Biomedical engineering in the reconstruction of the cardiovascular and skeletal systems

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https://www.youtube.com/watch?v=QONVYTPjZbI

Topics

1.) Eugenika i jej wpływ na losy świata

2.) Ewolucja mechanicznych zastawek serca

3.) Odpowiedź na pytanie „co było pierwsze jajko czy kura?”

4.) Wytyczne etyki w poborze narządów

5.) Rozwój anestezji w historii medycyny i problem korozji implantów.

6.) Holograficzne techniki obrazowania w medycynie

Biomaterial is any substance other than the drug either
a combination of natural or synthetic substances,
which can be used in any period,
and which task is to supplement
or replacement of organ tissues or parts
or fulfill their function.

[European Society for Biomaterials]

Biocompatibility is the ability to properly preserve the material
in contact with the tissue in a particular application

[Biomaterials Science An Introduction to Materials in Medicine-
ELSEVIER 2004 Academic Press]

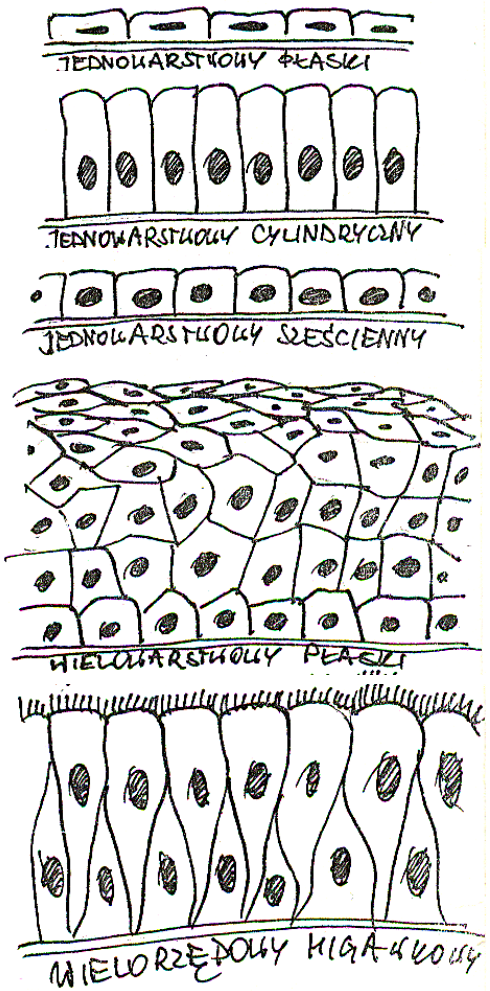
Part 1

Introduction to the human anatomy

Types of human tissue

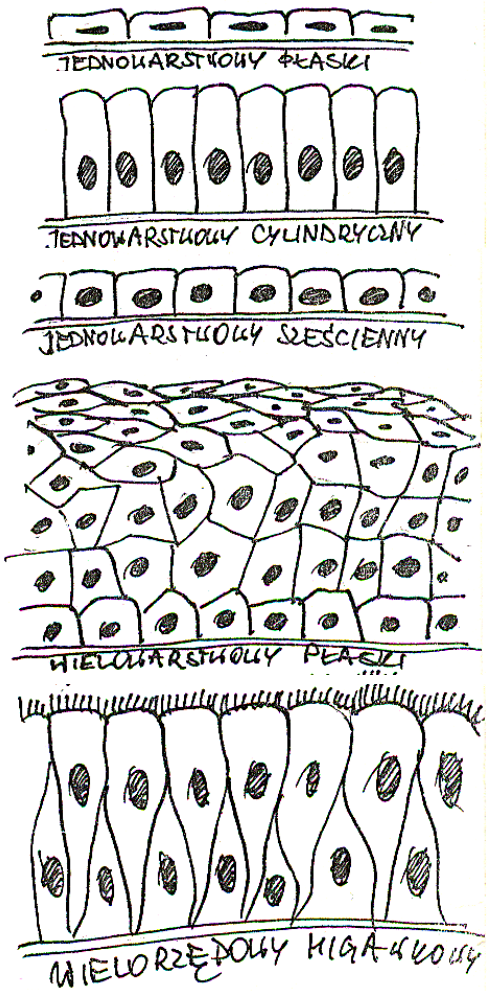
Tissue

- Epithelial (Nabłonkowa)
- Connective (Łączna)
- Muscular (mięśniowa)
- Nervous (nerwowa)



Epithelial tissue

Functions:
protective-cover
resorption
secretory
barrier
sensual



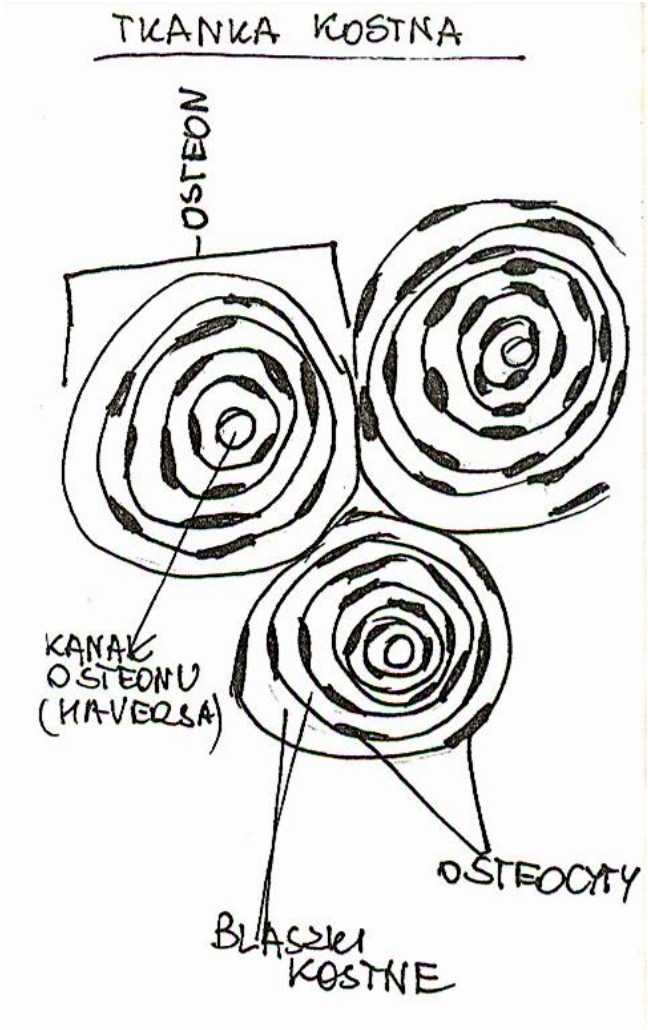
Connective tissue

Epithelial (Nabłonkowa)

→ Connective (Łączna)

Muscular (mięśniowa)

Nervous (nerwowa)



Connective tissue

Types:

-**Connective**

- Flaccid (wiotka)
- Compacted (zbita)
  - Adipose (Tłuszczowa)
  - Pigmentosa (Barwnikowa)
  - Mesh (Siateczkowa)

-**specialized in specific activities**

-Skeletal

- Cartilage (chrzęstna)
- bone (kostna)

Tissue

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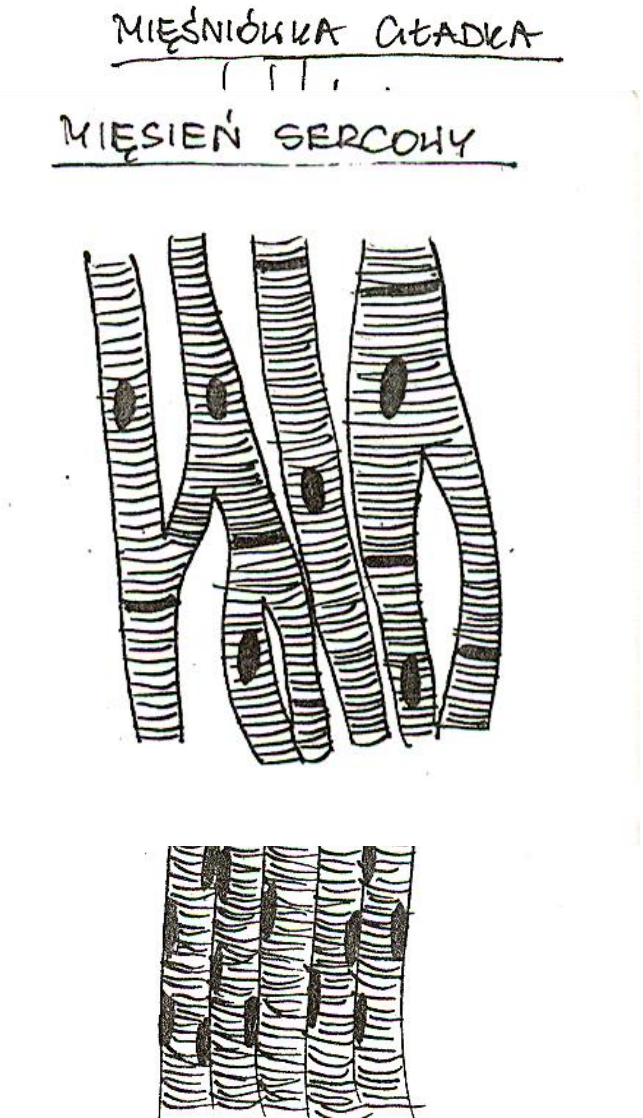


Tissue

Epithelial (Nabłonkowa)

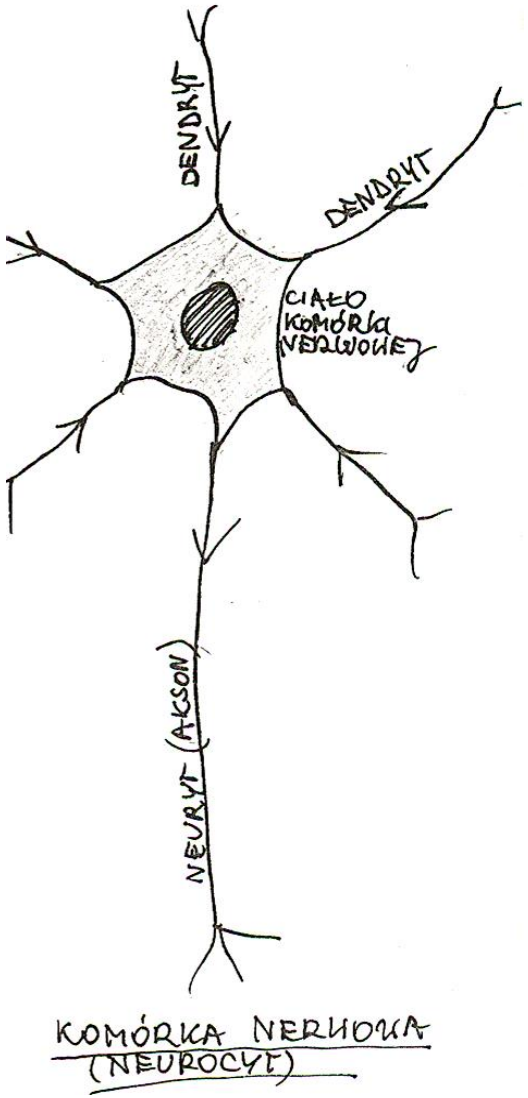
Connective (Łączna)

Muscular (mięśniowa)

→ Nervous (nerwowa)

Action potentials (MOV)

Neuron



Organ systems

Kostno- stawowy

Mięśniowy

Naczyniowy

Pokarmowy

Oddechowy

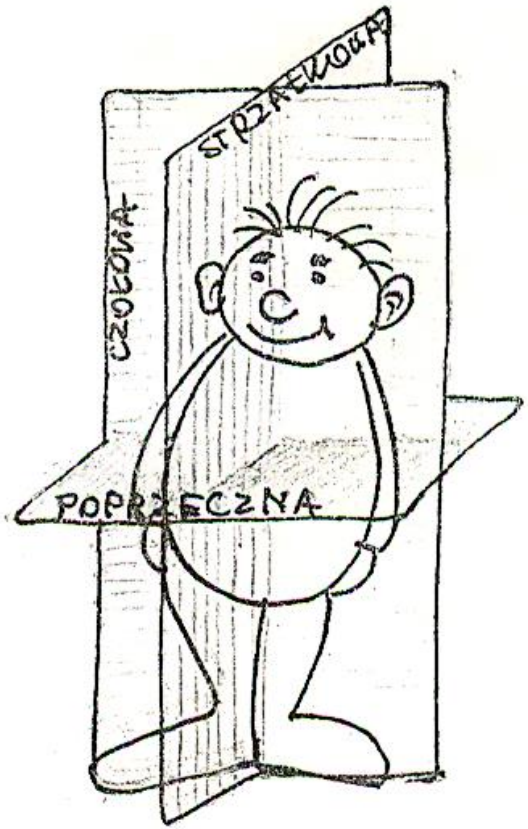
Moczowy

Płciowy

Nerwowy

Powłoka wspólna

Główne osie i płaszczyzny ciała



Part 2

Introduction to the human anatomy

Cardiovascular system

Cardiovascular system

Heart (cor)

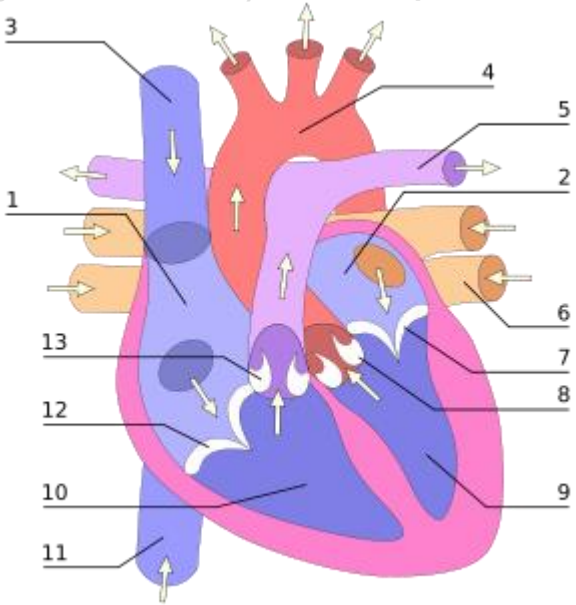
Naczynia krwionośne Blood vessels

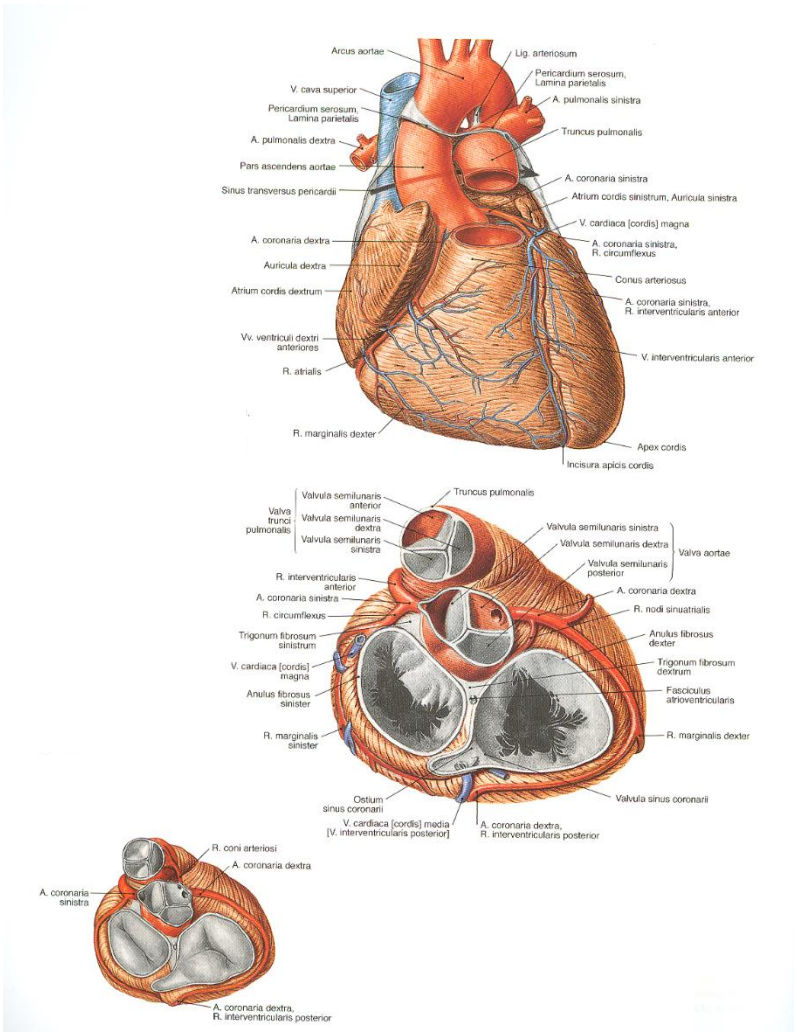
Tętnice krążenia dużego Large circulation arteries

Żyły krążenia dużego Veins of the large circulation

Naczynia chłonne Lymph vessels

Krążenie małe (czynnościowe) Small circulation (functional)





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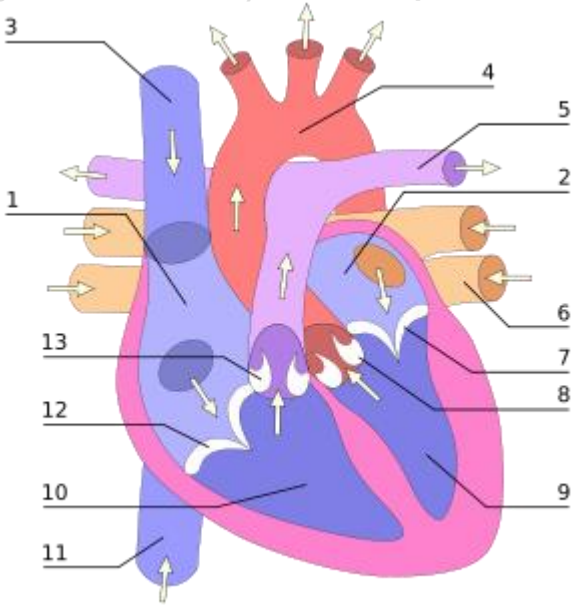
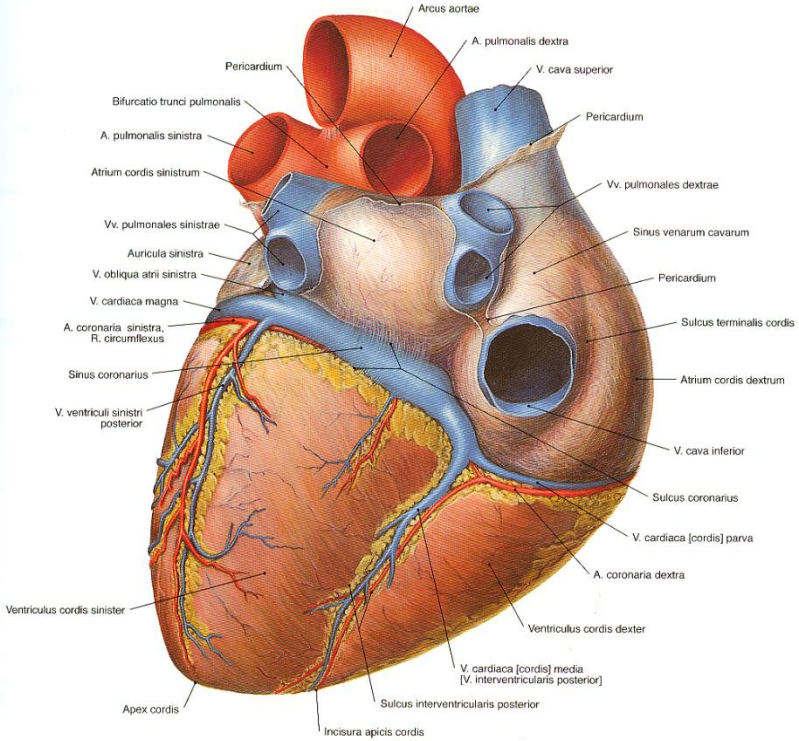
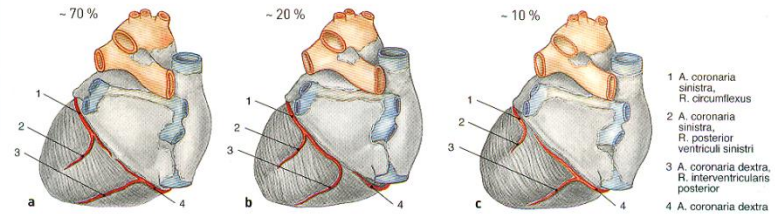




Ryc. 894 Żyły serca, *vv. cordis*.



Cardiovascular system

Naczynia krwionośne	Blood vessels
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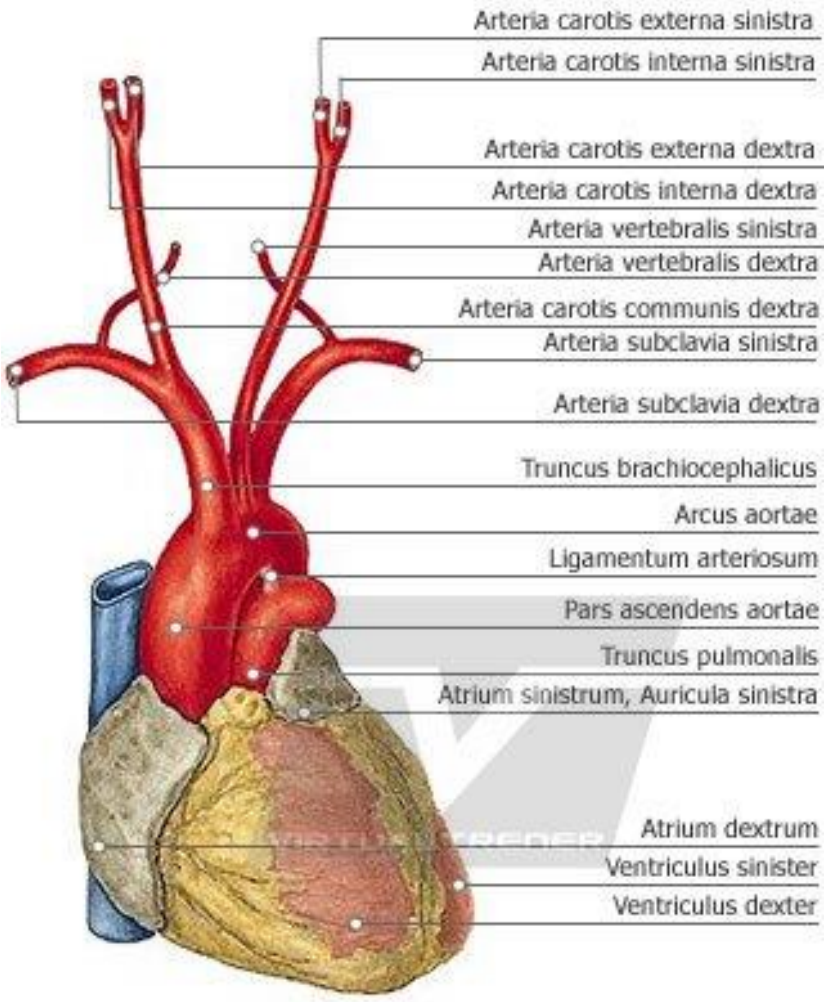


Biomaterials

Polymers

Metals

Ceramic (carbides, ceramic glass and glass)

Natural materials (greens and animal)

Composite materials (silica reinforced silicone rubber, carbon fibers, hydroxyapatite particle- reinorced poly (lactic acid))

Dedicated materials

Biodegradable polymers

Bioactive ceramics

Celuloze

Pyrolityc carbon

Modified materials, surfaces preventing clots

Natural tissues and polymers combined with living cells

Implant- definition

Implant- wszelkie przyrządy medyczne wykonywane z jednego lub więcej Biomateriałów, które mogą być umieszczone wewnątrz organizmu, jak również częściowo lub całkowicie pod powierzchnią nabłonka i które mogą pozostać przez dłuższy okres w organiźmie

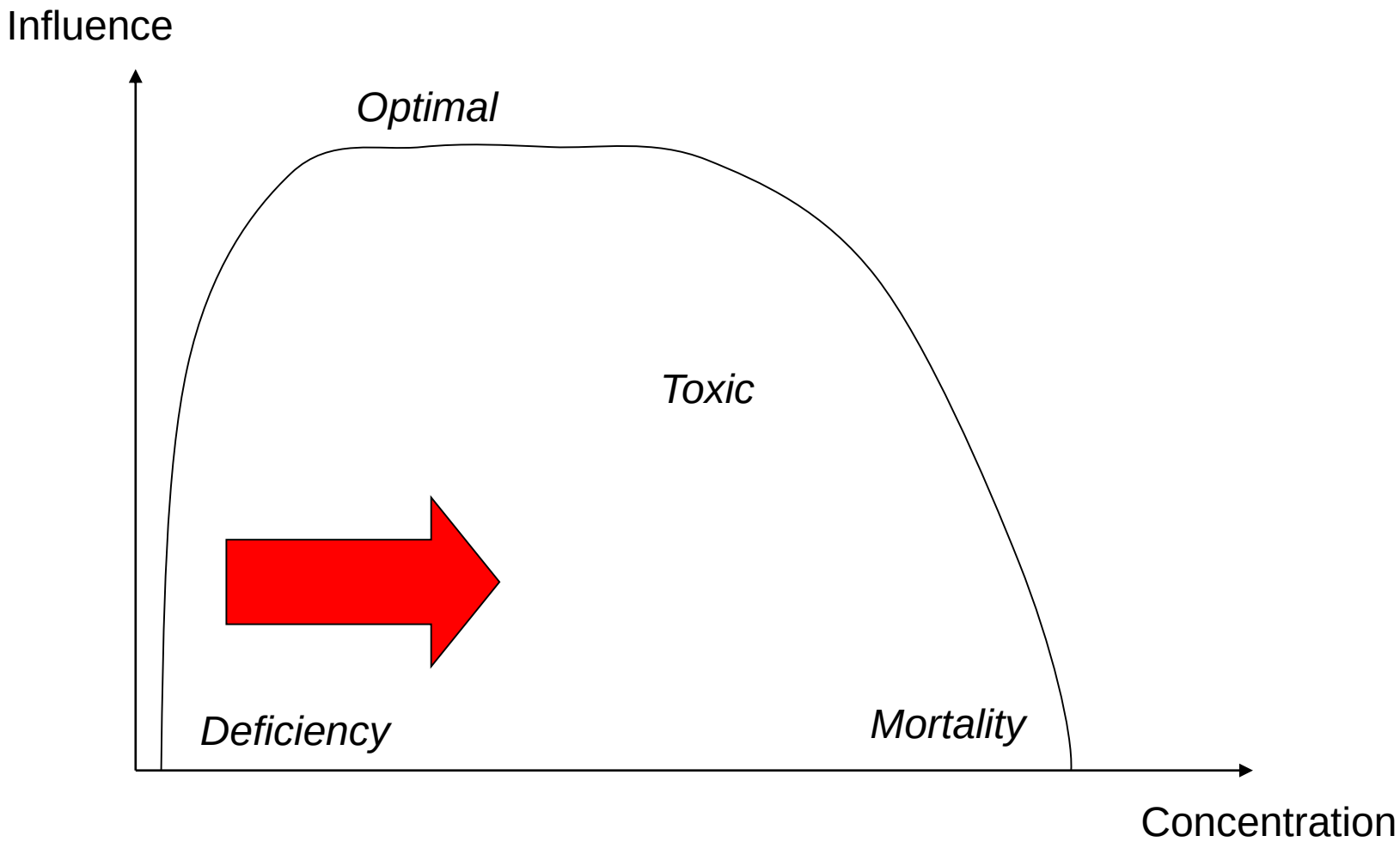
Implant chirurgiczny- używany jest w kontekście umieszczenia go w zamierzonym miejscu w procedurze chirurgicznej. Istnieją także implanty wprowadzane innym sposobem. Do nich zalicza się igły, dreny, sączki itp.

