

Wechselwirkung Material/Zelle-Oberflächeneigenschaften beeinflussen die Zelldifferenzierung

Von der Natur lernen: Biomimetische Beschichtungen auf Titanoberflächen

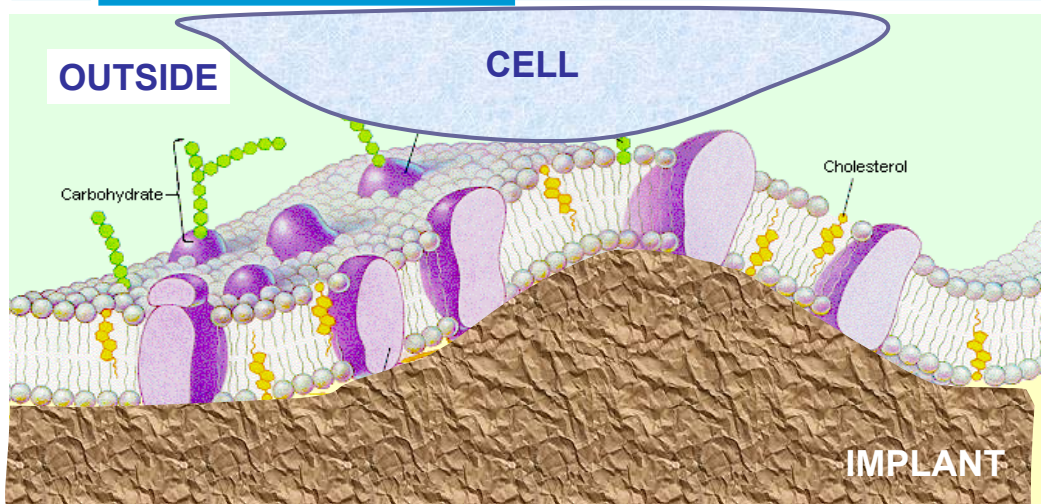
Regine Willumeit, F. Feyerabend, B. Luthringer, V. Haramus, D. Lott, A. Schreyer

Hanau, 24.02.2011

 **Helmholtz-Zentrum Geesthacht**
Centre for Materials and Coastal Research

The Idea

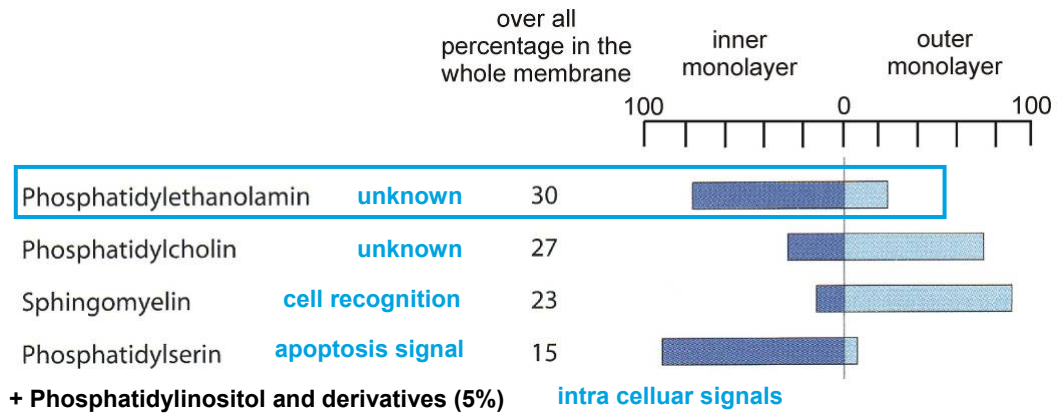
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- ➔ Do lipids have an effect and which is the best lipid?
- ➔ Structural characterisation of the surface

Which Lipid to Chose?

Composition of an Erythrocyte Membrane



POPE : 1 mM solution in chloroform/methanol (80:20)

Nelson & Cox, Lehninger Biochemie, Springer Verlag (2001)

Materials

VPS-Ti mirror polished Ti Ti6Al4V (LDC)



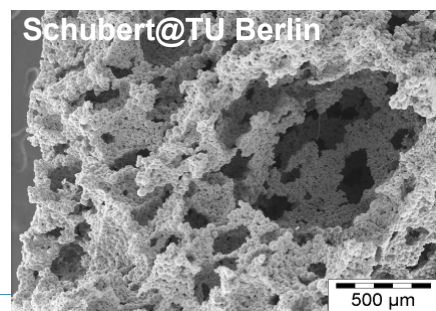
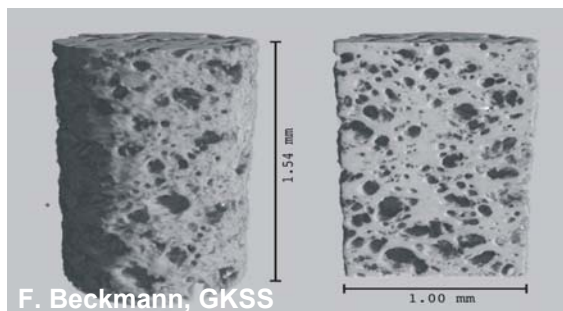
vacuum plasma sprayed Ti (courtesy of DOT GmbH, Rostock)

mirror polished Ti and Ti6Al7Nb/4V plates

LDC Ti6Al4V

34.4 % unconnected porosity

Ti6Al7Nb/4V Powder
'protein foaming'



