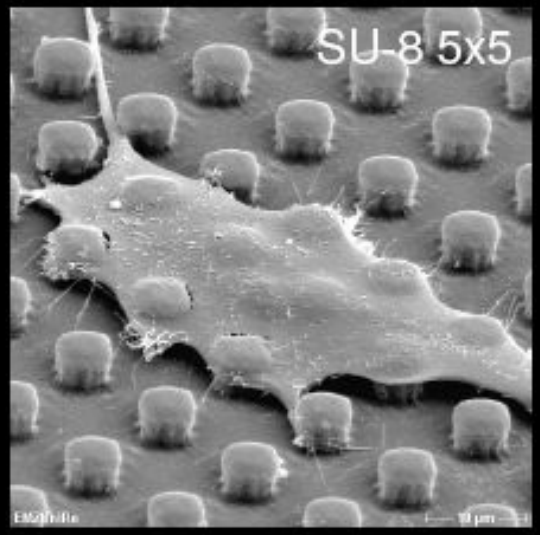
Morphologie der Osteblasten: REM

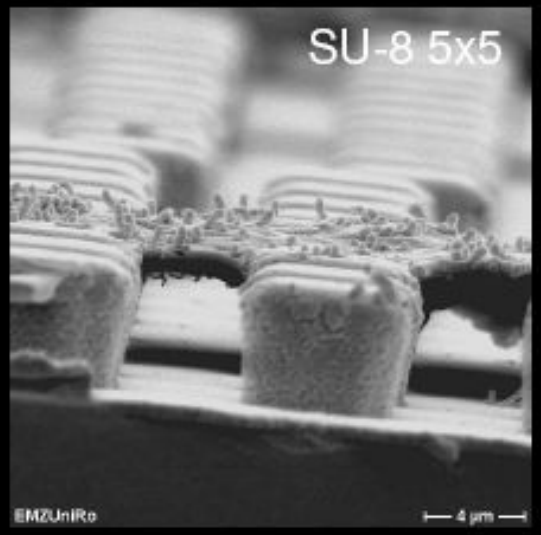


50°

z-sectioning







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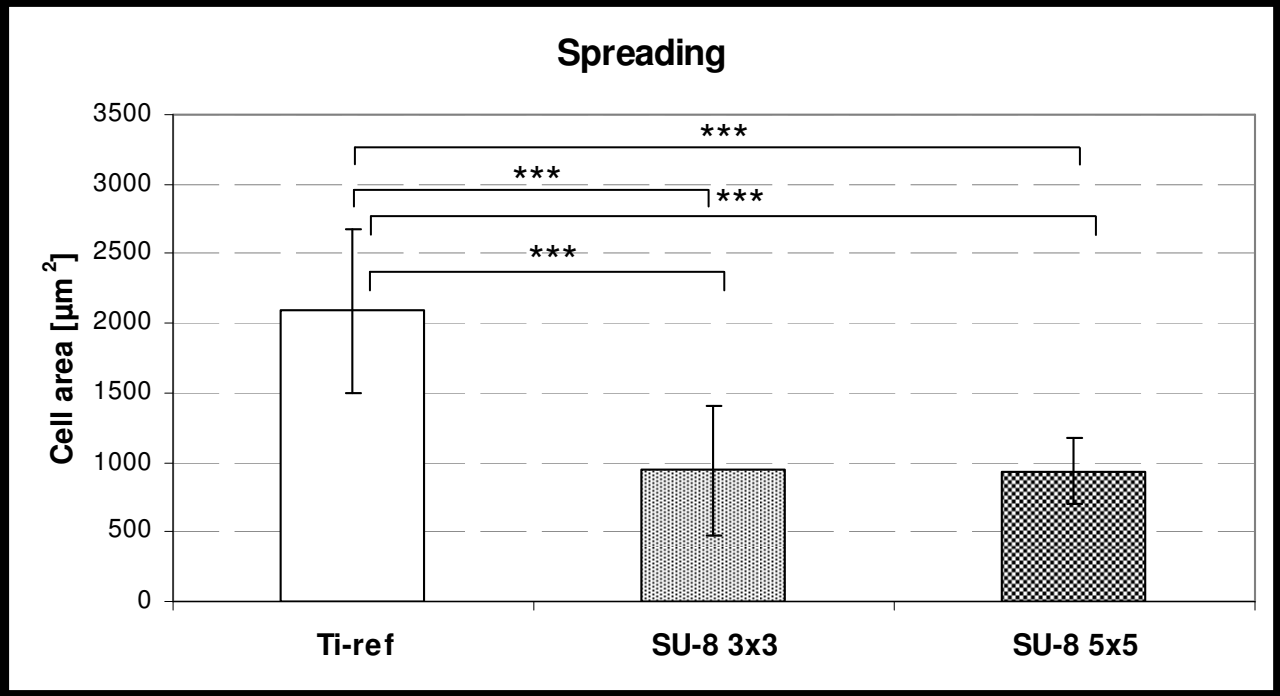
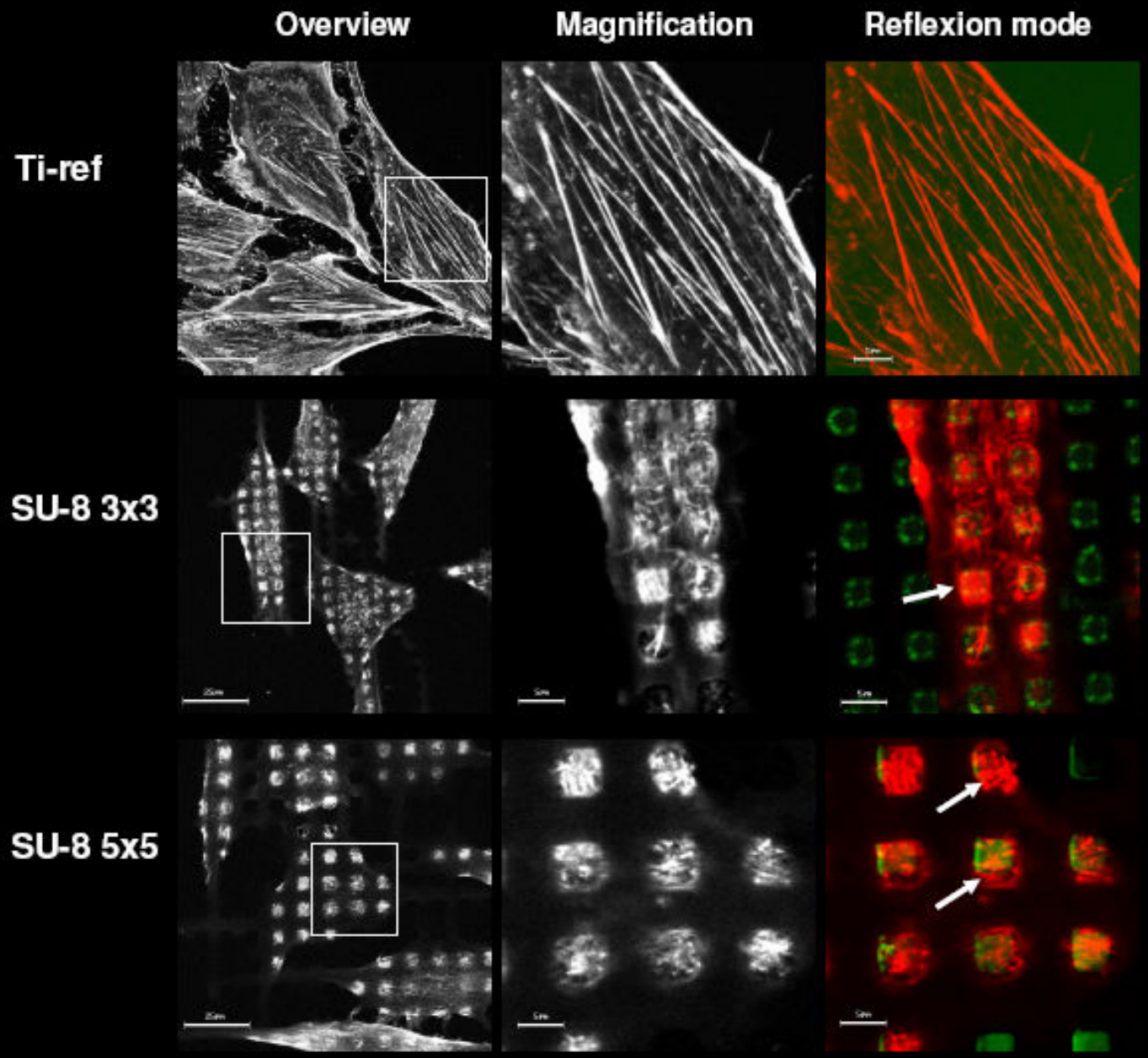
Spreading (24h) von Osteoblasten – reduziert auf SU-8



