



Biological Response at the Implant Interface

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Implantology

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Medizinische
Werkstoffwissenschaften
und Technologie

Biology of Implantation



<http://www.g-implant.com/article/15117227.html>



<https://www.toothspadental.com/dental-implants/>

Injury → Inflammation → Tissue proliferation → Integration or Rejection



Content of the lecture

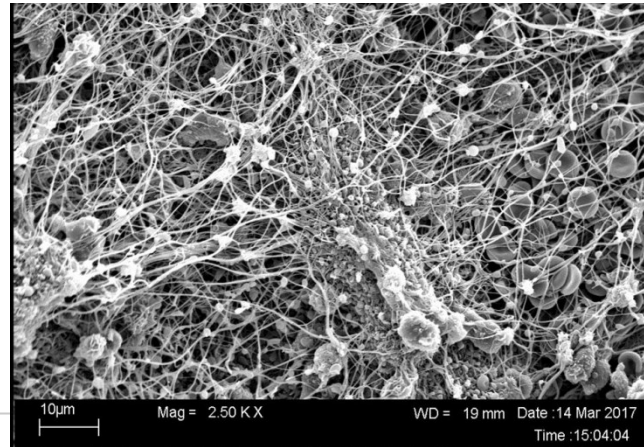
- General principles: implant – biosystem interactions
- Evolution of implant surfaces
- Biological evaluation of medical devices
- Biological reactions towards to implant surfaces
- How to influence these Processes to improve implantation?

Interactions Implant - Biosystem

All implants interact with the biological environment and vice versa

→ Biological effects of the **host towards implant**

- Adsorption of proteins and cells on the surface
- Enzymatic degradation
- Calcification
- Fibrosis

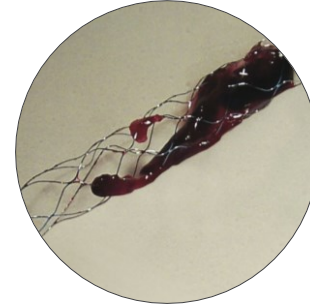


Scanning electron
microscopic image of
an implant surface
after blood contact

Interactions Implant - Biosystem

→ The implant can have local effects on the surrounding tissue

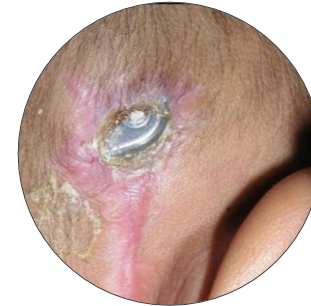
- Interaction with blood cells, coagulation cascade,
- Wound healing is influenced
- Infection
- Acute /Corrosion Toxicity
- Immunomodulating Properties



[<https://www.ahajournals.org/reader/content/16523b73b92/10.1161/STROKEAHA.112.663328/format/epub/EPUB/xhtml/fu1.xhtml>]]



[https://cdn.shopify.com/s/files/1/0072/4510/3213/products/IMG_3049_720x.jpg?v=1598625532]]



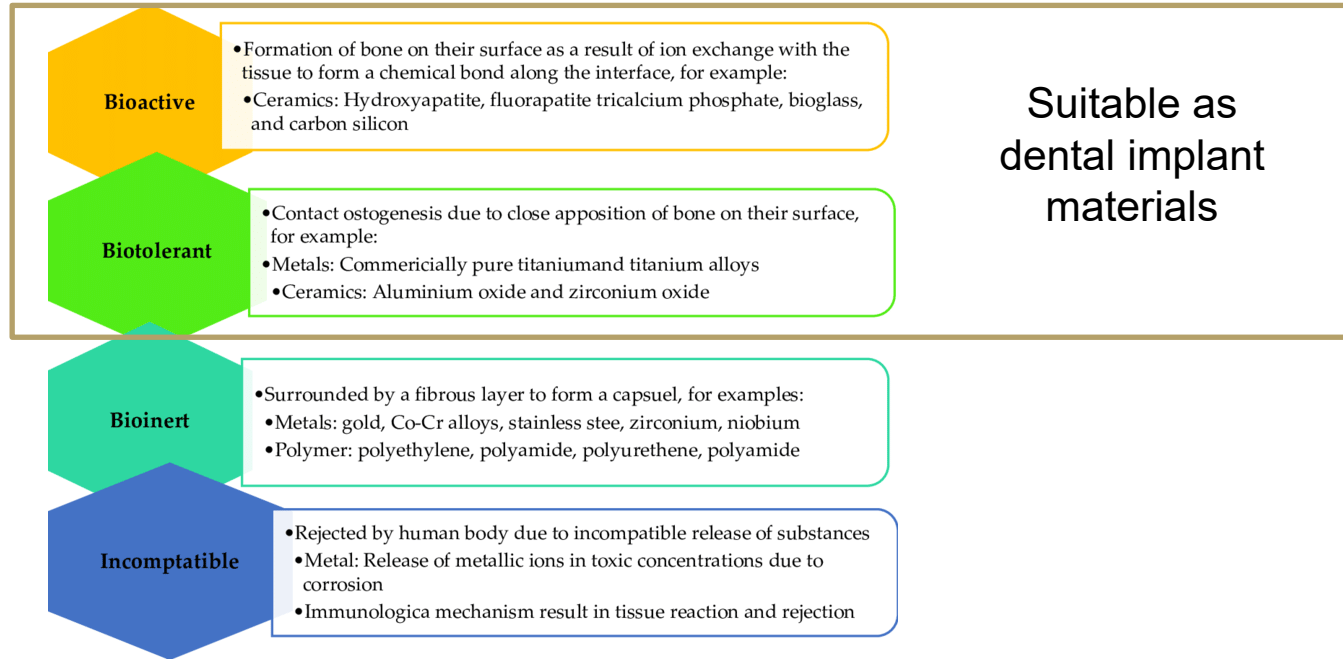
[https://media.springernature.com/original/springer-static/image/chp%3A10.1007%2F978-3-319-74831_8/MediaObjects/428121_1_En_8_Fig4_HTML.jpg]]



[<https://www.jandali.de/wp-content/uploads/2019/10/Kapsel-fibrose-1.jpg>]]



Evolution of implant materials



<https://doi.org/10.3390/coatings10060568>

Evolution of implant surfaces

Optimization strategies:



Change of material



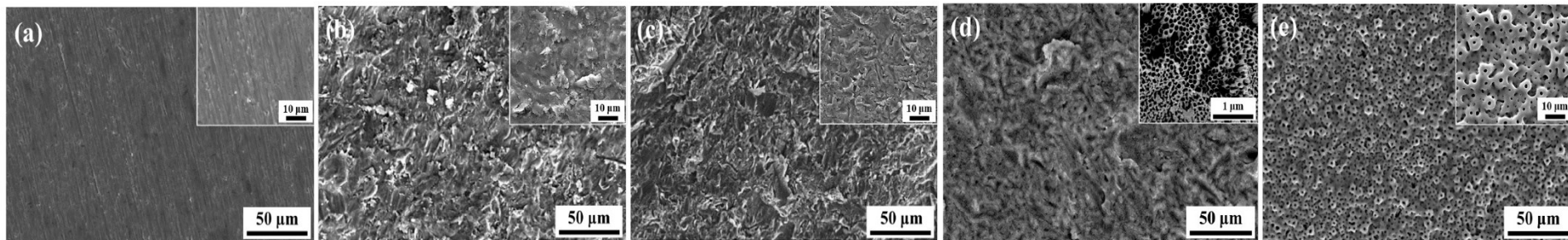
Technical surface
modifications



Biological
functionalization

Technical surface modification

<https://doi.org/10.3390/met11040618>



Machined Titanium

Hydroxyapatite-
blasted (HA)

Sand-blasted + acid-
etched (SLA)

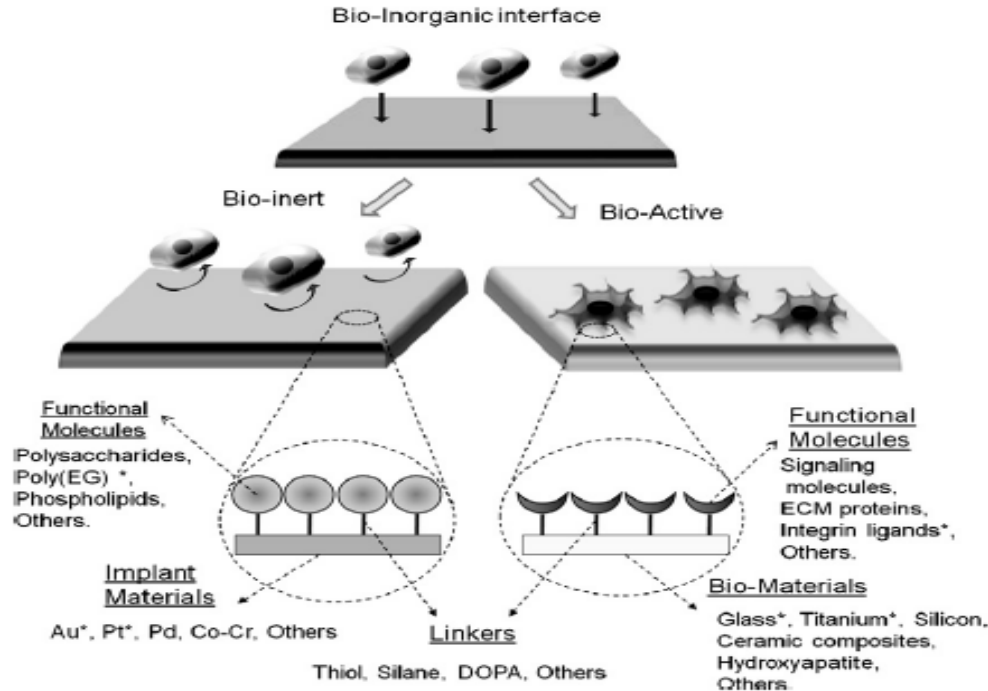
SLA + anodic oxidation
(AO)

SLA + plasma
oxidation (PO)

Variation of heights, roughness, wettability, surface chemistry...

Additionally: Hydrophilisation of the surfaces for activation (plasma cleaning, storage in saline ...)