Cellbiological Studies

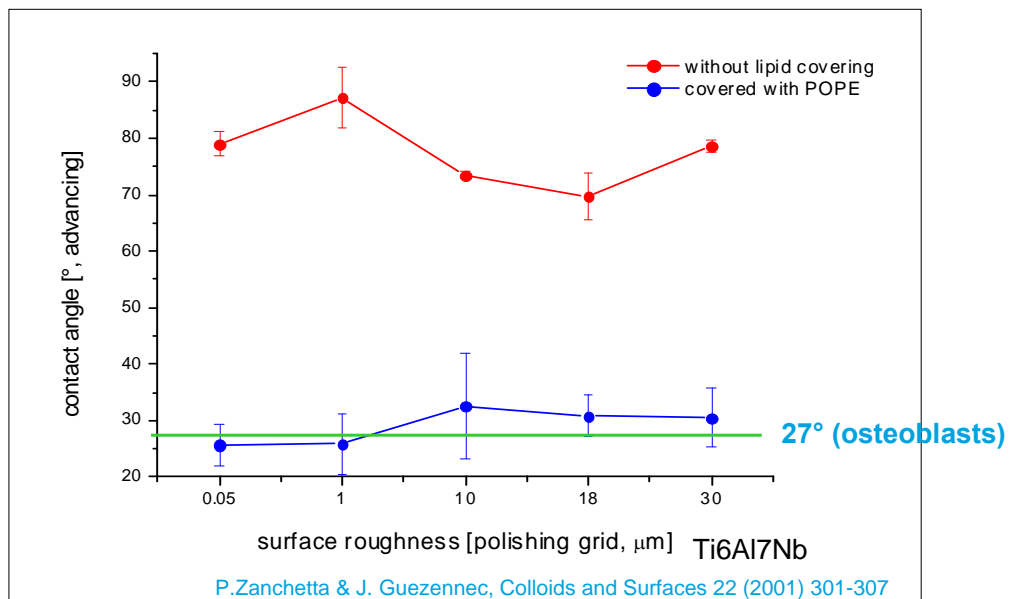
Cell types

- human articular chondrocytes
- human bone derived cells, MG63
- human mesenchymal stem cells (HUCPV)
- macrophages (RAW 264.7 mouse)
- Gram positive/negative bacteria

Parameters

- adhesion
- morphology
- proliferation rate / DNA / MTT
- differentiation
- chondroitinsulfate / osteocalcin / collagen.....
- inflammatory response (TNF- α , IL-1)

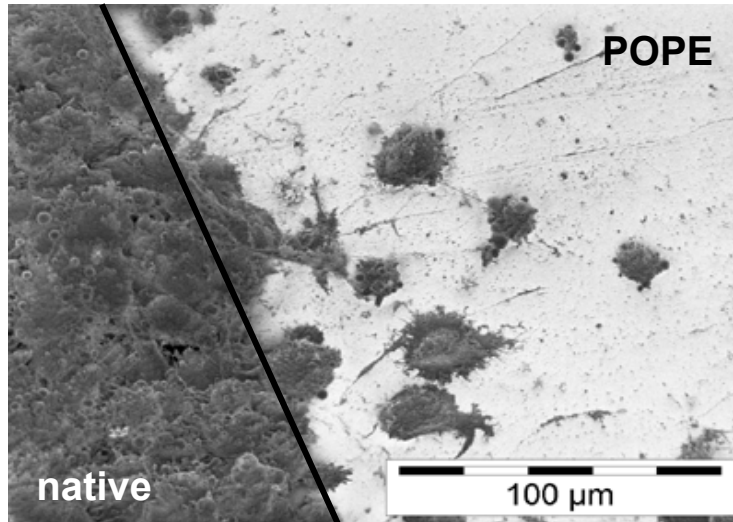
What happens with the Contact Angle?



Willumeit et al. *Mat. Res. Soc. Symp. Proc.* 734 (2003) B 8.6.1 - B 8.6.6

How do Cells react?

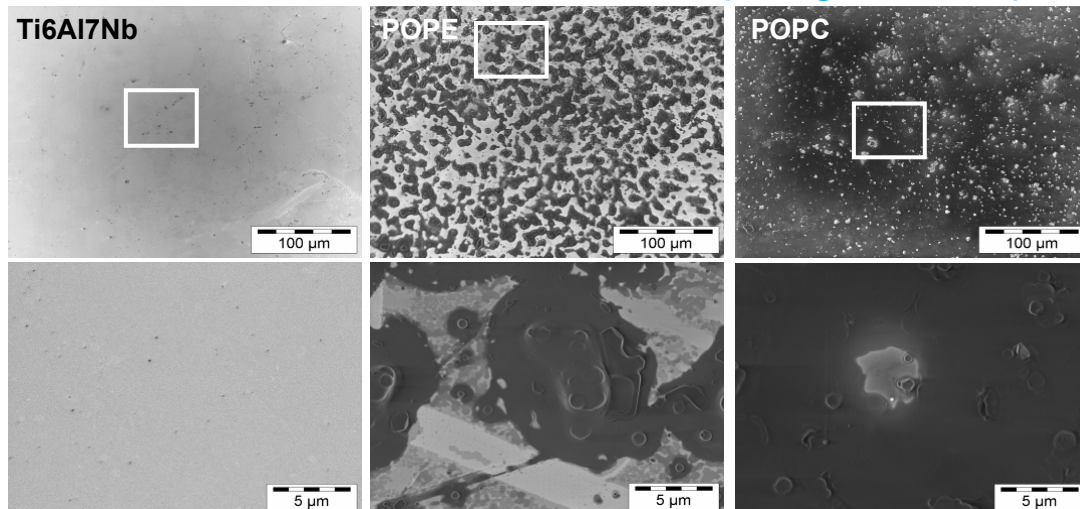
Chondrocytes on Lipid Layers (mirror polished Ti6Al7Nb)