Proteza implantowalna- (proteza wewnętrzna lub endoproteza)- przyrząd, który fizycznie zastępuje organ lub tkankę. W odróżnieniu, bioproteza stanowi implantowaną protezę wykonaną w całości lub częściowo z tkanek dawcy

Sztuczny organ- materiał medyczny, który zastępuje w całości lub częściowo funkcje jednego z głównych organów ciała. Sztuczne organy zastępują funkcje organów chorych, częściowo w nieanatomiczny sposób

Implant- influence

Implants- the concentration of elements influence on the reactions of vital processes



Metallic implants

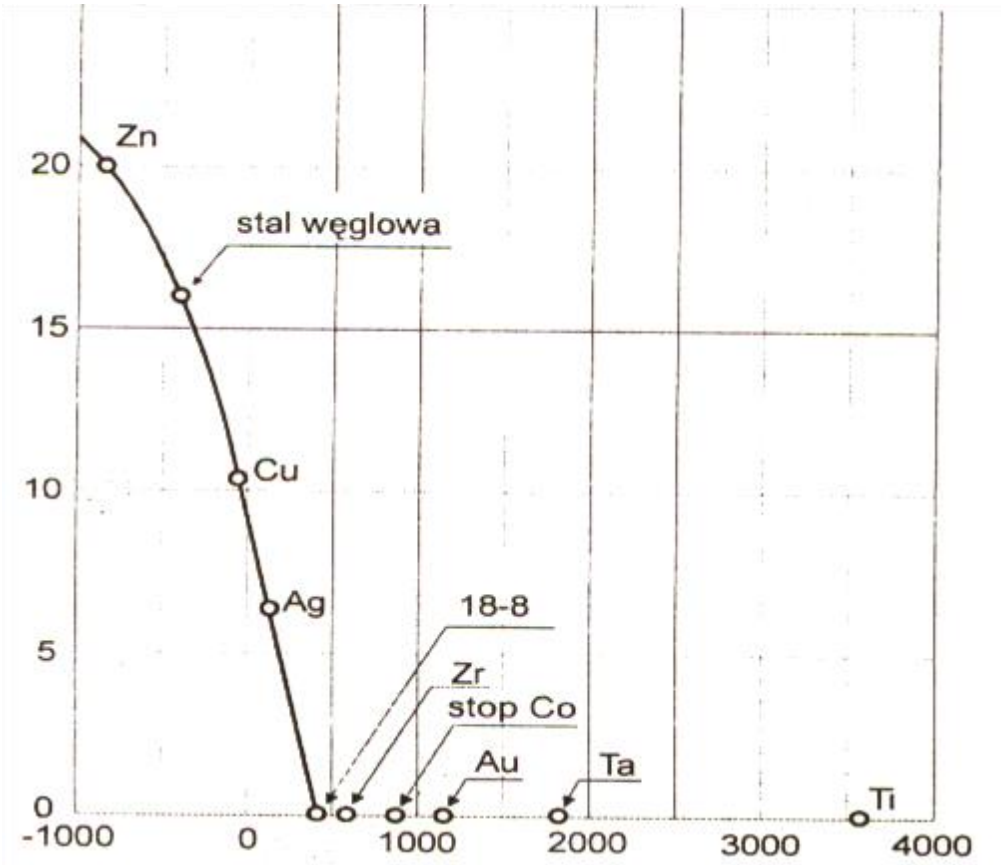
Mo:
Deficiency
- tooth brush
- bone crumbling
- disorders in the functioning of the gonads
Excess
-difference of enzyme diffusion through cell membranes
allergic reactions

Me: biochemical reactions and conversion of vitamin C
Deficiency
- disturbances in the development of bones and genital organs
Excess
- respiratory tract irritation and pneumonia
- OSN

Ti: in the spleen, liver and kidneys
Deficiency
-inert
Excess
-allergy or peri-implant reaction in inter-implantology as evidenced by the presence of macrophages and T-lymphocytes

-odczyny ale

Implants- corosion



Division of corrosive reactions

-quiet
-acute
-discreet

[Frank E., Zitter H.: Metallische Implantate in der Knochenchirurgie. Wien-New York, Springer Verlag 1977]
*[**Clarke E. G. C., Hickman J**.: J. Bone Jt. Surg. 35-A, 1953]*

Implants- layers

AIM

- **Biofunction (friction resistance)**
- **Diffusion barriers (increase of corrosion resistance)**
- **Improvement of osseointegration (ceramic bioactive layers)**

Types

- diffusion layers of titanium compounds
- diamond layers (NCD, DLC)
- hydroxyapatite coatings
- composite layers - new

Deposition methods

- methods using plasma, photons, ions:
- PDT processes - Plasma Diffusion Treatment
- RFCVD - Radio Frequency Chemical Vapor Deposition
- MWCVD - Microwave CVD
- PLD - Pulsed Laser Deposition
- the sol-gel method

Part 2.1

Executed projects

Cardiovascular system

Heart support

„My great desire to create a Polish Artificial Heart finally fully fulfills...”

prof. Zbigniew Religa

„POLISH ARTIFICIAL HEART”

Medicine

Modern technology

https://www.youtube.com/watch?v=7QFhxfVF7SA&list=PLMkpKh2ylbEa9OThOL0F6vQC_aKmHvgs8

„Moje wielkie pragnienie stworzenia polskiego sztucznego serca nareszcie w pełni się urzeczywistnia. Usilne starania o otoczenie tego przedsięwzięcia opieką państwa, uwieńczone uchwaleniem Wieloletniego Programu "Polskie Sztuczne Serce", uważam za ogromny sukces. Bardzo się z tego cieszę i życzę powodzenia wszystkim, którzy zaangażowani są w realizację prac”.

https://www.youtube.com/watch?v=71_E3Hh2Jqg *prof. Zbigniew Religa*

Heart support

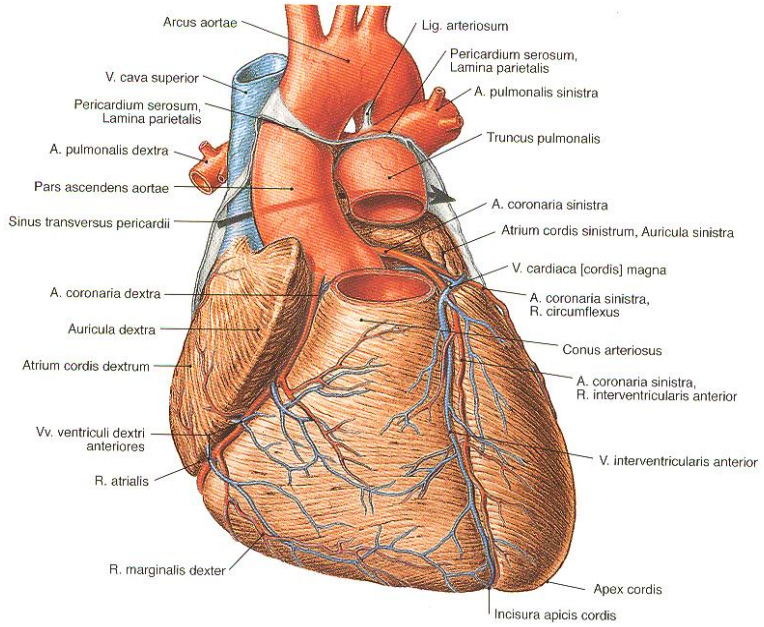
DIVISION BY SUPPORT TIME

Short-term

- An acute heart failure with a good prognosis of regeneration
- An infirmity with an uncertain prognosis for further treatment

Permanent support

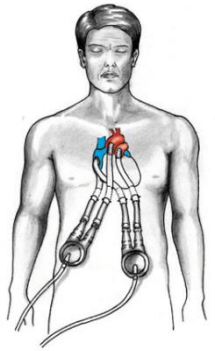
- chronic heart failure disqualifying for transplantation
- cardiomyopathy



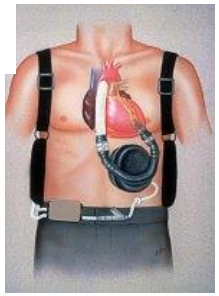
Heart support

DIVISION BY TYPE OF FAILURE

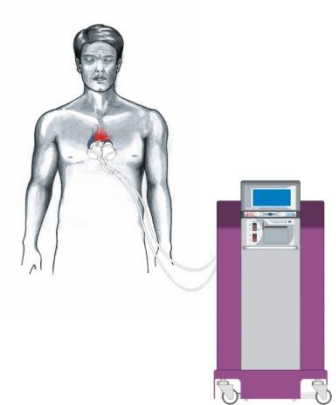
- **Acute failure**



- **Long-term failure**



- **Patients with cardiomyopathy**



Heart support

More than 40 teams from 35 scientific and research institutions, clinical centers and professional entities producing elements of heart prostheses participated in the implementation of the Program.
As part of 5 projects, 17 research tasks and 12 implementation tasks, covering over 200 stages, were implemented.

I STRATEGIC GOAL

Developed families of Polish heart prostheses, with a completely implantable permanent heart prosthesis as a final element

II STRATEGIC GOAL

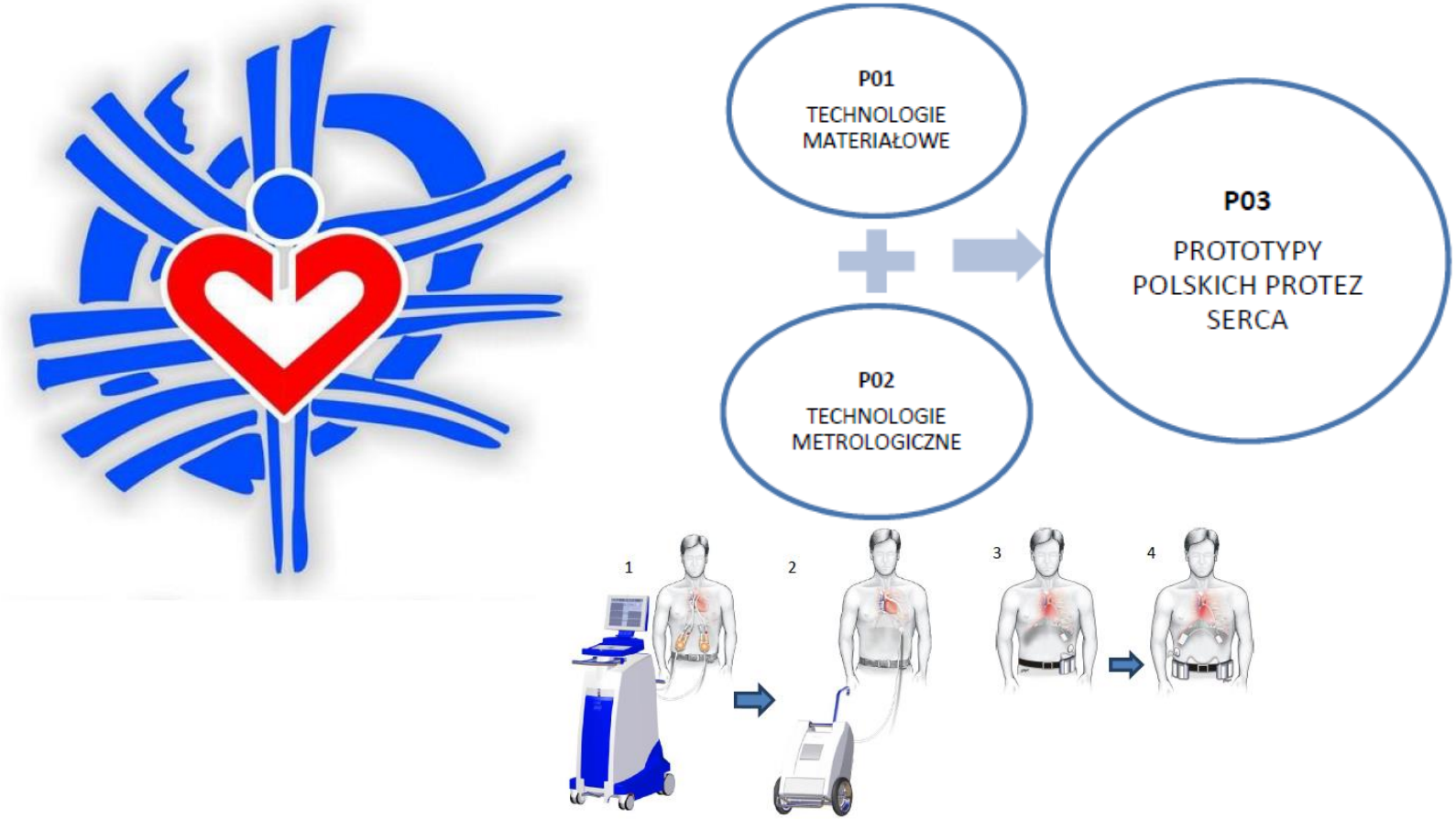
Clinical development of the use of Polish heart prostheses in the treatment of patients with critical heart failure

III STRATEGIC GOAL

Creation of a highly specialized scientific and technological platform for the purpose of conducting comprehensive research and development in the field of heart prostheses

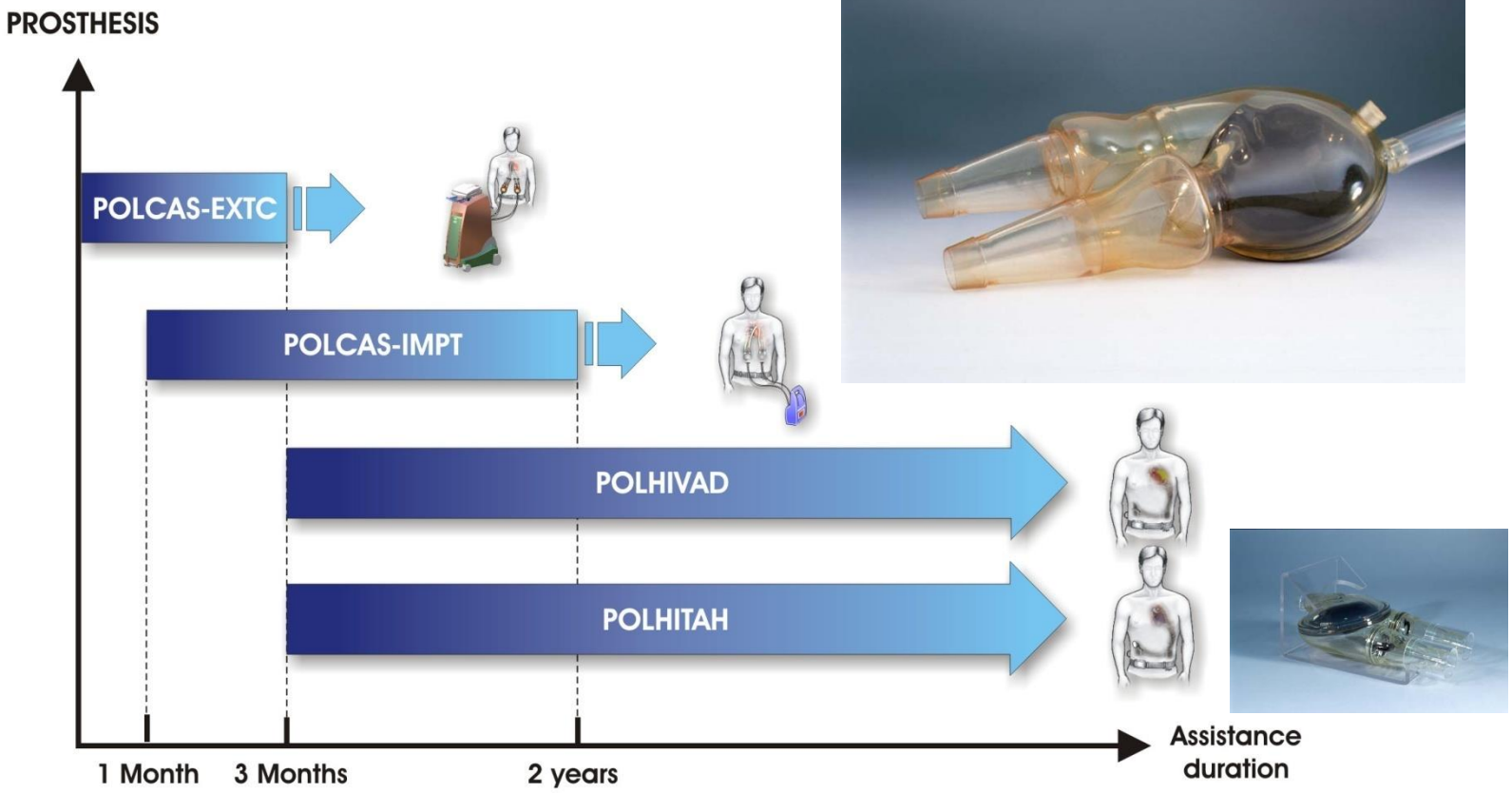
Heart support

Effects of the program

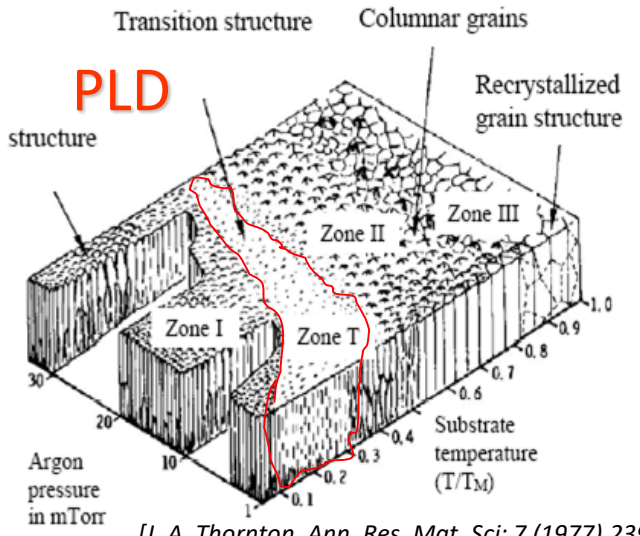


Heart support

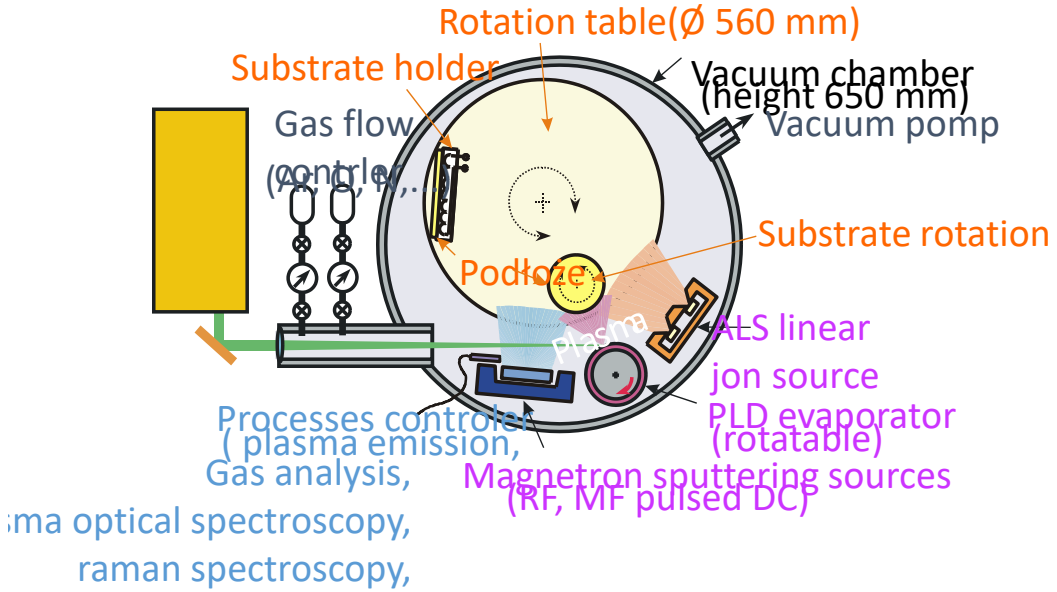
System POLVAD

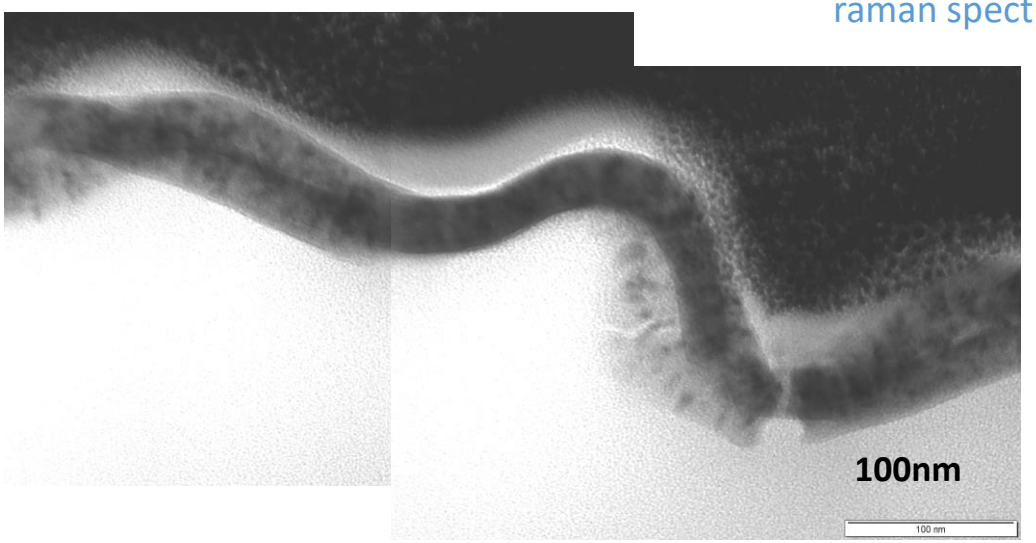


Heart support



[J. A. Thornton, Ann. Res. Mat. Sci; 7 (1977) 239]





Main assumptions

Good adhesion

Biocompatibility

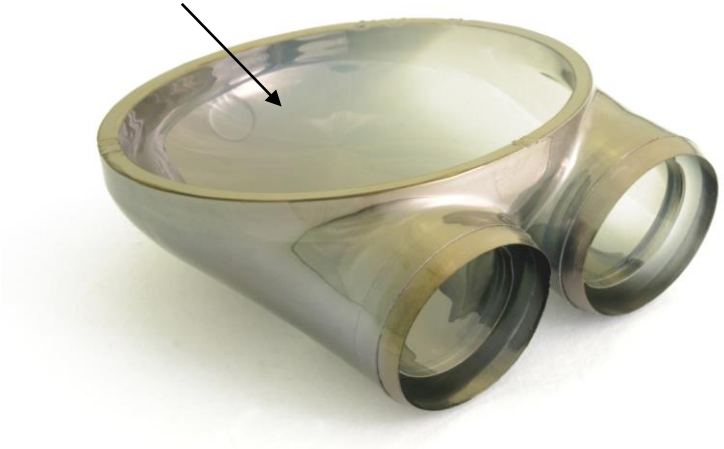
Lack of substrate degradation

TiN, TiO, Si-DLC, Ti(C,N), DLC

Heart support

Membrane

Chamber

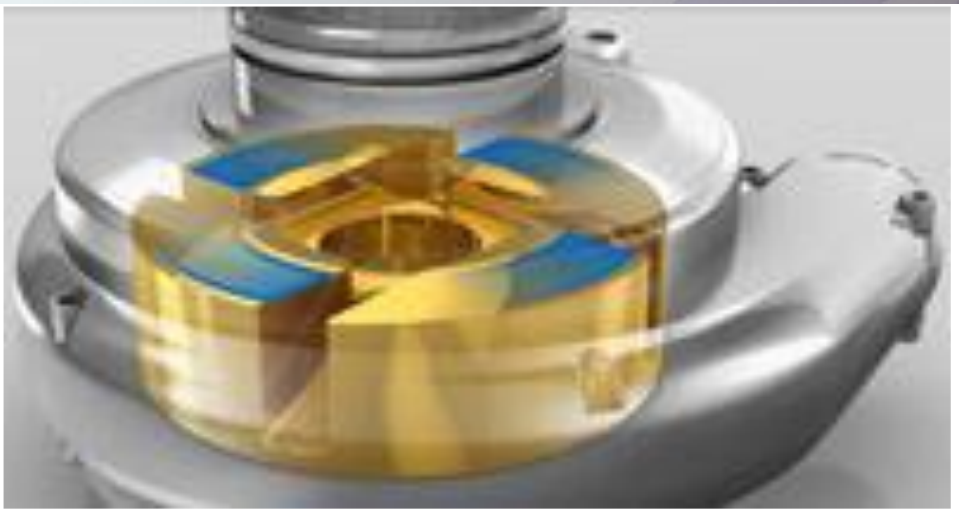


Valve

The goal of the work is the reduction of life-threatening thrombo-emboli formation in heart assist systems by a new material design

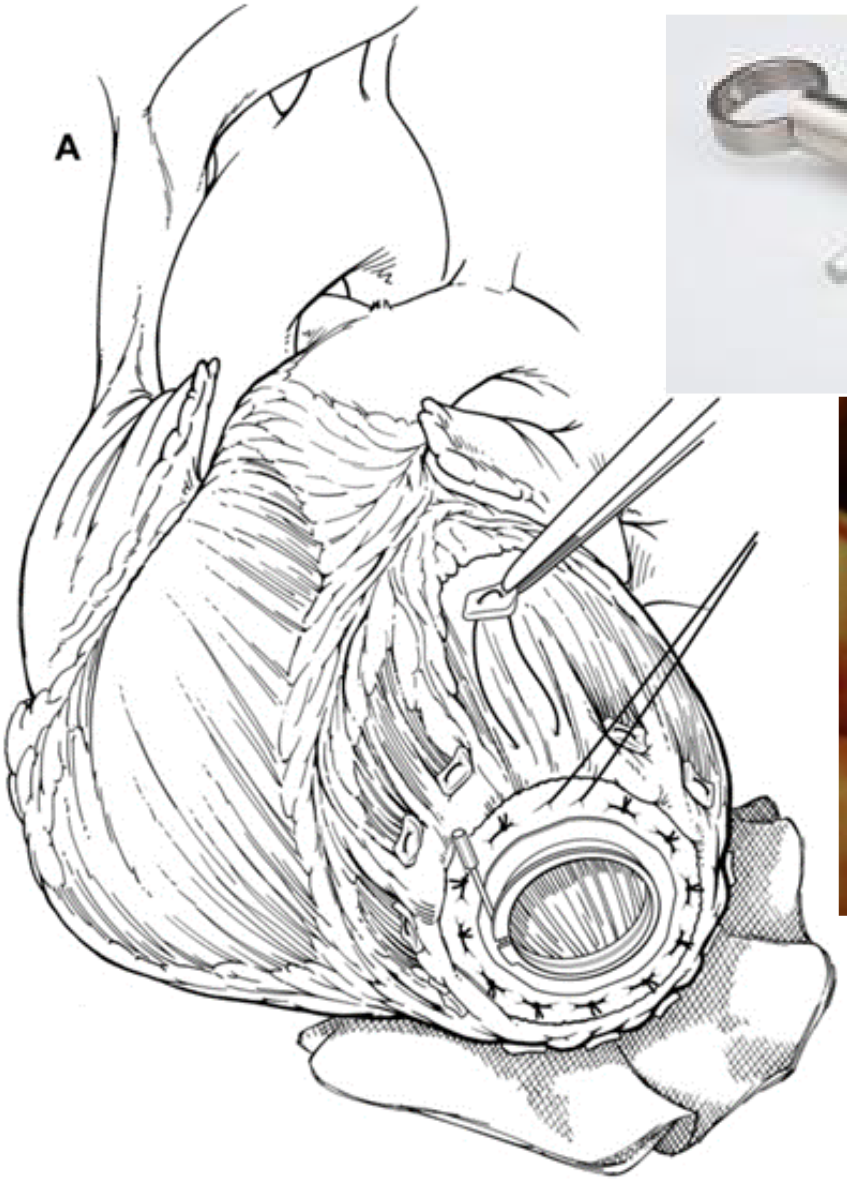
Heart support

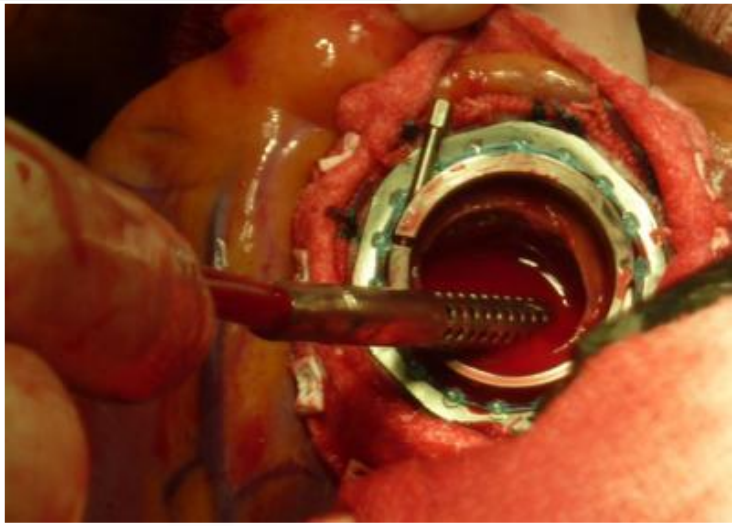
Heart support



Heart support

Heart support





Heart support

Material engineering mission in regenerative cardiac surgery

- Confokal Modul LSM 5 Exciter, two canals, RGB
- Laser HeNe 633nm 5mW
- Laser HeNe 543nm 1mW
- Laser argon 458/488/514nm, 25mW
- Laser diodowy V 405nm
- Main Beam Splitter turret PASCAL
- Software ZEN 2008 LSM 5 EXCITER
- Light division system (405, 458, 488, 514, 543 nm)
- Filtr BP 505-530
- Filtr BP 505-600
- Filtr BP 530-600
- Filtr BP 560-615
- Fitr LP 420
- Filtr BP 420-480
- System ECU LSM 5 EXCITER
- Moduł DIC I/0,9 z polaryzatorem
- Transmited light detectorT-PMT LSM 710

AB 120

Polish Artificial Heart

Nishe like structure

2014/13/B/ST8/04287
NCN

Silent heart mechanical valve

M-ERA.NET
Nonthrombogenic metal-polymer composites with adaptable micro and macro flexibility for next generation heart valves in artificial heart devices