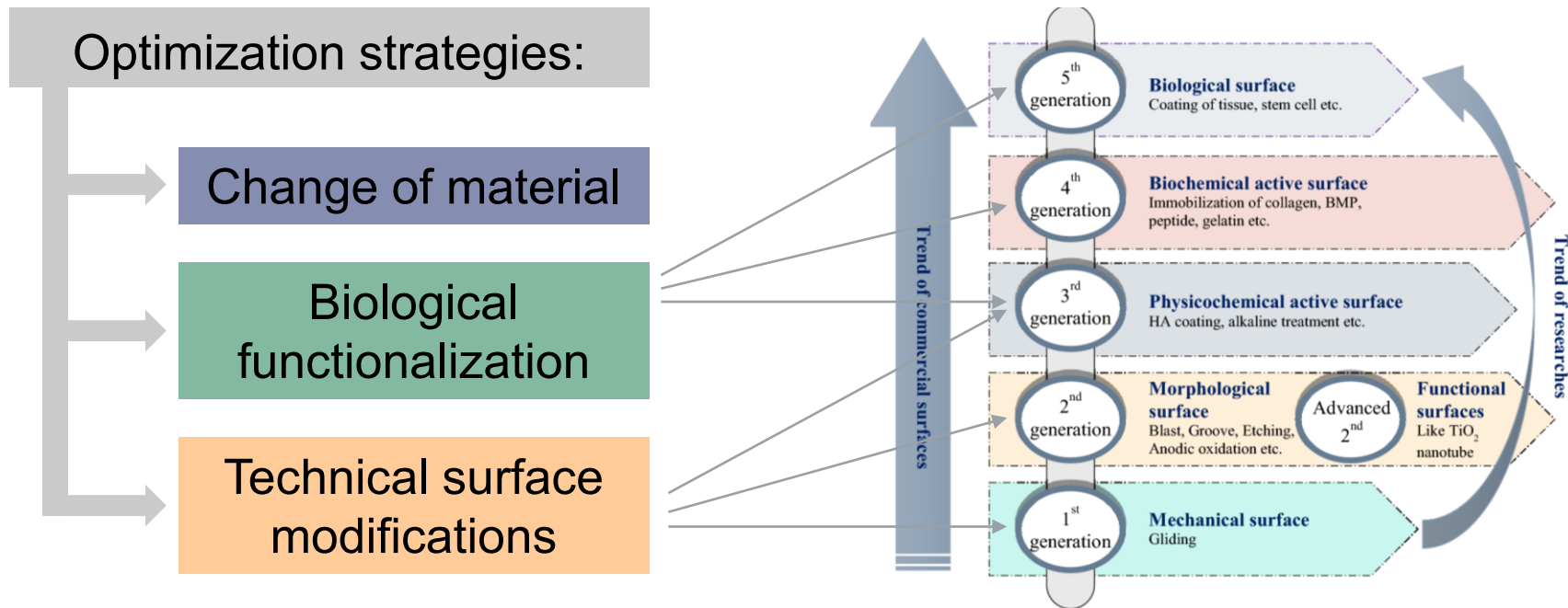
Biological functionalization



Tasks:

- Finding of respective functional signal molecule(s)
- Development of coupling methods for respective biomaterial

Evolution of implant surfaces



<https://doi.org/10.1038/s41598-022-11254-0>

Evolution of implant surfaces

R & D on dental implant surfaces

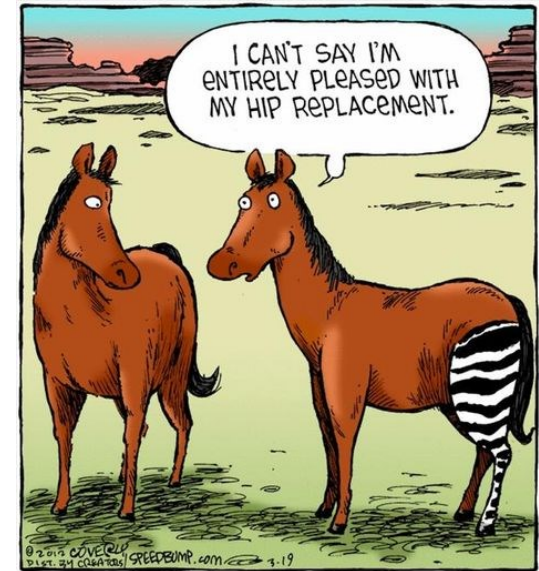
- Decisive factor: Clinical success
- Which biological mechanisms can be influenced to obtain improvement?
- Impossible to test all modifications in patients!

→ **Screening-tests for optimization**



Biological evaluation of medical devices – WHY?

- Continuous testing of further product developments
- **Assessment of safety and biocompatibility aspects**
- *Biocompatibility = ability of medical device to perform within a tolerable biological reaction*
- Using analytical chemistry, **in vitro tests** and in vivo models



<http://seriouslyfunnyhumor.blogspot.com/2017/11/horse-is-not-pleased-with-zebra-ish-hip-replacement-funny-photo.html>



Biological evaluation of medical devices – WHY?

Regulations for screening Implant surfaces in the development process:

ISO Standard 10993 Series:

„*Biological Evaluation of Medical Devices*“

- 20 Parts offer general guidelines:
- Part 4: Selection of tests for interactions with blood
- Part 5: Tests for in vitro cytotoxicity

→ animal and clinical studies obligatory for conformity assessment and optimization

Reference	Title
ISO 10993-1	Guidance on selection of tests
ISO 10993-2	Animal welfare requirements
ISO 10993-3	Tests for genotoxicity, carcinogenicity, and reproductive toxicity
ISO 10993-4	Selection of tests for interactions with blood
ISO 10993-5	Tests for cytotoxicity: In vitro methods
ISO 10993-6	Tests for local effects after implantation
ISO 10993-7	Ethylene oxide sterilization residuals
ISO 10993-8	Withdrawn: Clinical investigation of medical devices
ISO 10993-9	Evaluation of biodegradation of medical devices
ISO 10993-10	Tests for irritation and sensitization
ISO 10993-11	Tests for systemic toxicity
ISO 10993-12	Sample preparation and reference materials
ISO 10993-13	Identification and quantification of degradation products from polymers
ISO 10993-14	Static test to quantify in vitro degradation of ceramics
ISO 10993-15	Identification and quantification of degradation products from metallic materials used in medical devices
ISO 10993-16	Toxicokinetic study design for degradation products and leachables
ISO 10993-17	Glutaraldehyde and formaldehyde residues in industrially sterilized medical devices
ISO 10993-18	Characterization of materials
ISO 10993-19	Physico-chemical, morphological, and topographical characterization of materials
ISO 10993-20	Principles and methods for immunotoxicology testing of medical devices

*Helmus (2003); <http://www.iso.org/iso/en/StandardsQueryFormHandler.StandardsQueryFormHandler?scope=CATALOGUE&sortOrder=ISO&committee=ALL&isoDocType=ALL&title=true&keyword=10993>

In vitro studies

Advantages

- Adaptable
- Fast and simple
- Small amount of test material is required
- Relatively inexpensive
- Ethical issues can be avoided

Disadvantages

- Chronic effects cannot be tested
 - Systemic effects can not be measured
- Clinical relevance is questionable



Sgsgroup.de



ISO Standard 10993-4: Selection of tests for interactions with blood

- Guideline for performance of hemocompatibility testing
- Medical devices, medicines, blood products and diagnostic agents that contact circulating blood generally require a hemocompatibility assessment
- Evaluation of critical interactions of foreign material with blood to explore possible adverse effects to avoid serious health risk

→ Static, agitated or shear flow models: Dependent on intended device application

Hemocompatibility testing a shear flow model

“Chandler-Loop-Model”

- Positioning of the test material in the tube
- Filling tubes with blood
- Dynamic incubation in water bath
- Macroscopic analysis of the test material
- Preparation of blood and plasma samples
- Analysis of hematological markers

Chandler Loop System® in operation

<https://www.youtube.com/watch?v=XFqF3FmTON4>

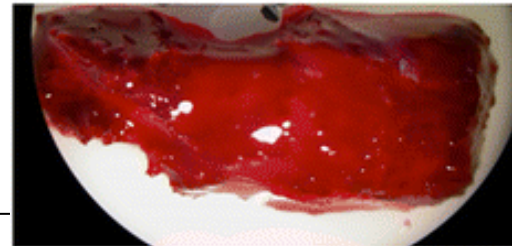
Hemocompatibility testing a shear flow model

“Chandler-Loop-Model”

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**Hemocompatible
test material**



**Coagulation
activation by
test material**

<https://www.youtube.com/watch?v=XFqF3FmTON4>

ISO 10993-5: Tests for *in vitro* cytotoxicity

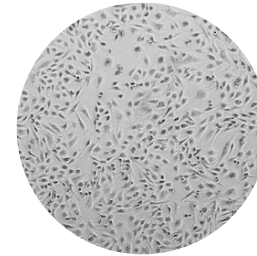
→ Test methods for the assessment of *in vitro* cytotoxicity of medical devices

Testing for different biological parameters

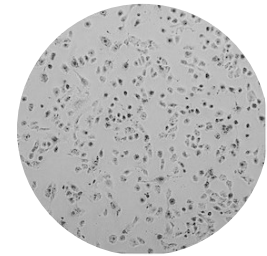
- Changes in cell morphology
- Membrane damage
- Inhibition of metabolic activity
- Inhibition of cell proliferation