Willumeit et al. *ECM* 13 (2007) 11-25

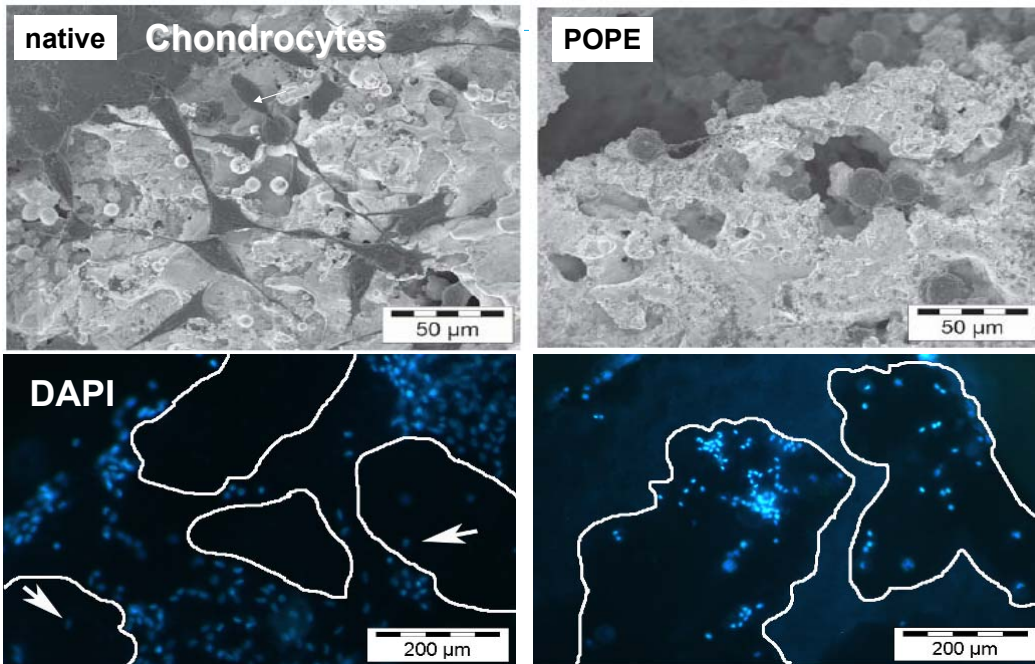
What does the surface look like?

SEM of lipid covered Ti6Al7Nb (mirror polished) after 2 hours of incubation in PBS (Charge Contrast)



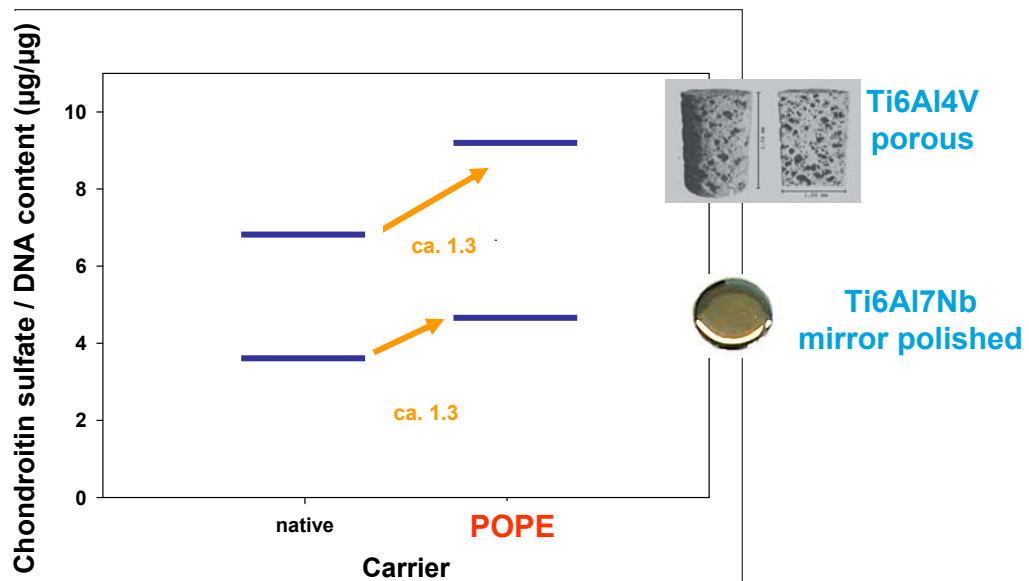
Willumeit et al. *J Mater Sci. Mater Med* 18 (2007) 367-380

How do Cells react?



Willumeit et al. *ECM 13* (2007) 11-25

How do Cells react?

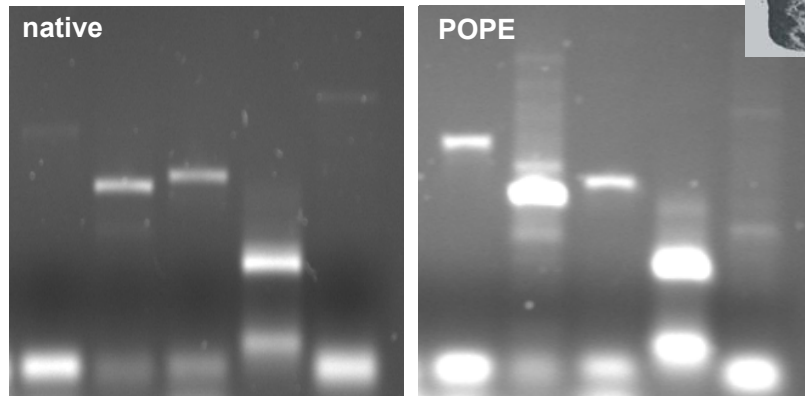


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Human Mesenchymal Stem Cells on POPE Lipids

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Gel-electrophoretic analysis of several protein encoding genes



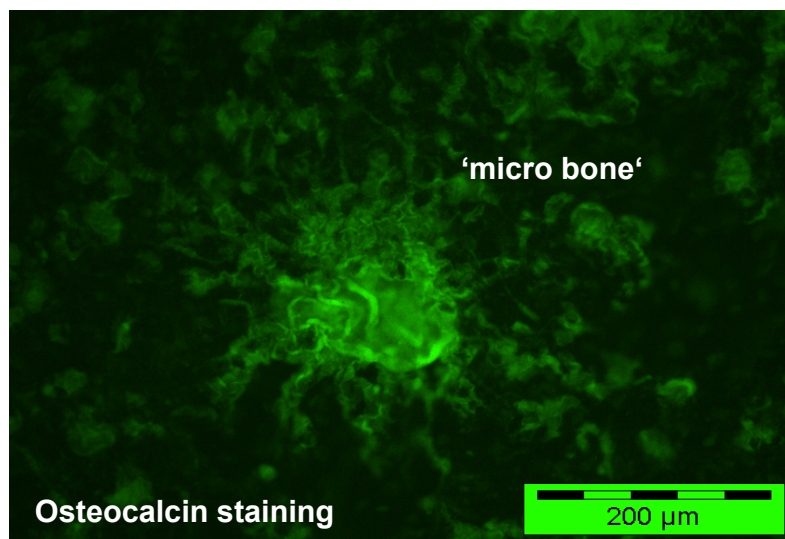
ALP OC OPN cbfa1 C I ALP OC OPN cbfa1 C I
ALP = Alkaline Phosphatase, OC = Osteocalcin, OPN = Osteopontin,
cbfa1 = bone cell marker, C I = Collagen I

Willumeit et al. *ECM* 13 (2007) 11-25.