Tissue precoursor

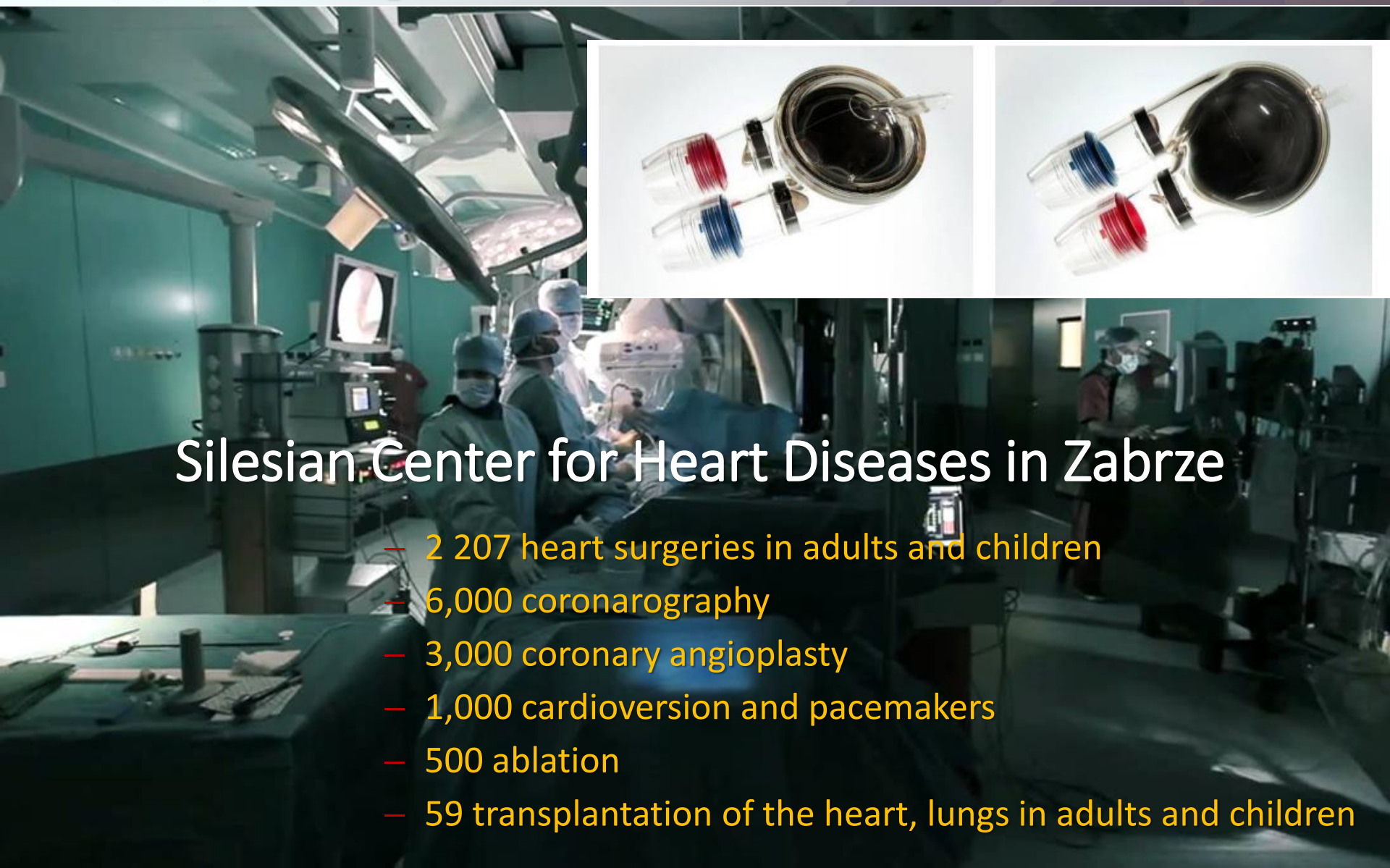
2011/03/D/ST8/04103
NCN

Bioactive heart valve

Program for Applied Research, Medical and

Pharmaceutical Science

Deplanted chambers

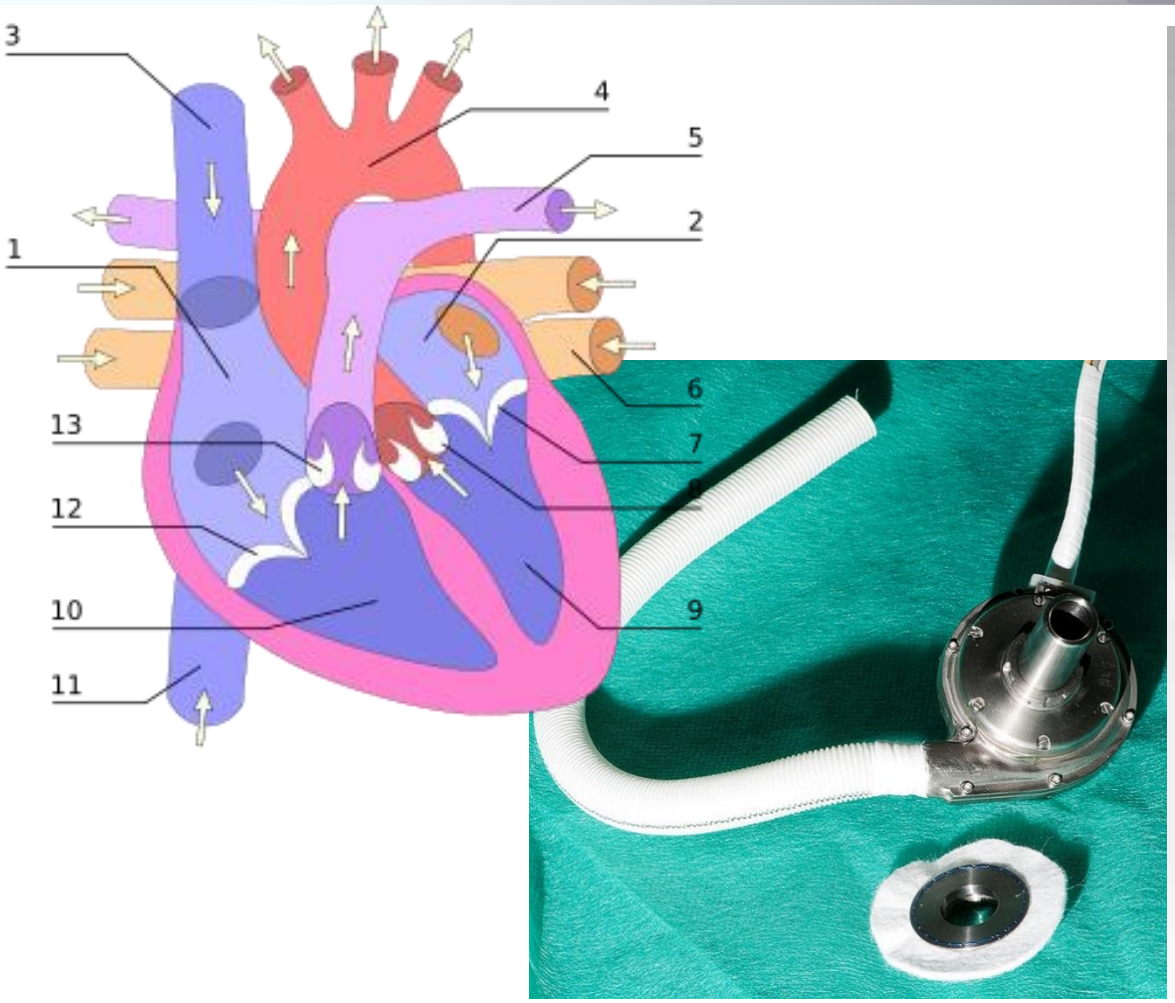


Silesian Center for Heart Diseases in Zabrze

- 2 207 heart surgeries in adults and children
- 6,000 coronarography
- 3,000 coronary angioplasty
- 1,000 cardioversion and pacemakers
- 500 ablation
- 59 transplantation of the heart, lungs in adults and children

Deplanted chambers

Deplanted chambers



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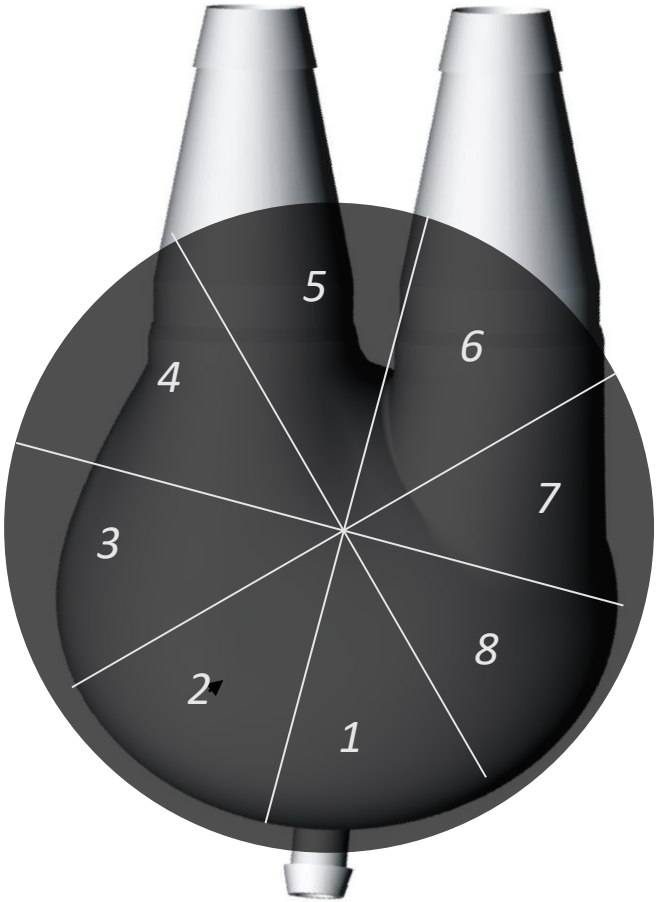
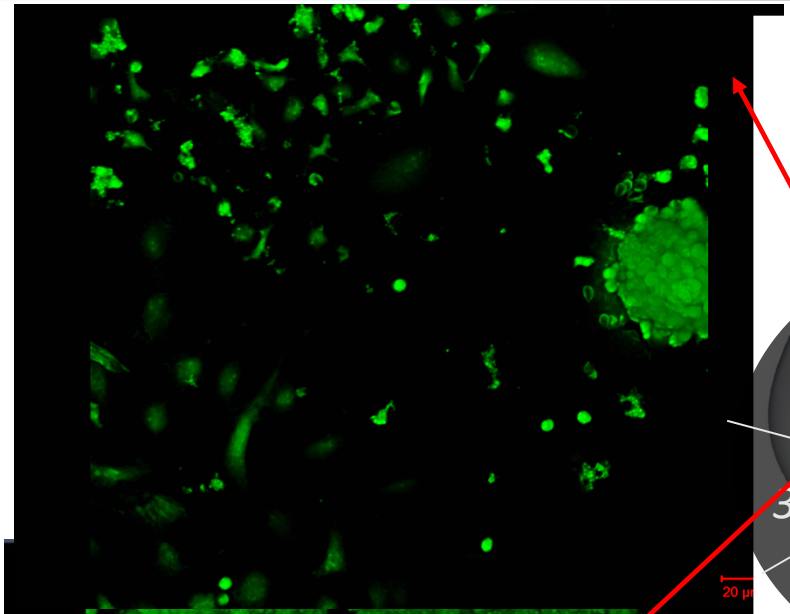
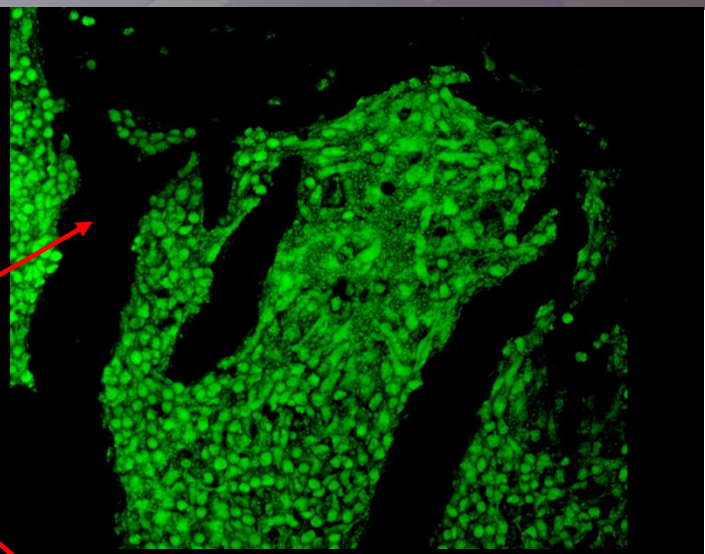
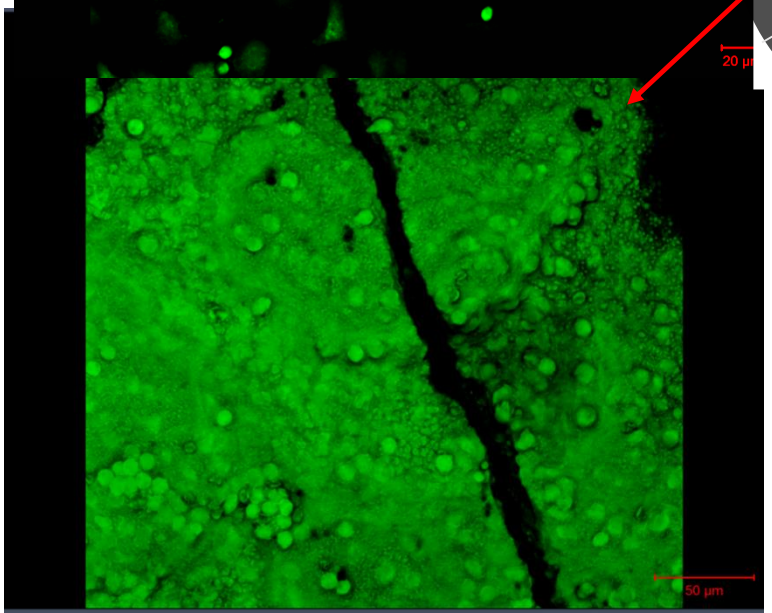
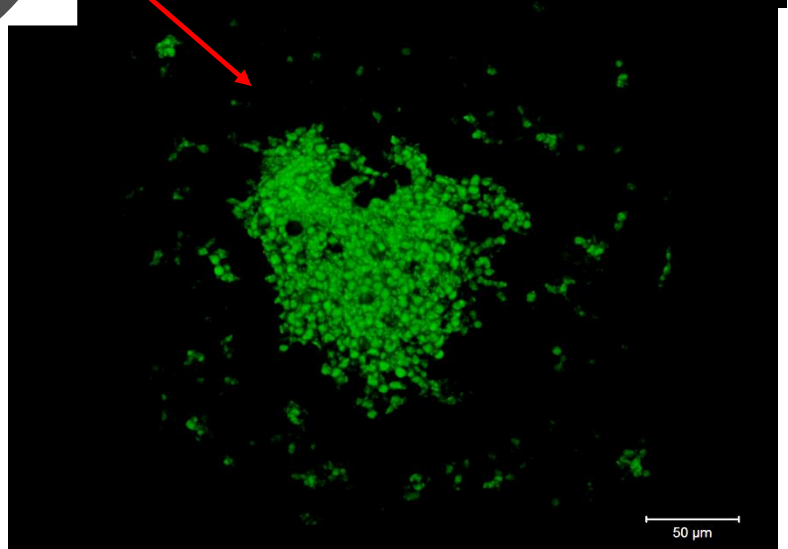


Deplanted chambers

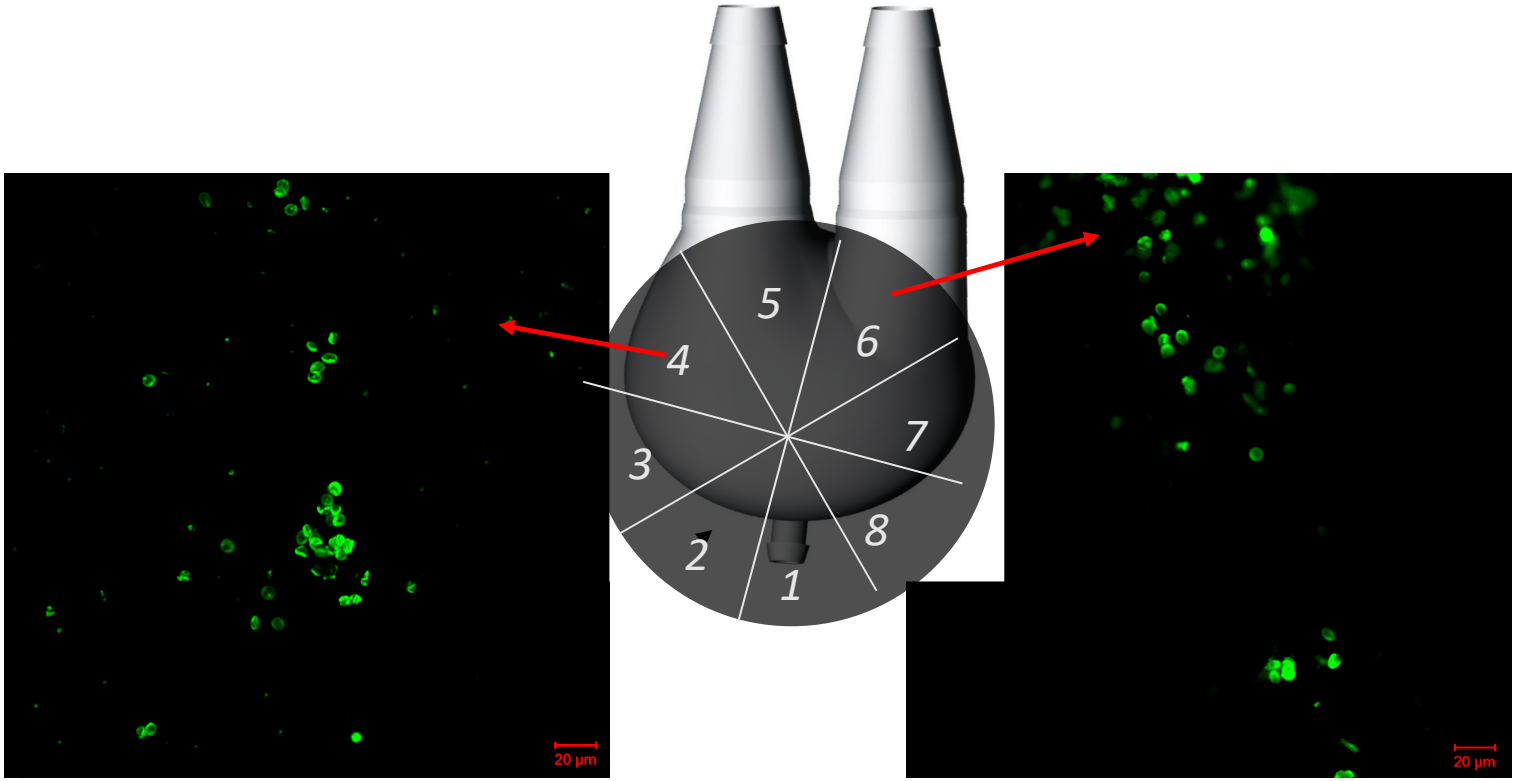




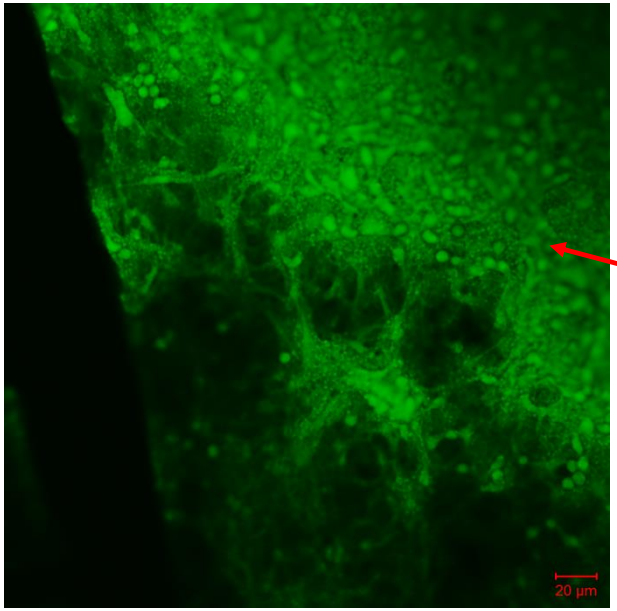


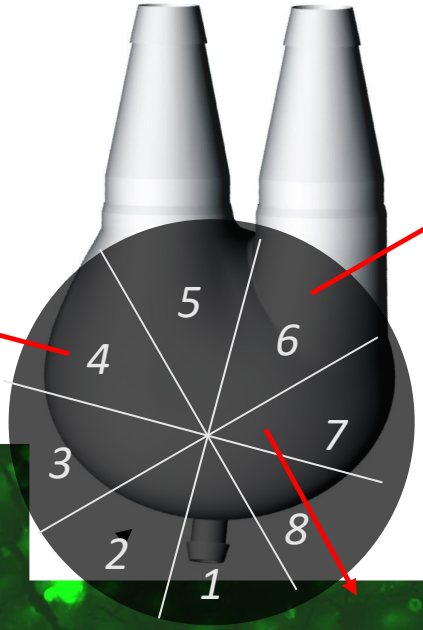


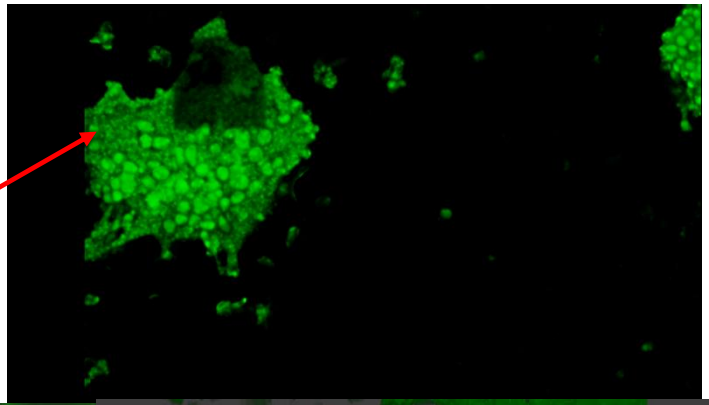
Deplanted chambers

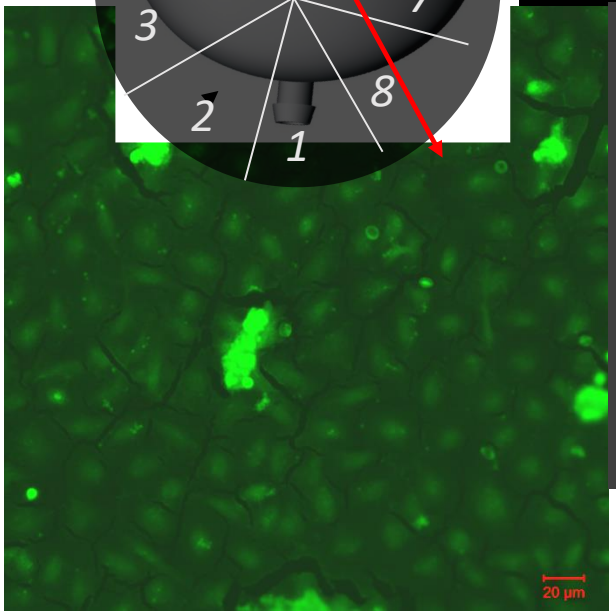


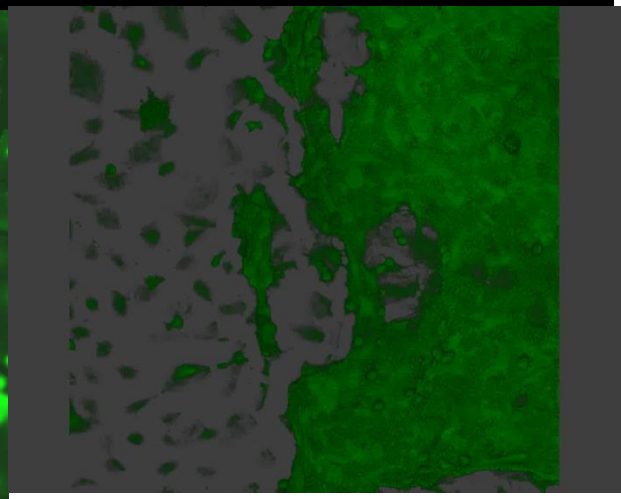
Deplanted chambers



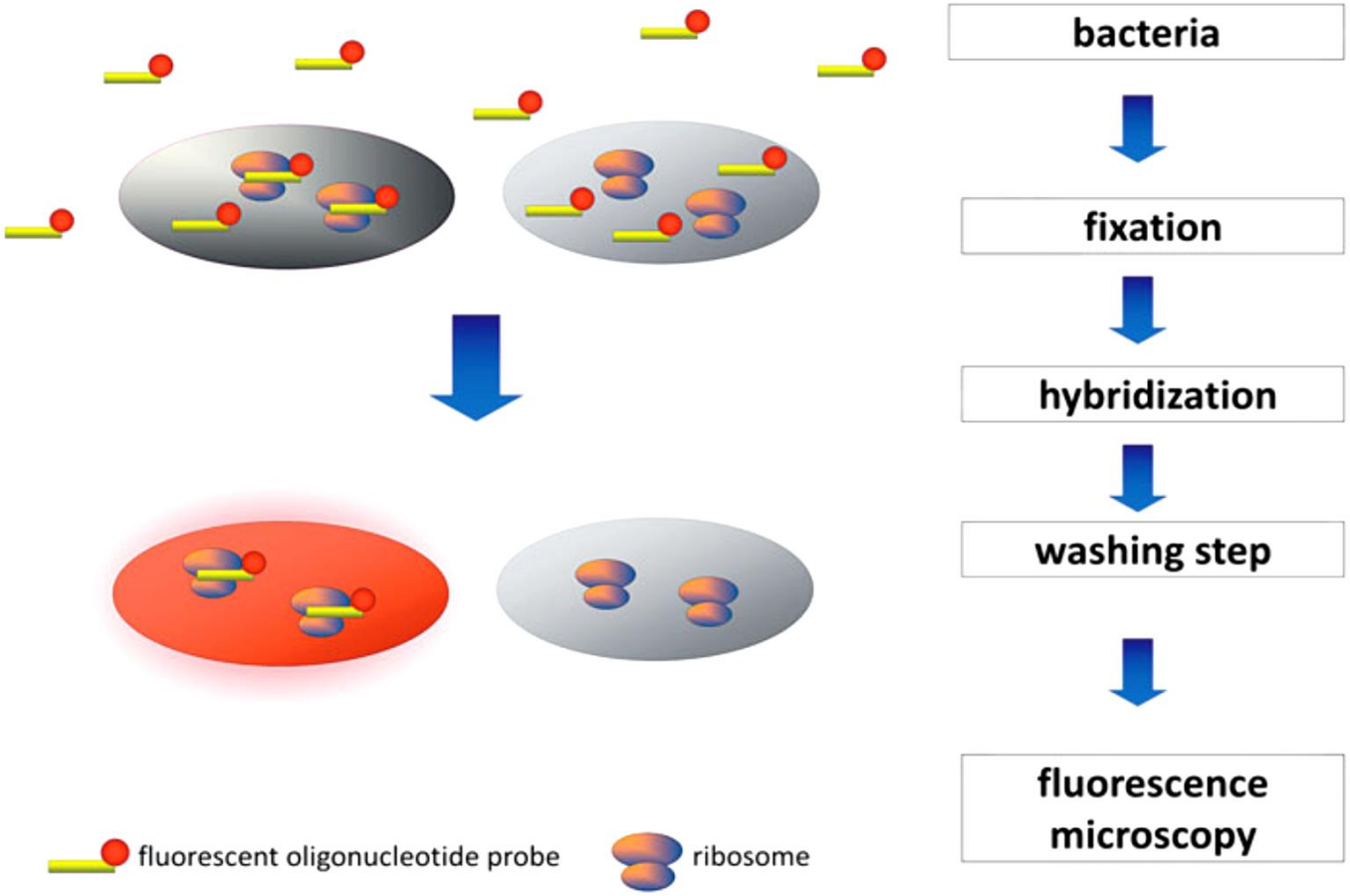








Deplanted chambers



Deplanted chambers

PACJENT NR 1

Patient: male aged 46, assisted due to left ventricular failure and developing in failure. Supported by two chambers from 4.07.2018. He underwent a neurological incident during the treatment, a small-blood hemorrhagic stroke. Currently, the patient is rehabilitating, being physically active, and the symptoms of stroke persisting.

No Antibiotics have been used throughout the heart support process. The antibiotic is only perioperatively. Patient treated with anticoagulants.

Sampling:

- sample No. 1- fragment of the valve with fibrin deposit

sample no. 2- fibrin deposits on the edge of the blood-red membrane near the chamber vent with a fragment of a blood-red canopy.

Deplanted chambers

PACTIENT NO 1

FISH analysis

Material 1 A few microorganisms were found on the basis of carried out staining of nucleic acids with the dye DAPI in the fibrin. FISH was not found to contain active bacteria, microorganisms or biofilms.

Material 2 Relative detection (uncertainty) of microorganisms with the application of nucleic acid staining with DAPI dye. FISH was not found to contain active bacteria, microcollies or biofilms.

16S rRNA GENE AMPLIFICATION TEST

(PCR study)

The study was carried out using the bacterium Sphingobium yanoikuyae.

Material 1	No bacterial DNA was found with 16S rRNA amplification
Material 2	No bacterial DNA was found with 16S rRNA amplification

Deplanted chambers

PATIENT No 1

No identification of active bacterial infection in the test samples. Due to the degradation of bacterial DNA and / or a small number of bacteria in the sample material, microorganisms detected microscopically were not effectively differentiated by PCR, amplification / sequencing.

Deplanted chambers

PATIENT No 2

Patient: a woman of 37 years. Diagnosed cardiomyopathy of an unknown cause. Two-chamber failure. There is no information about implantation. The chamber was replaced after 23 days after implantation.

Sampling:

test samples were taken from the right ventricle.

- sample 1 fragment of a blood-red membrane with fibrin deposit
- sample 2 fragment of a blood-red membrane with a large deposit of fibrin

Deplanted chambers

PATIENT No 2

BADANIE FISH

Materiał 1 Stwierdzono nieliczne mikroorganizmy na podstawie przeprowadzonych barwień kwasów nukleinowych barwnikiem DAPI, we włókniku. Nie stwierdzono metodą FISH aktywnych bakterii, mikroorganizmów ani biofilmów.

Materiał 2 Względna detekcja (brak pewności) mikroorganizmów przy zastosowanym barwieniu kwasów nukleinowych barwnikiem DAPI. Nie stwierdzono metodą FISH aktywnych bakterii, mikrokolonii ani biofilmów.

BADANIE AMPLIFIKACJI GENU 16S rRNA

(badanie PCR)

Badanie przeprowadzono przy użyciu szczepu bakterii Sphingobium yanoikuyae. Jest to charakterystyczny gatunek bakterii rozwijający się w środowisku cieczy, wskazuje na skażoną kolonizację na powierzchni próbek. W niektórych przypadkach, może być związany z procesem infekcji u niektórych pacjentów o obniżonej odporności immunologicznej.

Materiał 1 99,3% 447 (par zasad)

Materiał 2 99,3% 456 (par zasad)

Deplanted chambers

PACJENT NR 2

Brak identyfikacji aktywnego zakażenia bakteryjnego w badanych próbkach. Leczona lub wcześniejsza infekcja nie może być wykluczona.

Deplanted chambers

PACJENT NR 3

Pacjent: mężczyzna lat 28. Leczony od 14.07.2018 do 2.09.2018r. Zdiagnozowana kardiomiopatia o nieznanej etiologii. Przed wszczepieniem komór wspomagania POLVAD, stwierdzono frakcję wyrzutową na poziomie 10%, liczne skrzepliny w obu komorach serca. Dnia 2.09.2018 wykonano przeszczep serca z dobrym efektem.

Pobór próbek:

próbki do badań pobrano z prawej komory.

- próbka 1- fragment membrany krwistej ze złogiem włóknika
- próbka 2- fragment membrany krwistej z dużym złogiem włóknika

Deplanted chambers

PACJENT NR 3

BADANIE FISH

Materiał 1 Nie stwierdzono występowania mikroorganizmów przy zastosowaniu metody FISH ani barwień przy użyciu barwnika DAPI.

Materiał 2 Względna detekcja (brak pewności) mikroorganizmów przy zastosowanym barwieniu kwasów nukleinowych barwnikiem DAPI. Nie stwierdzono metodą FISH aktywnych bakterii, mikrokolonii ani biofilmów.

