Cell morphology and function in biomaterials & tissue engineering

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University of Basel
Switzerland

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Structure of presentation

1. Background : “setting the scene”
2. Examples from human cell culture systems
3. Future directions

summary





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1. Background : “setting the scene”

- relationship between morphology & function
- paradigm change(s) in biomaterial research

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What spectacle could be more impressive than the contemplation of the world ?

Erasmus of Rotterdam
[? 1469-1536]

portrait by
Hans Holbein the Younger, 1523

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correlation between STRUCTURE und FUNCTION

μορφη

Gr. = shape, form

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History of Implant Development

Sir John Charnley

First generation 1940s – 1960s

Technology transfer from existing technical fields

Second Generation 1970s – 1980s

„**inert** materials“ **Blood contact** etc.

Third Generation 1990s onwards

Controlling the „**biological dialogue**“ between tissue and biomaterial

biomimetics

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The Paradigm Change

previously

Focus on Replacement of diseased organs

Transplantation, Medical Devices

extracorporeal devices
implants

new approach

Promotion of inherent healing mechanisms

Development

Regenerative Medicine
Tissue Engineering [TE]

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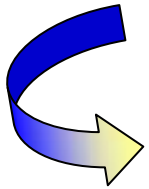
♦ responsive [„intelligent“] materials

➔ reaction to physical, chemical or biochemical stimuli :
ΔT, ΔpH, hυ, enzyme release

metal alloys
synthetic polymers

➔ simulation of the extracellular matrix [ECM]



remodelling ⇨ bioresorbable

♦ drug / gene delivery systems

growth factors, differentiating factors, angiogenic factors, anti-inflammatory drugs, nucleic acids (DNA, miRNAs)

♦ nanofabrication

self assembly, nanotubes, nanowires etc.

Biomolecular cues

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2. Examples from human cell culture systems





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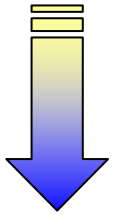
Research focus of REPAIR-lab

- To set up relevant *in vitro* models
using human endothelial cells
(e.g microvascular, endothelial progenitor cells [EPC])
alone or in co-culture with other relevant cell types
to study how three-dimensional biomaterials
can be vascularized



porous ceramics, porous titanium
silk protein fibroin, chitosan-based scaffolds
polymer blends

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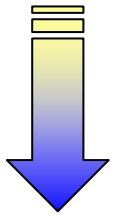
Research focus of REPAIR-lab

- To set up relevant *in vitro* models
  using human cells
  to study pathomechanisms of wear and corrosion products
  [metal ions, micro- / nanoparticles]



inflammatory and angiogenic phenotype of endothelial cells [micro, macro];
toxicity → more subtle changes of cell functionality

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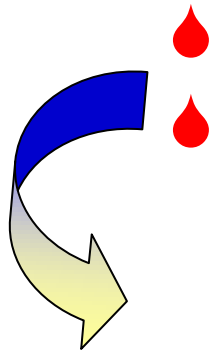
The importance of the endothelial cell [EC]



1. Essential element of the inflammatory reaction
2. Central to the angiogenic response
3. Pre-requisite for vascularisation of tissue engineered implants.

in vitro models of angiogenesis

- ➔ metal ion effects
- ➔ micro-/nanoparticle effects
- ➔ vascularization of synthetic and natural matrices [TE]

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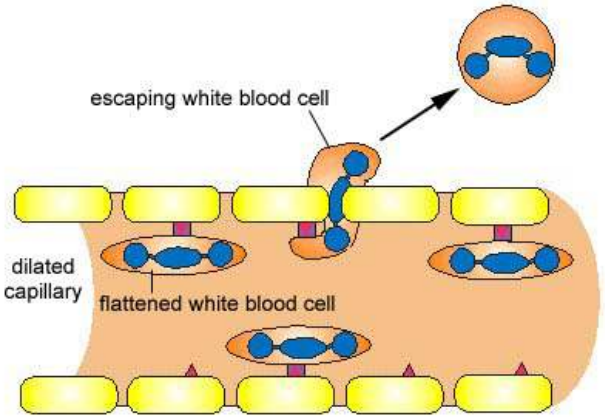
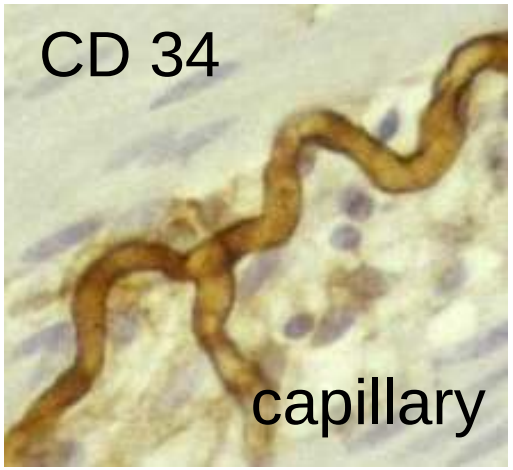
various endothelial phenotypes