►spreading on the pillar structures is significantly reduced
- due to the reduced cell's anchorage to the top of the pillars

Matschegewski et al. Biomaterials 2010

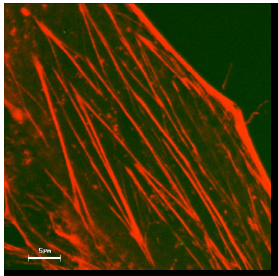
Formation der Aktinfilamente



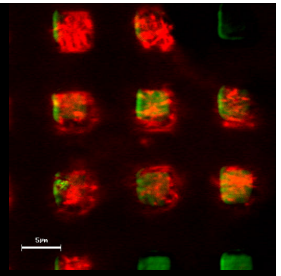
Matschegewski et al. Biomaterials 2010

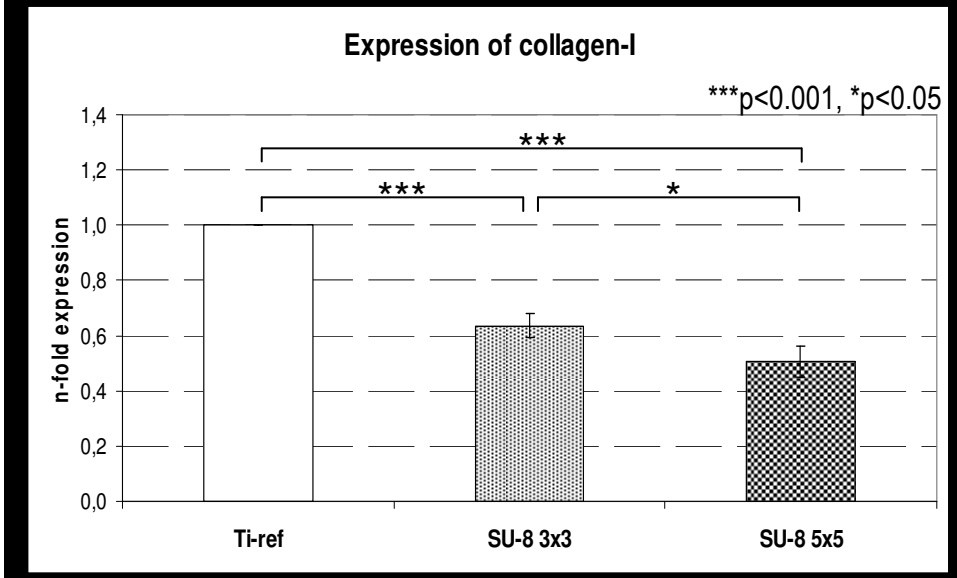
Structural changes of adhesion components in osteoblasts