BADANIE AMPLIFIKACJI GENU 16S rRNA

(badanie PCR)

Badanie przeprowadzono przy użyciu szczepu bakterii Sphingobium yanoikuyae.

Materiał 1 Początkowo hamowanie PCR przez materiał pacjenta. Po rozcieńczeniu (1: 2) nie wykryto bakteryjnego DNA za pomocą amplifikacji PCR 16S rRNA o obniżonej czułości.

Materiał 2 Amplifikacja genu 16S rRNA, a następnie sekwencjonowanie zaszło w krótkim fragmencie, dalsze różnicowanie było niemożliwe.

Deplanted chambers

PACJENT NR 3

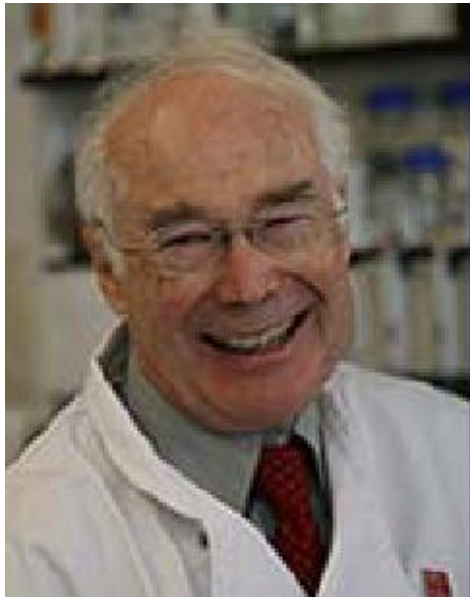
Brak identyfikacji aktywnego zakażenia bakteryjnego w badanych próbkach. Leczona lub wcześniejsza infekcja nie może być wykluczona. Z powodu degradacji bakteryjnego DNA i/lub małej liczby bakterii w materiale próbki 2, mikroorganizmy wykryte mikroskopowo nie zostały skutecznie zróżnicowane za pomocą PCR, amplifikacji / sekwencjonowania.

NICHE-LIKE STRUCTURES

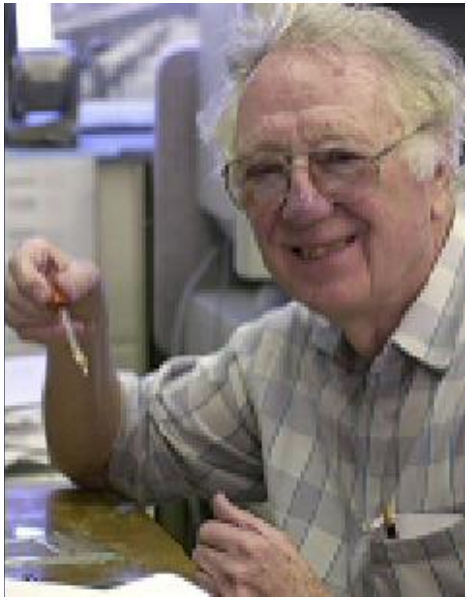
Surface modification

Mario Capecci
University Utah

Martin J. Evans
University Cardiff



Olivier Smithies
University Chapel Hill



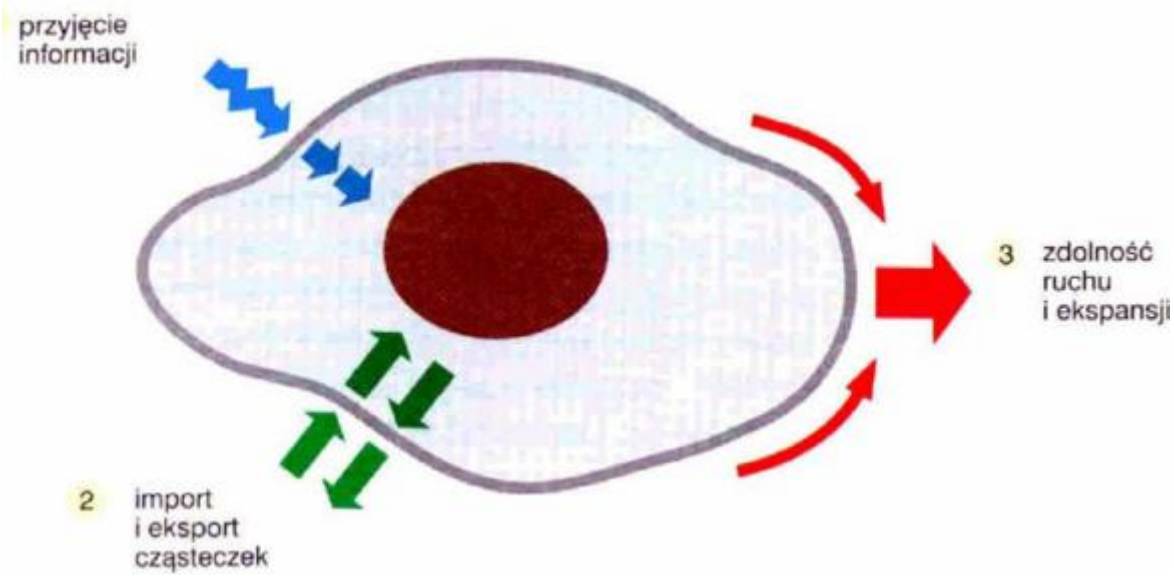
2007- Nobel Prize in Physiology and Medicine for discovery of the rules for use of embryonic stem cells for genetic modification of mice

Cellula

The smallest functional unit of life

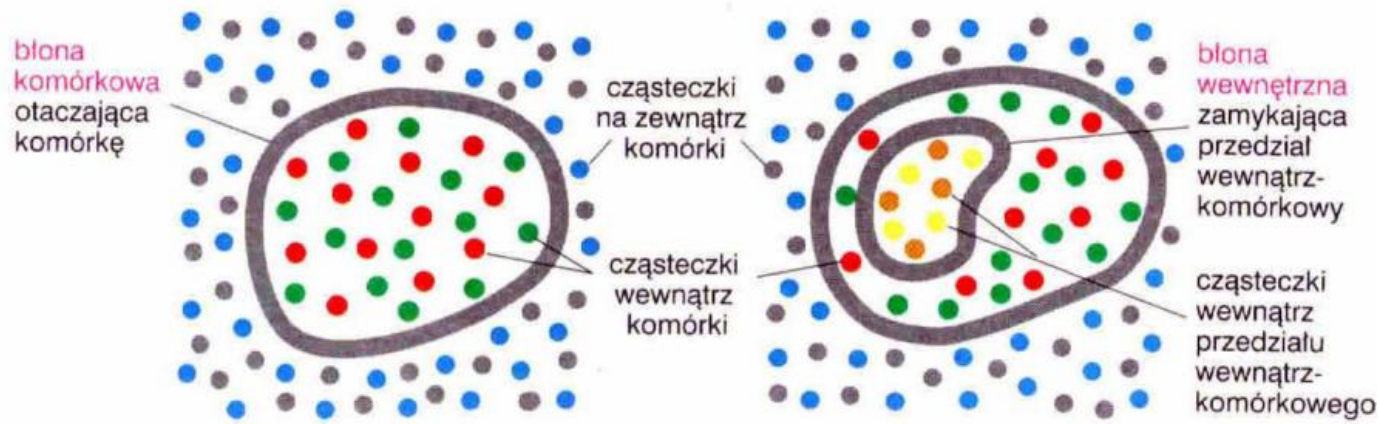
- Surrounded by a membrane
- Capable of creating its own copies (growth, division)
- Filled with thickened water gel of various molecules

Cell membranes as barriers



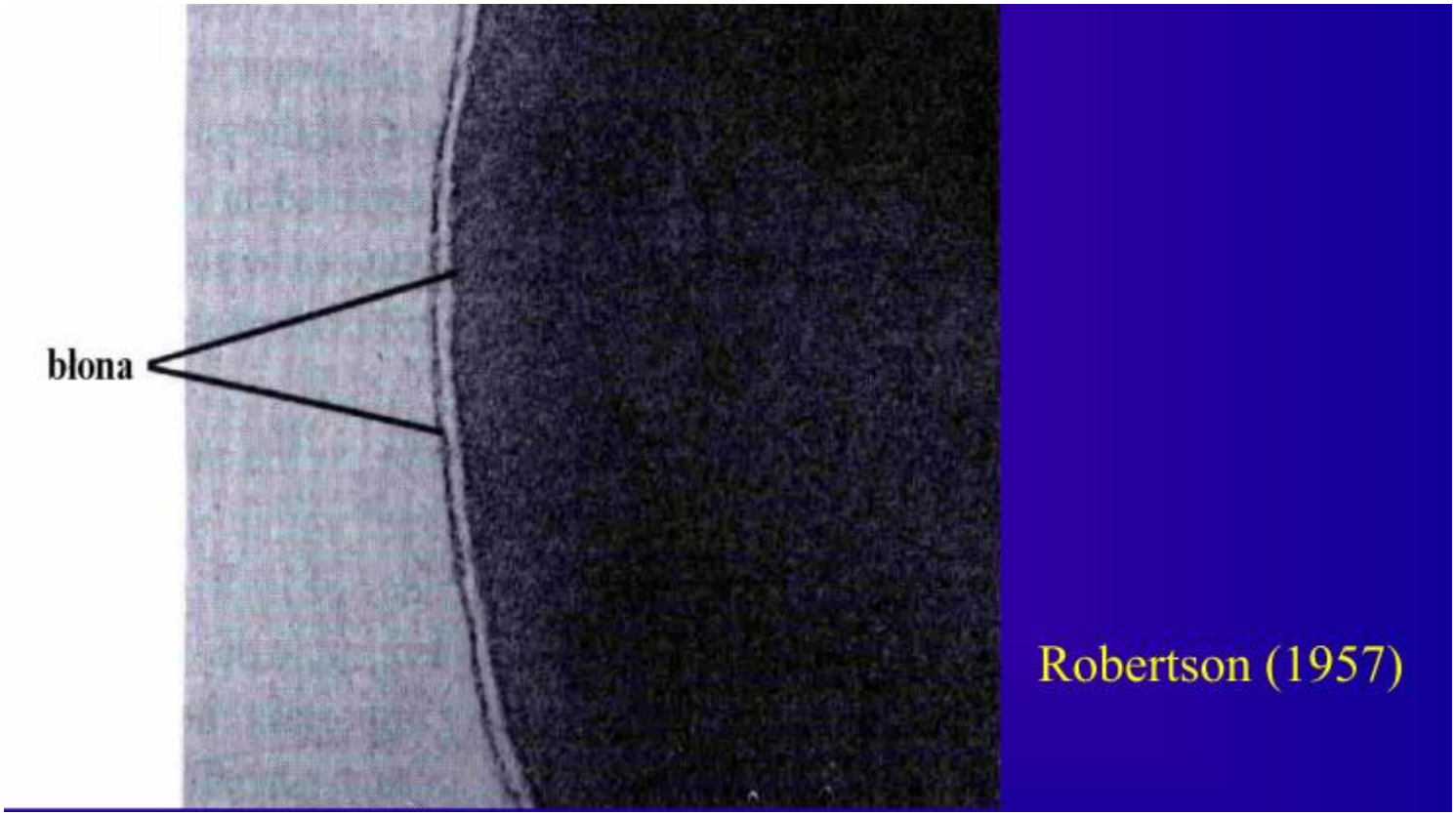
- Transport inside of the interior (selective permeability)
- Receiving stimuli
- Ability of movement and expansion
- The possibility of transforming energy processes (ATP synthesis)

Cell membranes as barriers

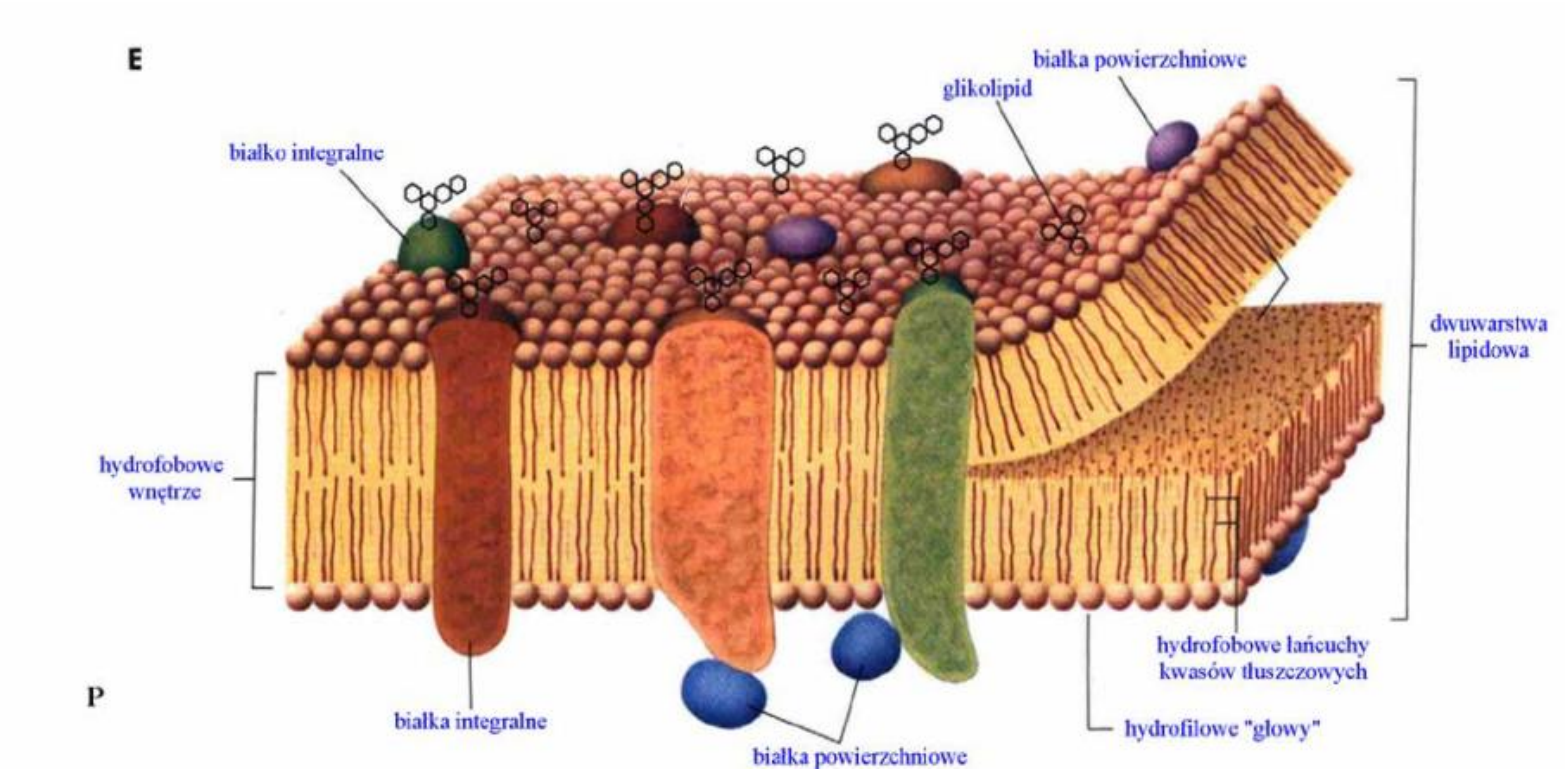


Barriers between compartments (components)
Prevent mixing of substances between compartments
Ensuring the maintenance of differences in the composition and function between organelles
Composition control
The membrane itself is a cellular compartment with unique functions
Dynamic structure (movement, synthesis and degradation of components)

The structure of the cell membrane



The structure of the cell membrane

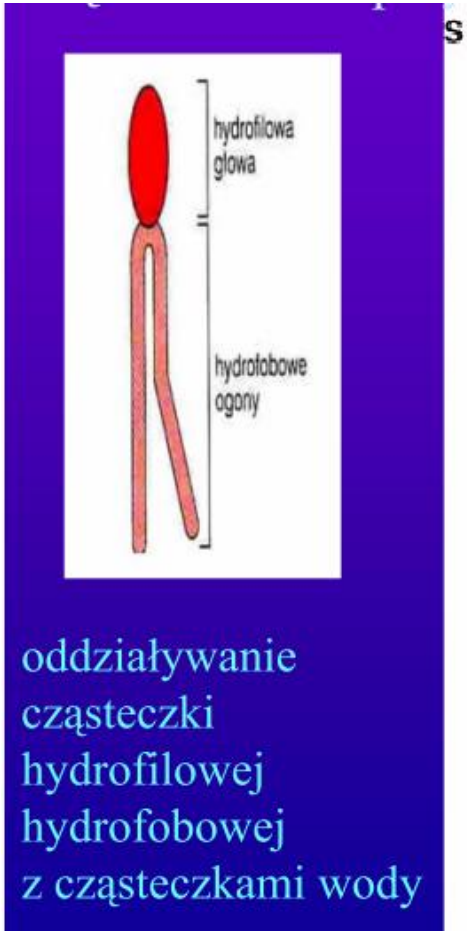


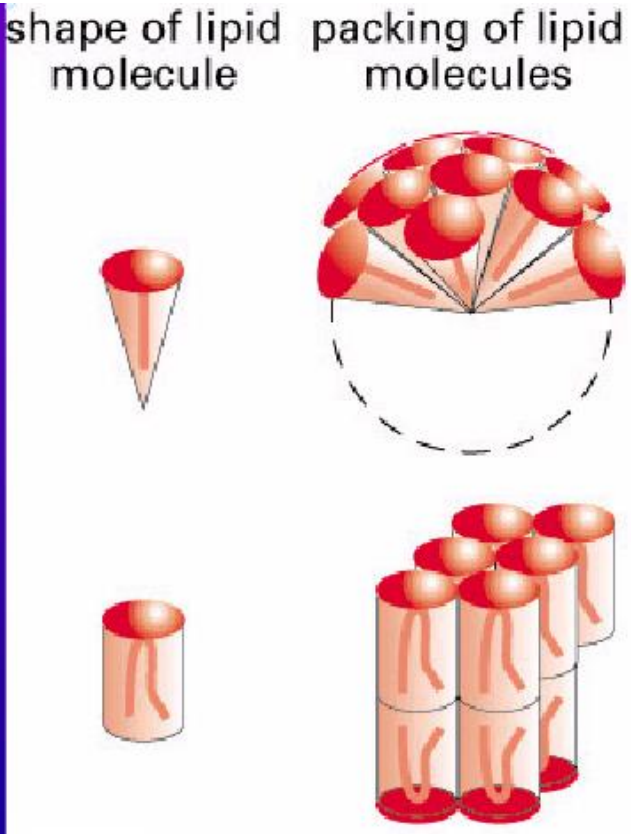
- lipid bilayer
- membrane protein

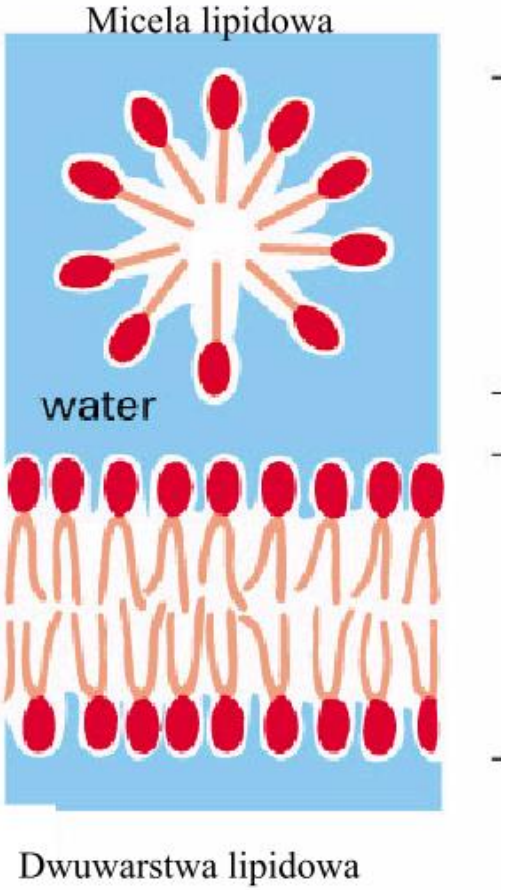
Membrane lipids

Membrane lipids

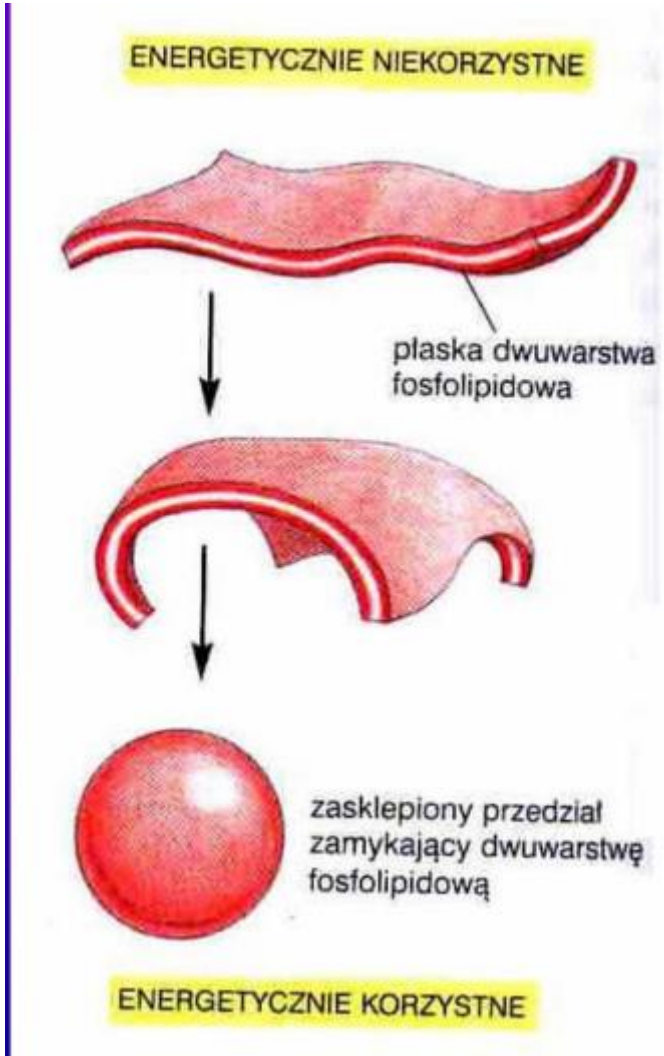
membrane molecules - amphipathic molecules





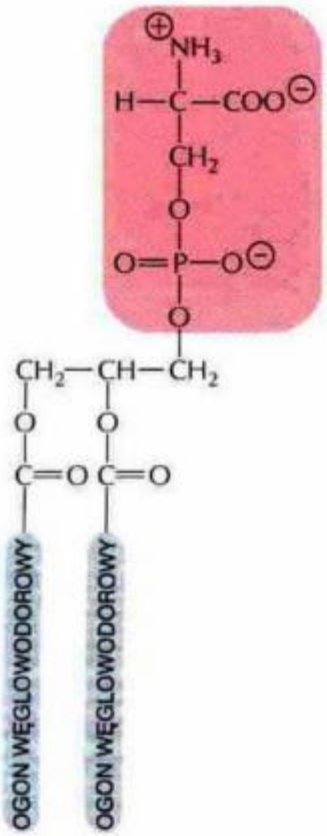


Membrane lipids

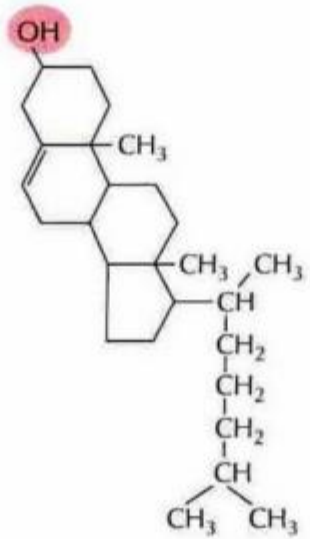


Membrane lipids

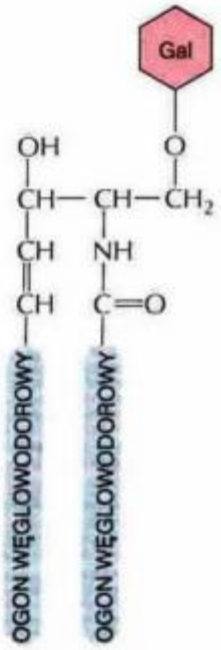
Membrane lipids
- phospholipids
- glycolipids
- steroids:
--sulfolipids
--lizolipids
--other



fosfatydyloseryna
(fosfolipid)



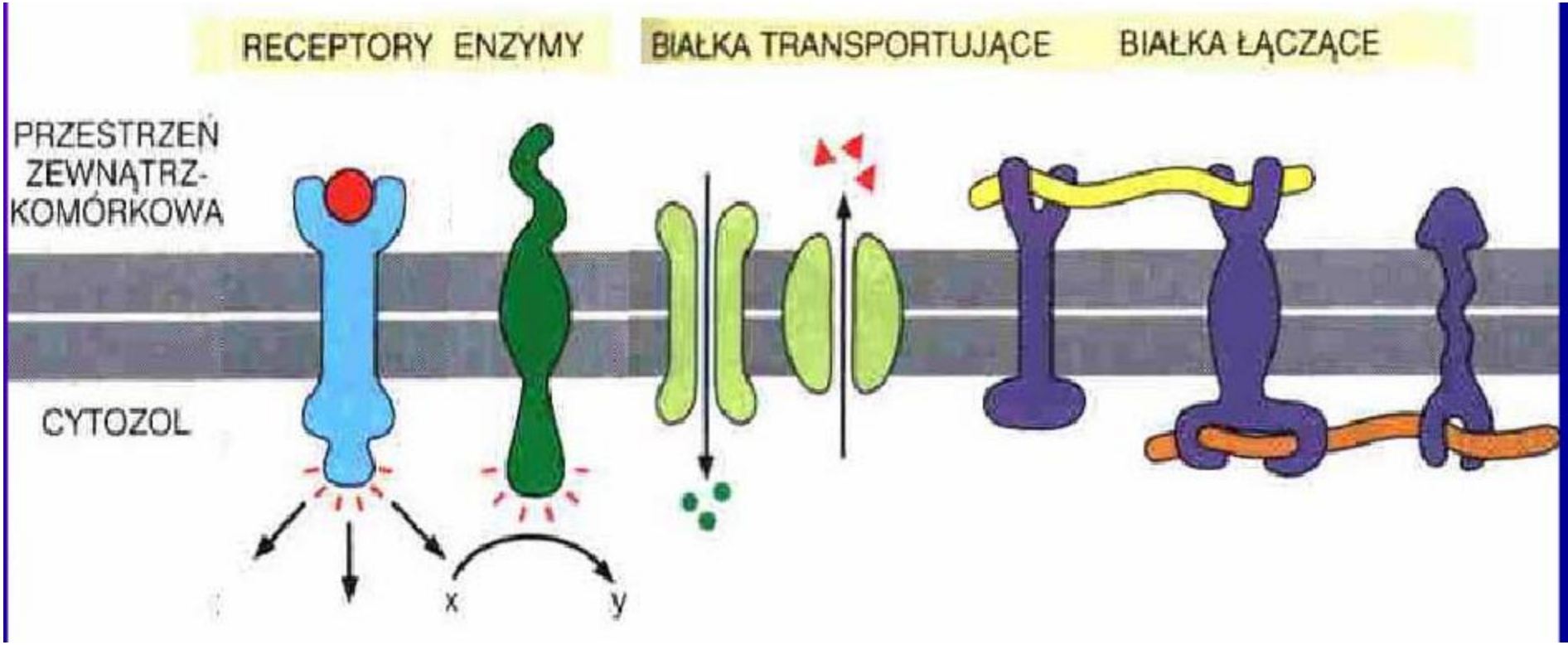
cholesterol
(steroid)



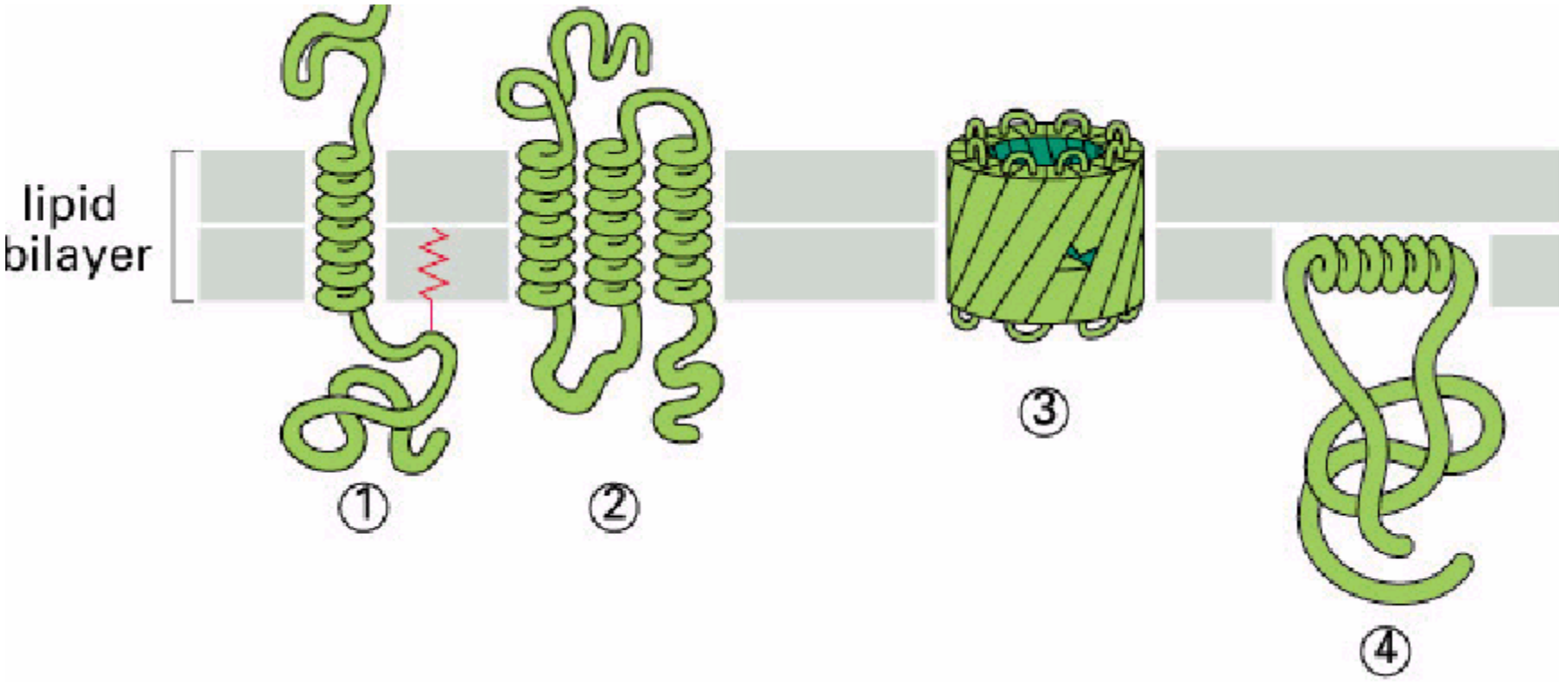
galaktocerebrozyd
(glikolipid)