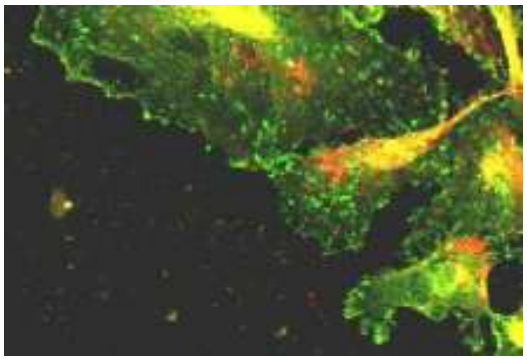
inflammatory phenotype



angiogenic phenotype



migrating phenotype



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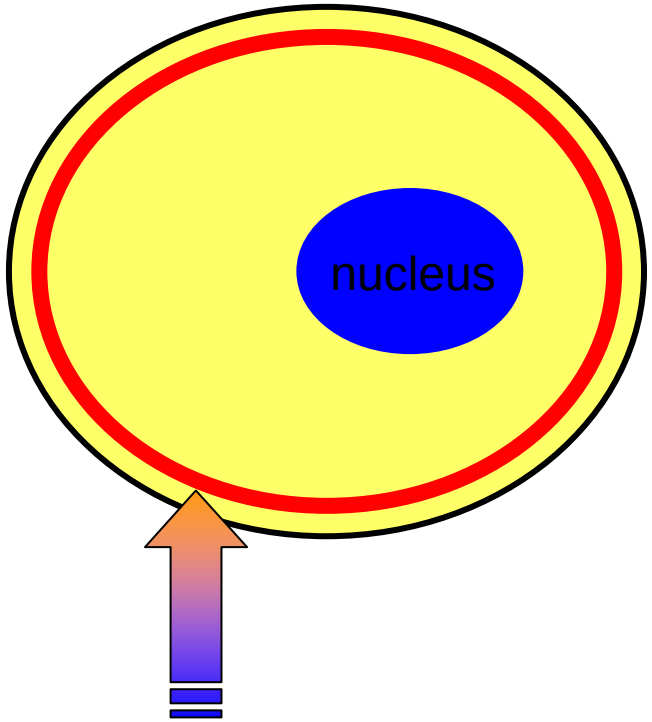
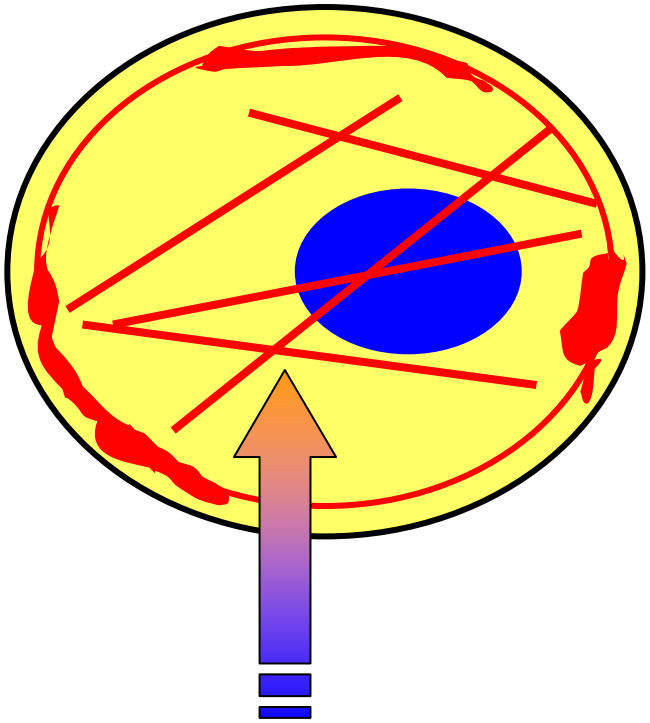
inflammatory phenotype

non-activated EC



F-actin : dense peripheral ring

activated EC
e.g. inflammation



F-actin : stress fibres





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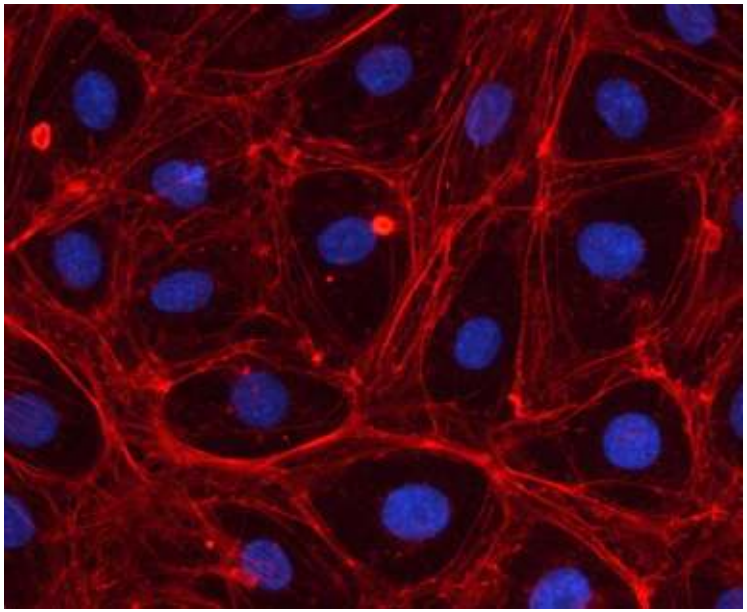
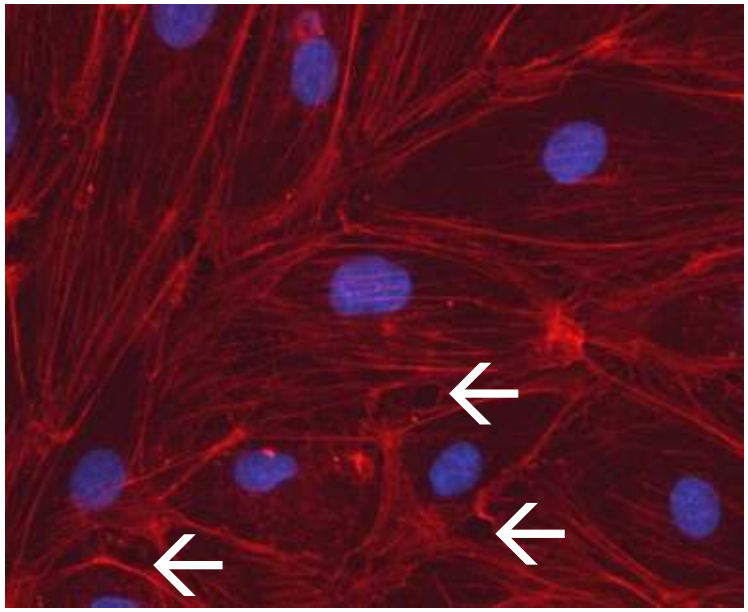
Cytoskeletal Changes in activated EC [HDMEC]