Test methods applicable

- ... after direct contact
- ... after indirect contact
- ... after extraction



Viability ~ 100 %

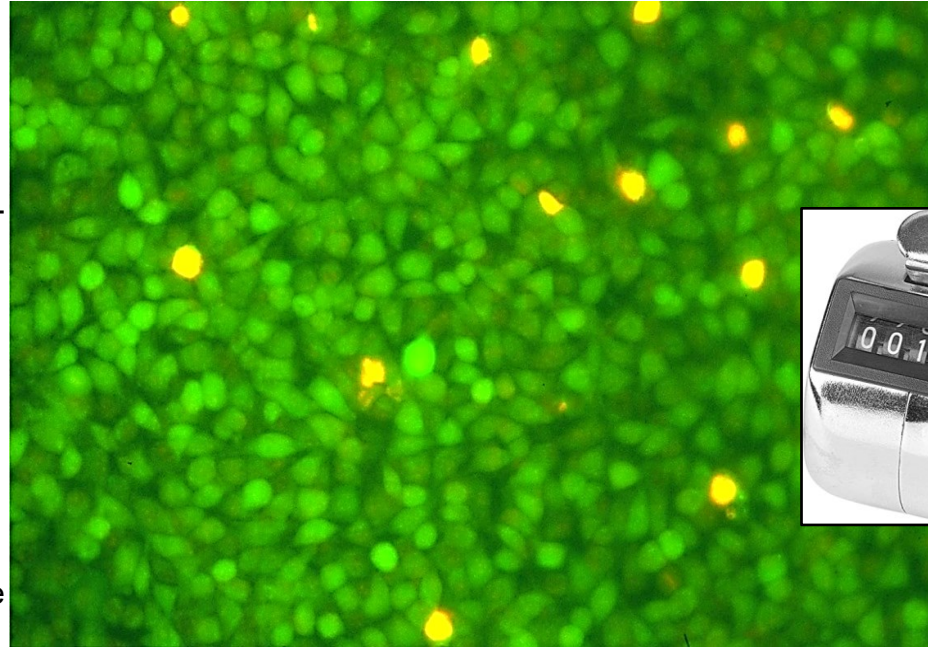


Viability ~ 60 %

<https://doi.org/10.1186/s40064-016-1910-5>

in vitro cytotoxicity: direct contact test

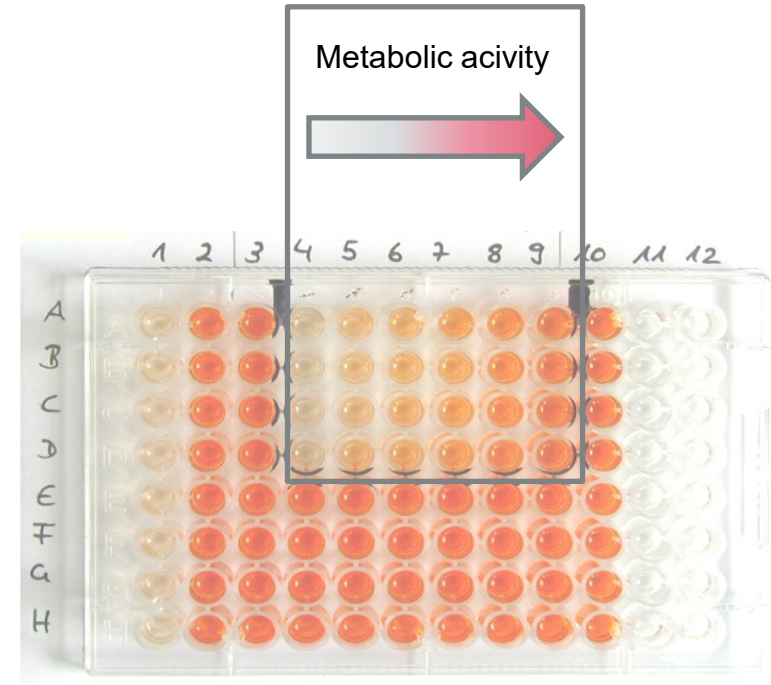
- Cell seeding on test material
 - Vital staining (e.g. *fluorescein diacetate* + *propidium iodide*)
 - Counting of dead cells
 - Quantification of cell viability
- Useful for the analysis of new materials, surface modifications



in vitro cytotoxicity: extract test

- Incubation of test material in extraction media for 24 h
- Cultivation of cells in the gained extract for 24 h
- Metabolic assay results in formation of coloured product (e.g. *XTT*, *CCK-8*)
- Analysis of the metabolic activity

→ Useful for corrosion studies, composite materials, evaluation of cleaning processes



Biological responses after implantation

Contact with blood
proteins

Contact with saliva
proteins

Interaction with
coagulation cascade

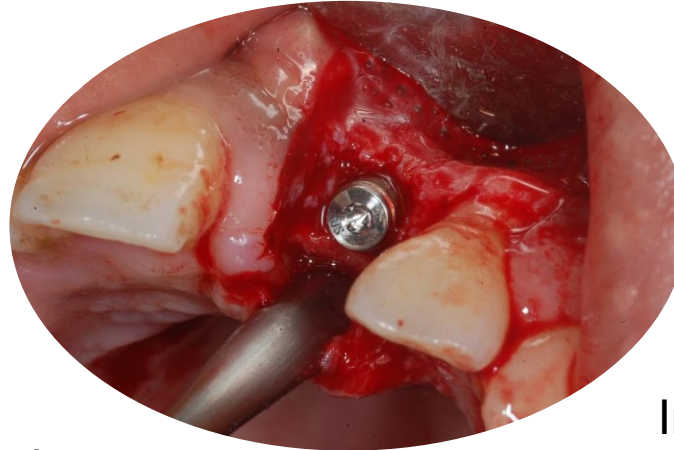
Adhesion of bacteria

Immune activation

Inflammatory response

Osseointegration

Adhesion of surrounding
tissue cells



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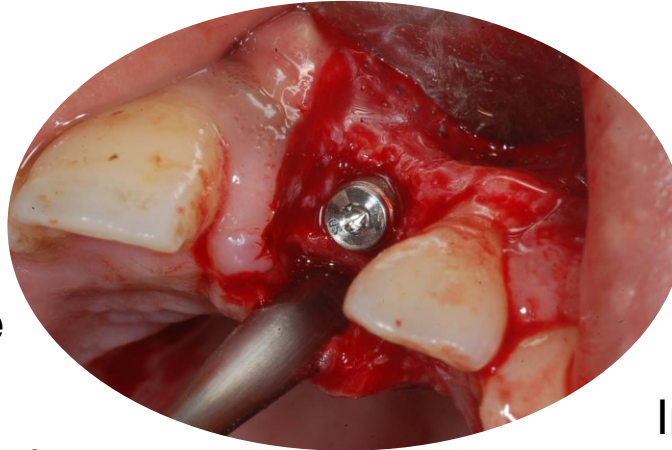
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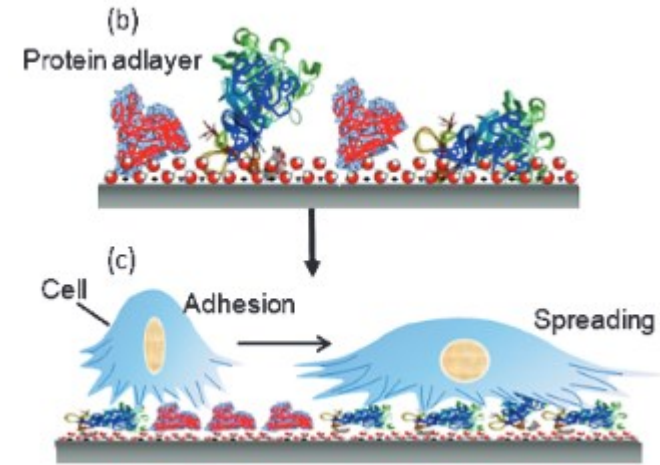
Osseointegration

Adhesion of surrounding
tissue cells



Protein adsorption on implant surface

- High surface affinity → seconds - minutes
 - Saliva: glycoproteins / proline-rich proteins
 - Blood: albumins, globulins, fibrinogen
 - Formation of the initial "conditioning film"
- Composition changes over time (Frequency < Affinity)*

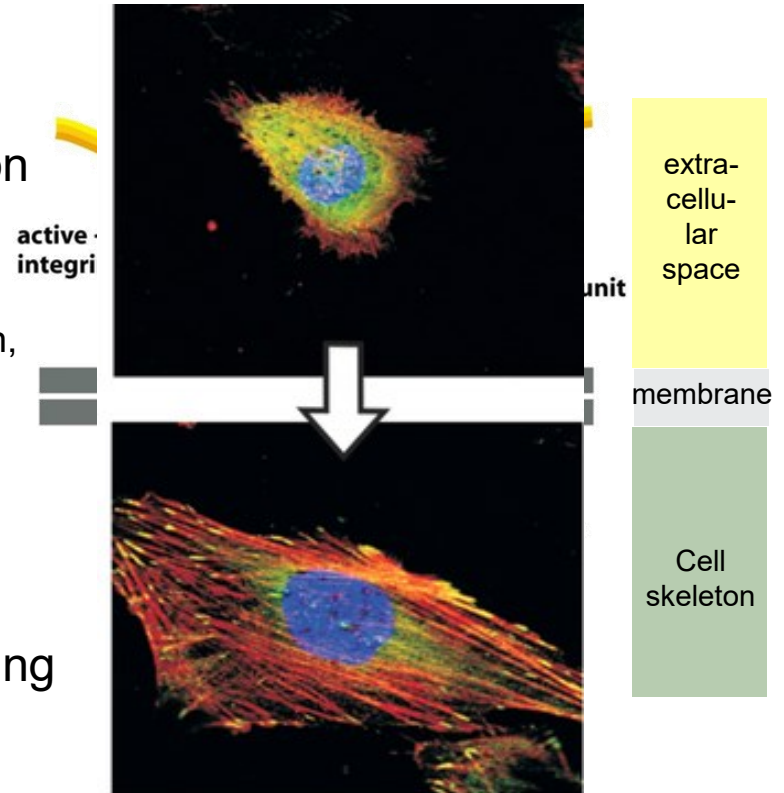


<https://doi.org/10.1166/mex.2012.1053>

- **Proteins provide structures for attachment of cells and bacteria**

Protein cell interaction

- Adsorbed proteins contain peptide recognition sequences
 - Short amino acid sequences (RGD, LDV)
 - Contained in fibrinogen, factor X, vWF, osteopontin, fibronectin
- Cellular integrin receptors
 - Dynamic "anchor" between cytoskeleton and extracellular matrix
 - Binding to peptide recognition sequence
- Integrin binding activates intracellular signalling cascade
 - Cell adhesion, spreading, proliferation



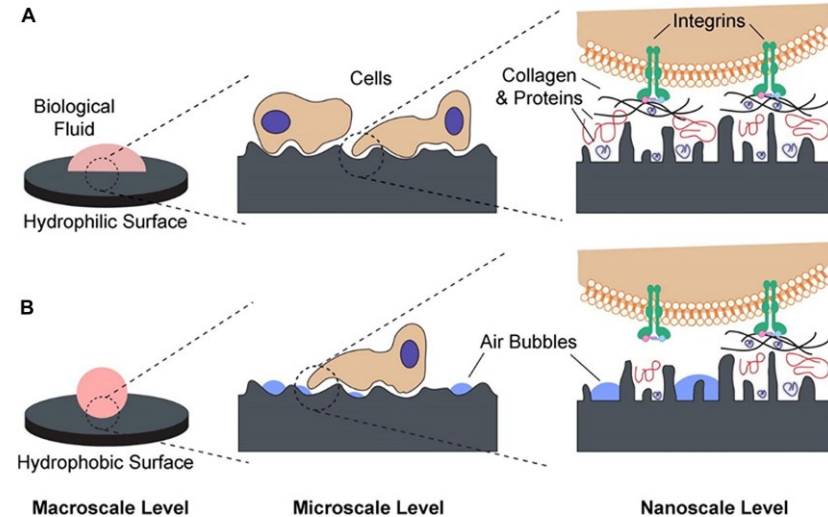
<https://doi.org/10.1002/ange.201511781>

Protein adsorption on implant surface

- **Surface characteristics strongly influence protein / cell adhesion:**

A: Surfaces with good wettability are evenly covered with proteins and allow good cell adhesion

B: Hydrophobic surfaces are prone to entrapment of air bubbles and interfere with protein adsorption / cell adhesion