Human Mesenchymal Stem Cells on POPE Lipids

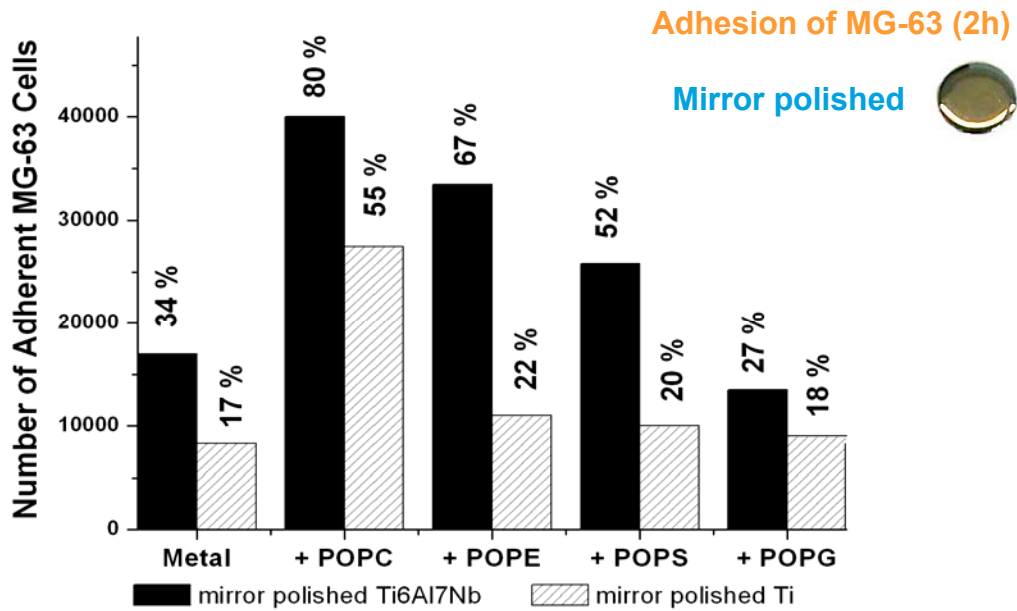
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Bone Nodule Formation on POPE

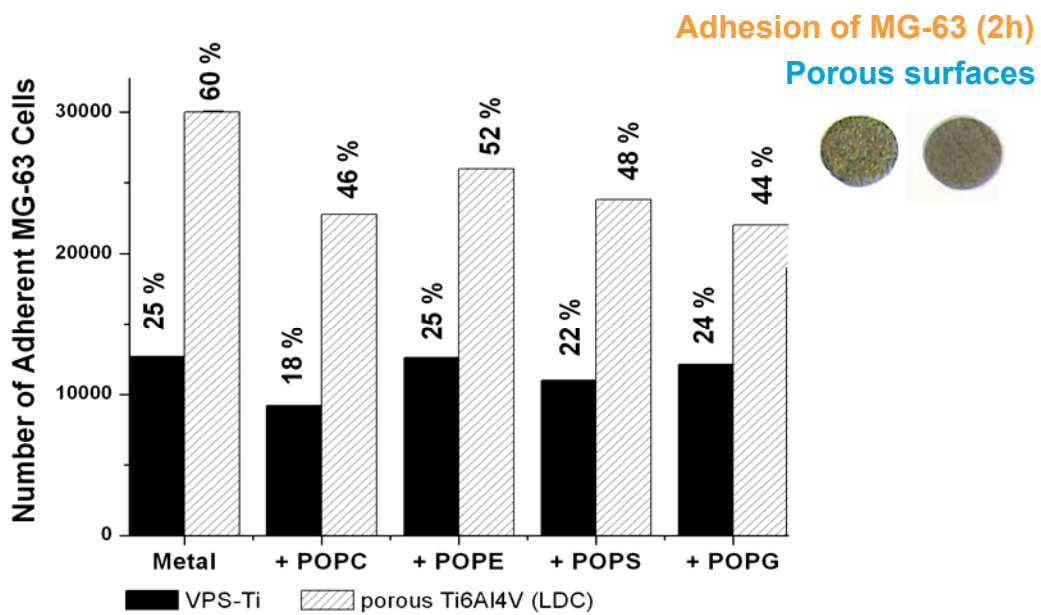


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Comparison of different lipids