control

F-actin = red
nuclei = blue

dense peripheral ring



TNFα (24 h, 300 U/ml)
[positive control]

arrow = interendothelial gap

stress fibres

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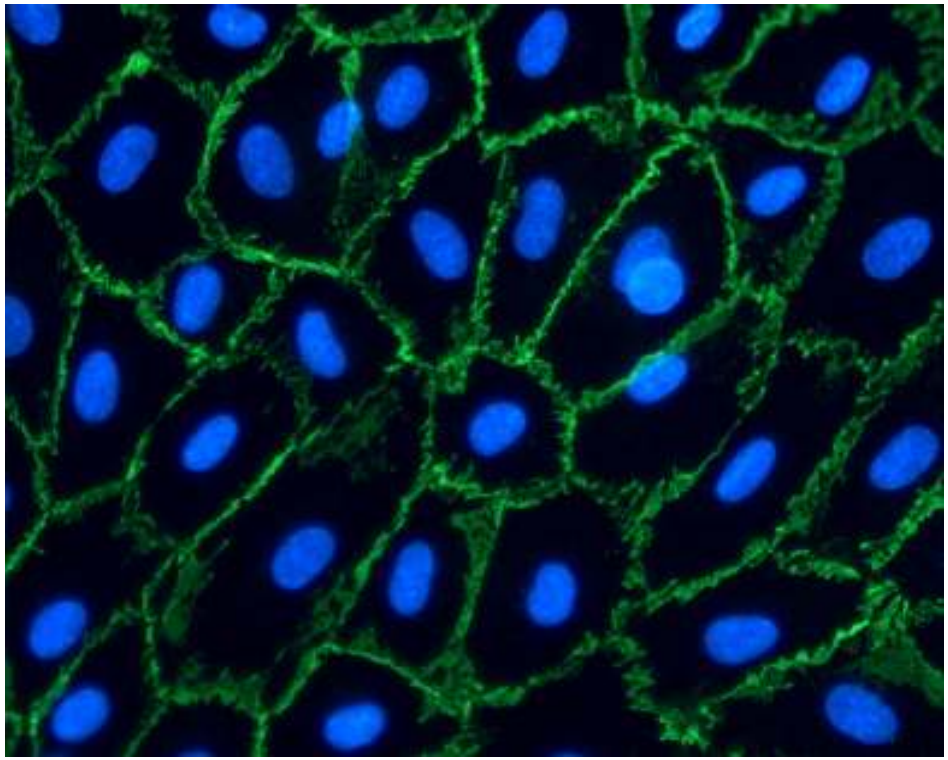
OEC : outgrowth endothelial cells

non-activated



VE-cadherin

CD31 ++ [PECAM-1]
vWF ++
VE-cadherin ++
caveolin-1 ++
VEGFR-2 ++
CD45 negative

Sabine Fuchs PhD

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Endothelial monolayer *in vitro* – proinflammatory phenotype