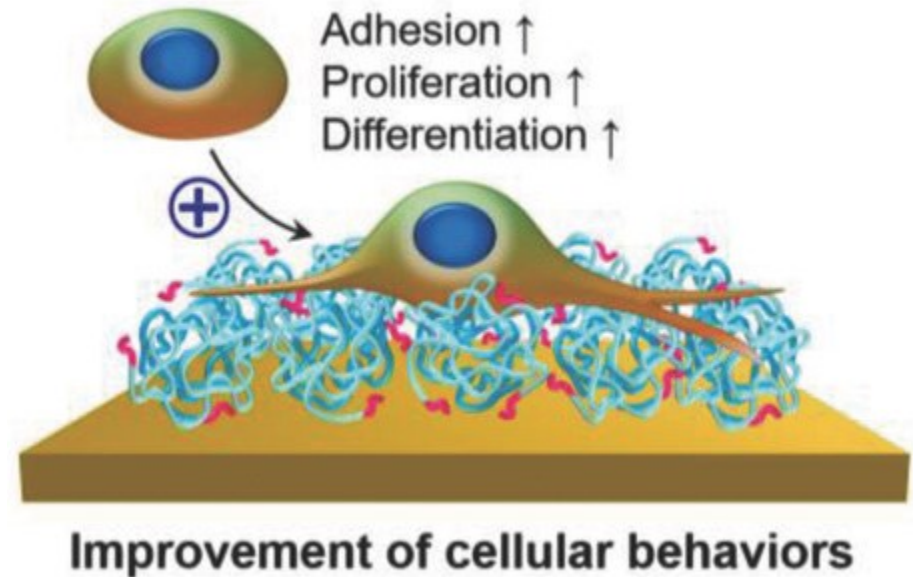
<https://10.1016/j.actbio.2014.03.032>

Biological response: Initial conditioning of implants

- **All biological responses start with adsorption of proteins**

→ *subsequent attachment of platelets and recruitment of tissue cells*

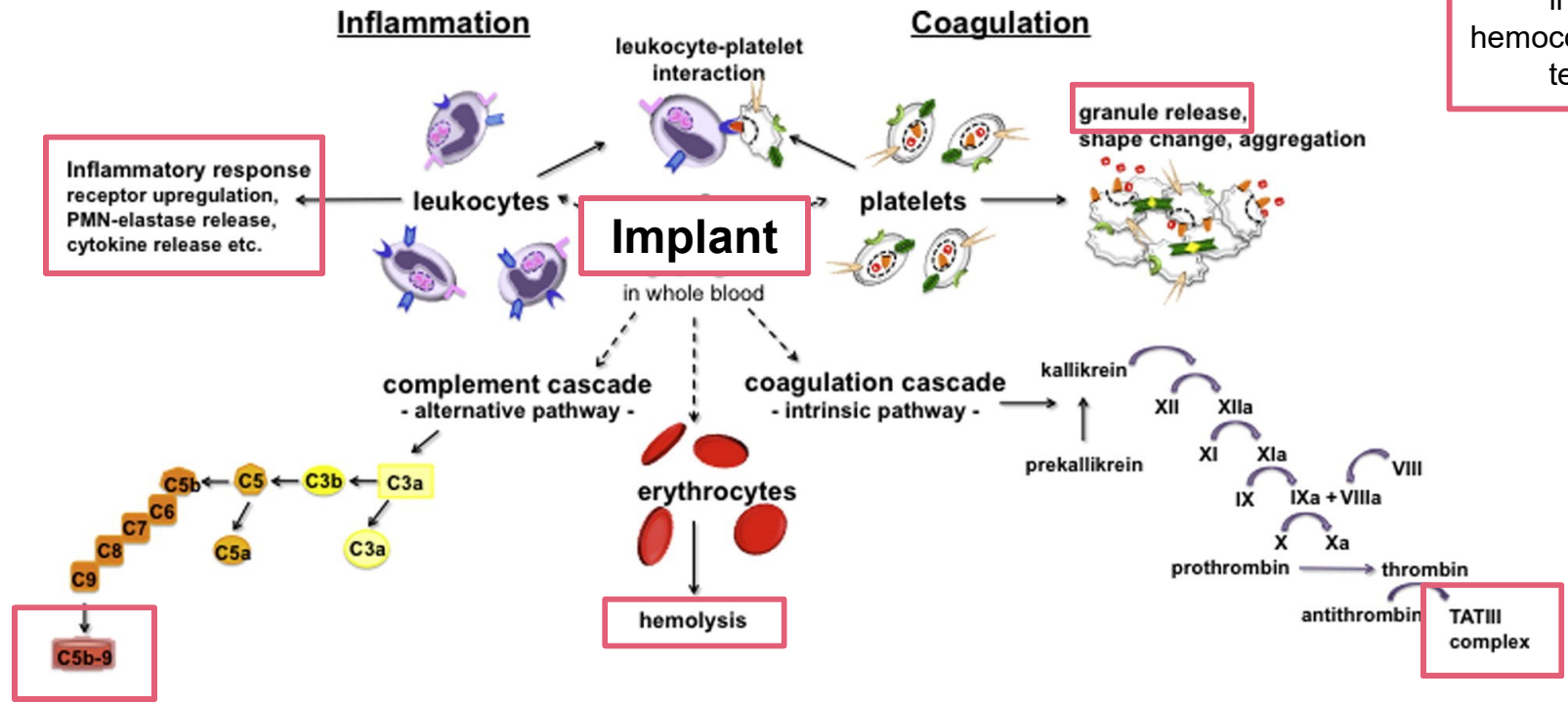
→ Appropriate cellular response to implanted surfaces is essential for tissue regeneration and integration



<https://doi.org/10.1002/admi.201800068>

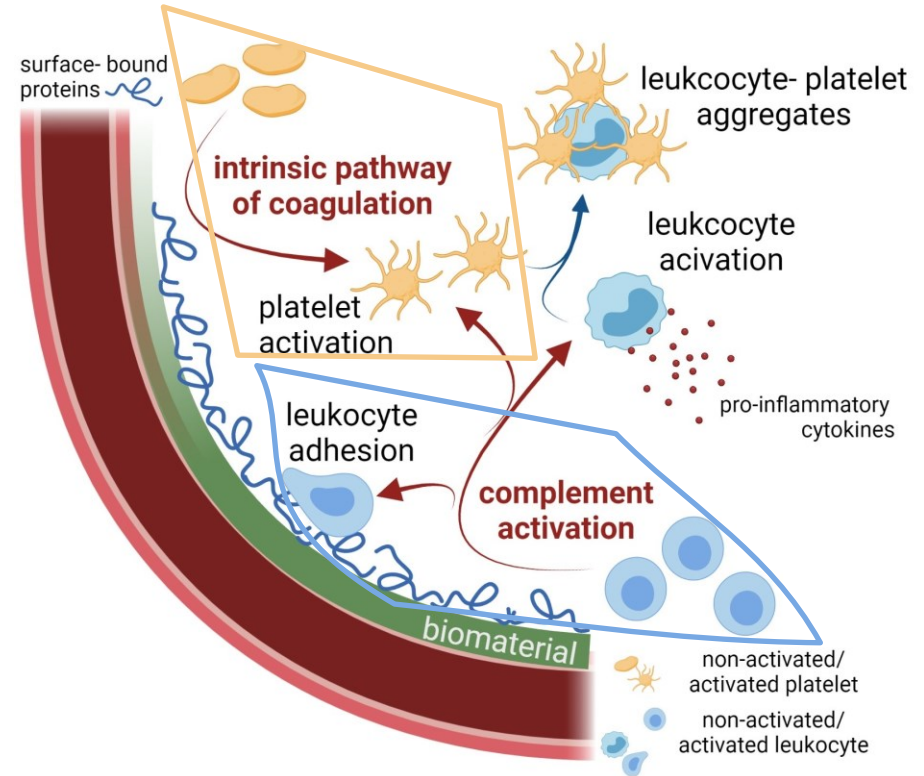
Blood material interaction

Values can be analyzed after in vitro hemocompatibility testing



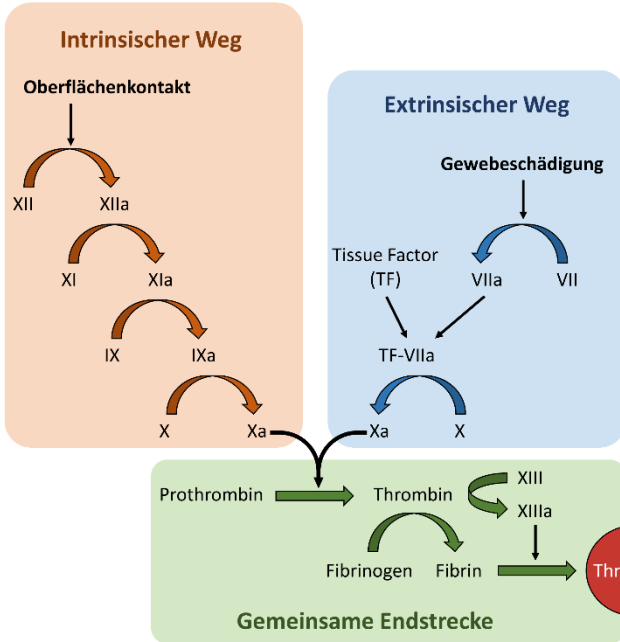
Adhesion of blood cells

- Protein conditioning of the material surface
- Platelet adhesion on proteins
 - activation and aggregation
 - formation of blood cell aggregates
 - secretion of aggregation-promoting agents
- Recruitment and activation of leukocytes
 - Activation of complement system

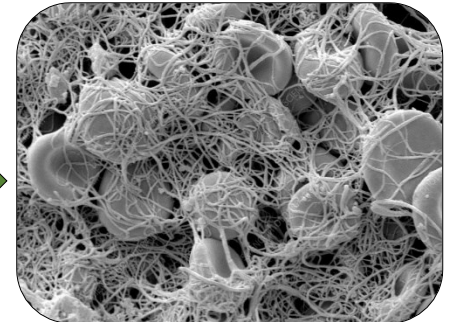
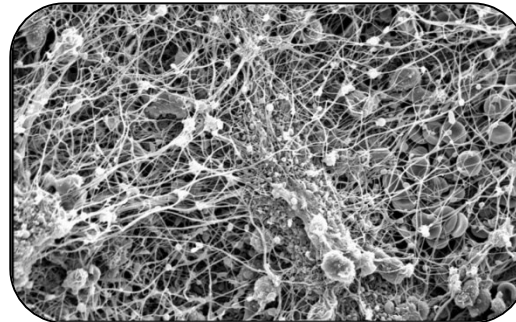


<https://doi.org/10.3390/ijms222111390>

Activation of coagulation cascade



- Activation after surface contact or injury
 - End product Factor Xa
 - Prothrombin → thrombin
 - Hydrolysis of fibrinogen into fibrin monomers
 - Polymerization/ cross-linking
- **Formation of a stable fibrin thrombus**



Biological significance of thrombus formation

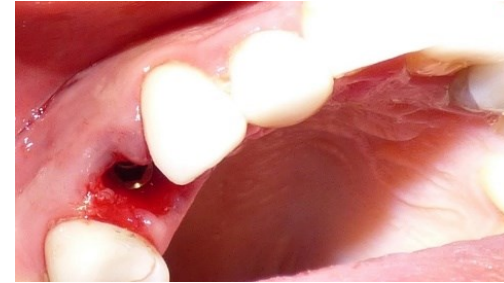
- Sealing of injured vessels
- Depot for cytokines and growth factors
- Scaffold for migrating cells and wound healing

Dental implants, orthopedic implants:

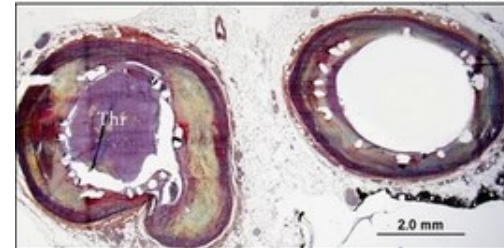
- Release of stimulating growth factors (VEGF, PDGF)
- Promote osteogenetic cell proliferation and differentiation

Vascular implants:

- Risk for thromboembolic events through lumen occlusion
- Platelet adhesion/ aggregation has to be prevented



<http://destinydentalimplant.com/wp-content/uploads/2016/10/P1030988-2.jpg>



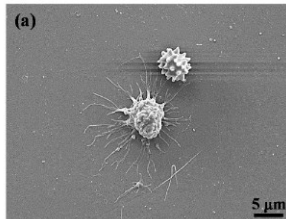
<https://doi.org/10.1155/2012/608593>

Mediation of thrombus formation on implant surface

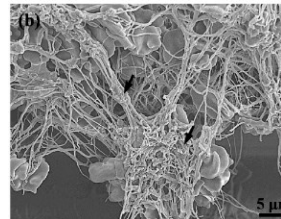
Platelet activation & fibrin formation are essential in dental implantations

Without thrombus formation: cytokine release ↓, cell recruitment ↓, scaffold structures
↓ soft tissue /osseointegration ↓

- „Problem case“ Titanium
 - Very high biocompatibility → Minimal coagulation activation
 - Additionally reduced platelet activation due to hydrophilic surface modifications
- Artificial increase of blood cell adhesion through biological coatings



Coating:
Gly-Arg-Gly-Asp
(GRGD-
Tetrapeptide)



<https://doi.org/10.3390/app11062616>

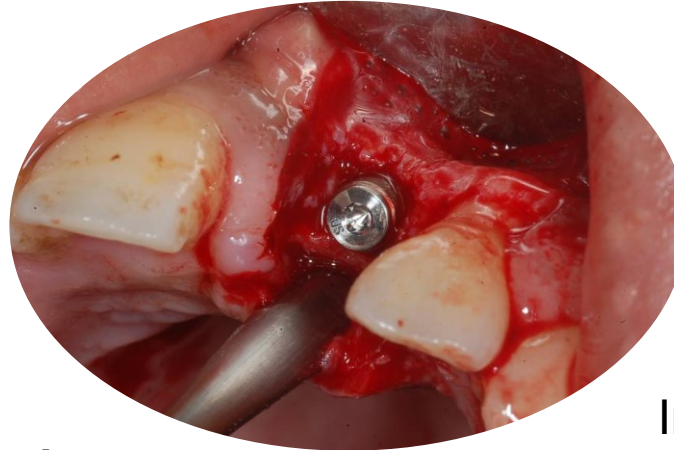
Biological responses after implantation

Contact with blood
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Interaction with
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Adhesion of bacteria



Inflammatory response

Immune activation

Osseointegration

Adhesion of surrounding
tissue cells

Osseointegration

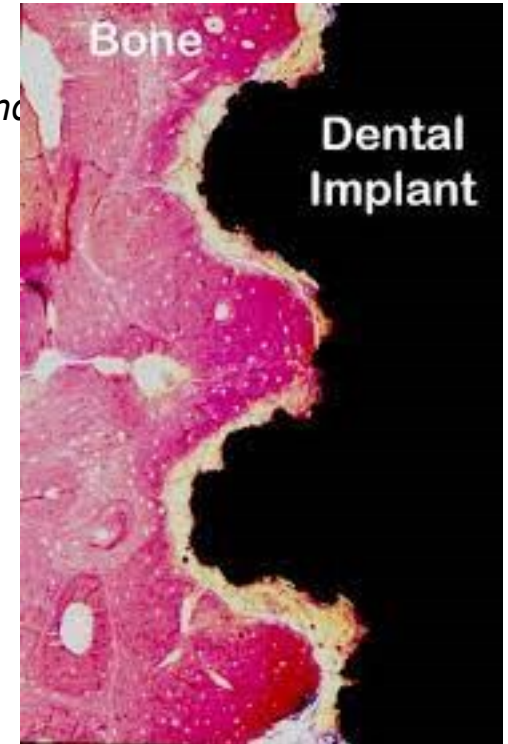
“...a direct structural and functional connection between ordered, living bone and the surface of a load-bearing implant.”

- Branemark et al., 1977

Today Osseointegration is seen as “...a time-dependent healing process”

Consisting of 3 overlapping steps:

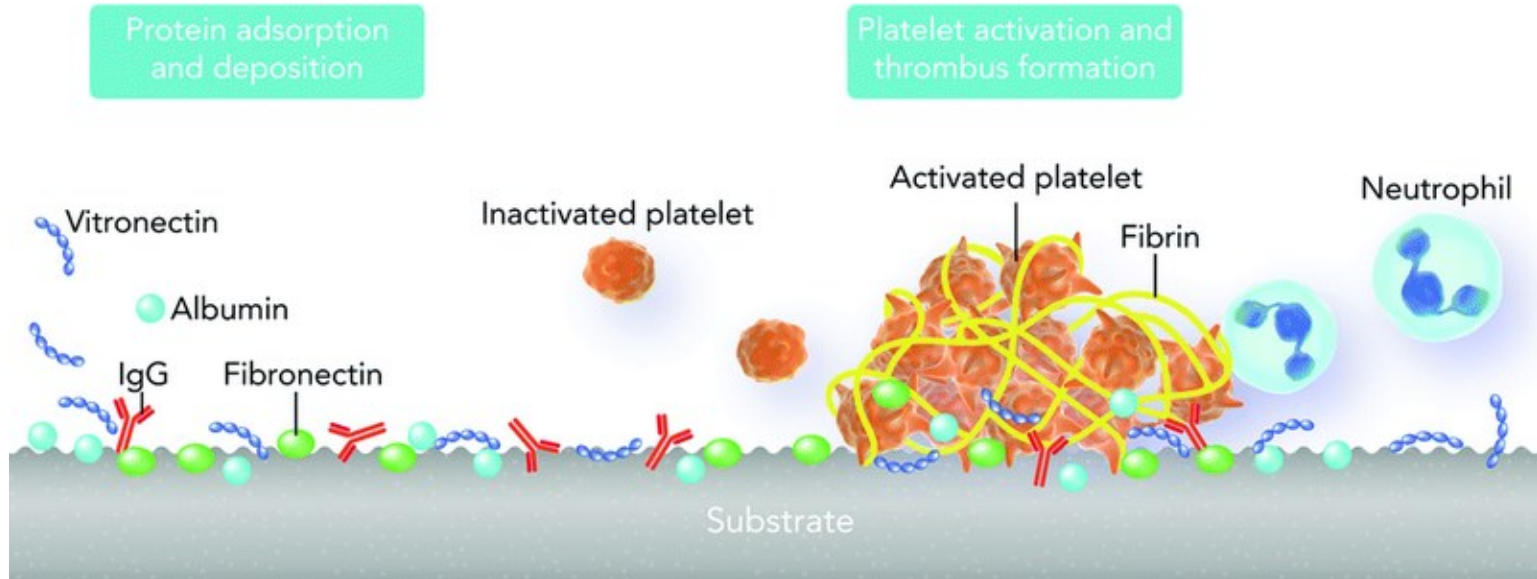
- Early immune-inflammatory response
- Angiogenesis
- Osteogenesis



<http://www.maryland-implants.com/dentalimplants.htm>

Foreign body response to implants

1) Material-blood-interaction

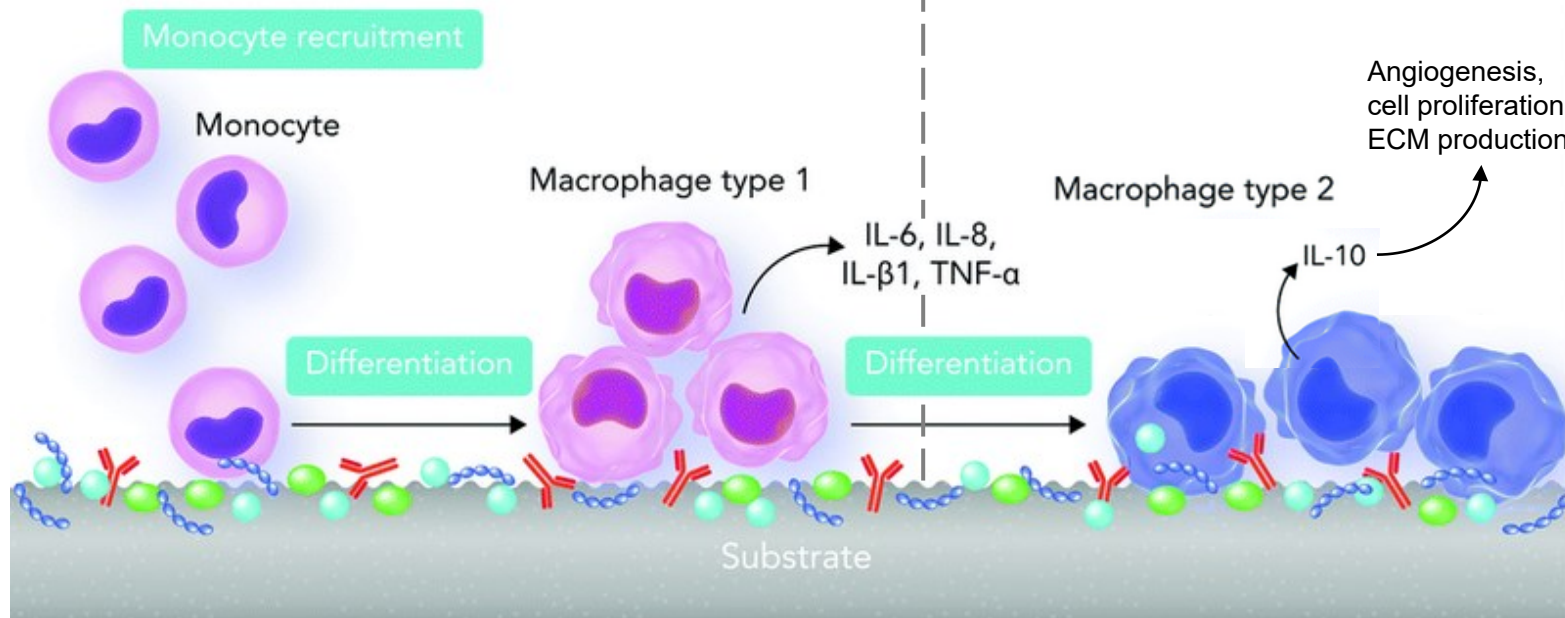


1) Protein adsorption: Coagulation activation → Cytokine release → Neutrophil recruitment → First responders