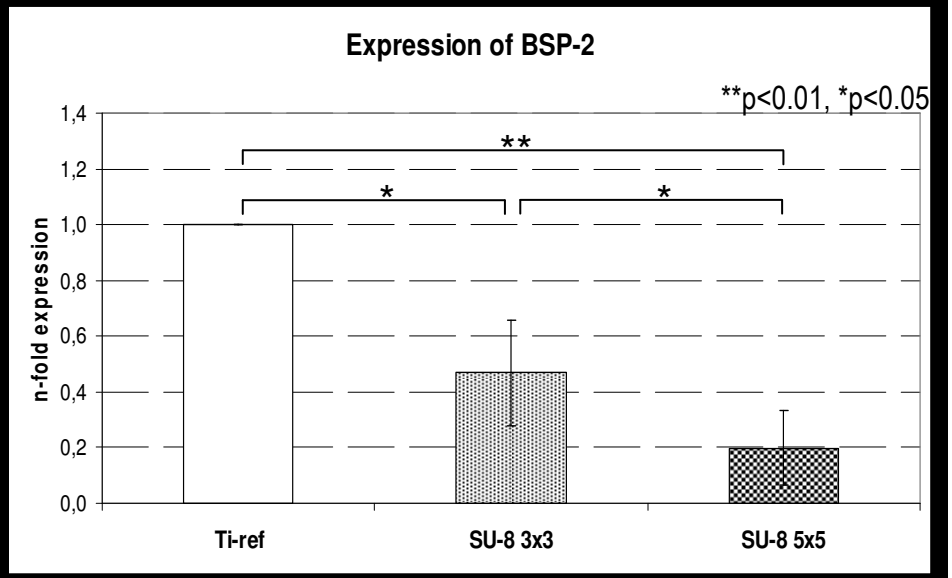
- responsible for an altered cellular function ?