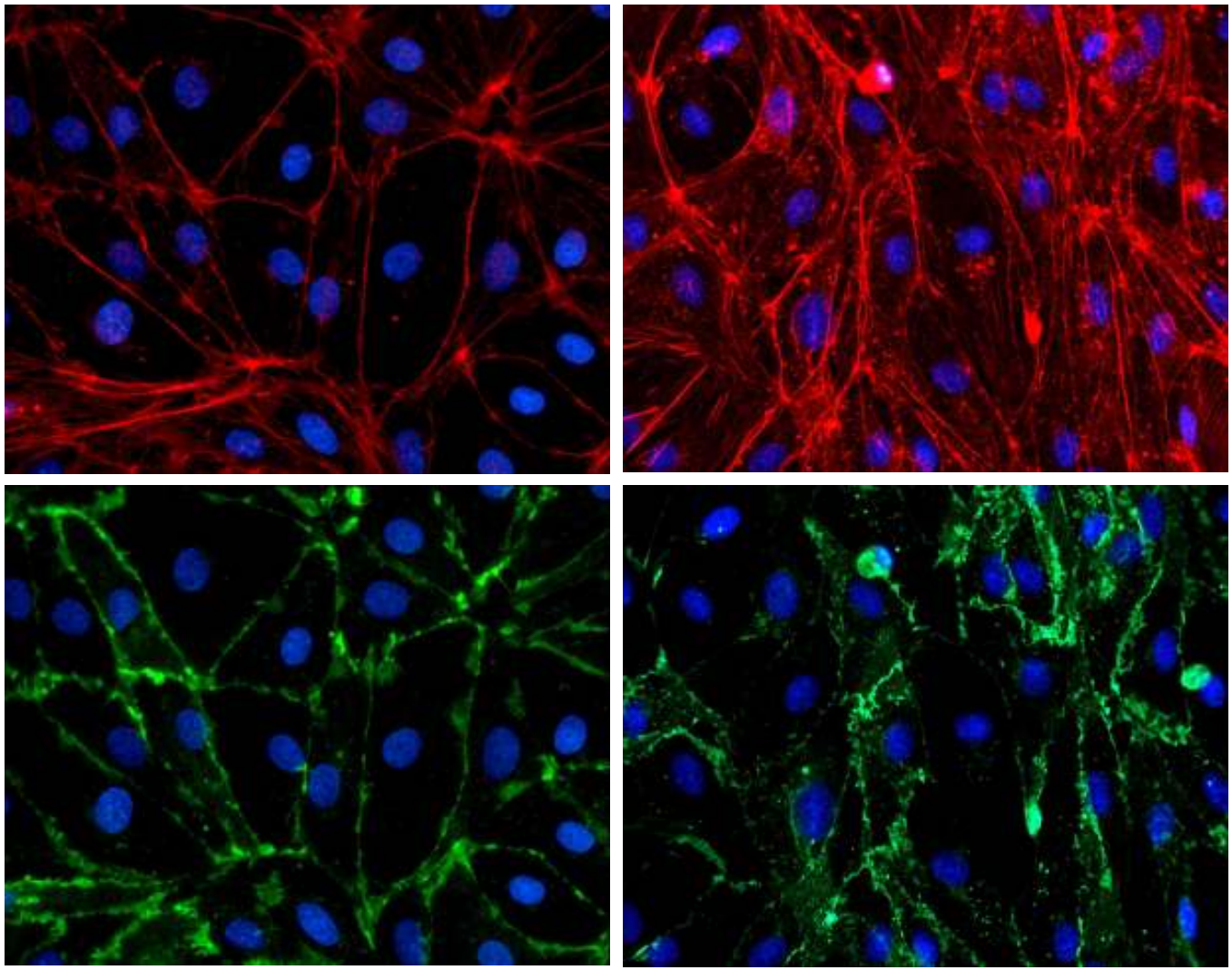
control

positive control TNFα

HDMEC

F-Actin
nuclei

PECAM-1
[CD31]
nuclei



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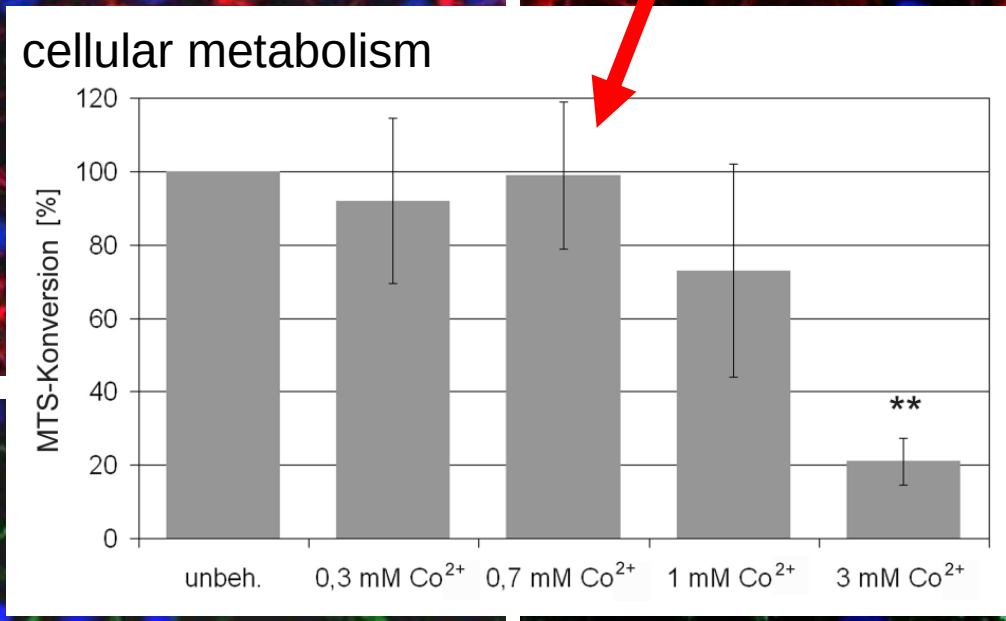
Endothelial monolayer *in vitro* : $CoCl_2$ 0.7 mM, 24 h