Foreign body response to implants

2) Acute Inflammation

3a) Chronic Inflammation + wound healing

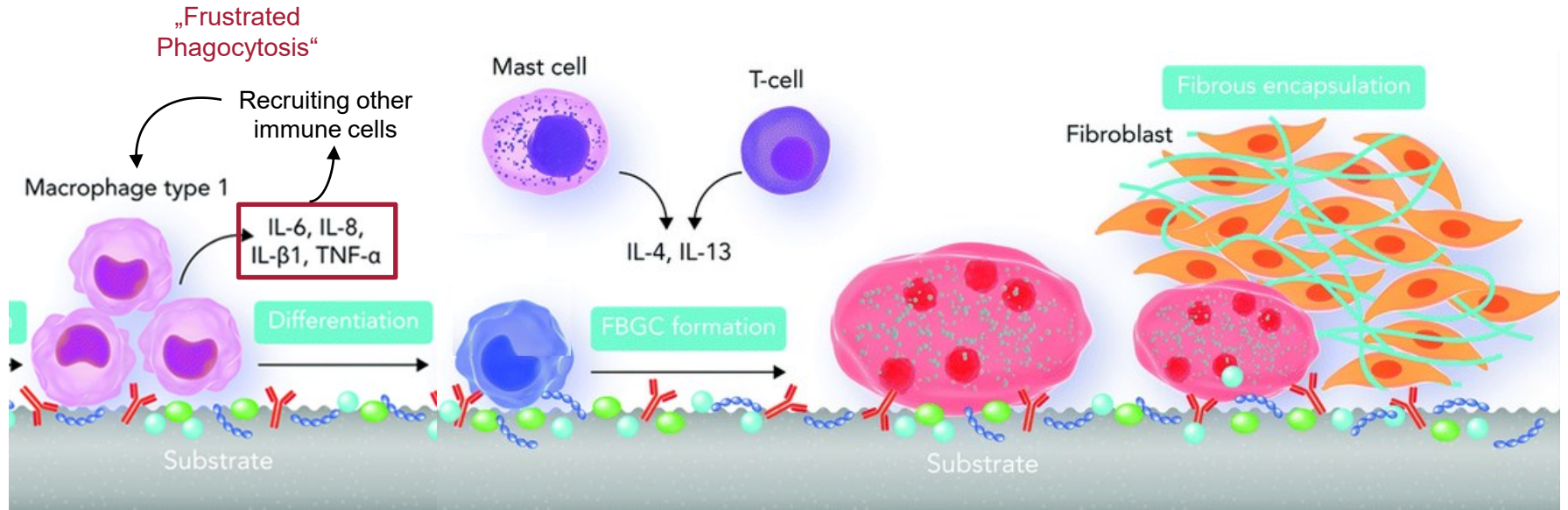


2) Acute inflammation: Monocytes/ M1 macrophages → proinflammatory cytokines → pathogens / tissue debris

3a) Chronic Inflammation: M2 macrophages → cytokines → regenerative processes

Foreign body response to implants

3b) Chronic Inflammation + Fibrosis

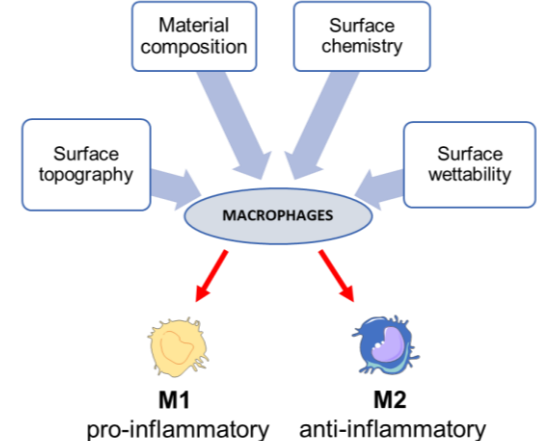


<https://doi.org/10.1039/D0CS00103A>

3b) Frustrated Phagocytosis: M2-based foreign-body-giant-cell-formation → fibroblast proliferation → Encapsulation

Immune modulation by implant materials

- **Inflammatory reactions are essential** for the integration of implants
 - Acute: degradation of destroyed tissue, antibacterial + antiviral activity (M1 macrophages)
 - Chronic: cytokine release, cell recruitment, regenerative processes (M2 macrophages)
- **Goal:** Elimination of excessive reactions (“frustrated phagocytosis”)
 - Abolishing persistent M1 phagocytosis and formation of FBGCs
 - Monocytes → M1 + M2
- Possible coping strategies
 - Structures surfaces: M2 ↑
 - Hydrophilic, anionic surfaces: M1 ↓
 - Anti-inflammatory agents (dexamethasone, heparin, IL-10,...)



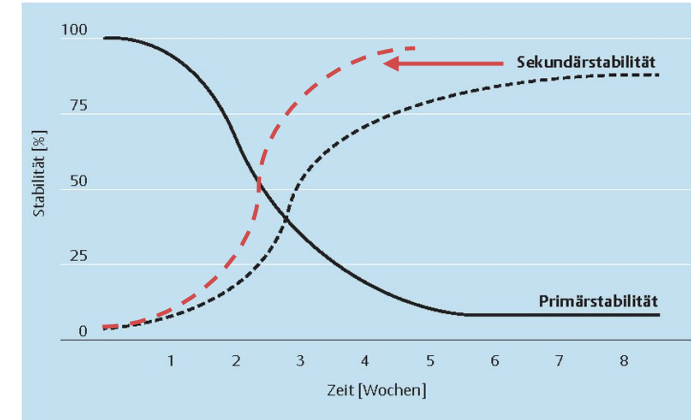
Importance of osseointegrating processes

Essential for implant stability

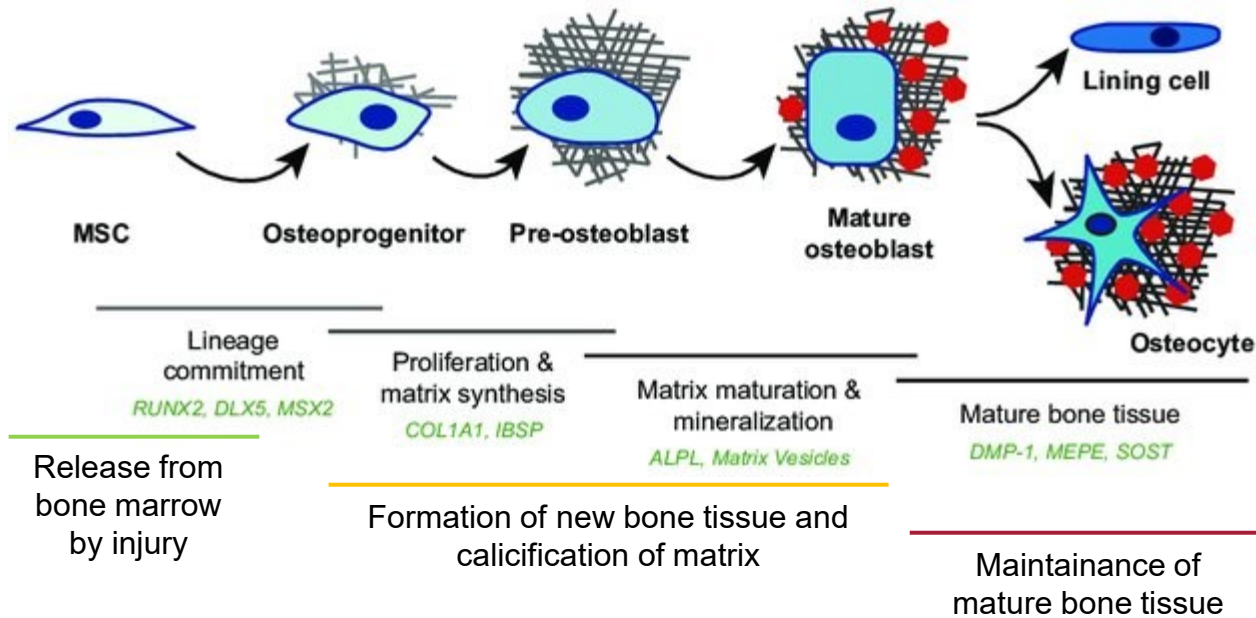
- Primary stability: Anchorage of the implant in the bearing bone (force-fit).
- Over time: degradation of the damaged bearing bone (peri-implant remodelling)
- Decrease in primary stability
- Improving secondary stability due to osseogenesis
- Tight implant fit in regenerated bone tissue

Aim of biomaterial research:

- Shortening the vulnerable phase between primary and secondary stability

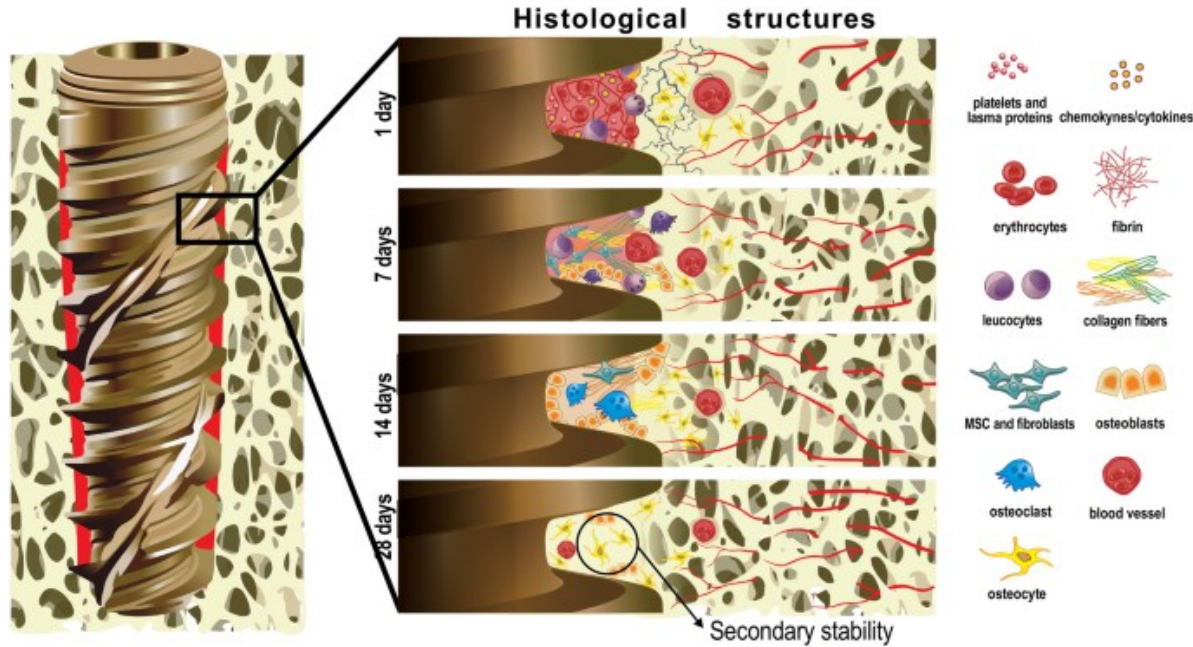


Background: Osteoblast differentiation



https://www.researchgate.net/publication/241868391_Osteoblast_Differentiation_and_Bone_Relevant_proteins_regulatory_processes_and_the_vascular_connection

Osteogenesis



Day 1-7:

Thrombus formation, angiogenesis and stem cell recruitment from surrounding tissue

Day 7-14:

Osteoblast differentiation, woven bone formation, matrix calcification

Day 14-28 :

Resorption of damaged bone, transition from woven to lamellar bone

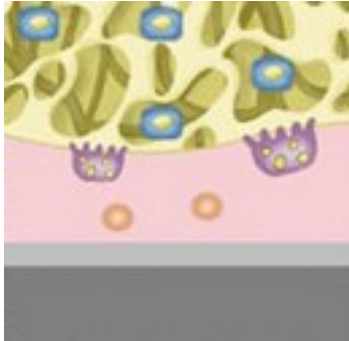
Day 28 and beyond:

Maintenance of mature bone tissue through osteocytes → secondary stability

<https://doi.org/10.1038/s41598-021-94886-y>

How to promote bone formation ?