Membrane proteins

Functions of proteins



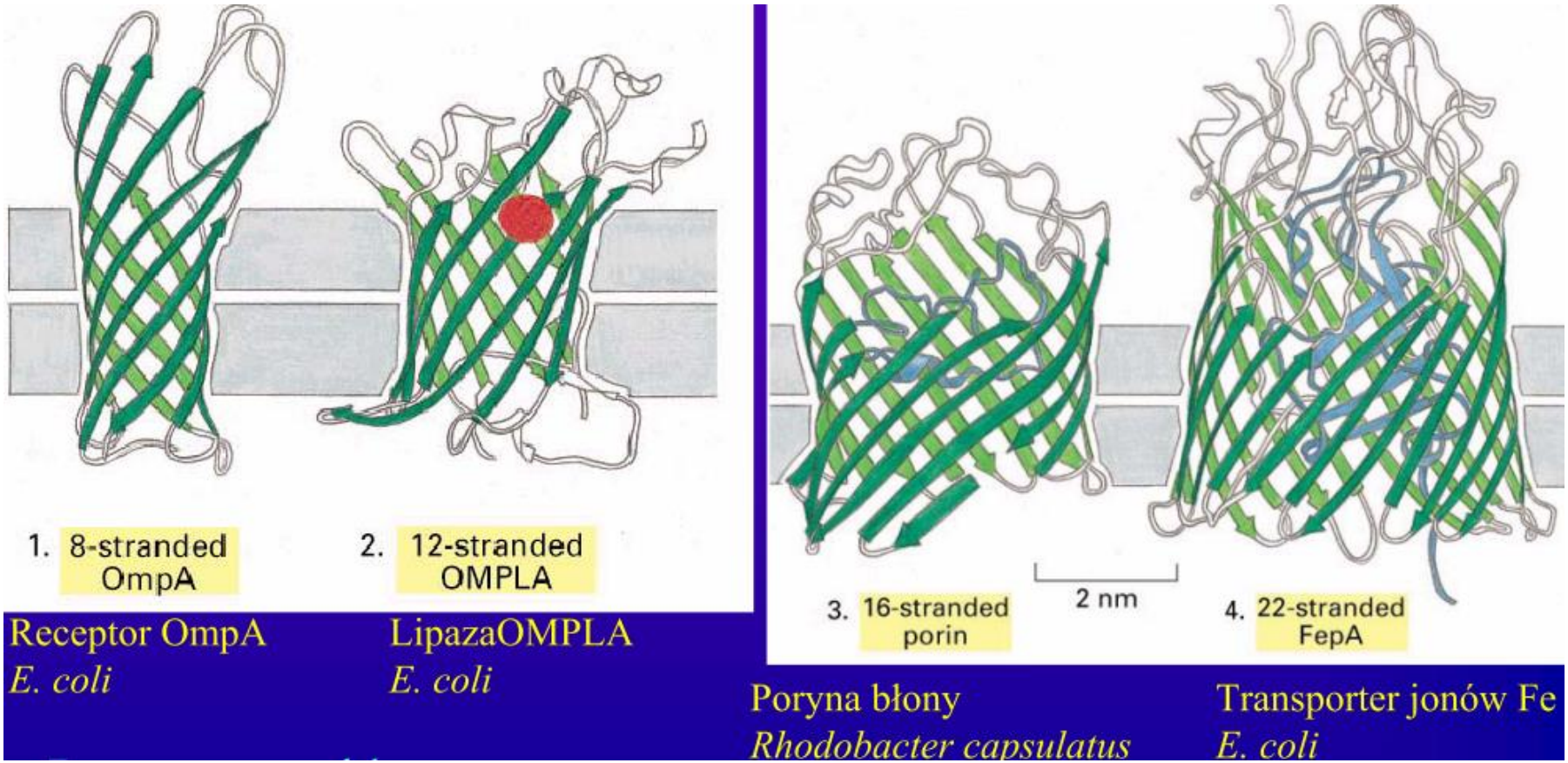
Functions of proteins



- Trans-membrane placement - transmembrane proteins
- Anchoring through covalent lipids

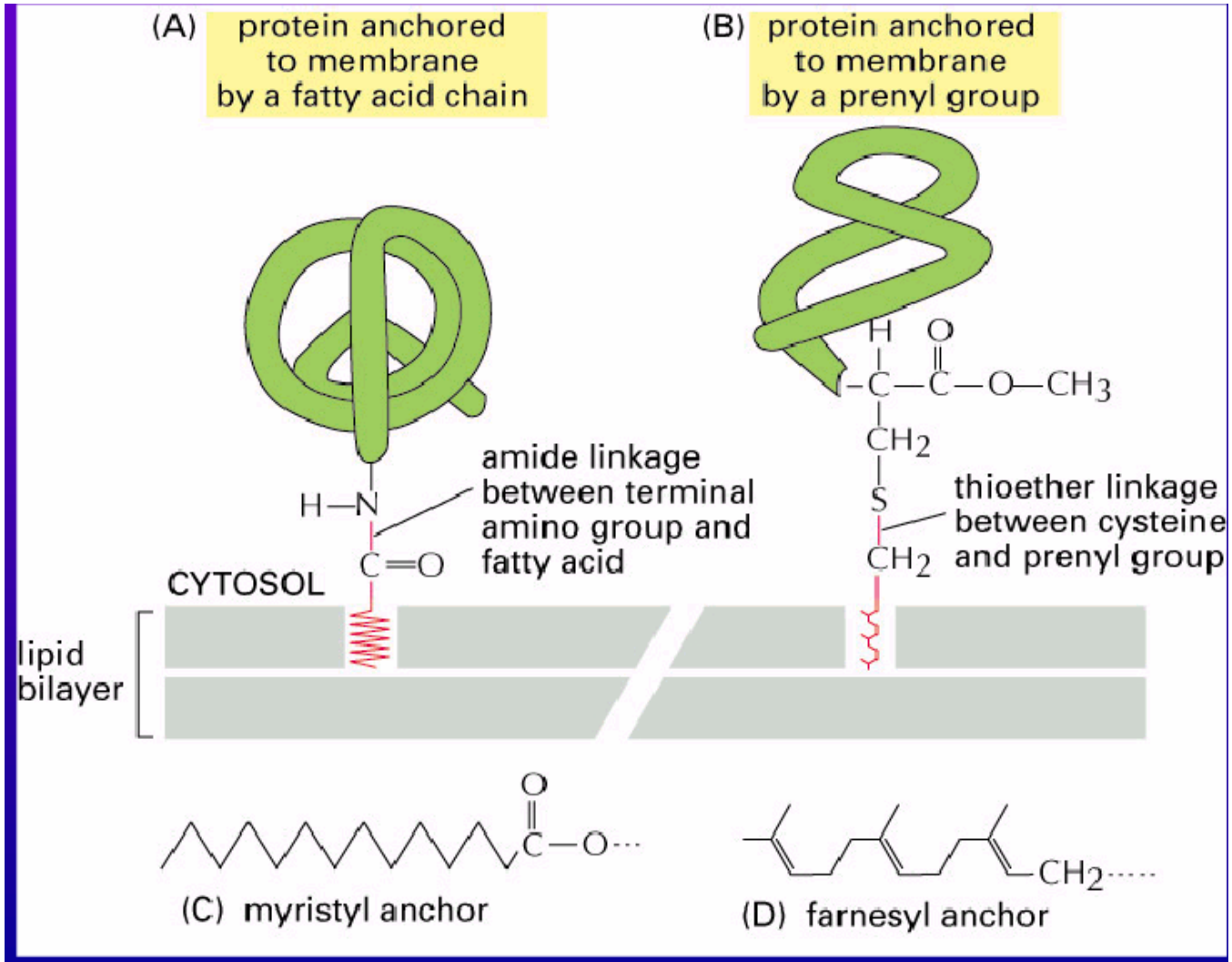
} Integral proteins

Integral-transmembrane proteins



Integral proteins - anchored through lipids

Białka integralne, zakotwiczone przez lipidy



Membrane proteins - surface proteins

- Membrane bound by interacting with other proteins
- Different on both sides of the membrane
- Release (by extraction) without destroying the structure of the lipid bilayer

Membrane properties

Fluidity (mov)

Asymmetry

Selective permeability

Membrane properties

Fluidity (lipid bilayer)

- film cohesion (fusion)
- membrane flexibility
- displacement of proteins (cell signaling)

Płynność błon/ Szczypce laserowe film 1, 2

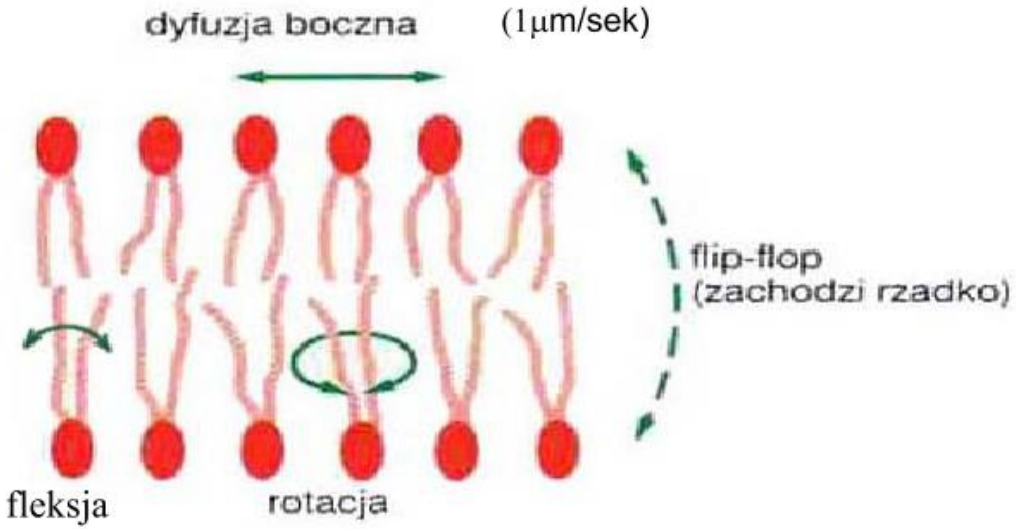
Membrane properties

Rotational movements:

- Intramolecular
- rotating around its own axis

Translational movements

- lateral movement = lateral diffusion
- transversal movement = flip flop movements



Part 2.2

Executed projects

Cardiovascular system

„Bio-inspired thin film materials with the controlled contribution of the residual stress in terms of the restoration of stem cells microenvironment”

2014/13/B/ST8/04287

Bio-inspired materials

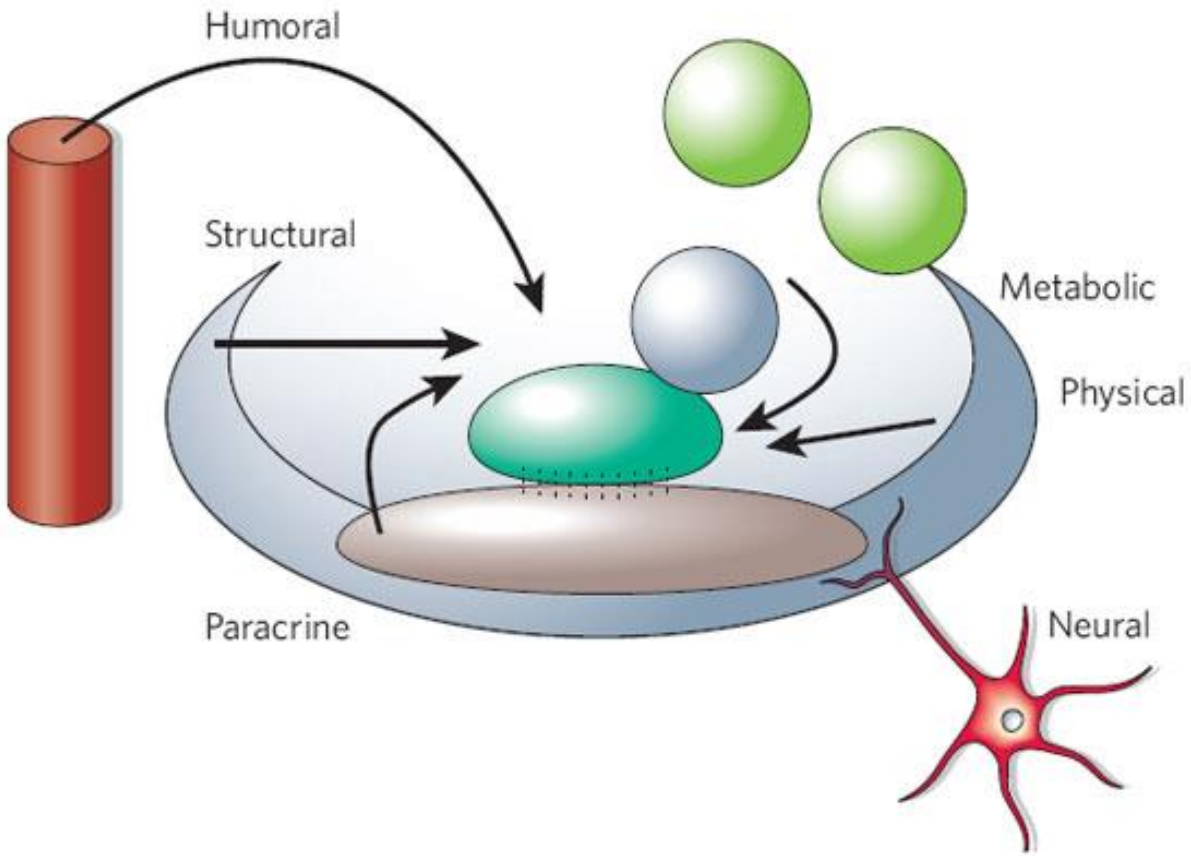
Artificial materials modification

Bio-inspired materials

Coating material / process		Thickness
a-C:H	Magnetron sputtering	110nm
		60nm
a-C:H low gas flow sputtering		100nm
a-C:N		105nm
a-C:H (DLC) (Ti)	Plasma-enhanced chemical vapour deposition	85nm
		43nm
		65nm
		86nm

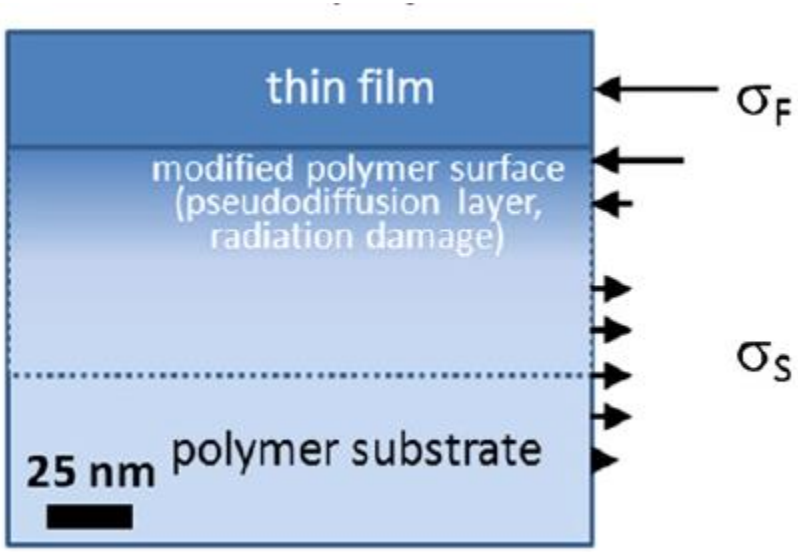
Bio-inspired materials

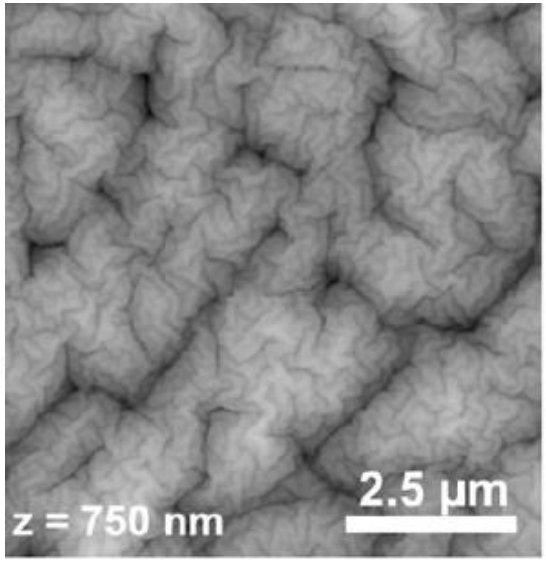
"The concept of a niche as a specialized microenvironment housing stem cells" **first proposed by Schofield.**

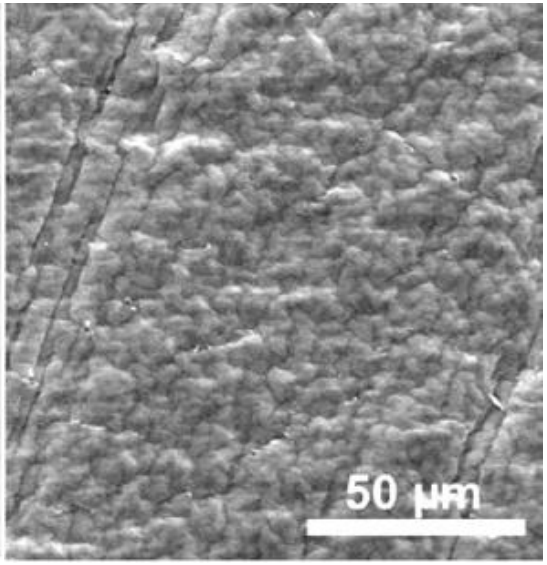


David T. Scadden, The stem-cell niche as an entity of action, NATURE|Vol 441|29 June 2006|doi:10.1038/nature04957

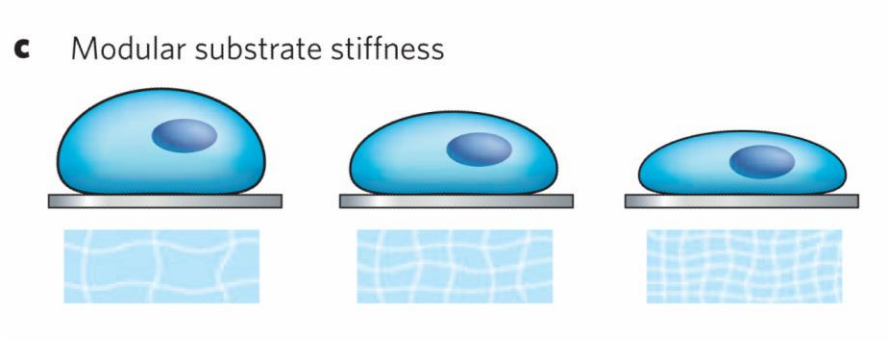
Bio-inspired materials



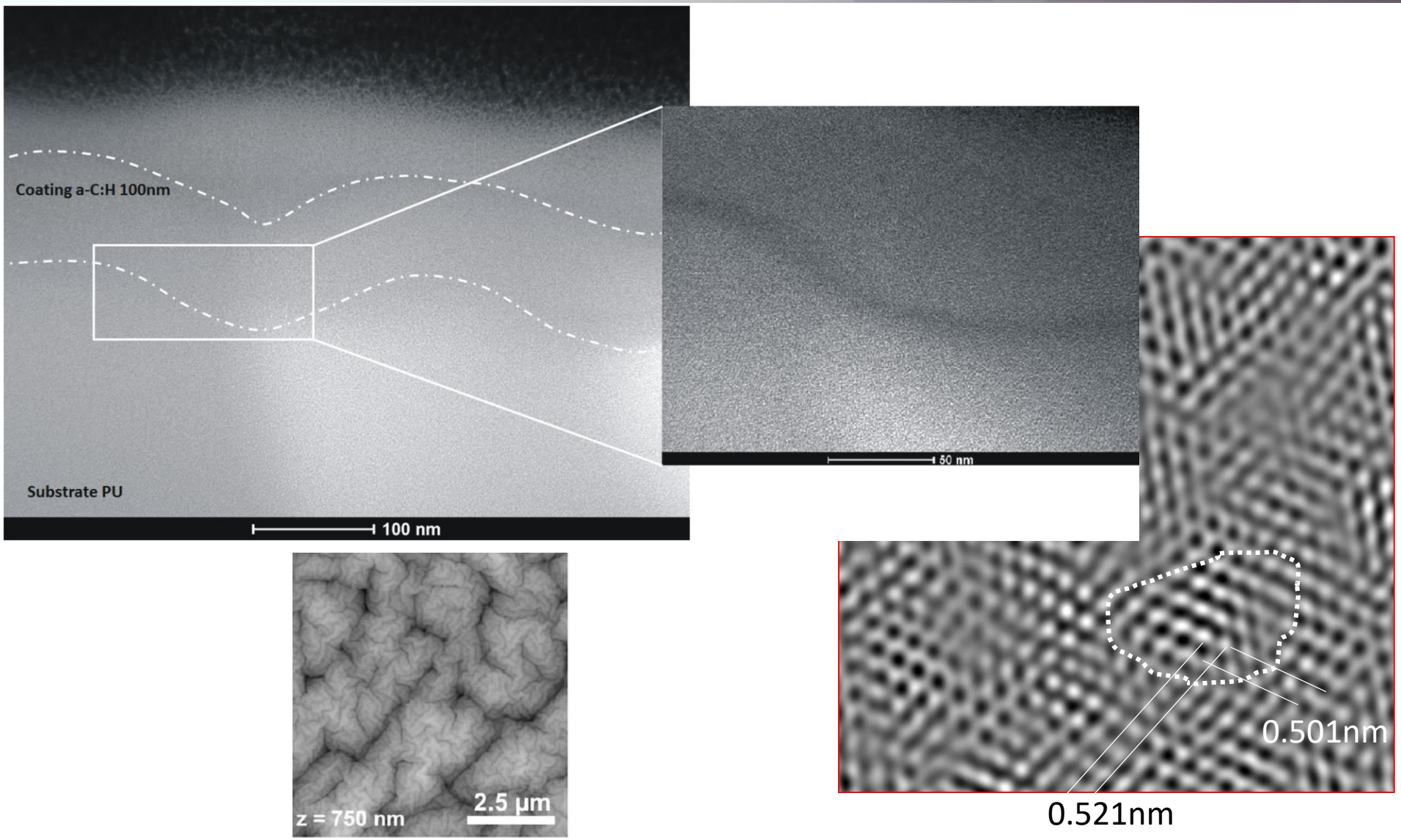




$$\lambda_c = 2\pi h \left[\frac{\left(1-\nu_f^2\right) E_s}{3\left(1-\nu_s^2\right) E_f} \right]^{1/3}$$



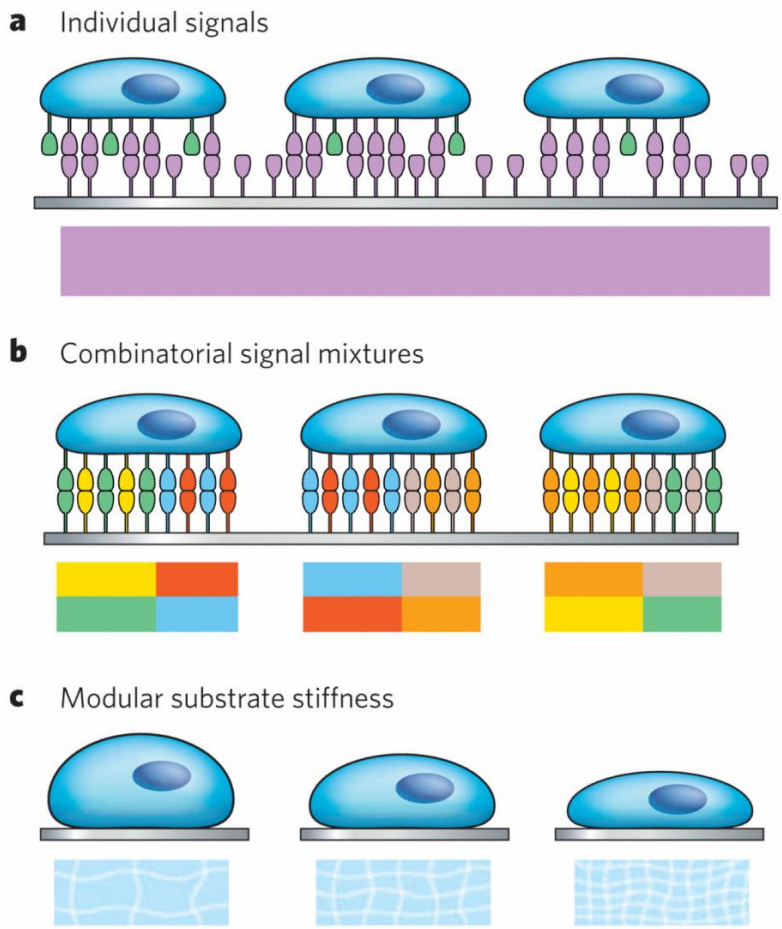
Bio-inspired materials



Bio-inspired materials

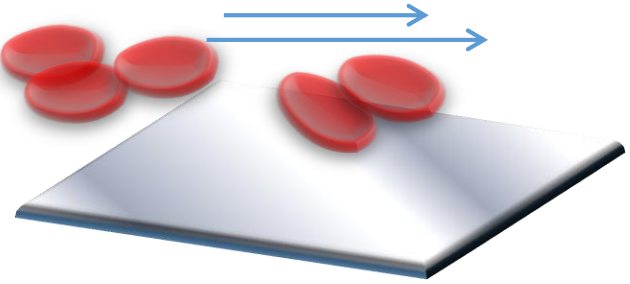
M-ERA.NET
Nonthrombogenic metal-polymer composites with adaptable micro and macro flexibility for next generation heart valves in artificial heart devices

„Artificial Heart Laboratory”



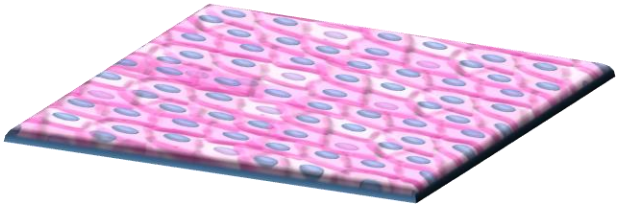
Bio-inspired materials

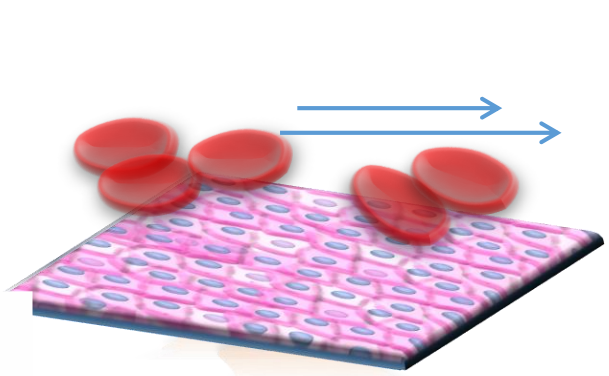
Hemocompatibile

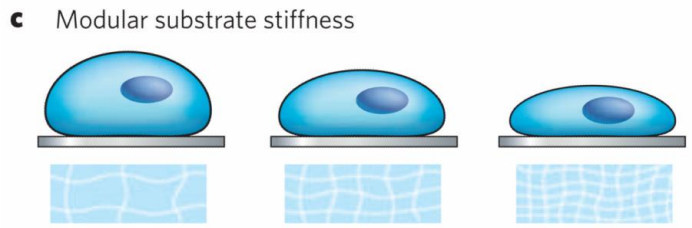


- Surface topography
- Mechanical properties of the material
- The structure of the niche (2D or 3D)
- Residual stresses
- The chemical composition of the substrate