HDMEC

F-Actin
nuclei

PECAM-1
[CD31]
nuclei



control

Co^{++}-ions

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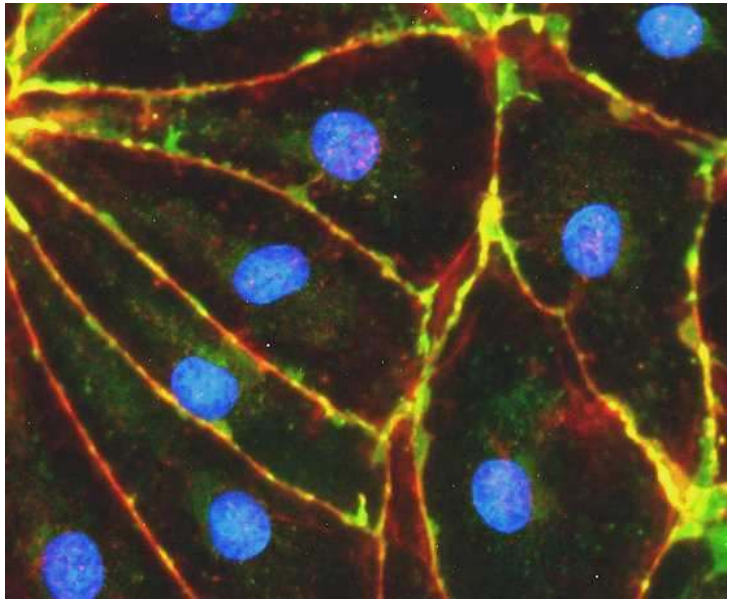
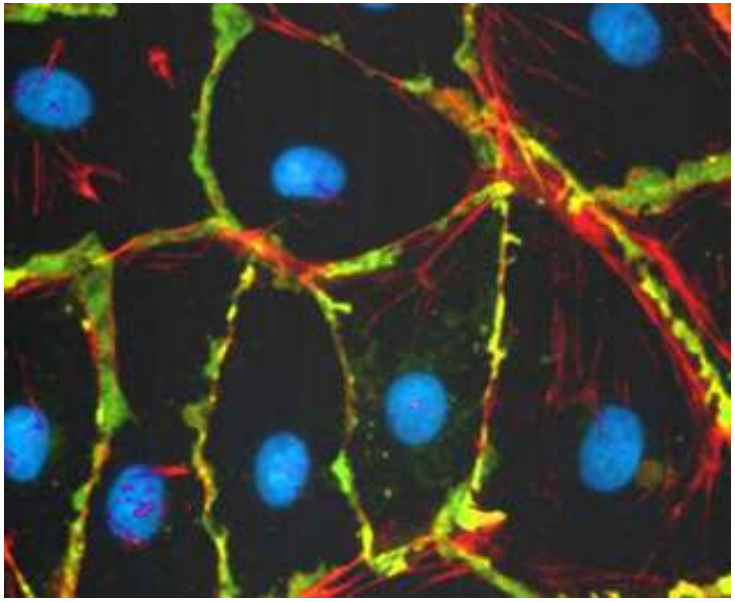
Endothelial cells [EC] on metal surfaces



control (glass)



cp-titanium

green = EC surface marker, PECAM-1 (CD31)
red = F-actin cytoskeleton
blue = nuclear fluorochrome

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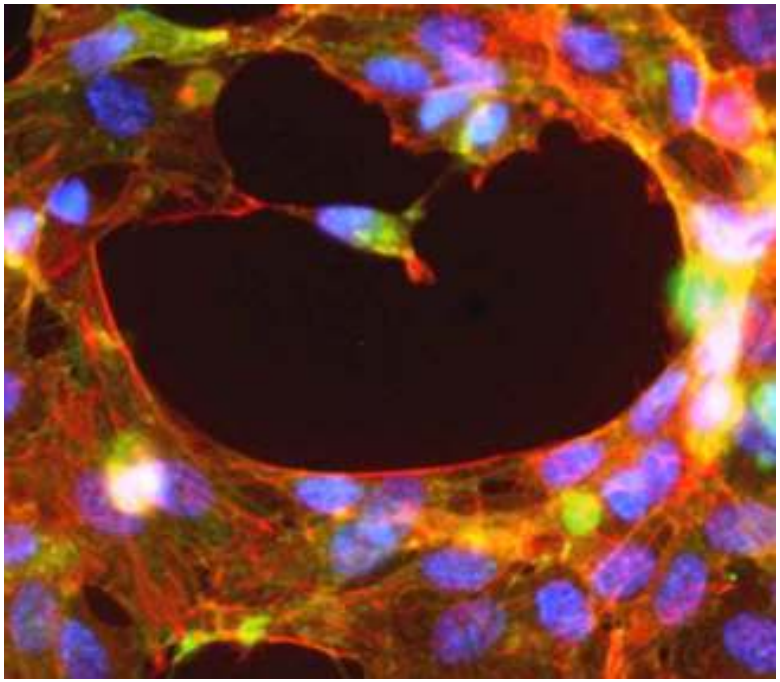
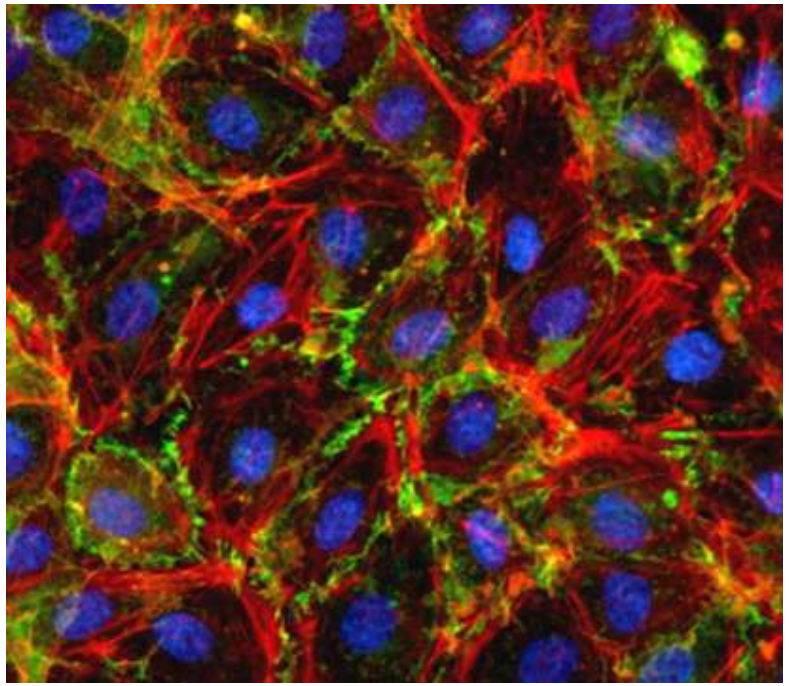