Damaged Bone



Surface structuring

Blasting, etching, anodization ...

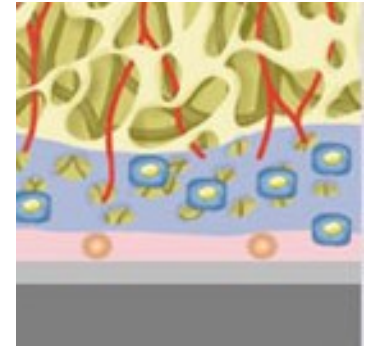
Biomimetic Surface

Hydroxyapatite, magnesia, graphene ...

Bioactive Coating

Bone morphogenic proteins, fibronectin, interleukine 4 ...

New Bone



Common target:

Increase cell adhesion, promote bone cell differentiation, regulate immune response

Biological responses after implantation

Contact with blood
proteins

Contact with saliva
proteins

Interaction with
coagulation cascade

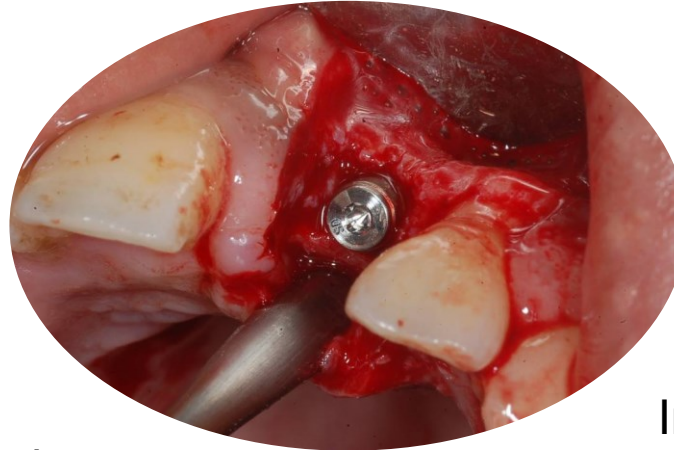
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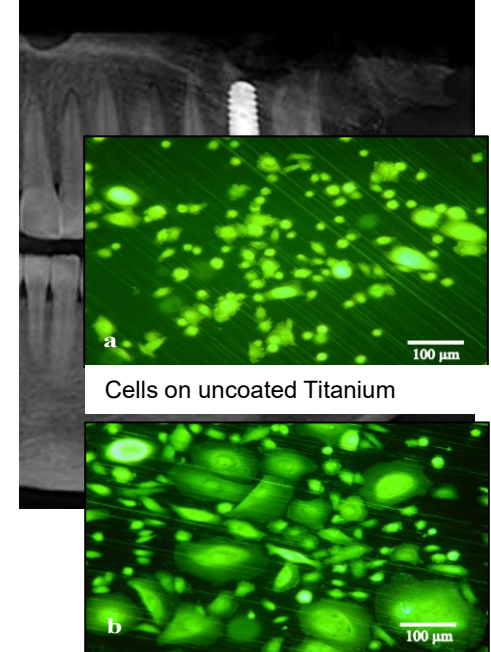
Osseointegration

**Adhesion of
surrounding tissue
cells**



Importance of cell adhesion to implant surfaces

- Essential for healing and regeneration
- **Gingival cells** and **mesenchymal stem cells (MSC)**
 - Tight peri-implant seal towards dental implant prevents bacterial colonisation of implant/bone
 - Enhanced MSC adhesion supports osseointegration
- Improvement possible through surface modification:
 - Biological: coupling of signalling molecules/proteins
 - Physical: Hydrophilization, micro-/ nano-structuring



Cells on uncoated Titanium

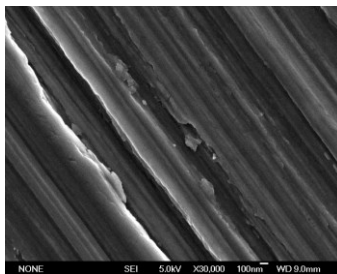
Cells on fibronectin-coated Ti

<https://doi.org/10.1016/j.dental.2006.03.005>

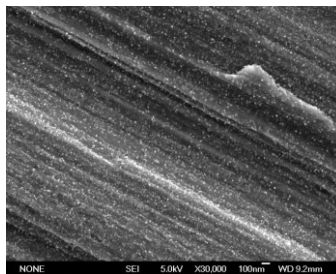
Cell coverage on implant surfaces

Scanning
electron
microscopy

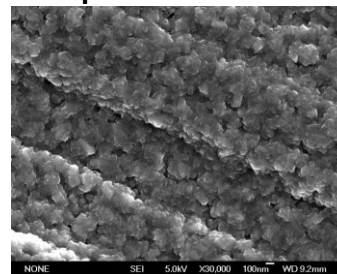
Smooth Ti



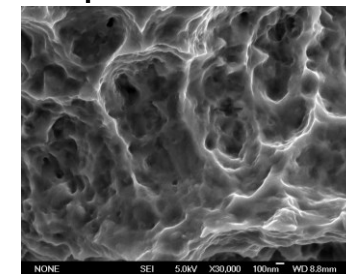
Smooth nanostructure



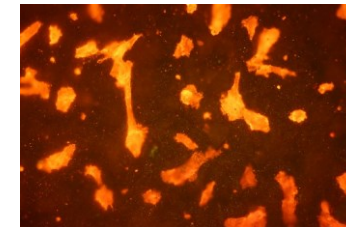
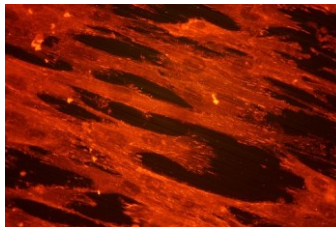
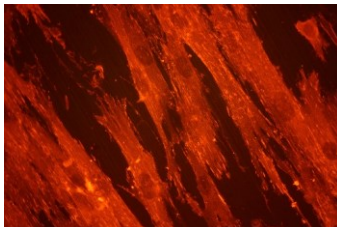
Complex nanostructure



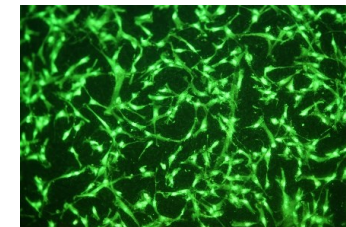
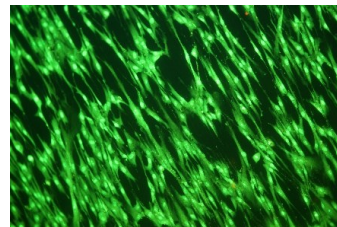
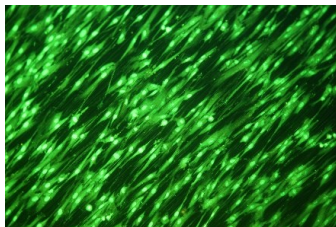
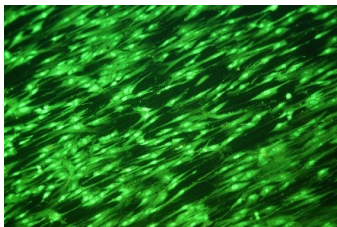
Complex microstructure



Human
gingival
fibroblasts

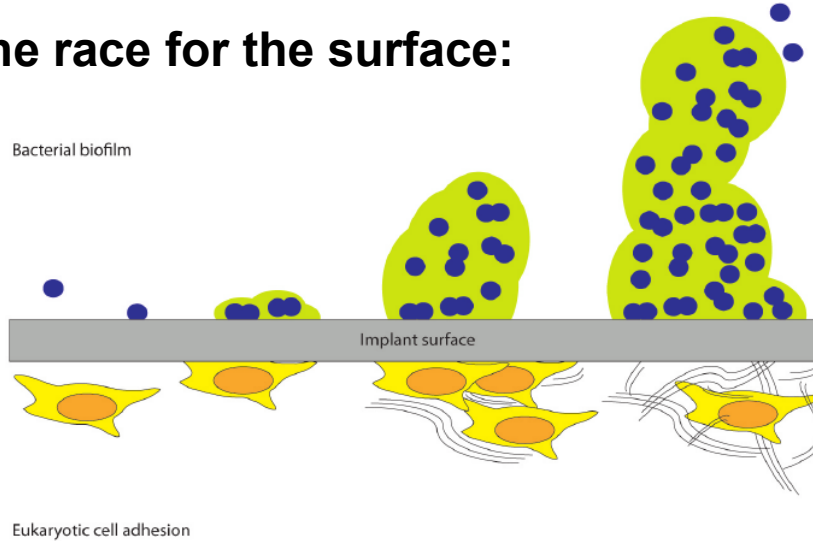


Human
oral
keratinocytes



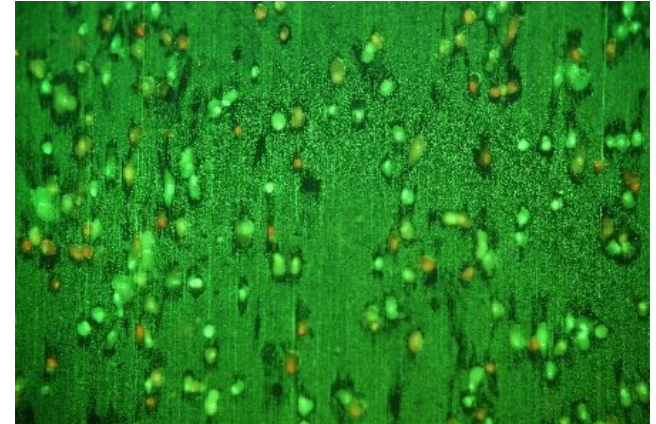
Bacterial adhesion to biomaterial surface

The race for the surface:



<https://doi.org/10.5772/55976>

Titanium surface after 2 h cultivation with bacteria and tissue cells



Tissue cells compete with bacteria for the same protein-conditioned surface

- If tissue cells “win” → surface is occupied and less available for bacterial colonization
- If bacteria “win” → unimpeded proliferation and biofilm formation