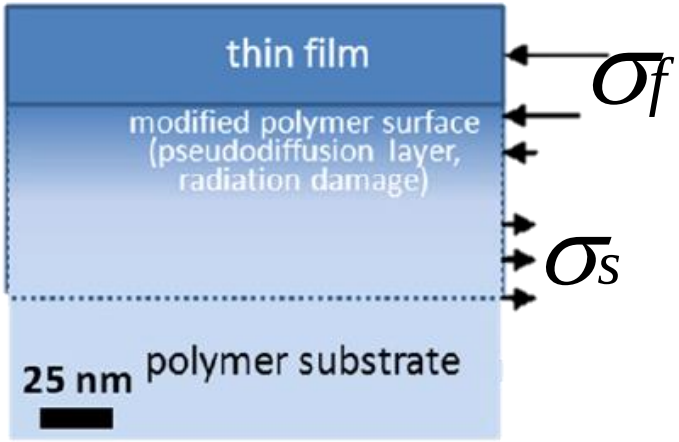
Pro-endothelial

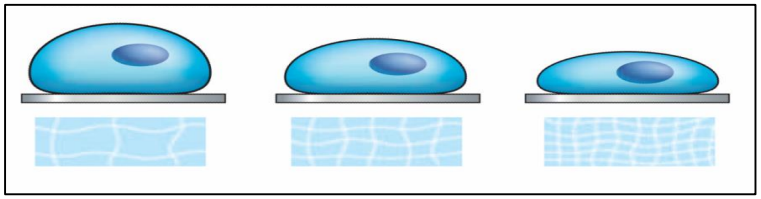






Bio-inspired materials

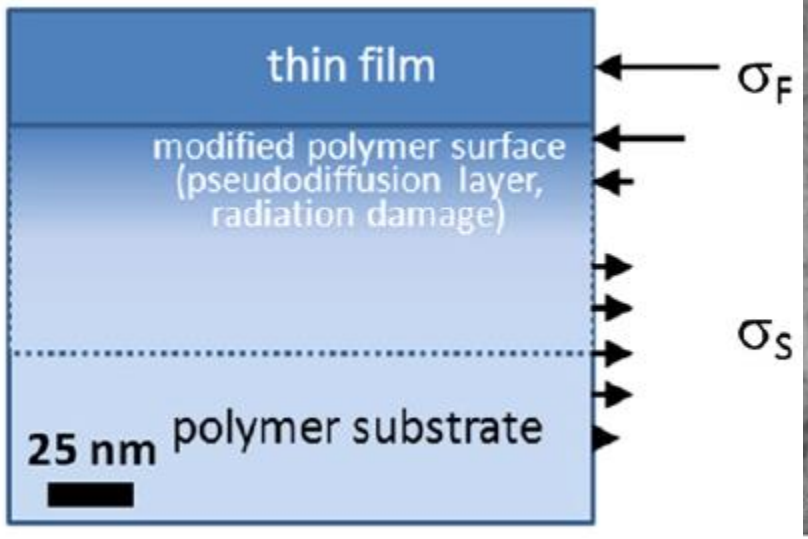


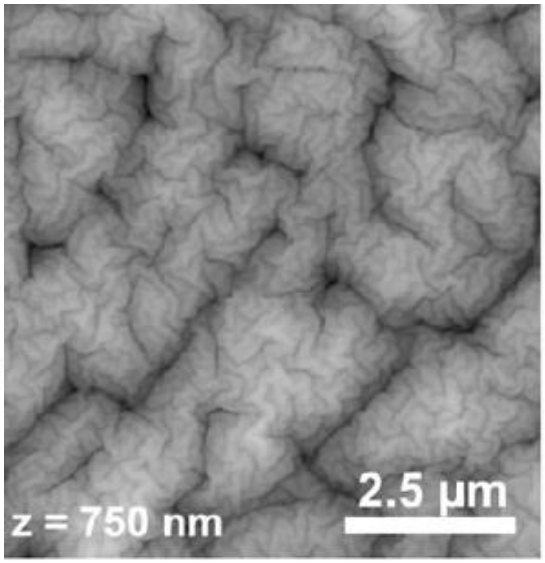


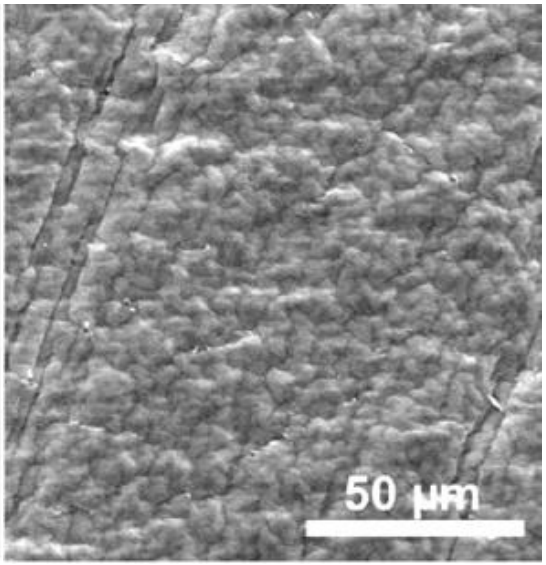
Layer	Thickness
a-C:H (sputtered)	110nm
a-C:H (sputtered)	60nm
a-C:H (lower gas flow; sputtered)	100nm
a-C:N (sputtered)	105nm
DLC (ALS)	85nm
DLC (ALS)	43nm
DLC (ALS)	65nm
DLC lower gas flow (ALS)	86nm

ALS- Anod Laser Sorce

Bio-inspired materials

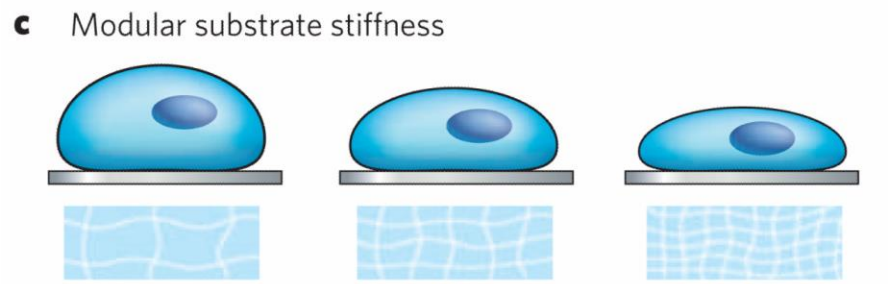






Surface reconstruction:

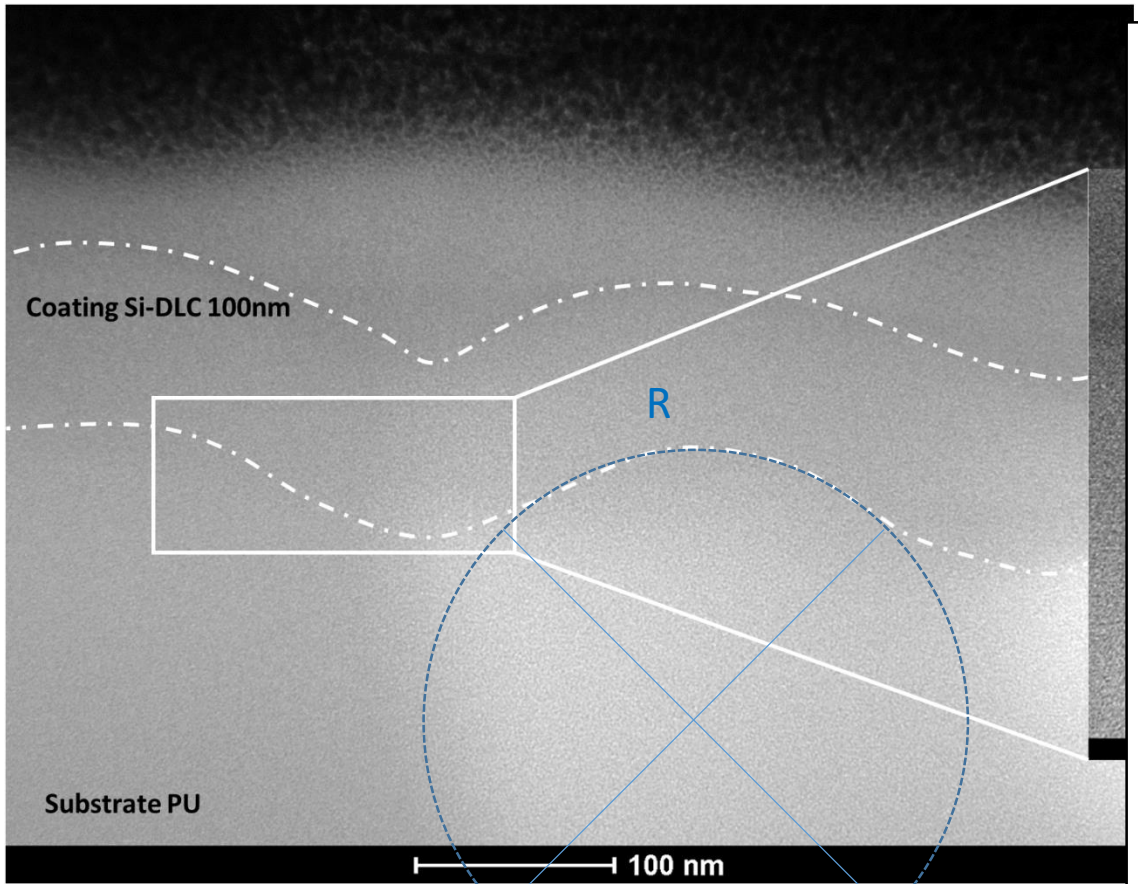
$$\lambda_c = 2\pi h \left[\frac{\left(1-\nu_f^2\right) E_s}{3\left(1-\nu_s^2\right) E_f} \right]^{1/3}$$



Bio-inspired materials

Transmission electron microscopy analysis:

TEM BF



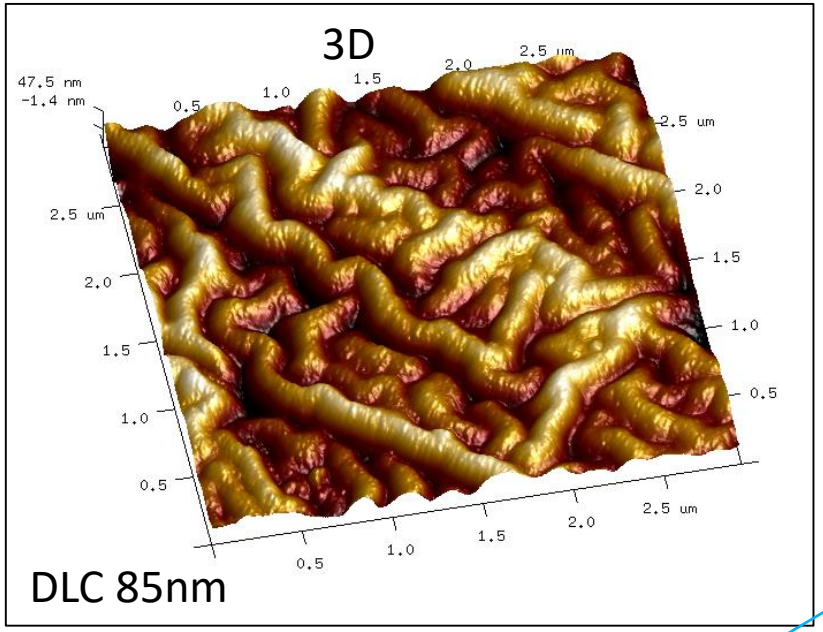
Cross section

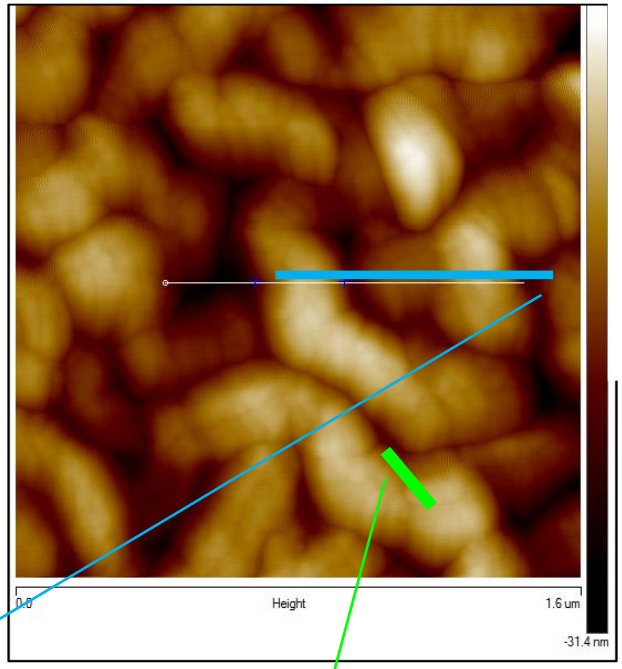
E_s-Young modulus
ϑ_s -Poisson ratio
t_s- substrate thickness
t_f - layer thickness
R- curvature of the coating

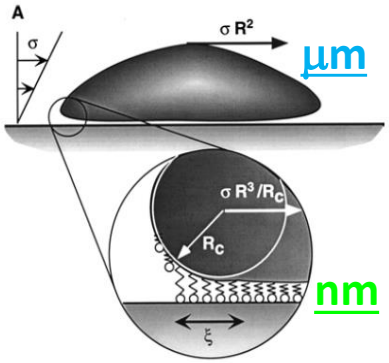
$$\sigma = \frac{1}{6}\left(\frac{E_s}{1-\vartheta_s}\right)\left(\frac{{t_s}^2}{t_f R}\right)$$

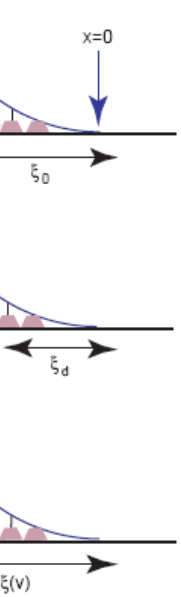
σ=2.79GPa

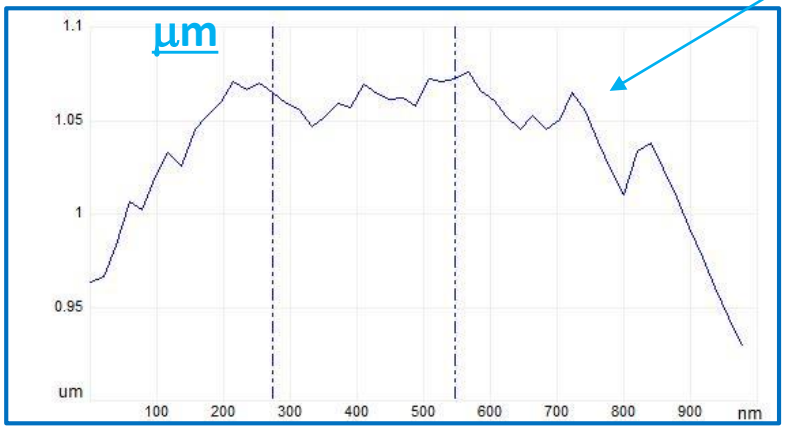
Bio-inspired materials











„Bio-inspired thin film materials with the controlled contribution of the residual stress in terms of the restoration of stem cells microenvironment”

2014/13/B/ST8/04287

CELL-MATERIAL INTERACTION

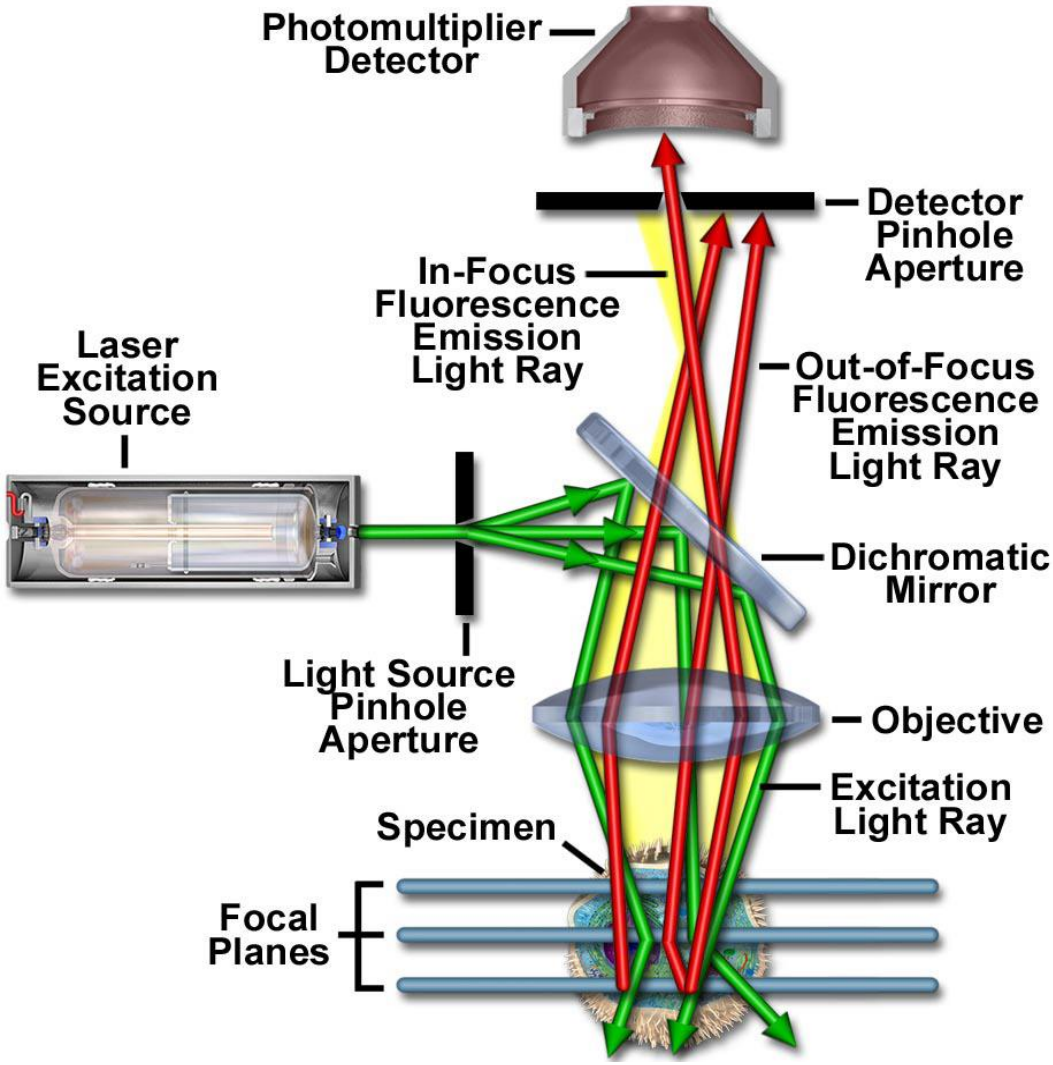
Confocal microscopy

- Confokal Modul LSM 5 Exciter, two canals, RGB
- Laser HeNe 633nm 5mW
- Laser HeNe 543nm 1mW
- Laser argon 458/488/514nm, 25mW
- Laser diodowy V 405nm
- Main Beam Splitter turret PASCAL
- Software ZEN 2008 LSM 5 EXCITER
- Light division system (405, 458, 488, 514, 543 nm)
- Filtr BP 505-530
- Filtr BP 505-600
- Filtr BP 530-600
- Filtr BP 560-615
- Fitr LP 420
- Filtr BP 420-480
- System ECU LSM 5 EXCITER
- Moduł DIC I/0,9 z polaryzatorem
- Transmited light detectorT-PMT LSM 710

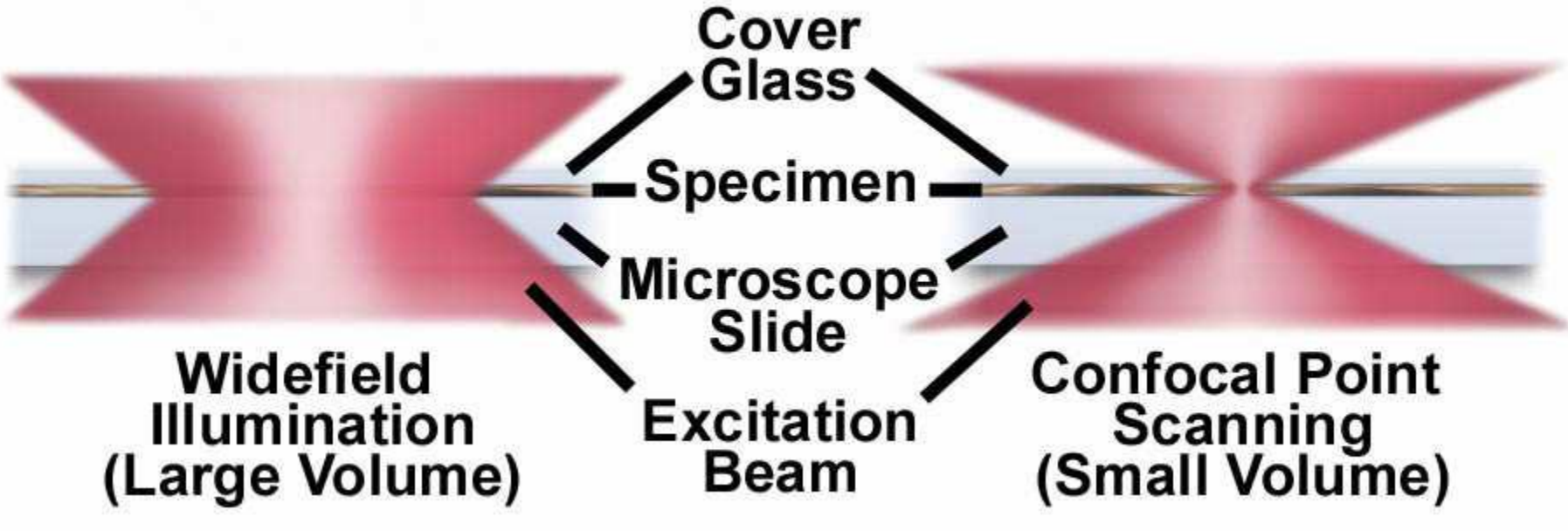
AB 120

Confocal microscopy

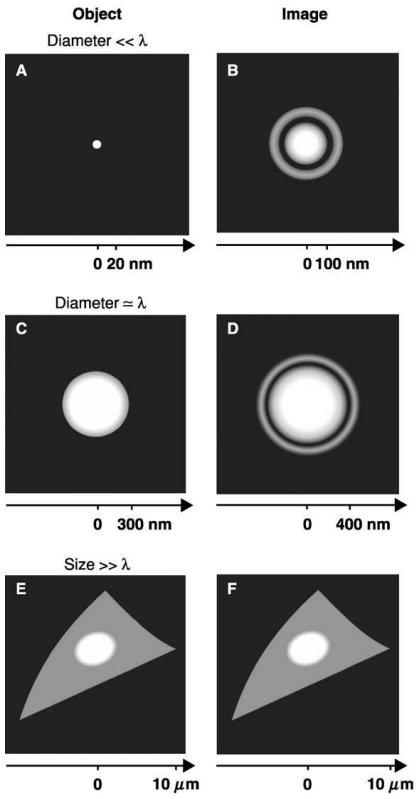
Layout

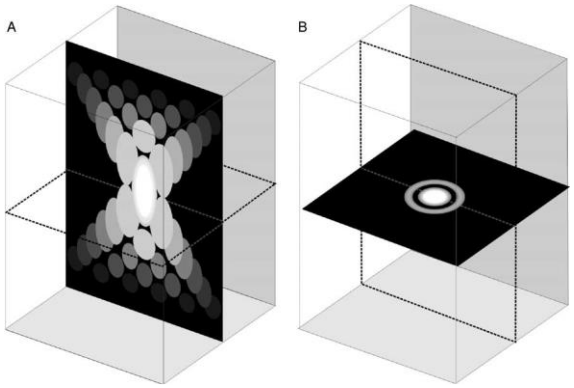


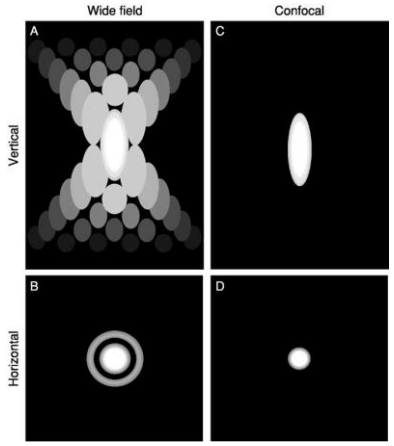
Confocal microscopy

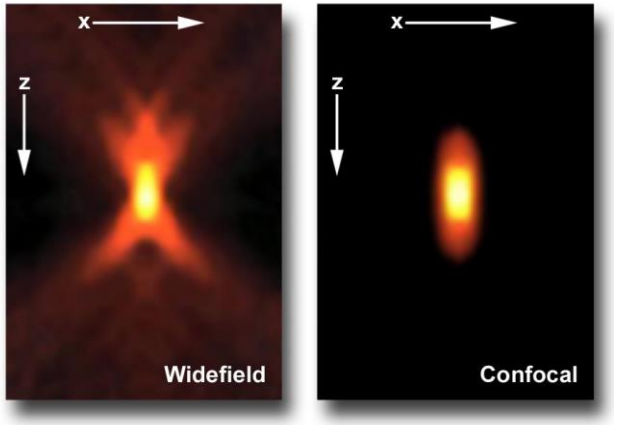


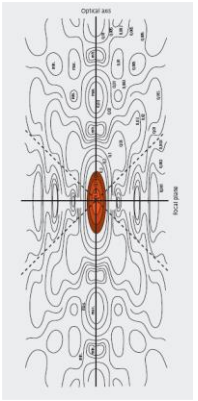
Confocal microscopy





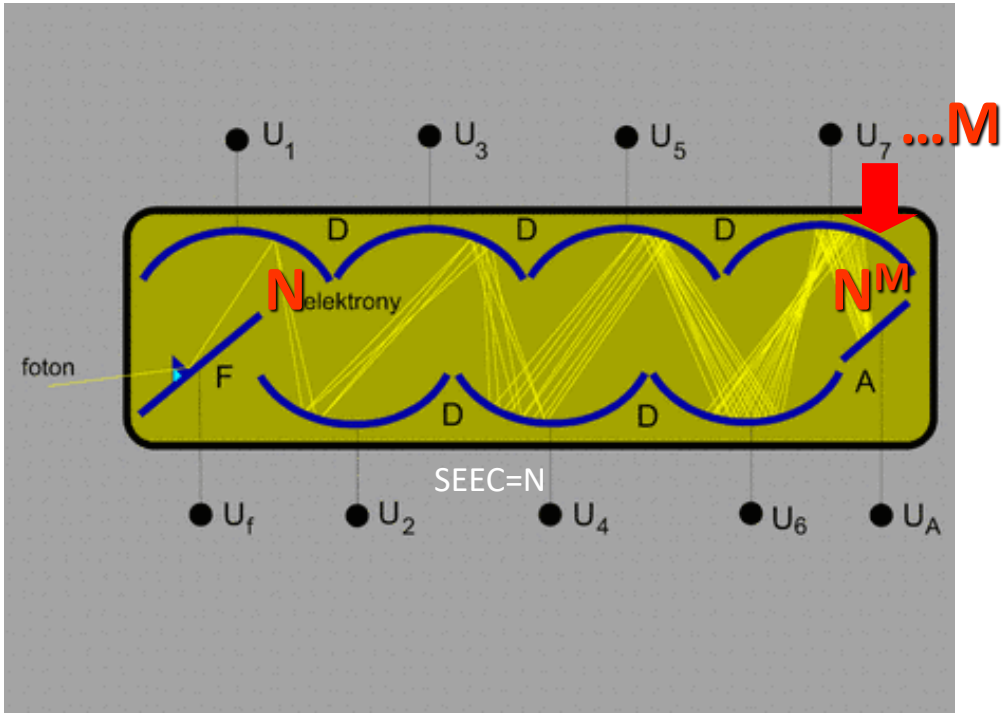






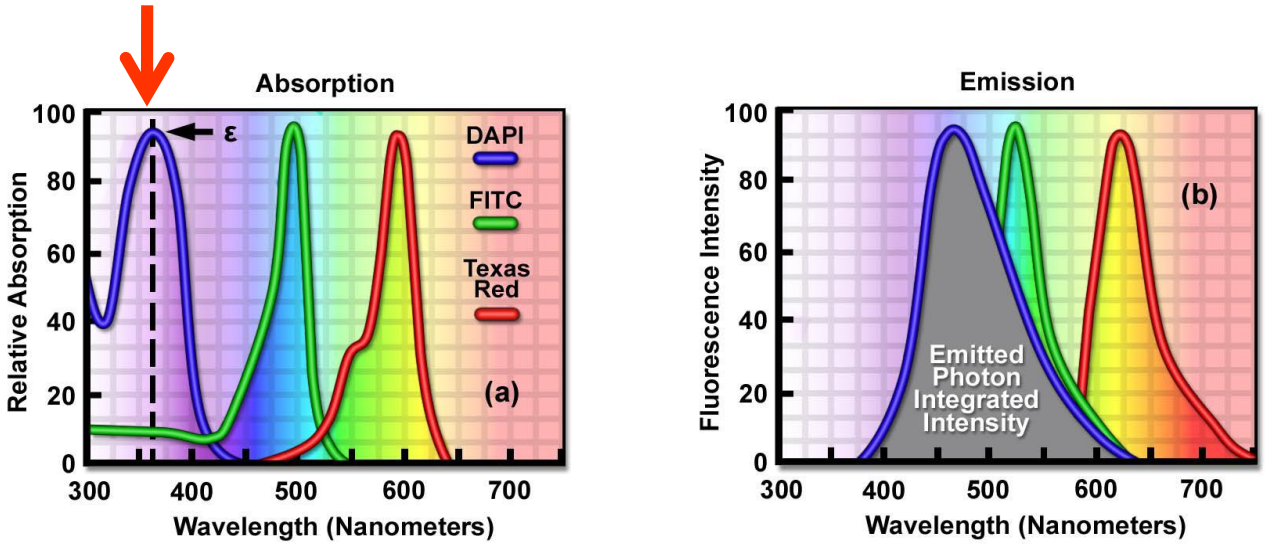
Confocal microscopy

Photomultiplier



Confocal microscopy

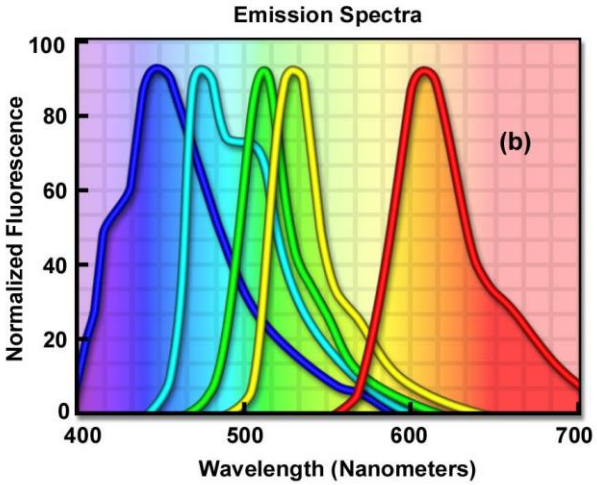
Fluorochromes

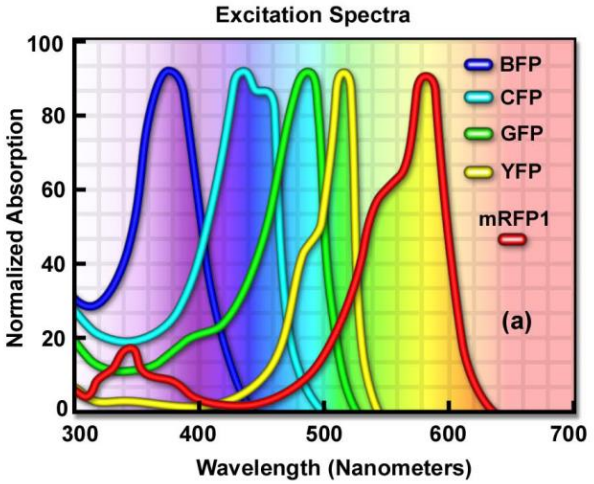


Absorptive and fluorescent properties
Wavelength of maximum absorption and emission
Intensity of fluorescence of emitted light