Co28Cr6Mo



Ti6Al4V

green = EC surface marker, PECAM-1 (CD31)
red = F-actin cytoskeleton
blue = nuclear fluorochrome

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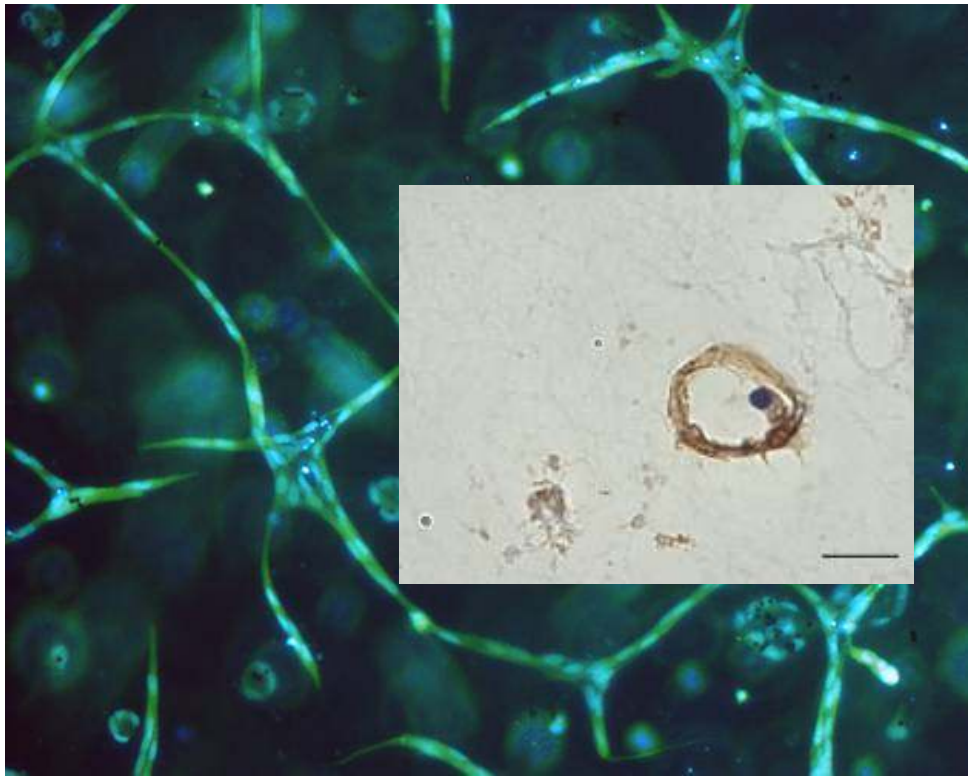
angiogenic phenotype

3D-angiogenesis / vasculogenesis model *in vitro*

calcein AM



human dermal microvascular endothelial cells [HDMEC]

- suspension of EC
- gel of fibrin/type I collagen
- proangiogenic factors
  VEGF [50 ng/ml]
  + bFGF [5 ng/ml]
- 6 days of culture