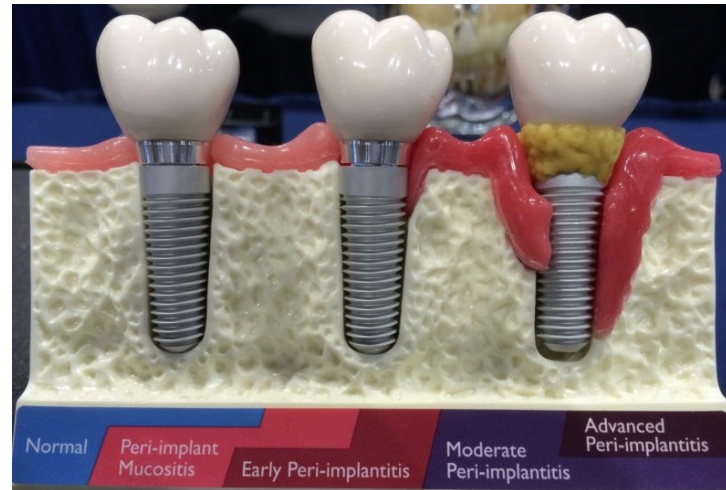
Clinical relevance of bacterial colonisation

**Bacterial attachment/
proliferation at the sulcus**

**Bacterial toxins
→ Immune response
→ peri-implant mucositis**

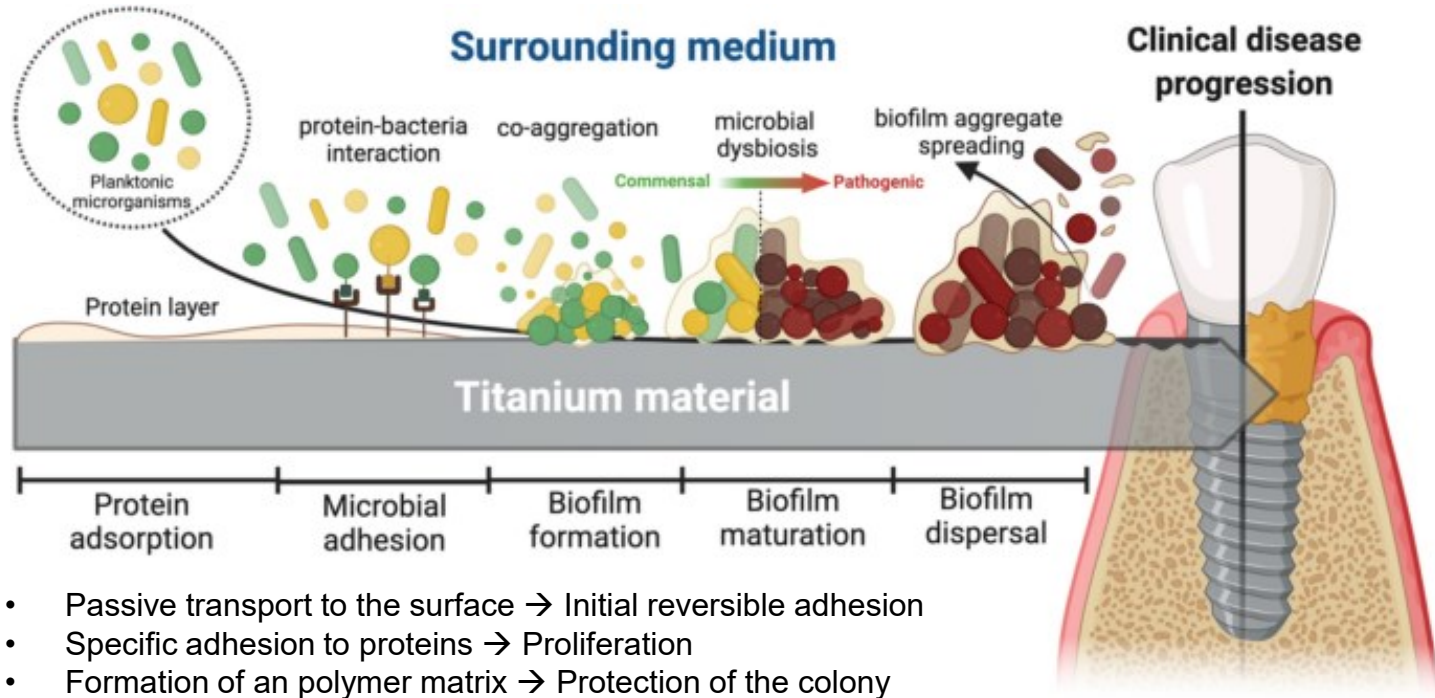
**Activation of
osteoclasts
→ peri-implantitis**

**Manifestation of bone
defect
→ Implant loss**



<https://www.cosmetic-dental.de/mukositis-und-periimplantitis/>

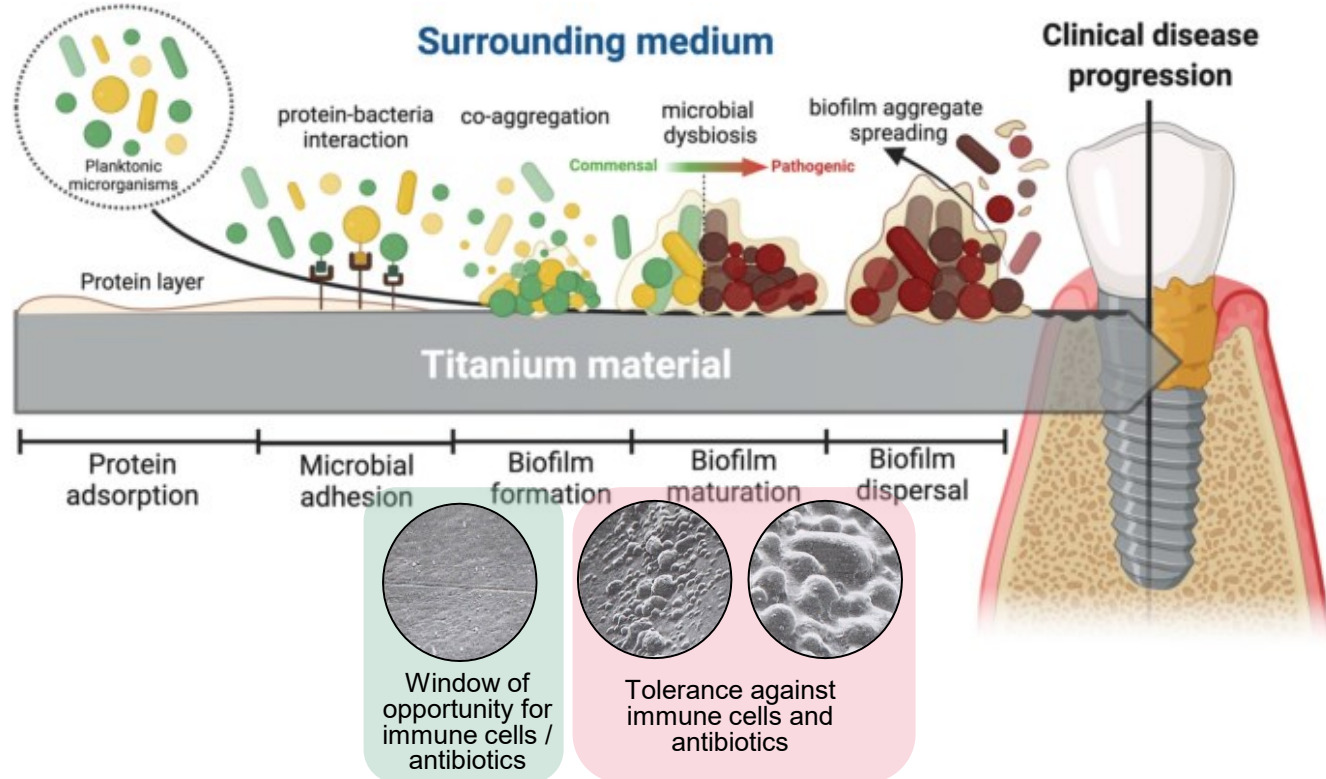
Biofilm formation on implant surfaces



- Passive transport to the surface → Initial reversible adhesion
- Specific adhesion to proteins → Proliferation
- Formation of an polymer matrix → Protection of the colony
- Dysbiosis → Proliferation of secondary (pathogenic) species
- Biofilm rupture → widespread distribution

<https://link.springer.com/article/10.1007/s40735-021-00575-8>

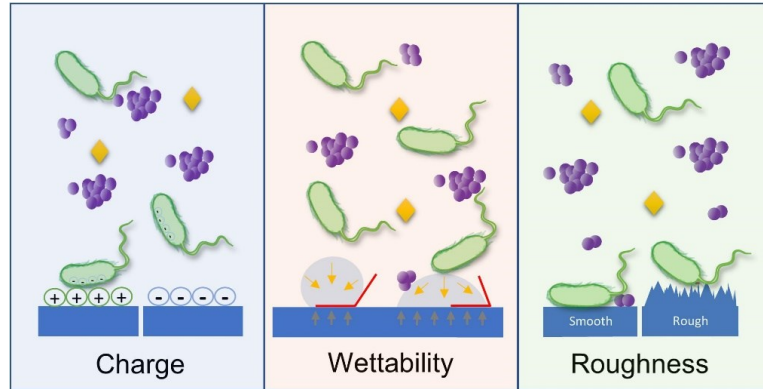
Biofilm formation on implant surfaces



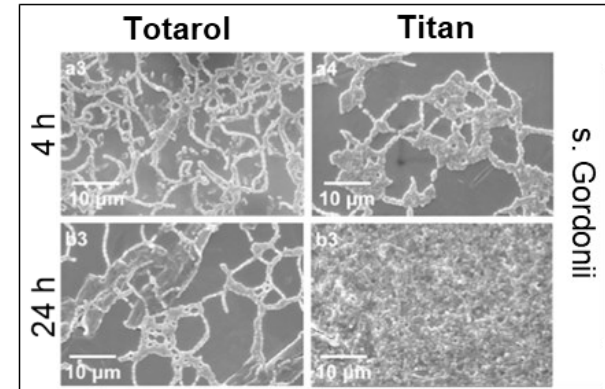
<https://link.springer.com/article/10.1007/s40735-021-00575-8>

How to cope with bacterial adhesion?

- **Reduction of early colonization** through modified surfaces to control biofilm formation and colonization of pathogens in the long term



<https://doi.org/10.3389/fbioe.2021.643722>



<https://doi.org/10.1016/j.msec.2020.110701>

Take Home Message

A wide spectrum of biological interactions exists between the implant and the patient

- The adsorption of proteins on the surface is the initial point for every following reaction
- The variety of biological and technical modifications enables us to systematically influence biological processes occurring
- For quick and reproducible screening of surface modifications standardized in vitro tests are used





Thank you for your
attention