Differenzierte Zellfunktion

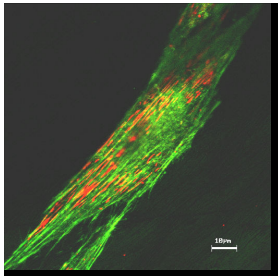


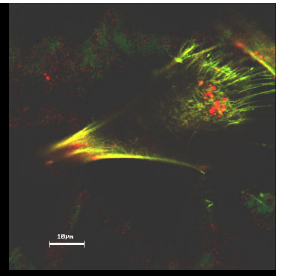


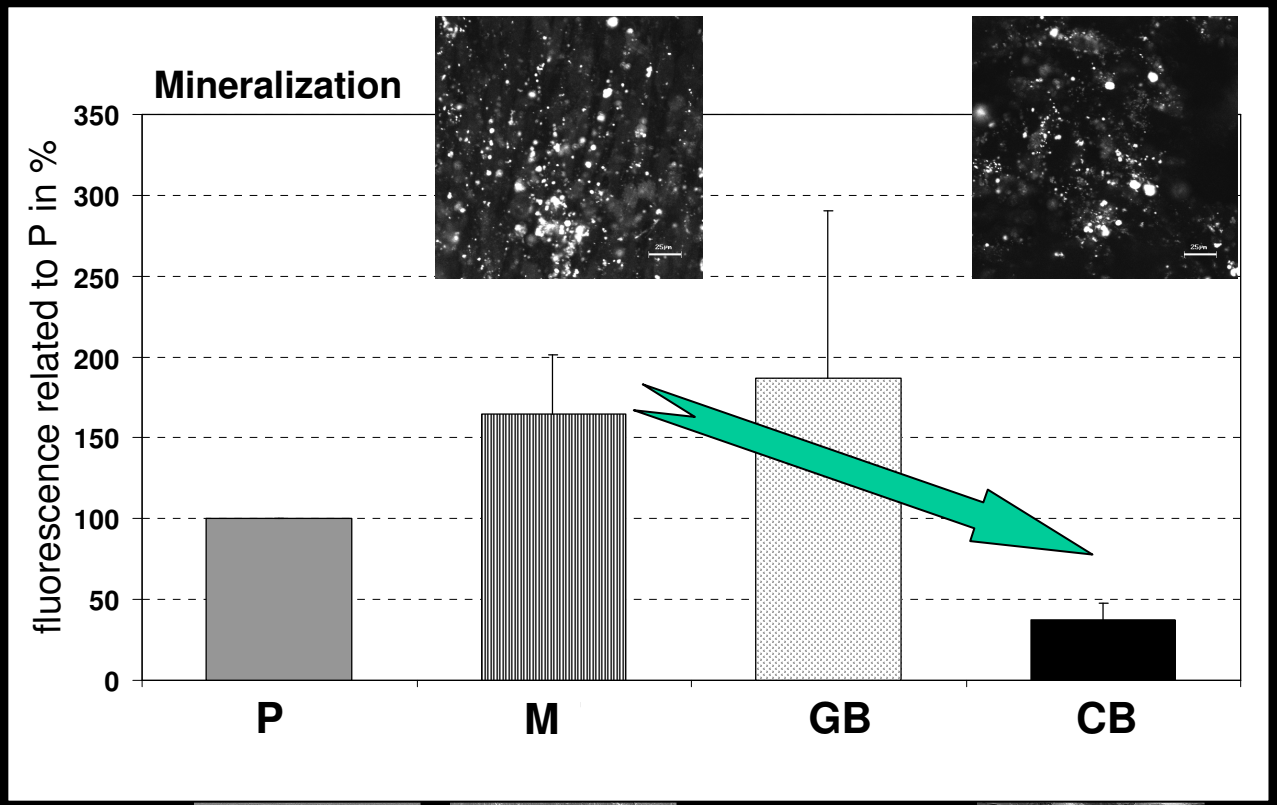


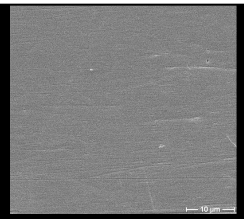
Matschegewski et al. Biomaterials 2010

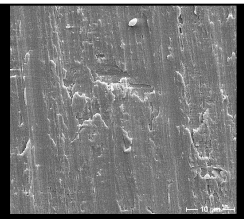
Differenzierte Zellfunktion

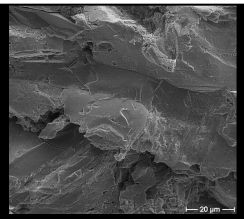












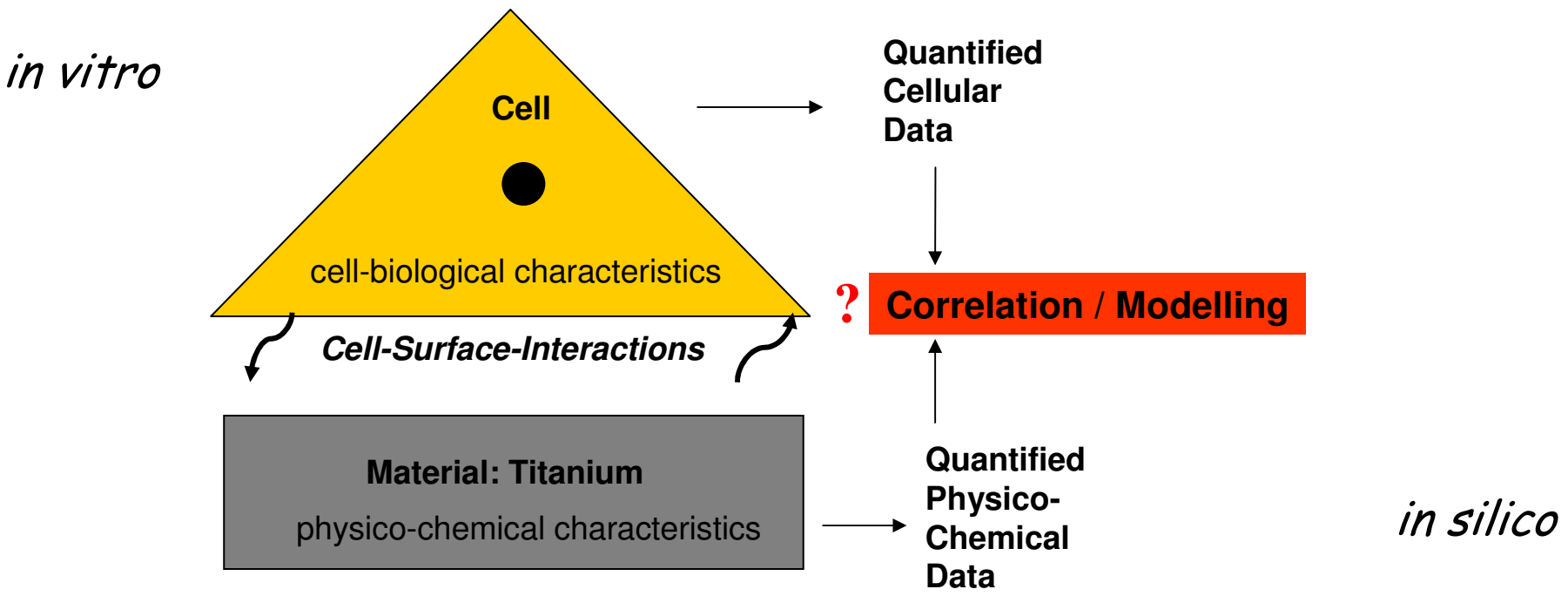
P polished M machined CB corundum blasted

Nebe et al. Mat Sci Engin C 2004

Graduiertenkolleg welisa: Analyse und Simulation elektrischer Wechselwirkungen zwischen Implantaten und Biosystemen

DFG GRK *welisa*: Projektbereich A - Grundlagen:
Einfluss von strukturierten Implantatoberflächen auf angrenzende Biosysteme und dessen mathematische Modellierung

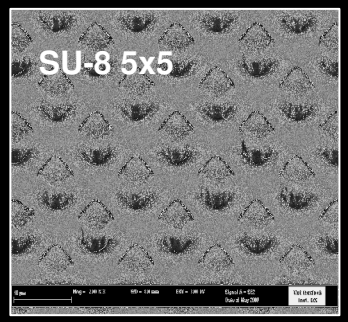
Grafik: Projektbereiche



Korrelation der Zelldaten mit Material-Parametern

►The cell parameters adhesion, spreading, integrin expression, and synthesis of matrix proteins are positively associated with the material surface characteristics wettability and surface energy and CPE-exponent from the EIS.

Hydrophilicity ↓	5 min cell adhesion ↓
Surface energy ↓	24 h cell spreading ↓



on SU-8 pillar structures *vs.* unstructured, flat titanium

Matschegewski et al. 2010, Biomaterials

Quantification of actin filament organization by estimating graph structures in confocal microscopic images

H. Birkholz[1], C. Matschegewski[2], J. B. Nebe[2] and K. Engel[1]

[1] University of Rostock, Institute of Mathematics, Universitätsplatz 1, 18055 Rostock, Germany
[2] University of Rostock, Biomedical Res. Center, Dept. of Cell Biology, Schillingallee 69, 18057 Rostock, Germany