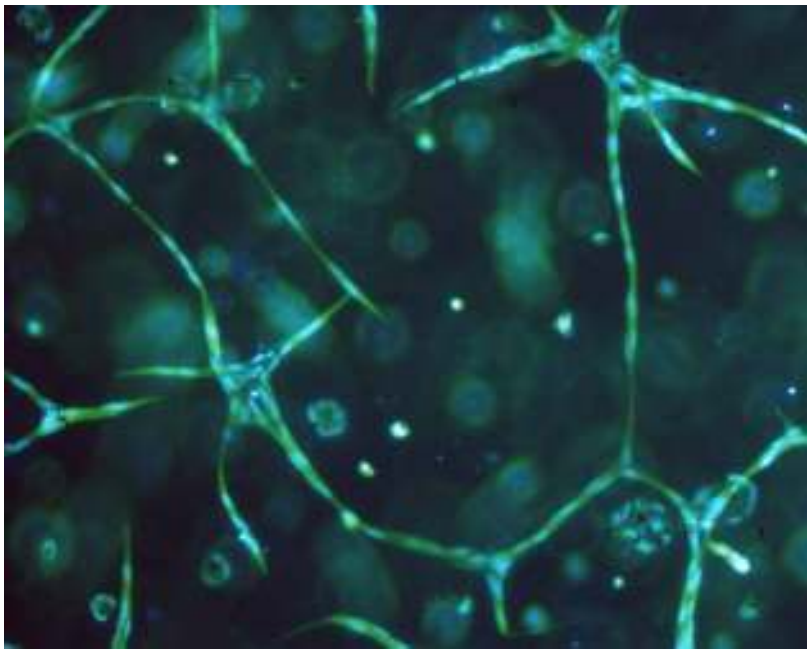
Kirsten Peters PhD

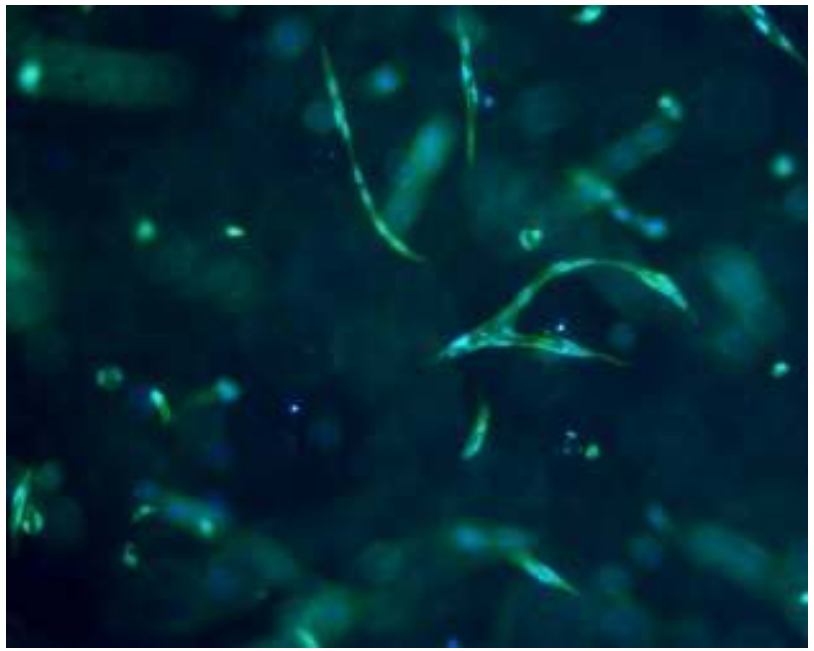
3D-angiogenesis model *in vitro* : effects of cobalt

HDMEC embedded in a gel of fibrin/type I collagen with growth factor stimulation [VEGF + bFGF] for 6 days.

Digital overlay images of a vital (green) and a nuclear stain (blue)

calcein AM





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control

Co^{2+}-treated (0.1 mM)

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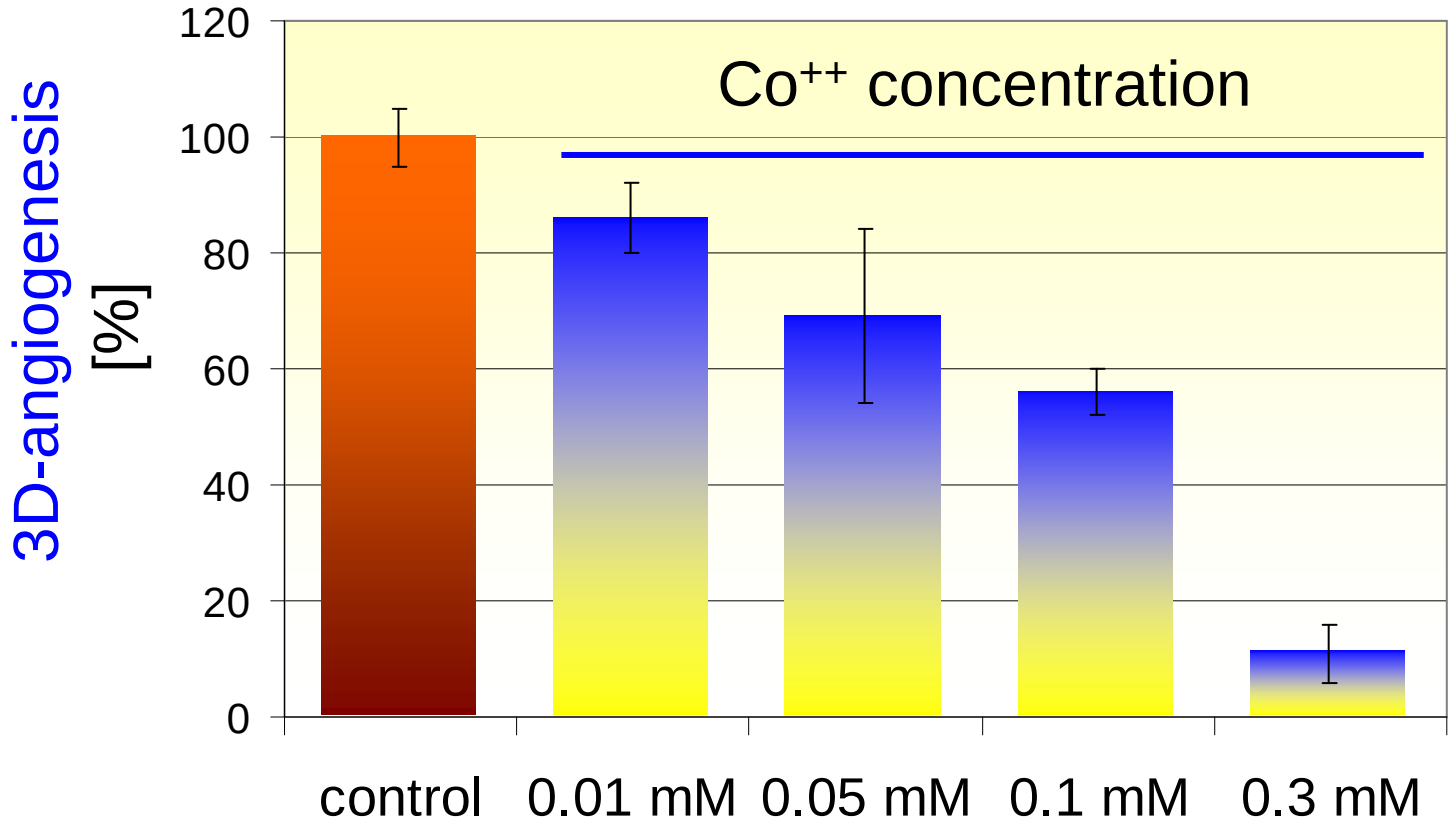
Quantitation of 3D-angiogenesis