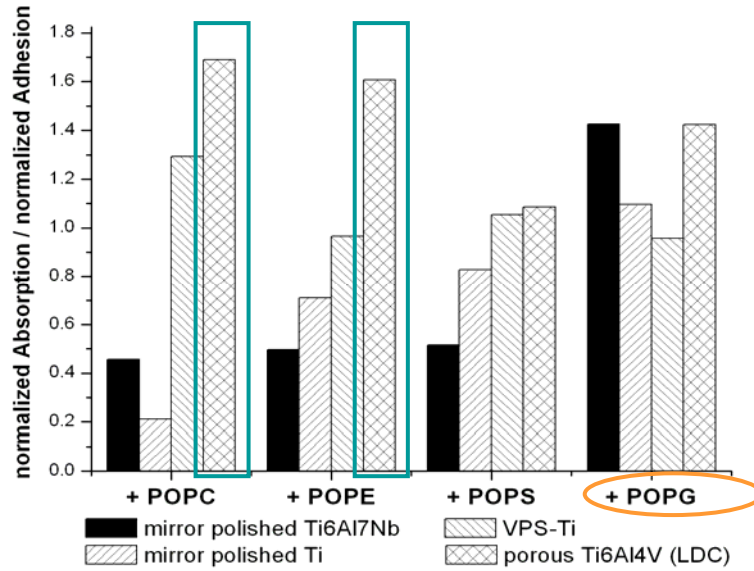


Comparison of different lipids



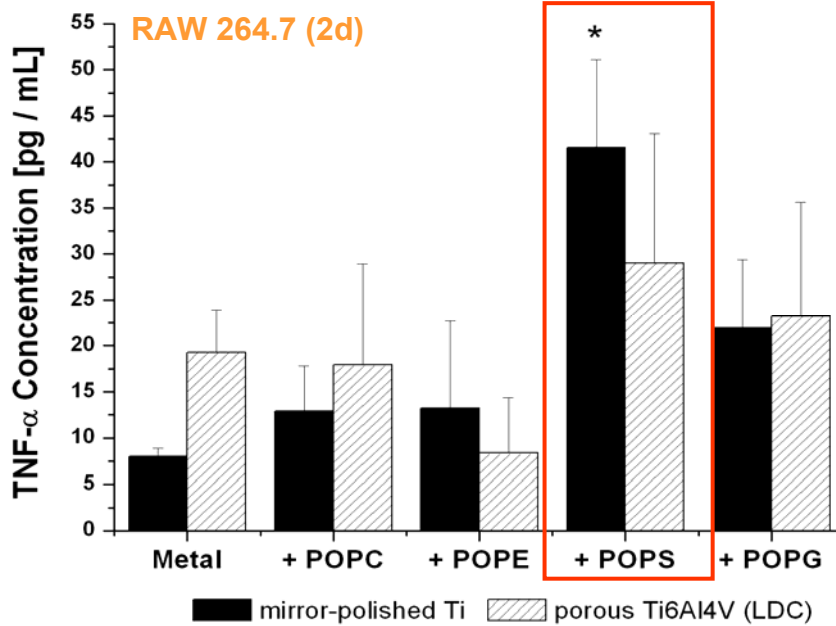
How do Cells react?

Correlation: viability per adherent cells (MG-63)



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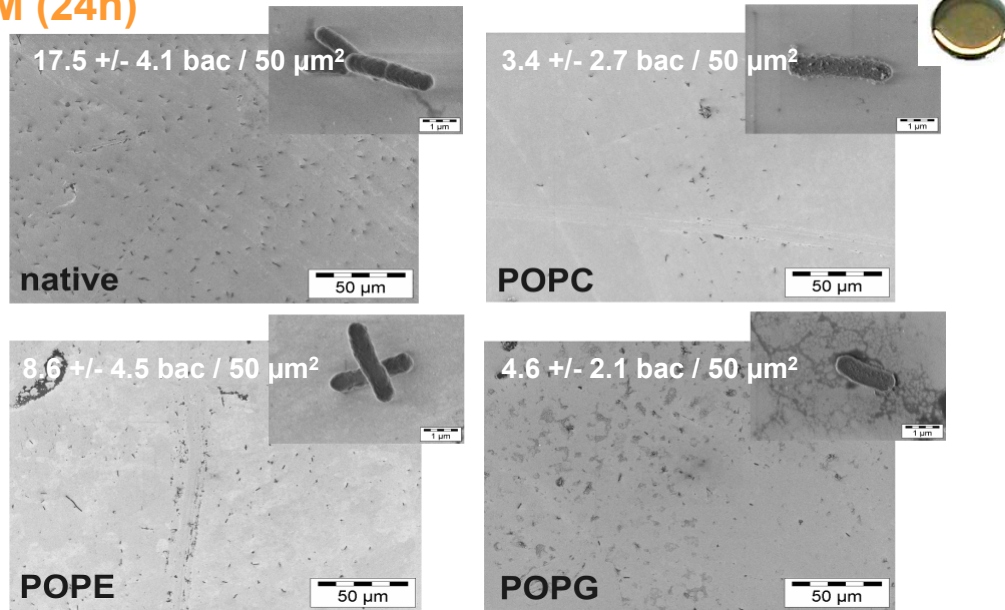
Influence of Lipids on TNF- α



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Influence of Lipids on Bacteria

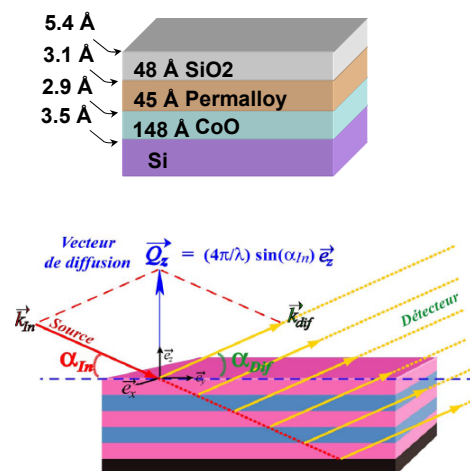
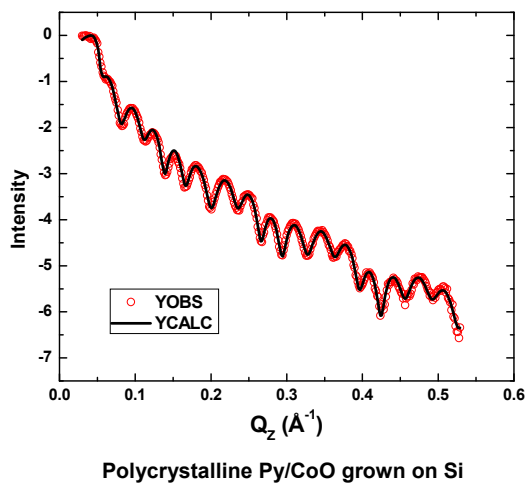
SEM (24h)



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Introduction to Reflectometry