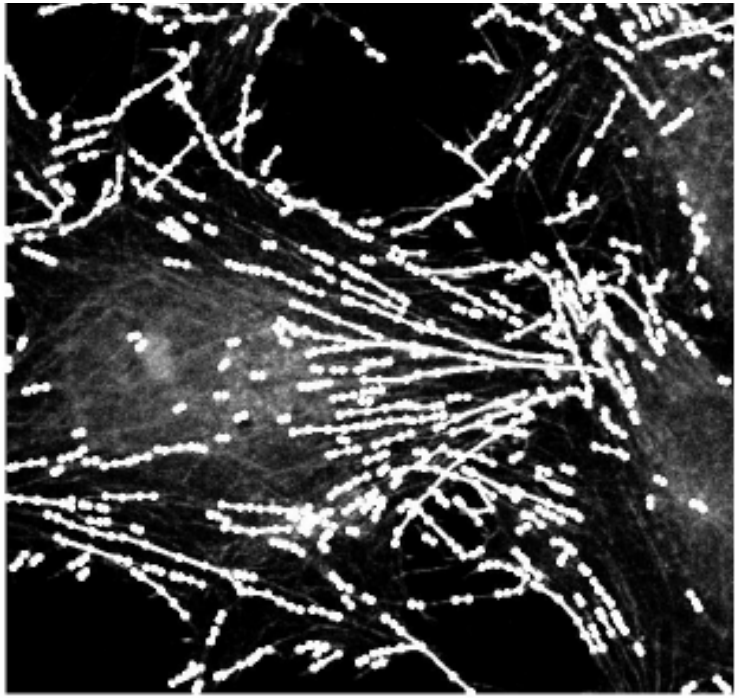


Figure 3 Feature graph (white dots, white lines) representing the estimated filament layout of the underlying gray-valued image shown in Figure 1 (b)

11th International Congress of the IUPESM - World congress on medical physics and biomedical engineering;
07.09.-11.09.2009, Munich, Germany;
ISBN 978-3-642-03881-5, IFMBE Proceedings 25/4 (2009) 1932-1935.

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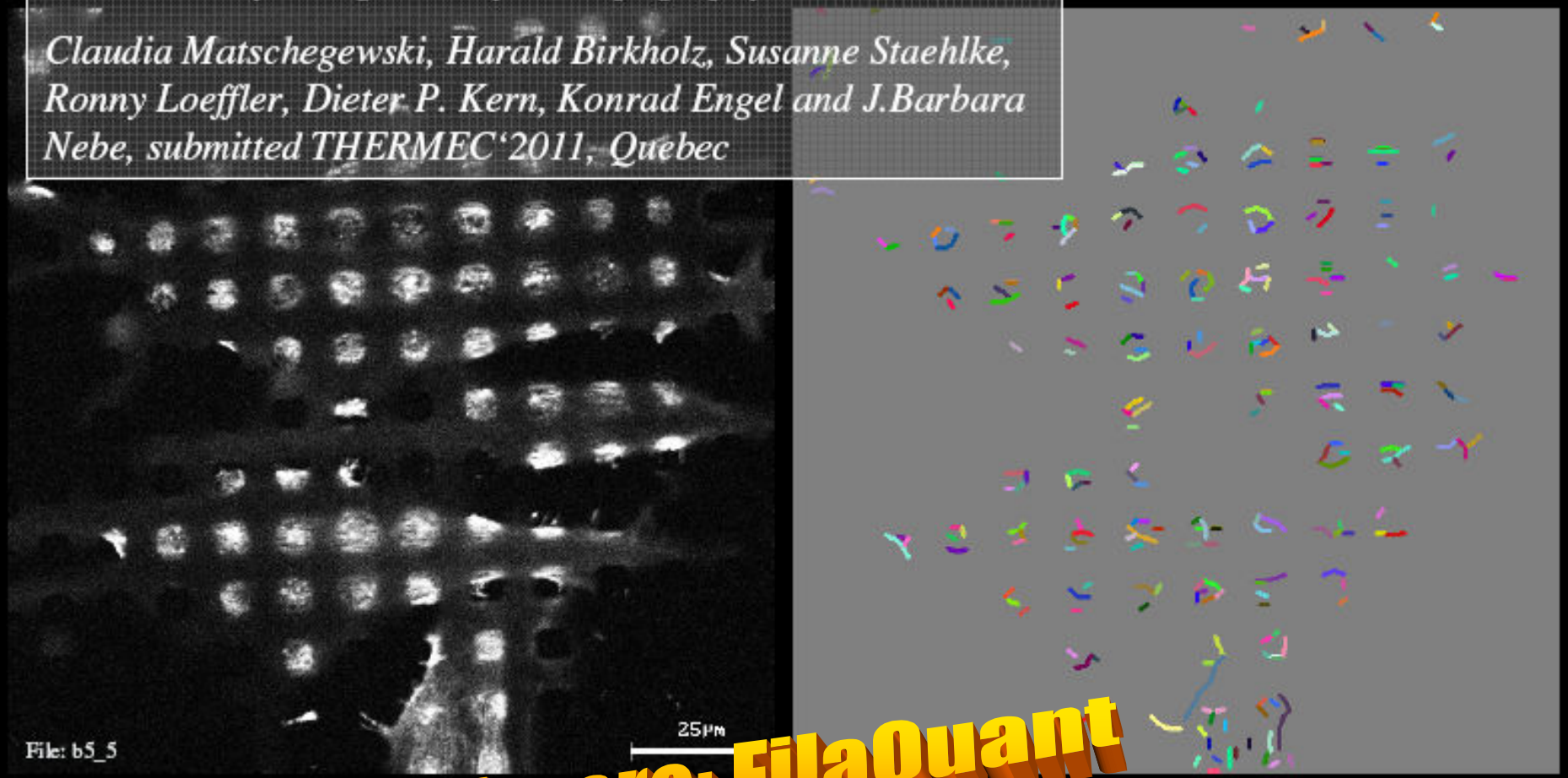
Mathematische Analyse der Aktinzytoskelett-Organisation