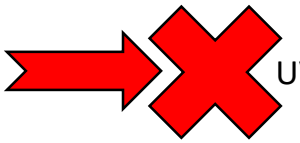
Confocal microscopy

Excitation and imiging





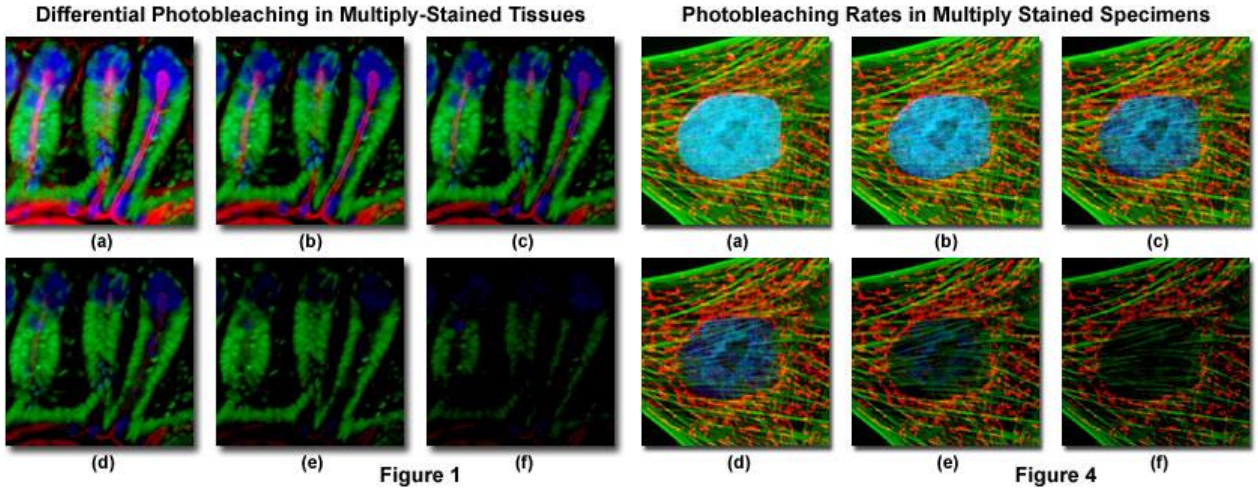
- 488 nm blue-green argon laser
- 543 nm helium-neon green laser
- 633 nm helium-neon red laser

405nm  UV

Confocal microscopy

1. Cytotoxicity

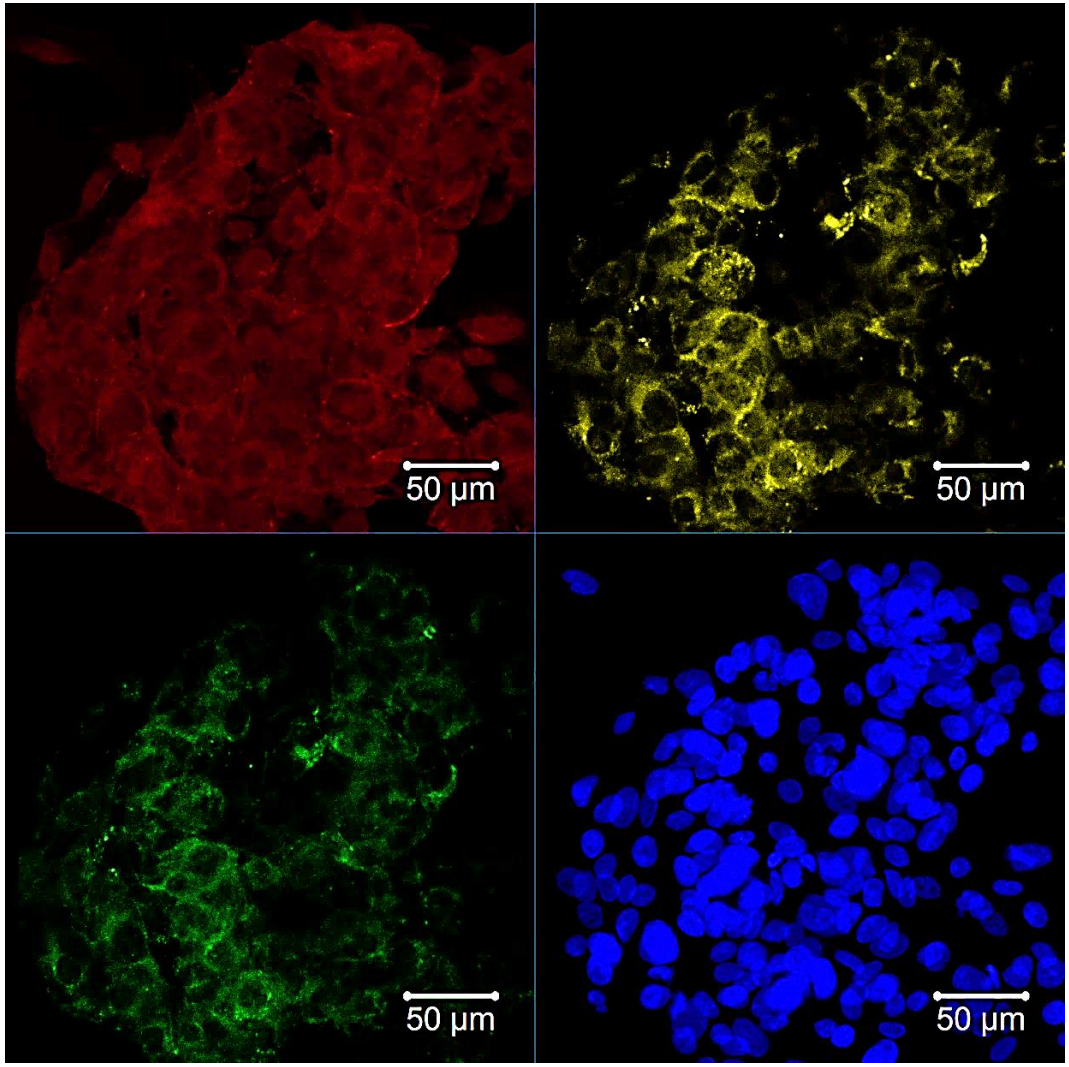
2. Photobleaching

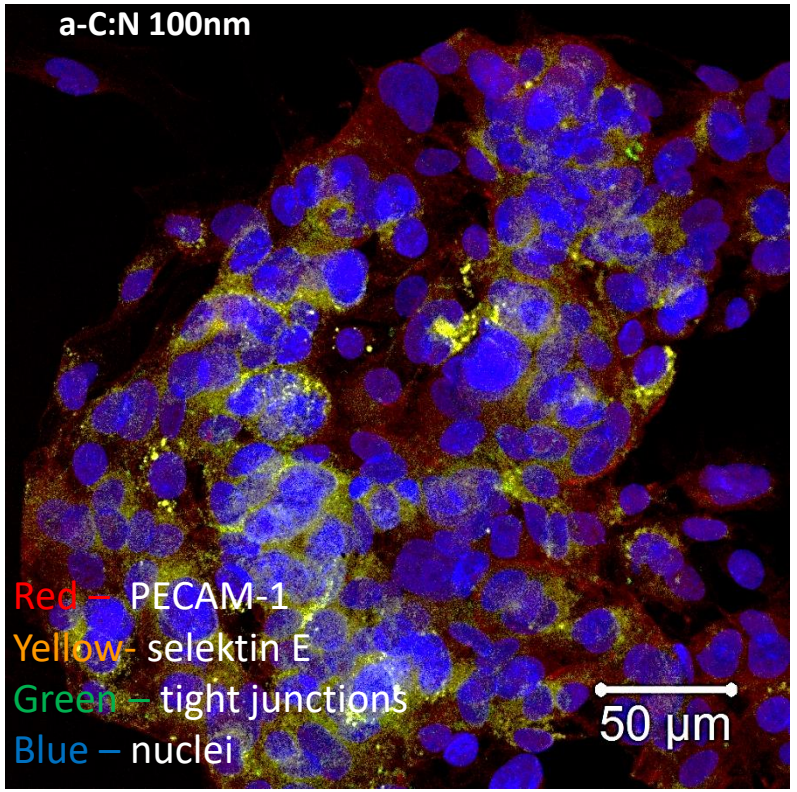


3. Phototoxicity

FRAP (mov) Laser twisers (mov)

Bio-inspired materials



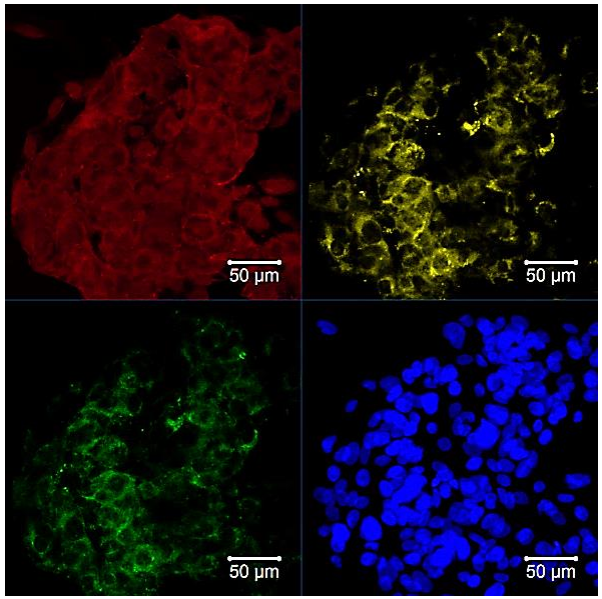


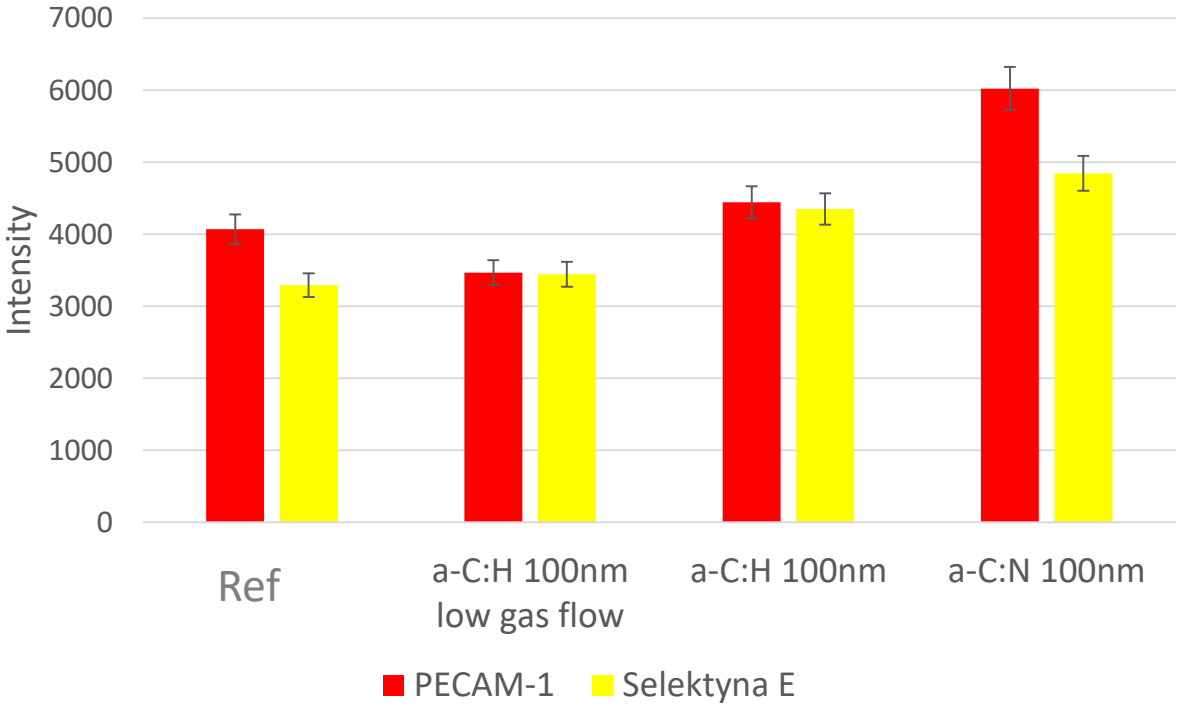
Bio-inspired materials

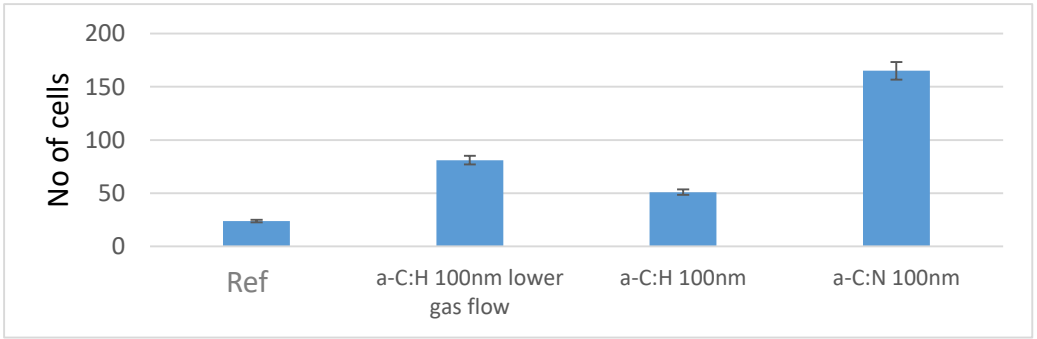
PLT-Endoth. Cell adhesive molecule

Klaster of differentiation 31

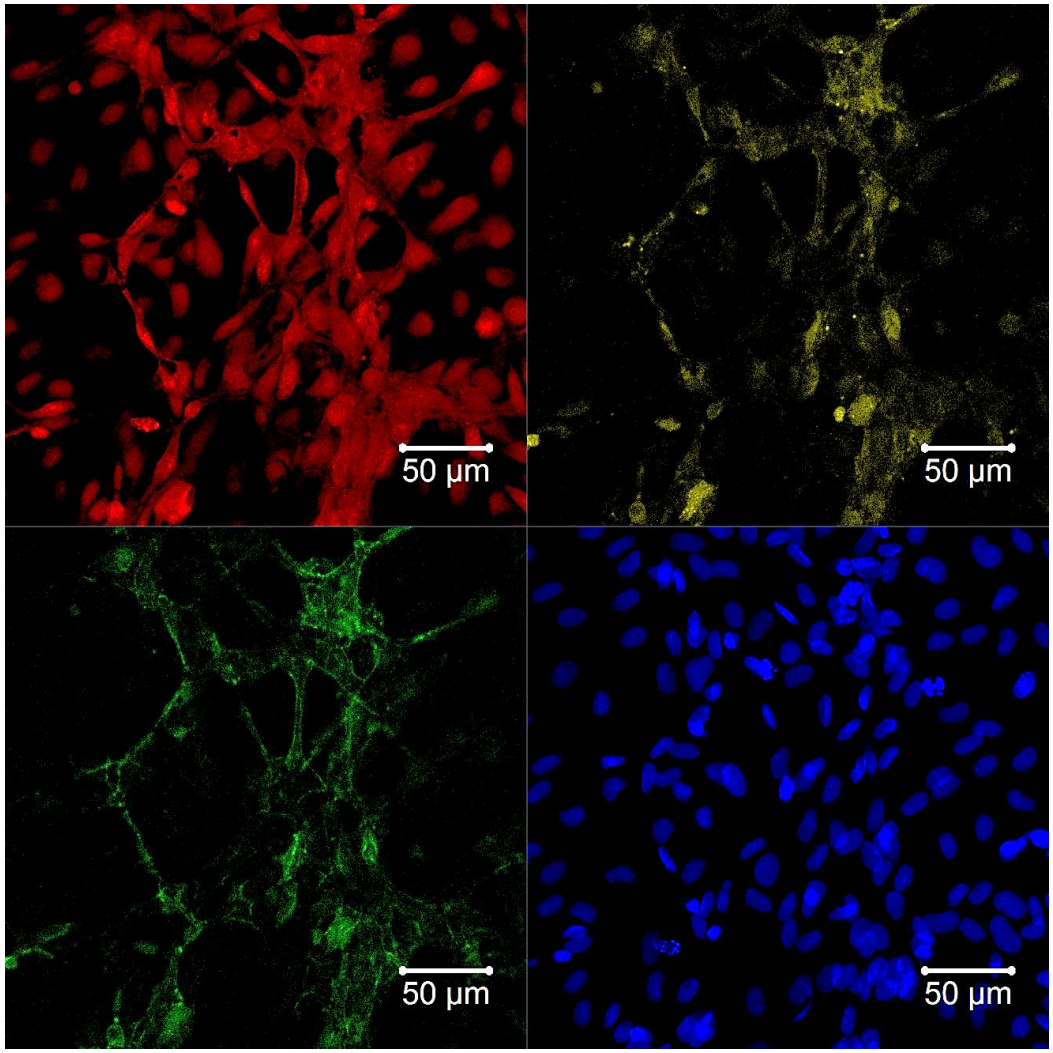
E-selektyna, antybody **CD62** (CD62E), Endoth.- leuc. Cell adhesive molecule







Bio-inspired materials



Optimal

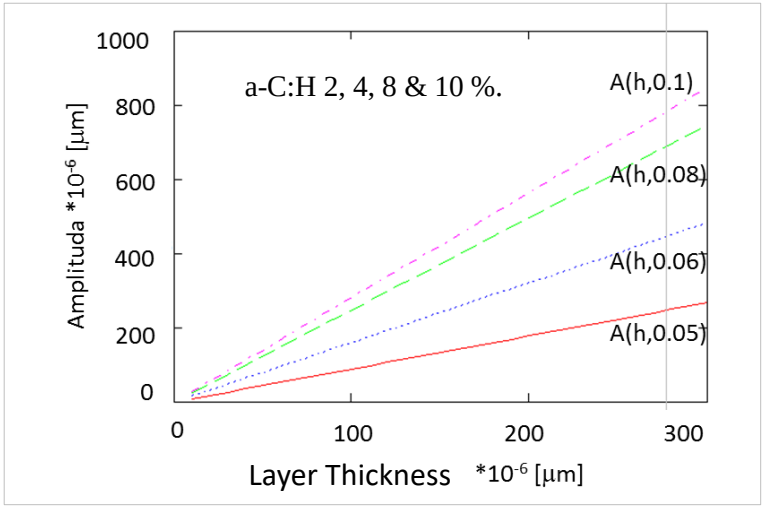
Red – PECAM-1
Yellow- selektin E
Green – tight junctions
Blue – nuclei