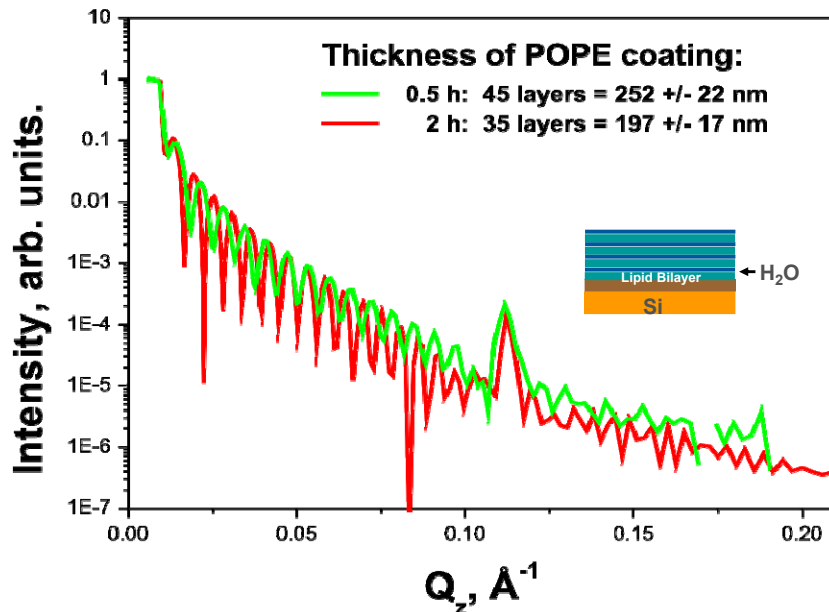
Reflectivity of a multilayer



Thomas Gutberlet, HZB, Berlin

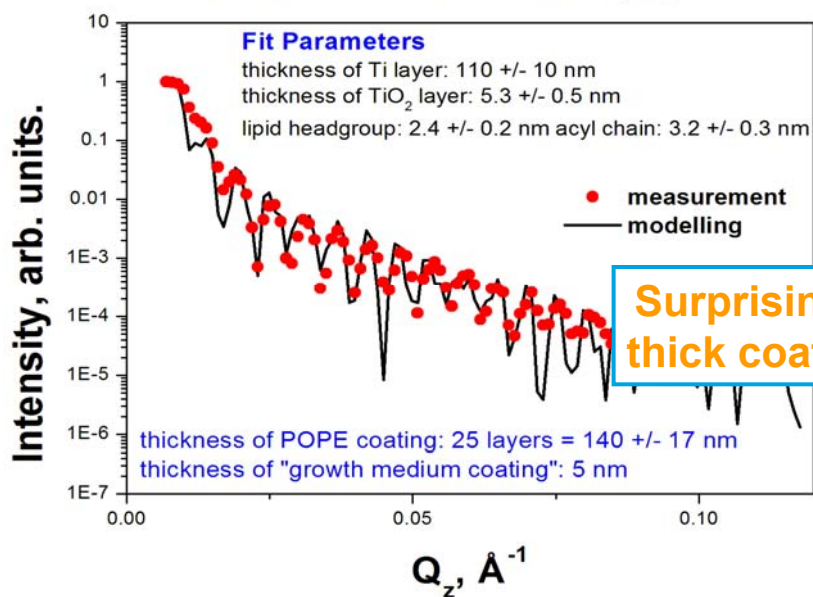
What does the coating look like?

Ti-6Al-7Nb + POPE: Incubation in Water

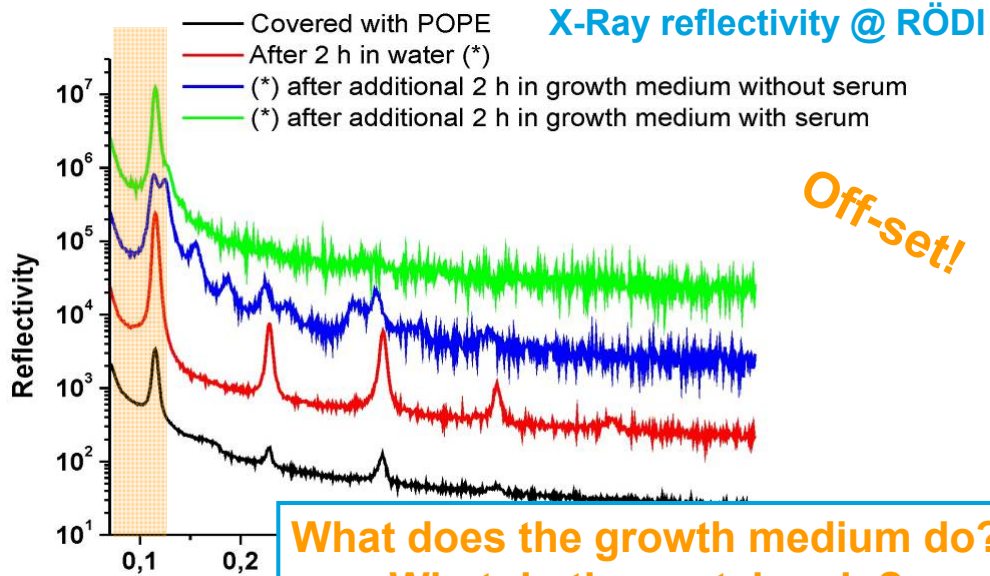


What does the coating look like?

Ti-6Al-7Nb + POPE + DMEM (3h)



What does the coating look like?

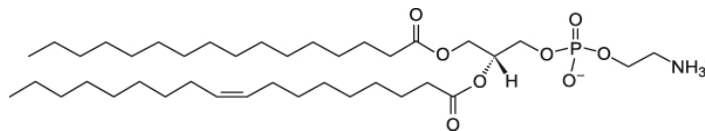


Covalent linkage of the lipids

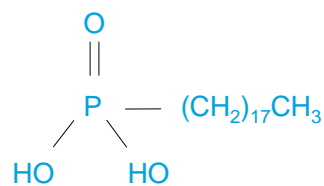
Materials

Mirror polished Ti6Al7Nb and Ti or sputtered Ti (on Si)