Software: Prof. Dr. K. Engel, H. Birkholz, Inst. f. Mathematik, Uni Rostock: konrad.engel@uni-rostock.de

Quantitative Analysis of the Cellular Actin Cytoskeleton on Geometrically Designed Surface Topography

Claudia Matschegewski, Harald Birkholz, Susanne Staehlke, Ronny Loeffler, Dieter P. Kern, Konrad Engel and J.Barbara Nebe, submitted THERMEC'2011, Quebec



Software: FilaQuant

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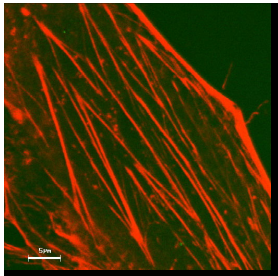
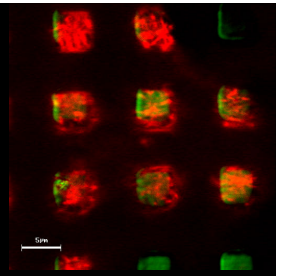
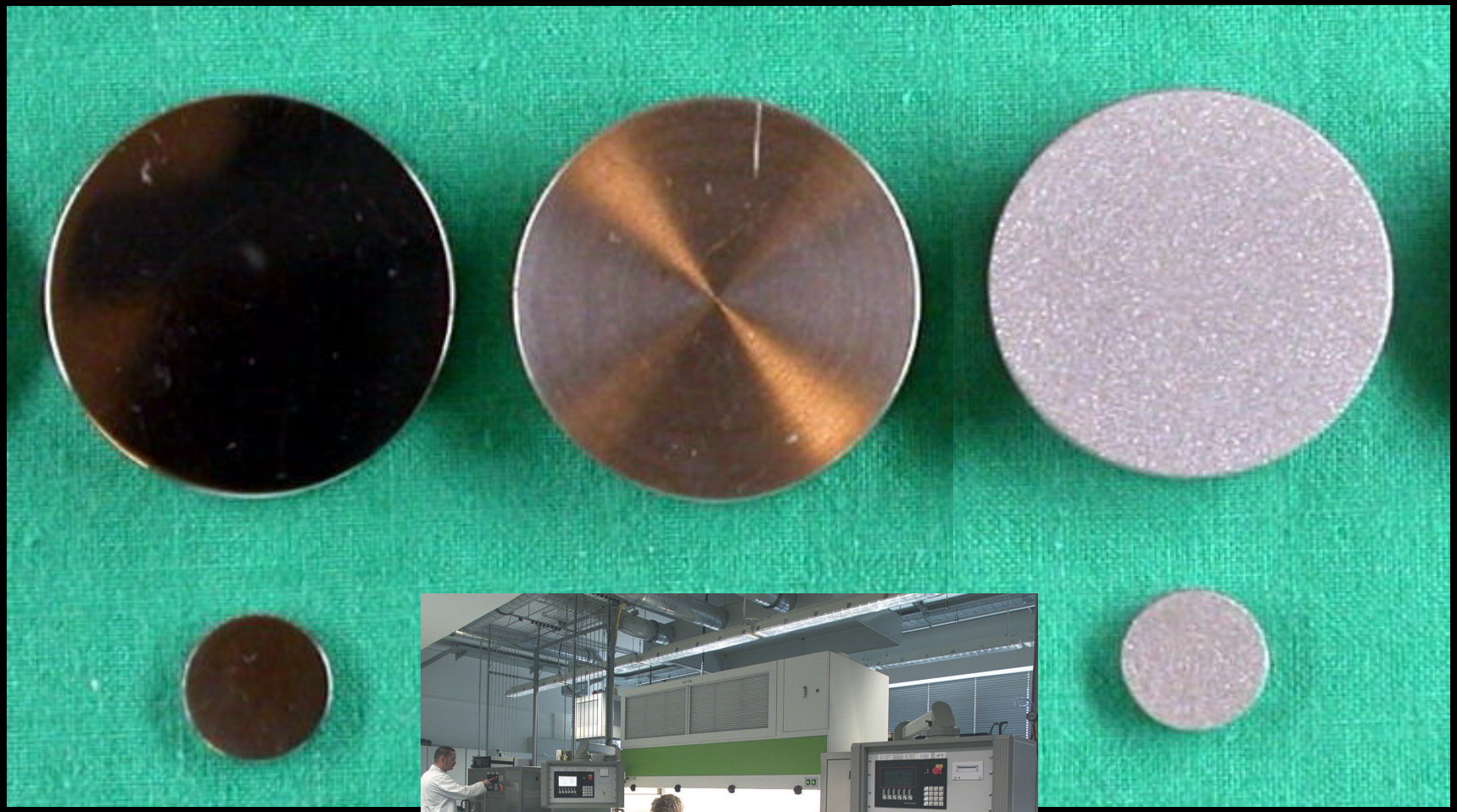




Was ist dominant – die Topographie oder die Chemie einer Oberfläche?