Effects of cobalt ions



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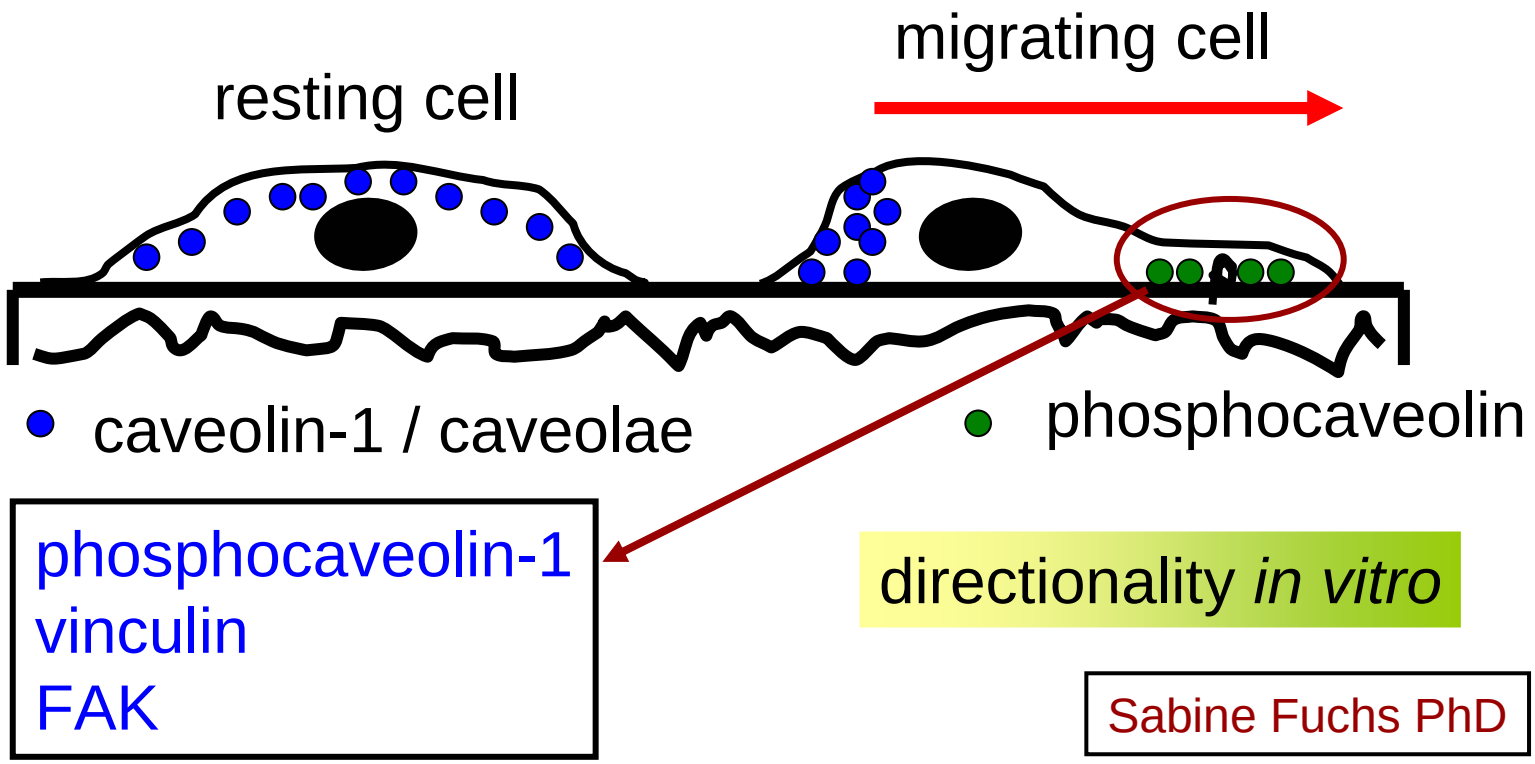
New aspects of OEC interaction with biomaterials

morphological markers of cell functionality

caveolin-1 and phosphocaveolin-1 involved in cellular polarization and migration



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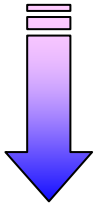
Functionality changes when heterotypic cell interactions take place



angiogenic phenotype in co-culture with osteoblasts ± biomaterials

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3. Future directions

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- developing the field of functional morphology

  → immunocytochemical distribution of epitopes

  → combining microscopy and molecular biology

laser capture microdissection + qRT-PCR

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summa summarum…….

- choice of structural parameters can be an excellent monitor of functional status :
  → CAMs, cytoskeleton, phosphorylated proteins

- 3D- and/or co-culture systems essential for future strategies in TE & RegMed

- LCM + qRT-PCR a major tool for functional morphology

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Deutsche Forschungsgemeinschaft DFG

Bundesministerium für Bildung und Forschung

AUTOBONE

EXPERTISSUES

Hippocrates

Marie Curie Actions
Human resources and mobility

REPAIR

Institute of Pathology, JGU, Mainz

http://www.repair-lab.org


Many thanks !
JOHANNES
GUTENBERG
UNIVERSITÄT
MAINZ