POPE

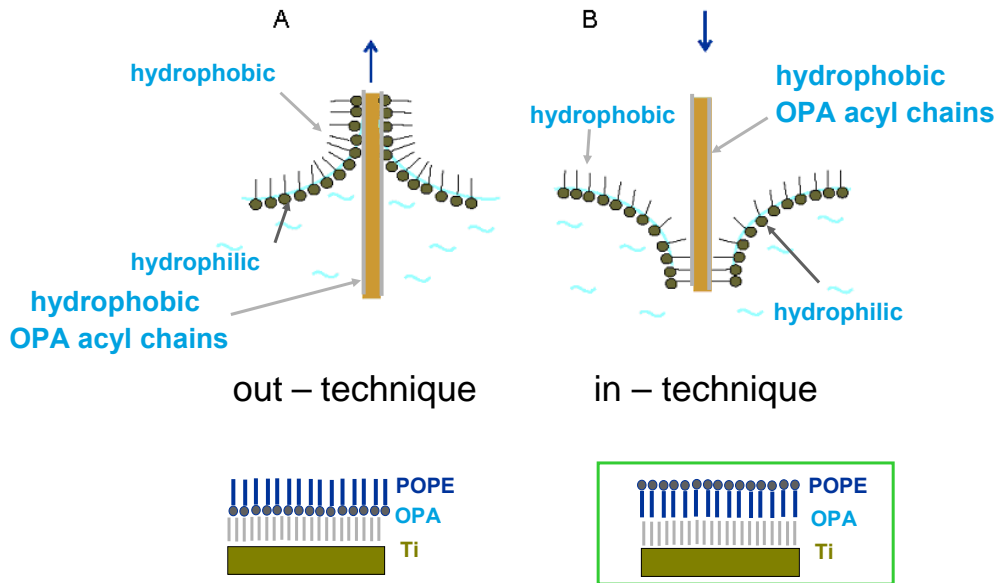


Octadecylphosphonic acid (OPA)



Covalent linkage of the lipids

Langmuir-Blodgett transfer

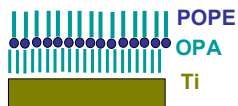


Covalent linkage of the lipids

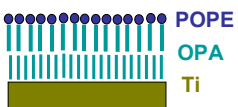
Langmuir-Blodgett transfer

available surface = 2,25 cm²

POPE out transfer = 2,25 cm² (100%)

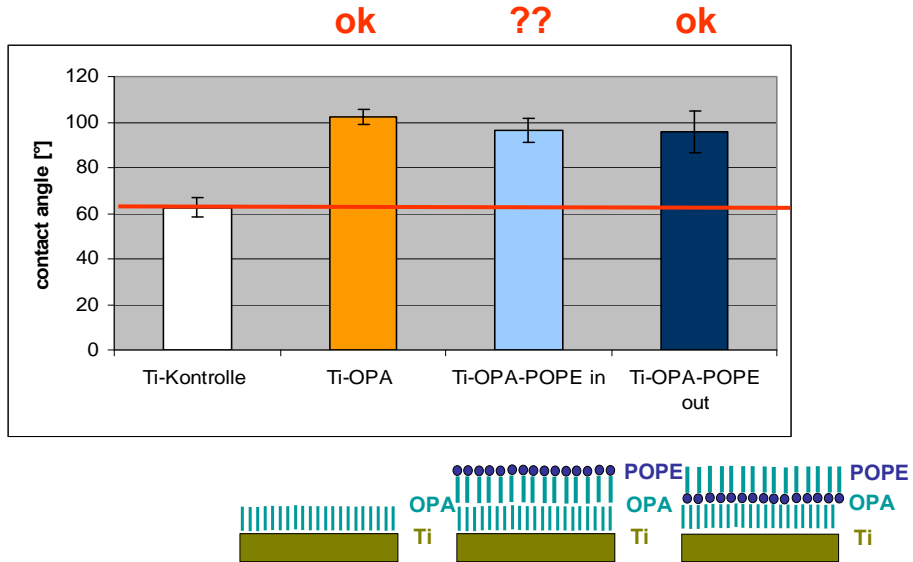


POPE in transfer = 1,48 cm² (66%)