Aminofunktionalisierung der Titanoberflächen



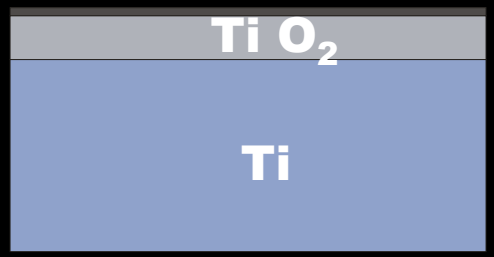
INP Greifswald

CAMPUS PlasmaMed

Finke et al. Biomaterials 2007; Nebe et al. Biomol Eng 2007; Rebl et al. Adv. Biomat. 2010

Aminofunktionalisierung der Titanoberflächen

Ti mit nativem Oxidfilm

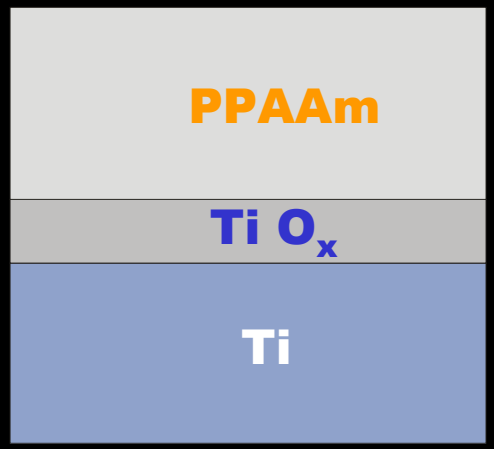


Wasser-Kontaktwinkel
77,7 ± 3,4 ° (Digidrop, GBX, Romans, France)

XPS:
O:Ti = 2:1 (TiO_2)

Allylamin-Plasma-Beschichtung
CH_2=CH-CH_2-NH_2 Plasma
Mikrowellenplasma 2,45 GHz, 500 W, 50 Pa (Niederdruck)
0.1 g/min AAm, 50 sccm Ar, 1000 s (gepulst)

Ti nach Reinigung mit Sauerstoffplasma und Beschichtung mit Plasmapolymer

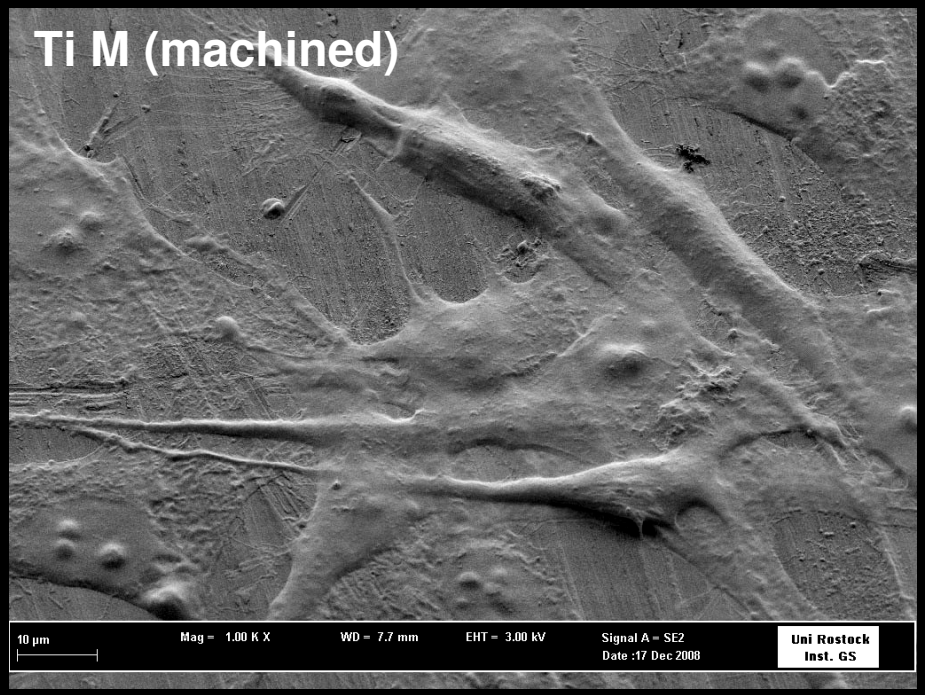


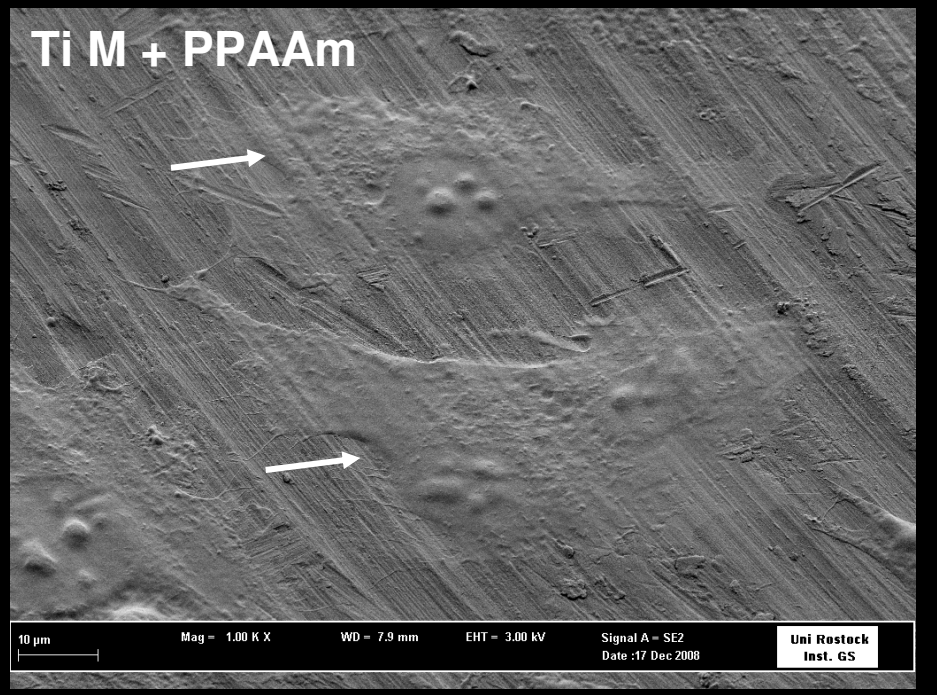
ca. 100 nm dünner Polymerfilm

Wasser-Kontaktwinkel
48,2 ± 3,4 °

PPAAm = Plasma Polymerisiertes Allylamin

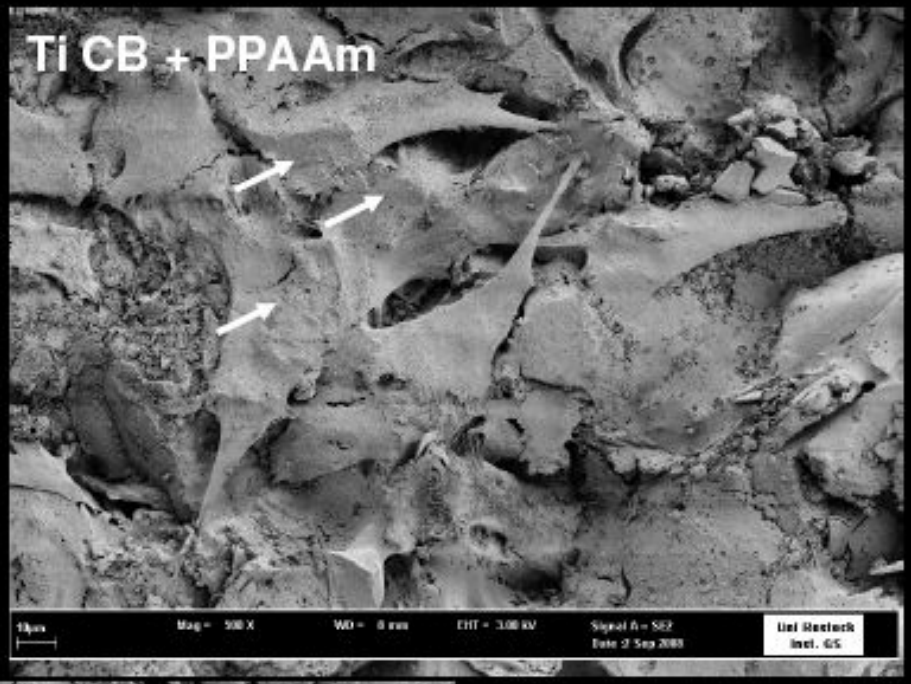
Zell-Titan (Ti)-Kontakt verstärkt durch Plasmapolymer

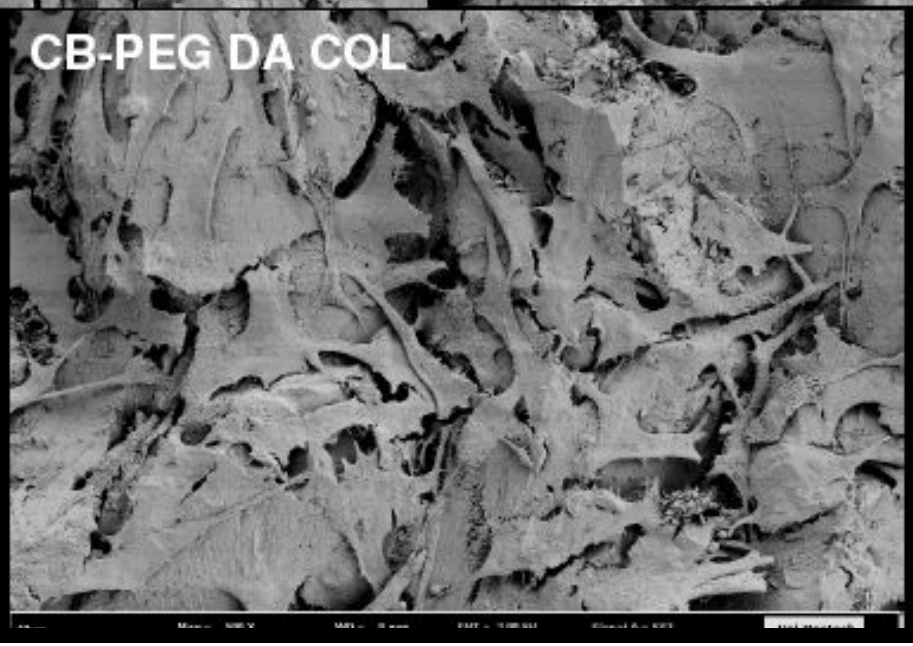




Finke et al. Biomaterials 2007

Zell-Titan (Ti)-Kontakt verstärkt durch Plasmapolymer

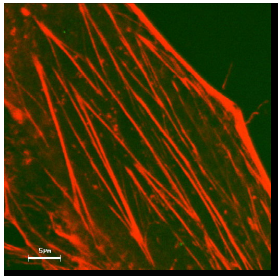




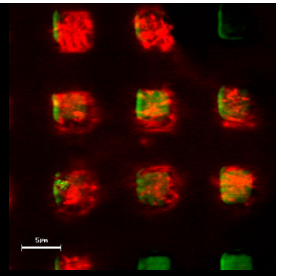
Kollagenanbindung zum Vergleich →

REM: R. Lange; Uni Rostock

CAMPUS PlasmaMed



Zusammenfassung



- Topography induced cell architecture–cell function dependencies !

- Influence of stochastic and regular surface geometry on cell behavior.

- Detailed knowledge about the correlation of cellular parameters with physico-chemical surface characteristics – could facilitate the design of biomaterial surfaces.

  ► Future experiments: determine the local physico-chemical characteristics of the surface

- The plasmachemically modified material surface could be dominant vs. their topography concerning cell orientation and attachment

Cooperation:

G.Fulda, M.Laue, EMZ Uni Rostock
F.Chai, University of Lille, France

Financial Support:

DFG, NE 560/3-3, 3-4; NE 560/7-1,7-2
DFG graduated school welisa, 1505/1
BMBF, Campus PlasmaMed, 13N9779

Deutsche Forschungsgemeinschaft DFG

Universität Rostock welisa DFG-Graduiertenkolleg 1505/1

CAMPUS PlasmaMed

Biomedical Research Center

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