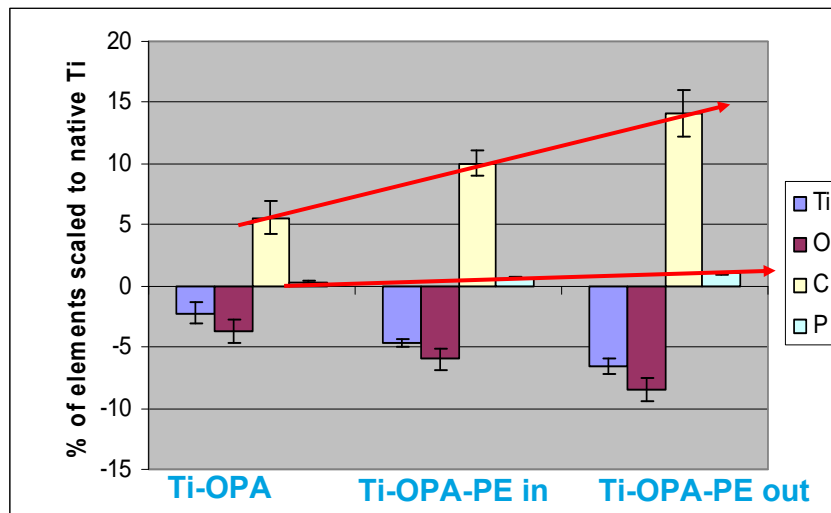
Covalent linkage of the lipids

Contact Angle Measurements



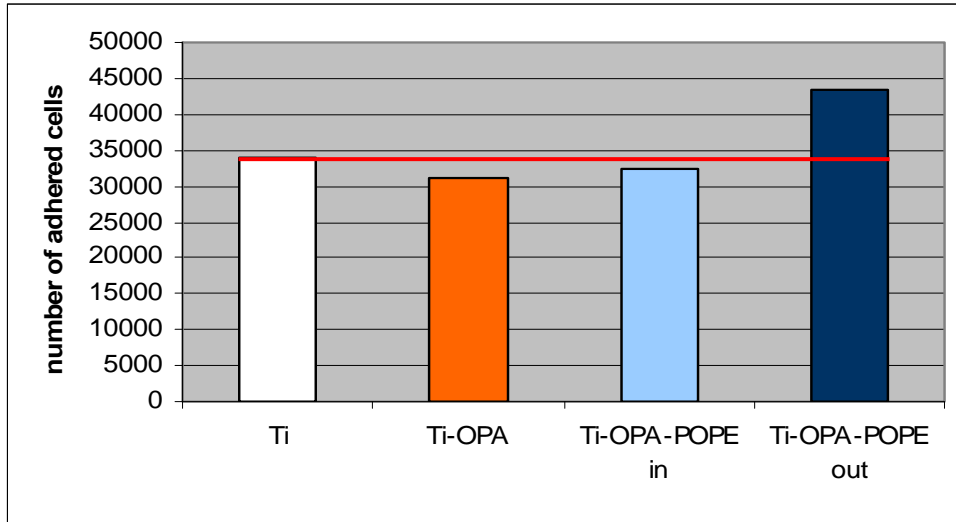
Covalent linkage of the lipids

Element analysis of coated samples via XPS



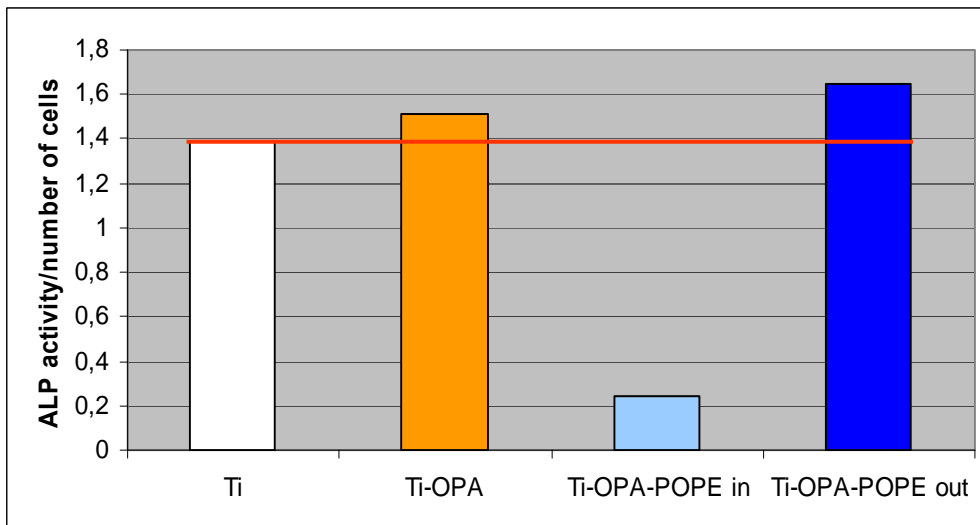
Covalent linkage of the lipids

Adhesion of HBDC on Ti-OPA-POPE out → + 28%



Covalent linkage of the lipids

Matrix formation of HBDC on Ti-OPA-POPE out → + 20%



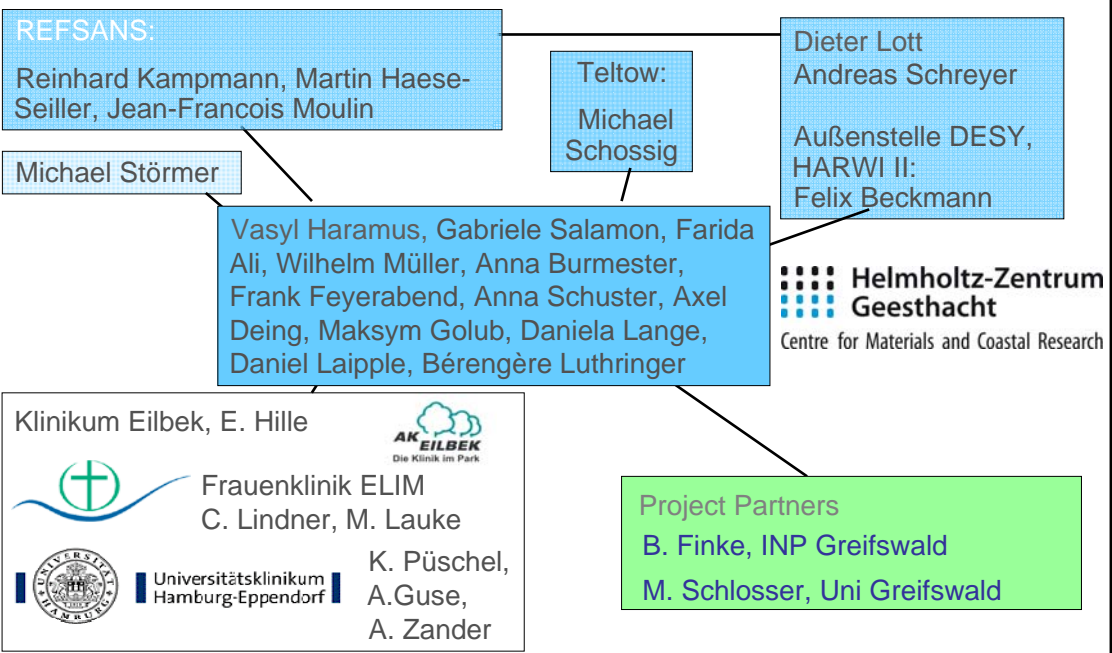
Conclusion

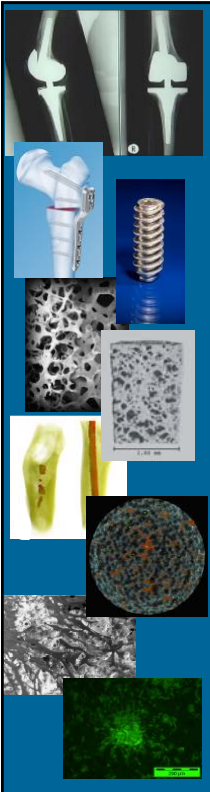
- ➔ Lipids do have a (strong) effect on cellular performance
- ➔ Severe reorganisation of the lipid coating in growth medium and in the presence of BSA

Outlook

- ➔ Surface characterisation also for monolayer
- ➔ Study of gene/protein expression

Acknowledgement





2. Workshop

Neue Horizonte für metallische Biomaterialien

Geesthacht

02. und 03.05.2011