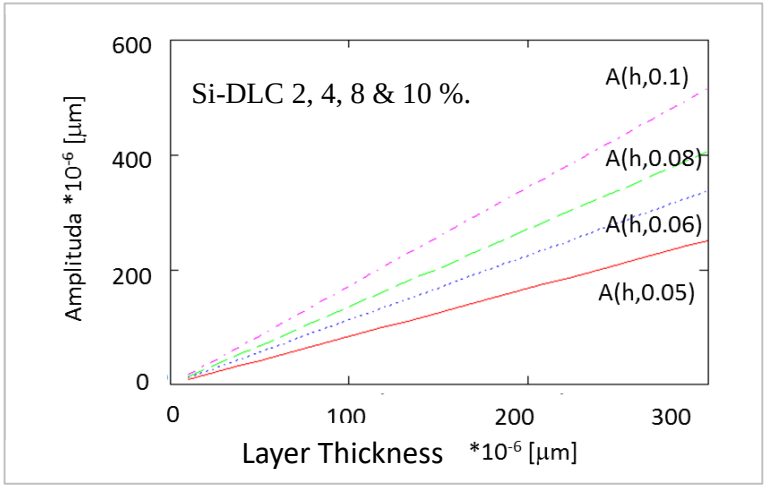
Bio-inspired materials

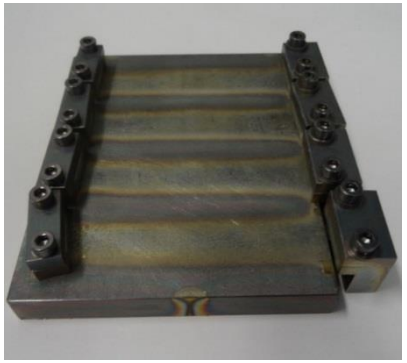
Wrinkles by strain

$$A(h, ed) = h\sqrt{\frac{ed}{ec} - 1}$$

$$ec = 0{,}25\left(\frac{3E_s}{E_f}\right)^{\frac{2}{3}}$$

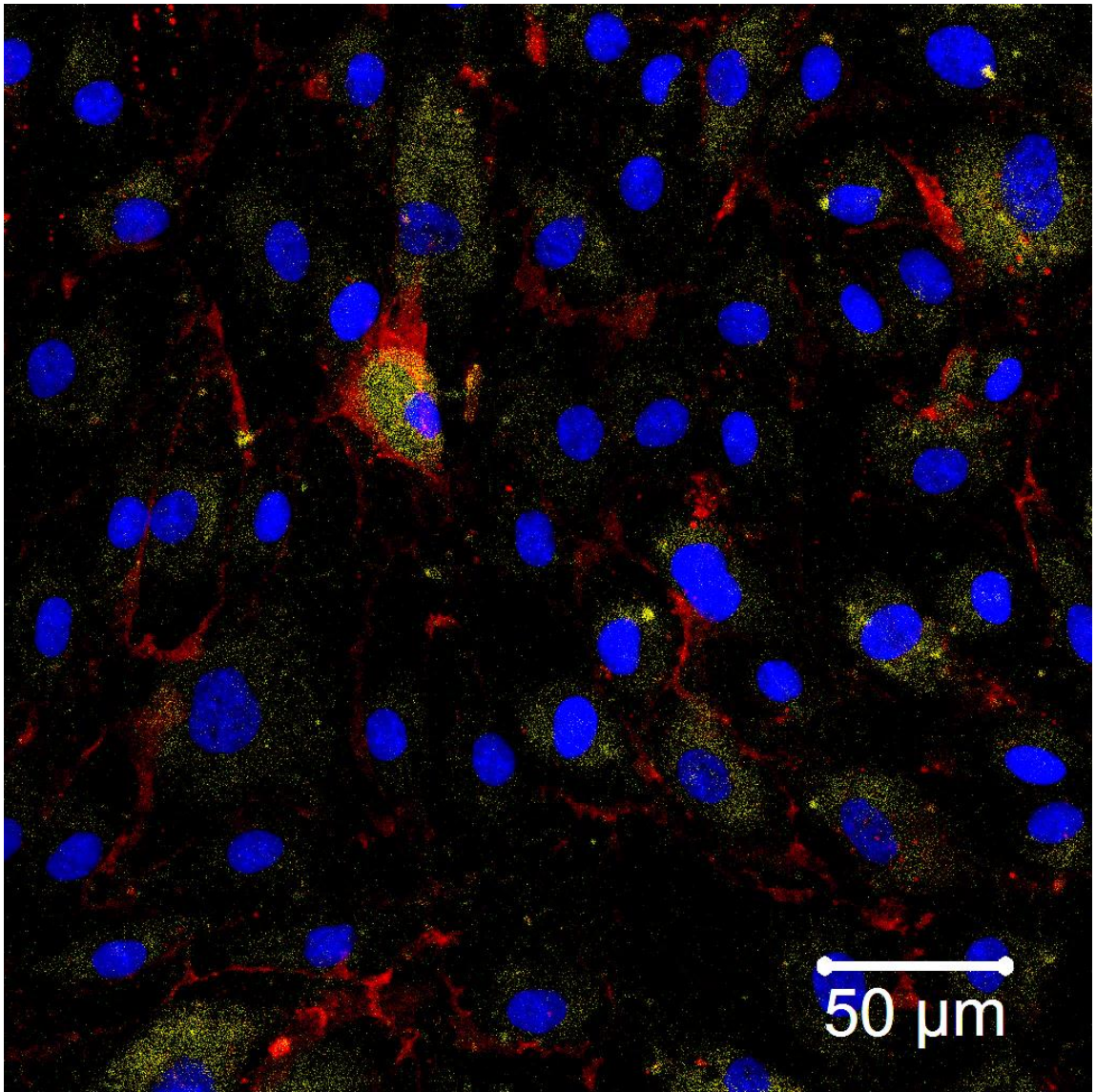






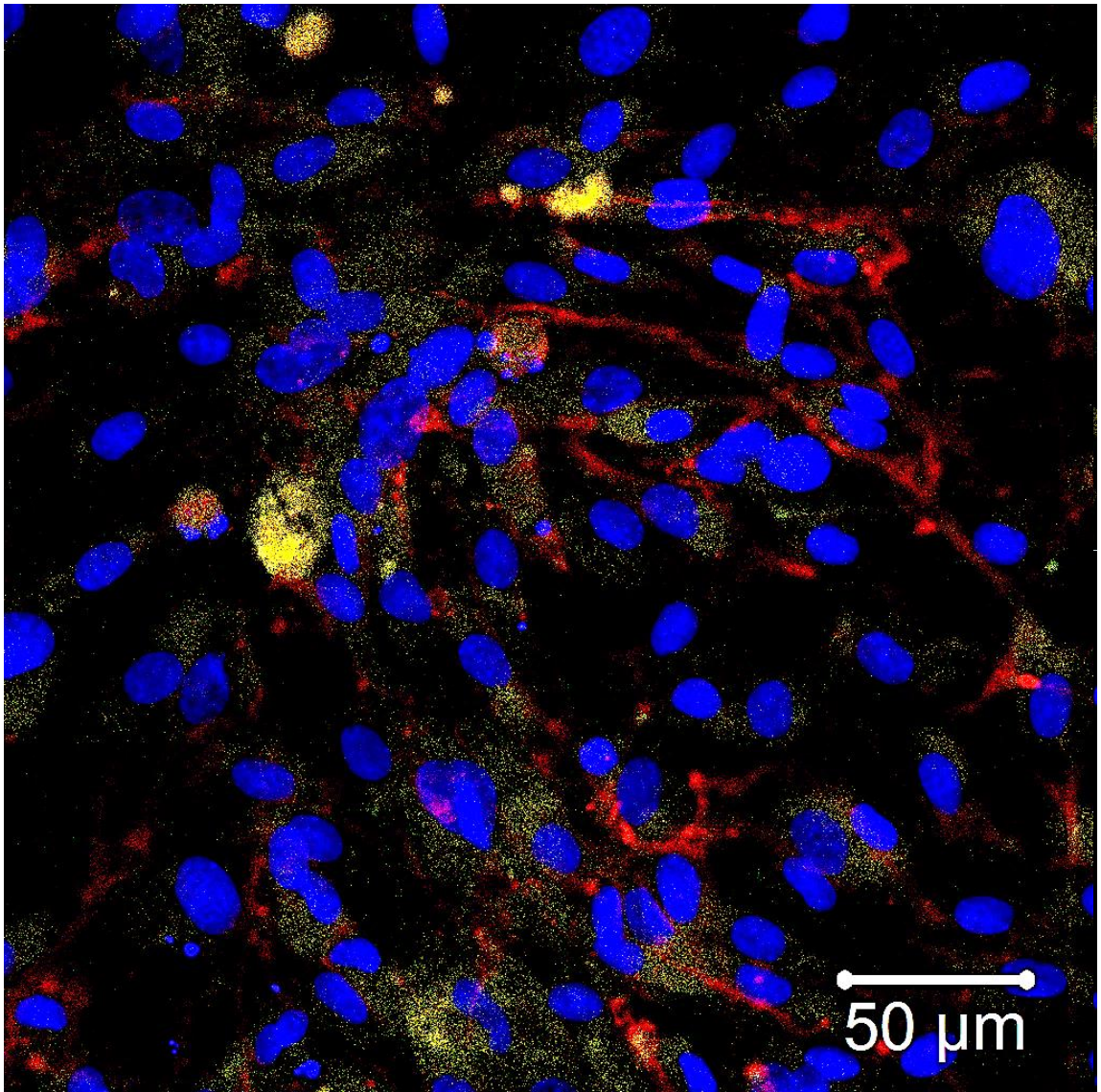
Wrinkles by strain

0%



Wrinkles by strain

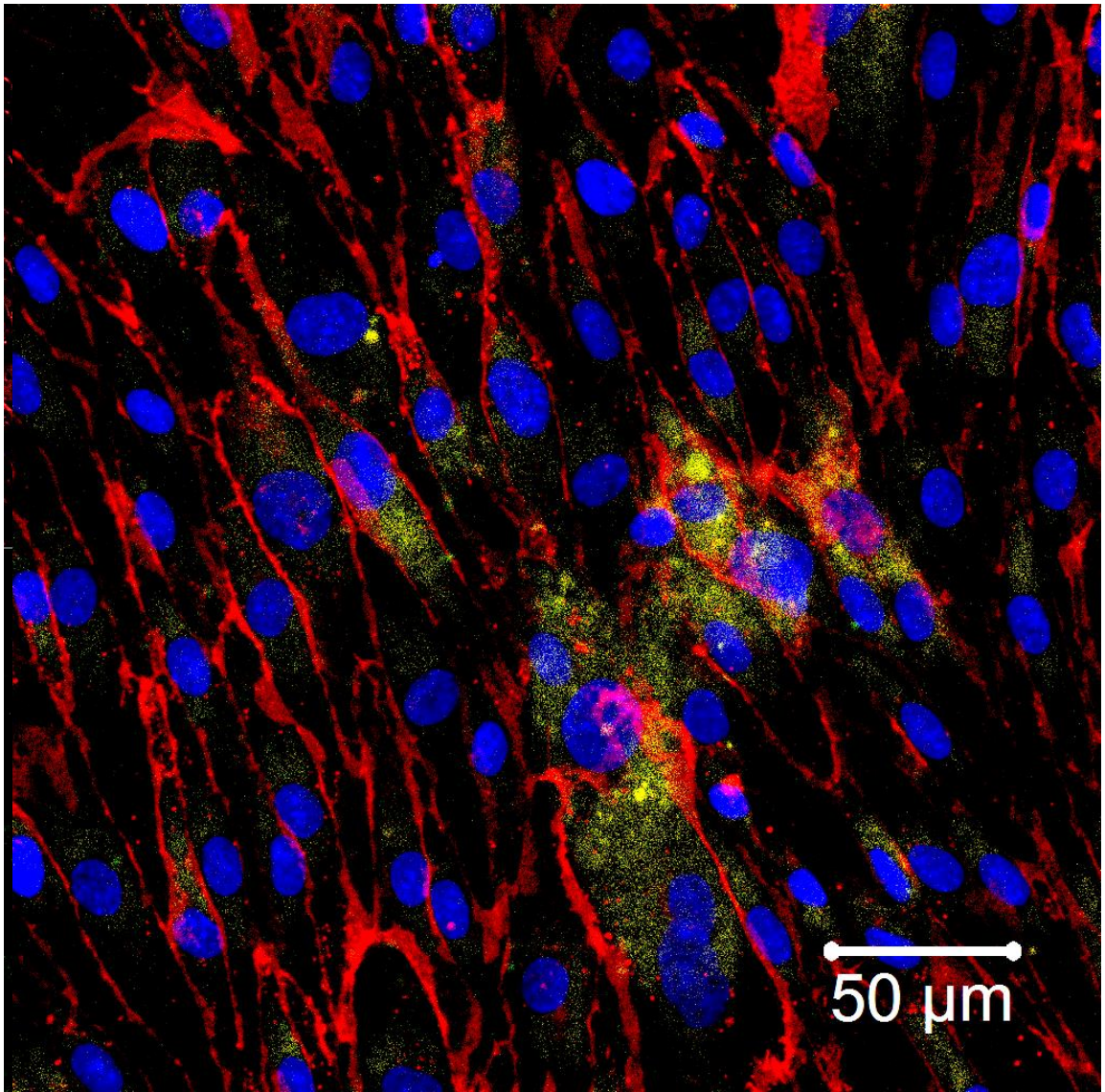
3%



Bio-inspired materials

Wrinkles by strain

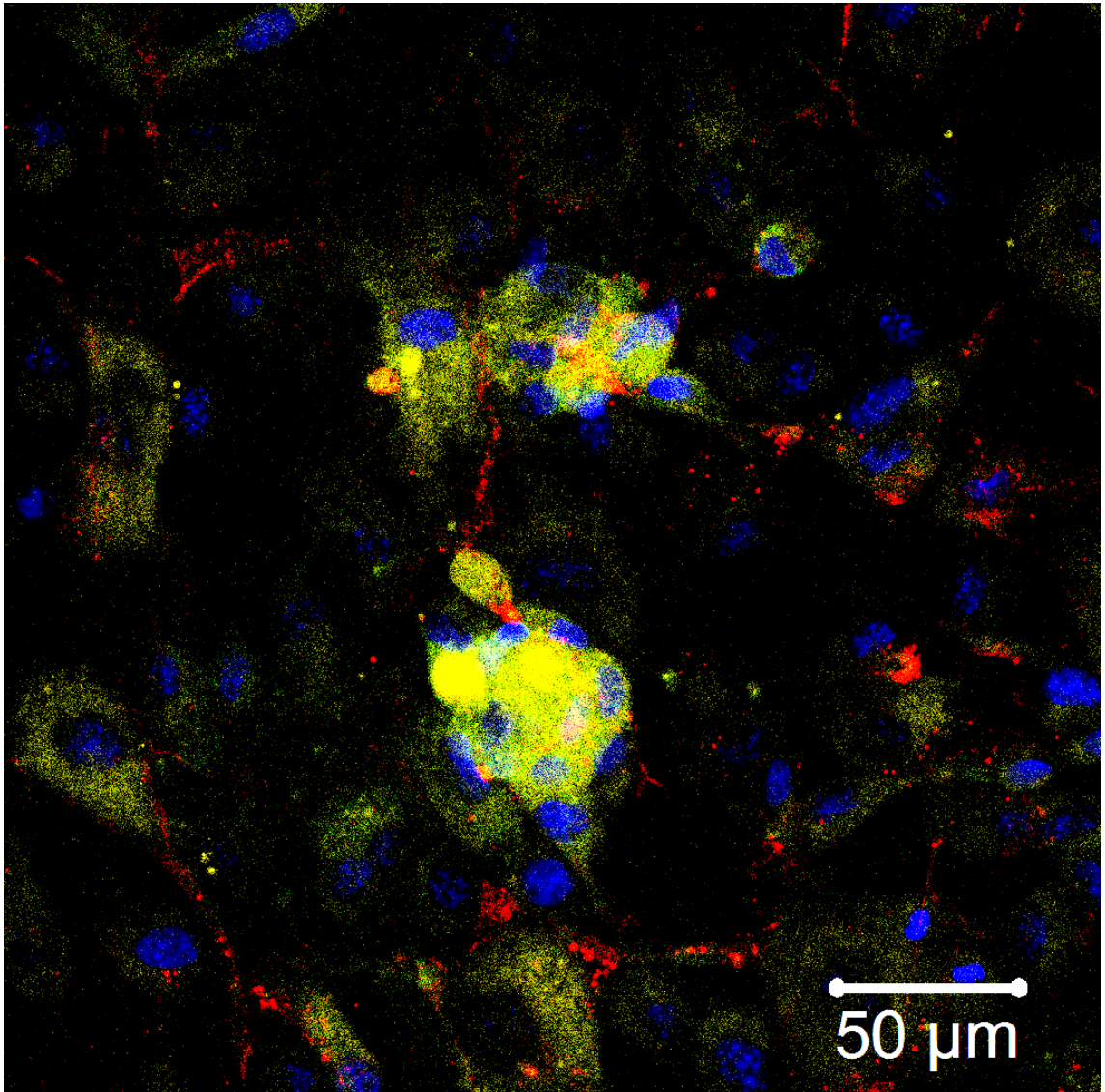
5%



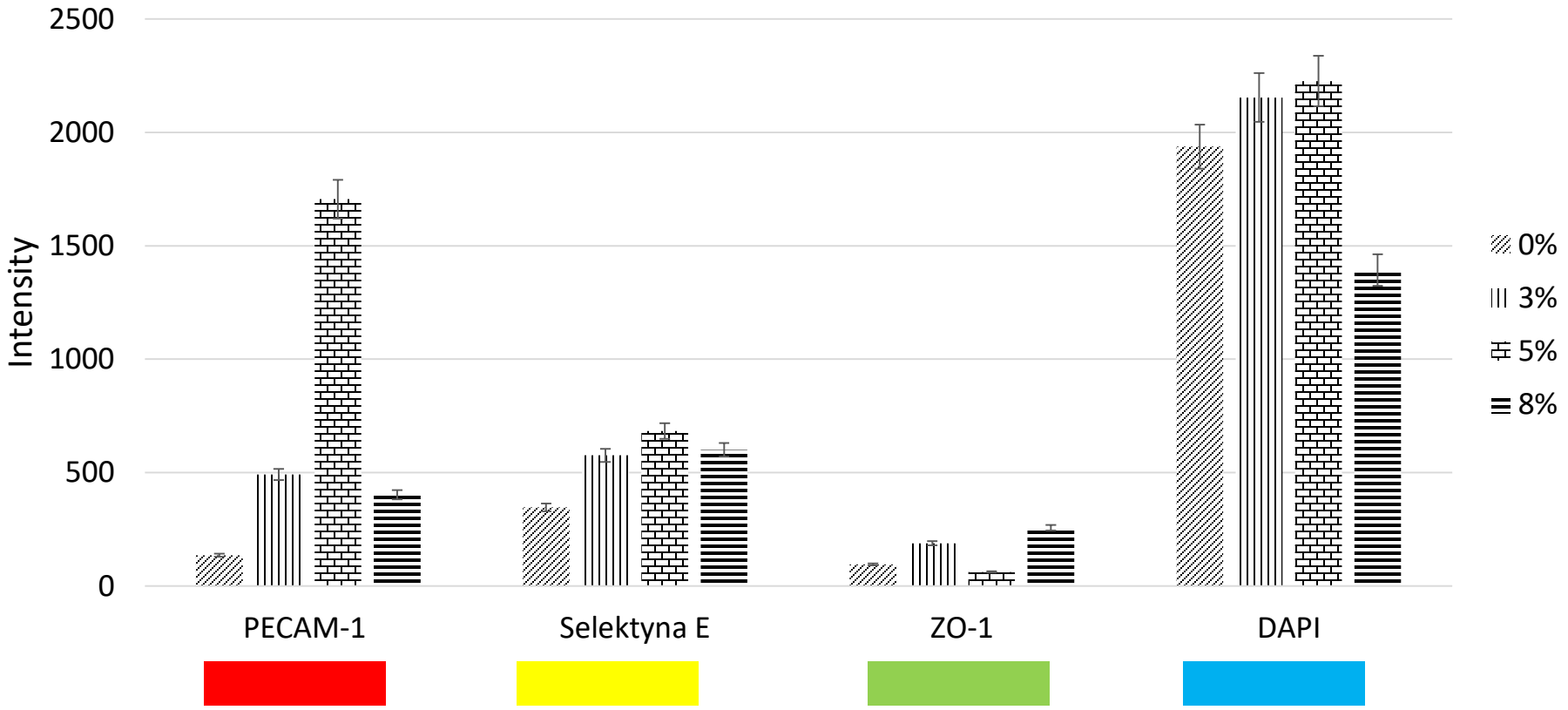
Bio-inspired materials

Wrinkles by strain

8%

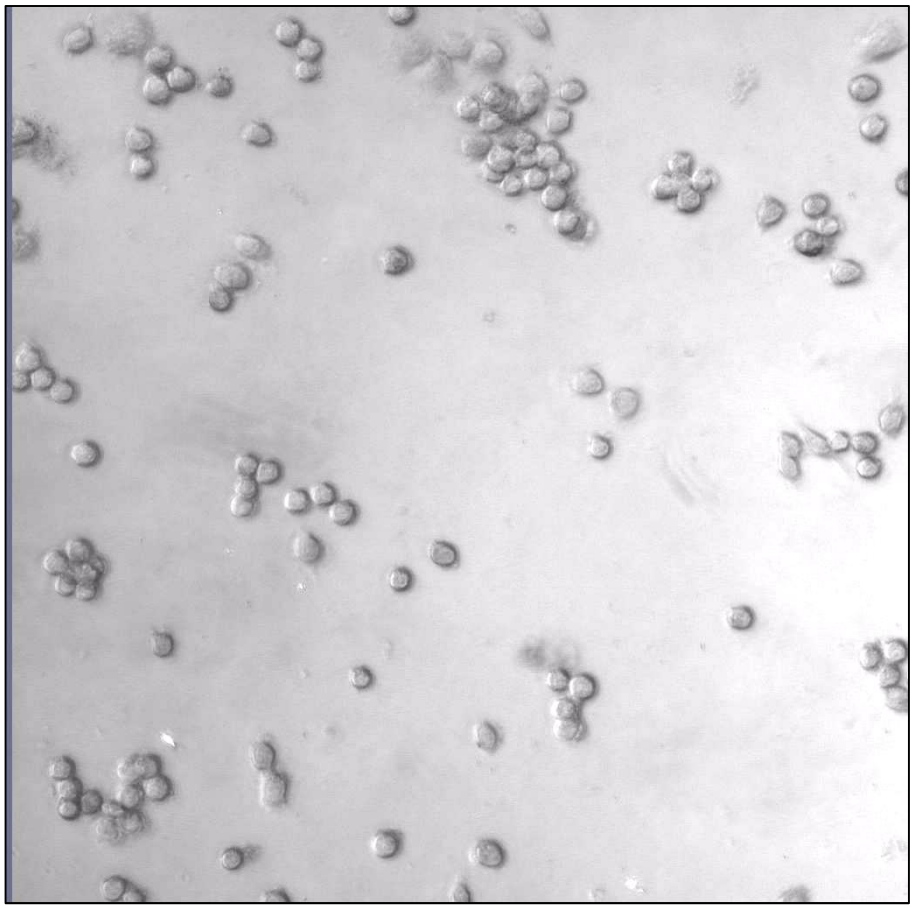


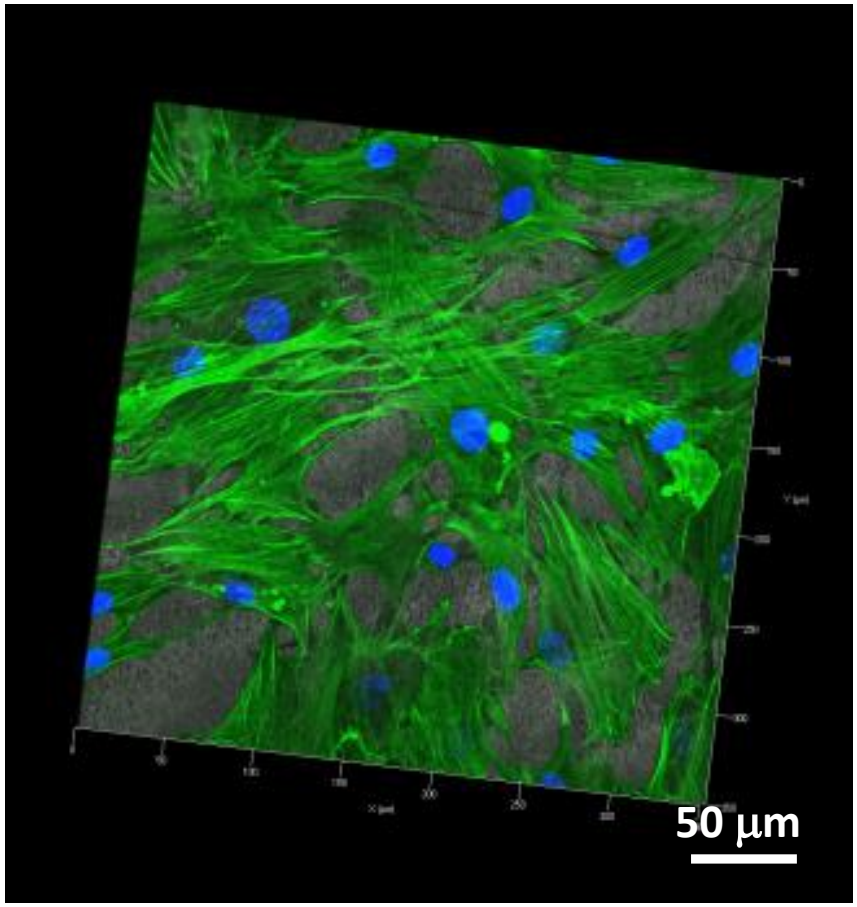
Wrinkles by strain



Bio-inspired materials

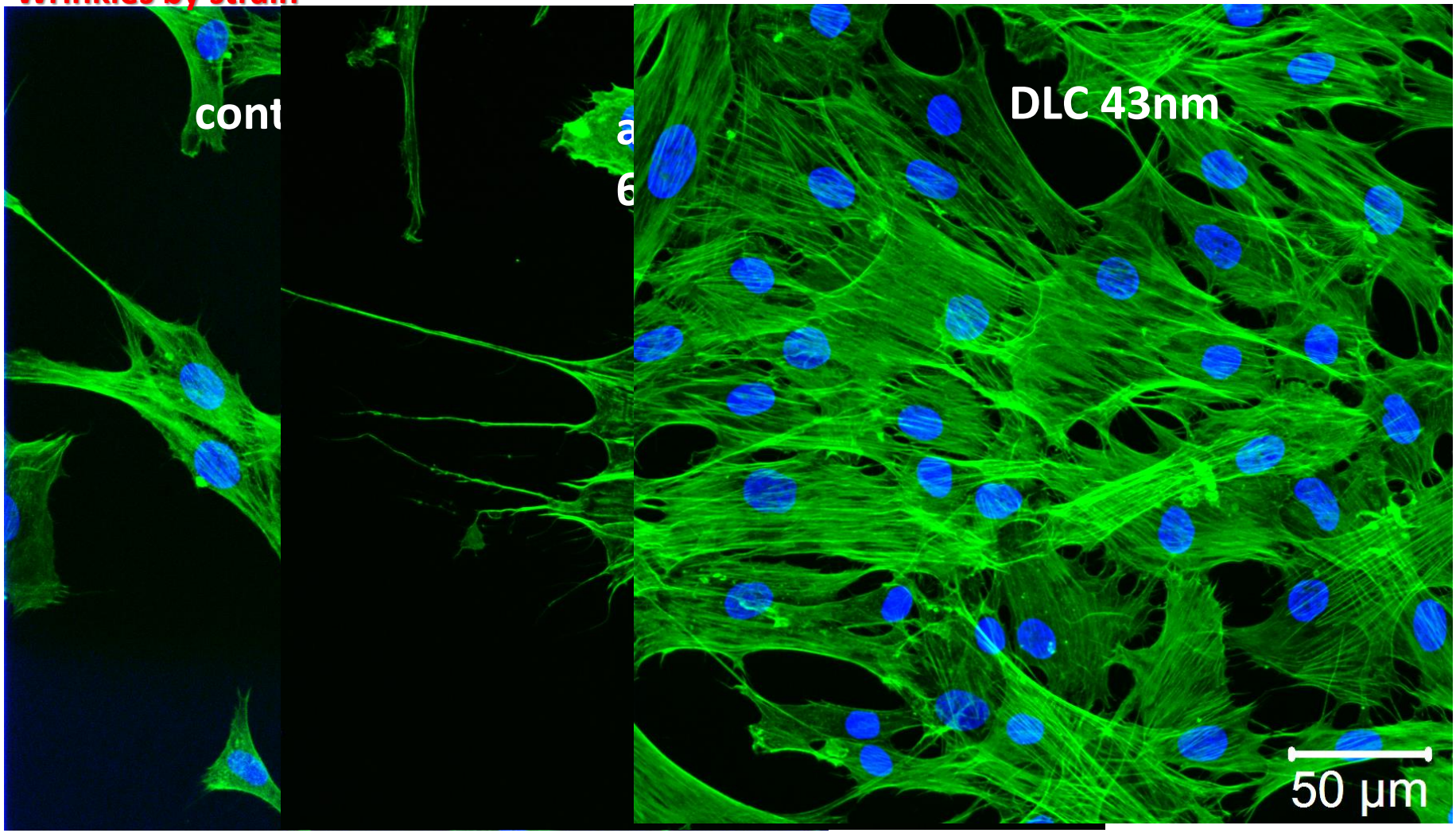
DLC 85nm



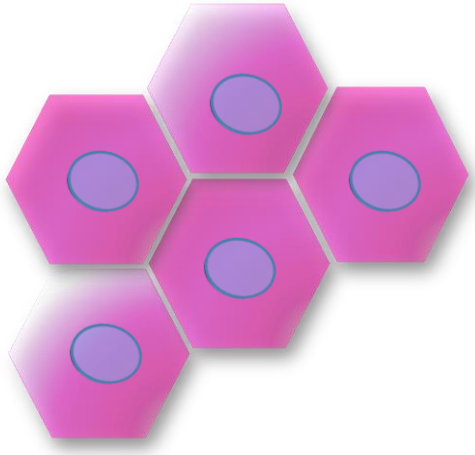


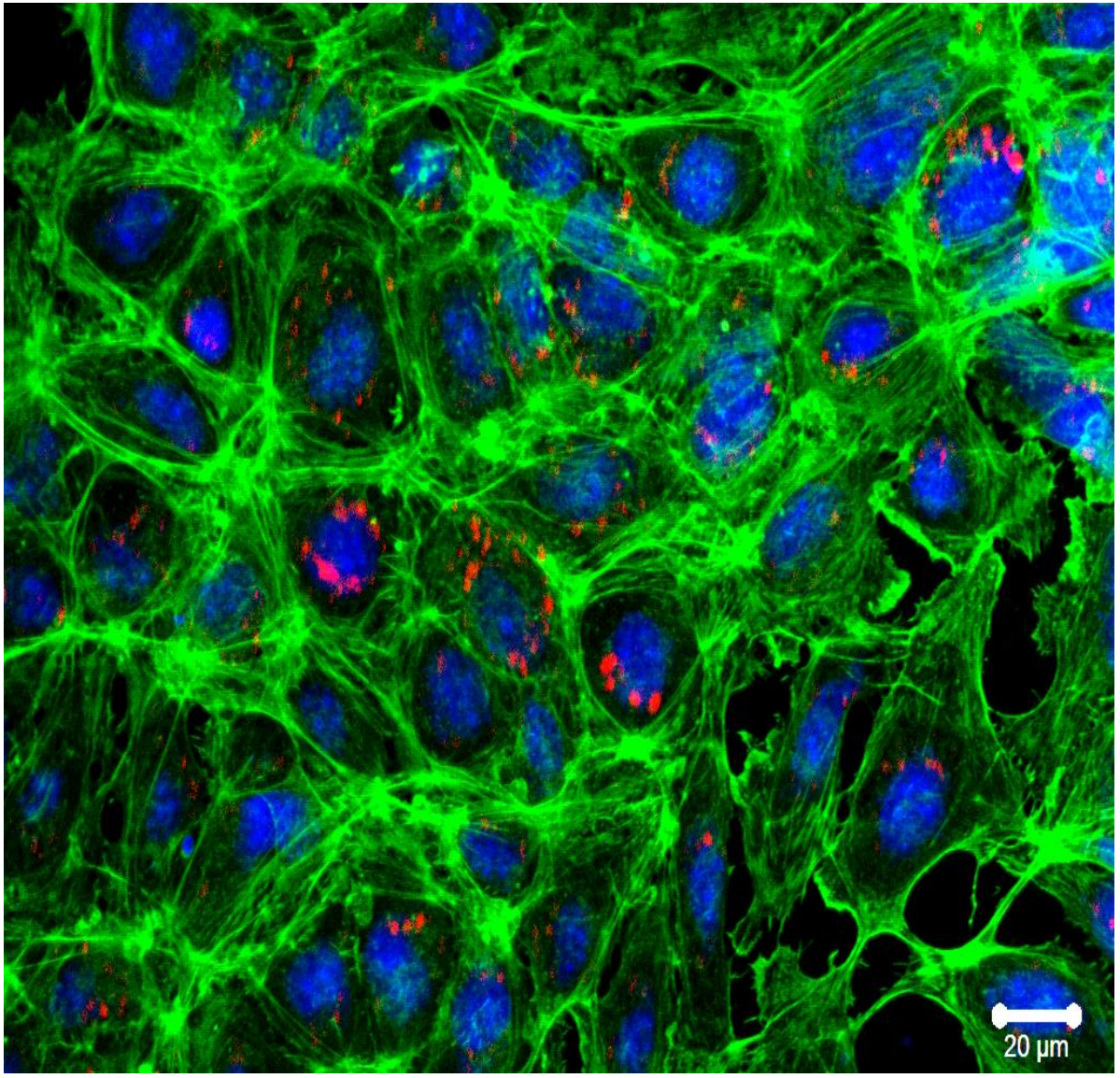
Bio-inspired materials

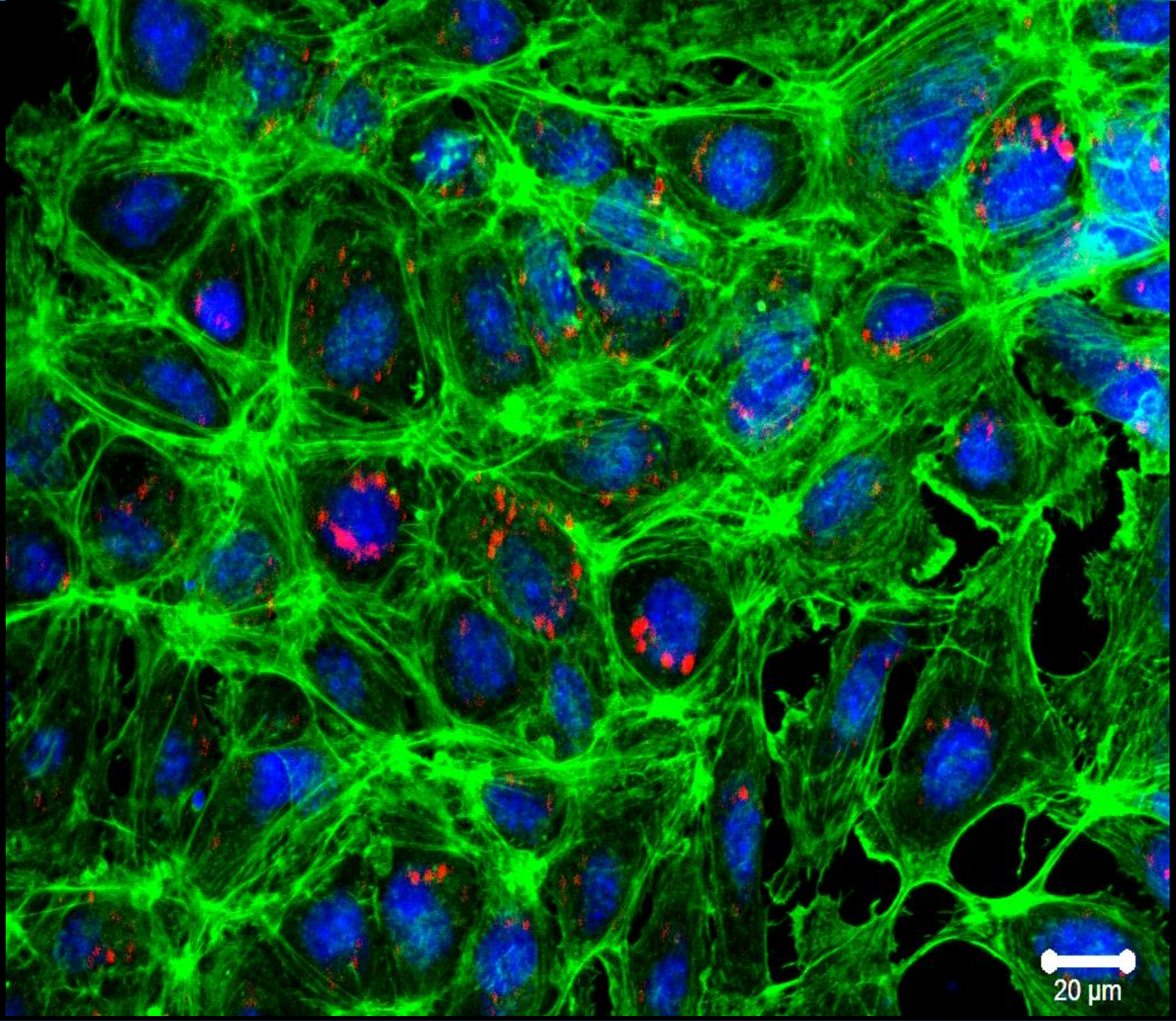
Wrinkles by strain



Bio-inspired materials






20 µm

„Bio-inspired thin film materials with the controlled contribution of the residual stress in terms of the restoration of stem cells microenvironment”

2014/13/B/ST8/04287

BLOOD-MATERIAL INTERACTION

Bio-inspired materials

Whole human blood dynamic tests

ELSEVIER

Biomolecular Engineering 19 (2002) 91–96

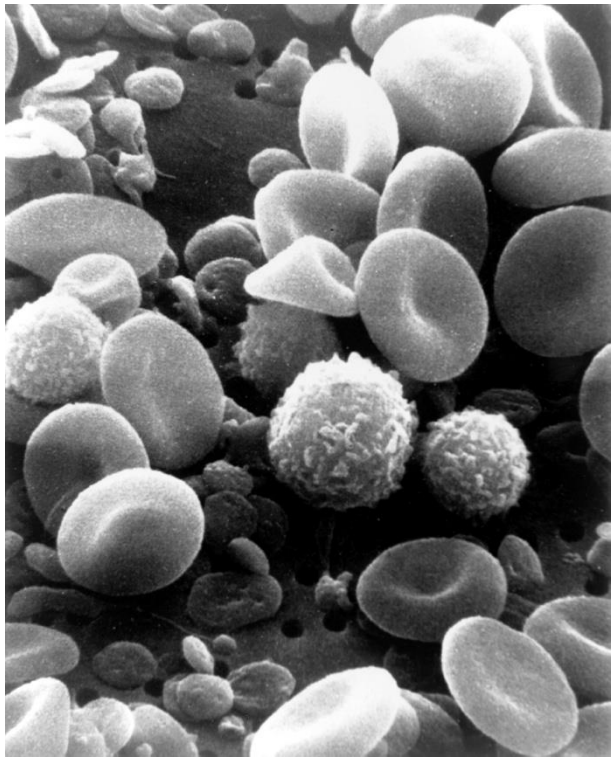
Biomolecular Engineering

www.elsevier.com/locate/geneanbioeng

In vitro hemocompatibility testing of biomaterials according to the ISO 10993-4

Ulrich Theo Seyfert [a,*], Volker Biehl [b], Joachim Schenk [a]

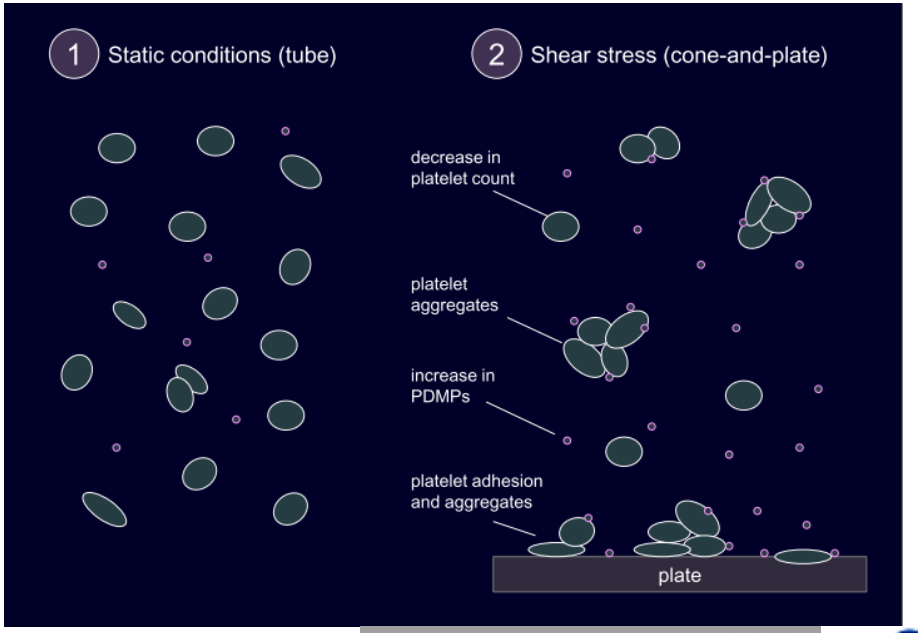
[a] *Abteilung Klinische Hämostaseologie und Transfusionsmedizin, Haus 75, Universitätskliniken, D-66421 Homburg, Germany*
[b] *Universität des Saarlandes, Lehrstuhl für Metallische Werkstoffe, Im Stadtwald, D-66123 Saarbrucken, Germany*

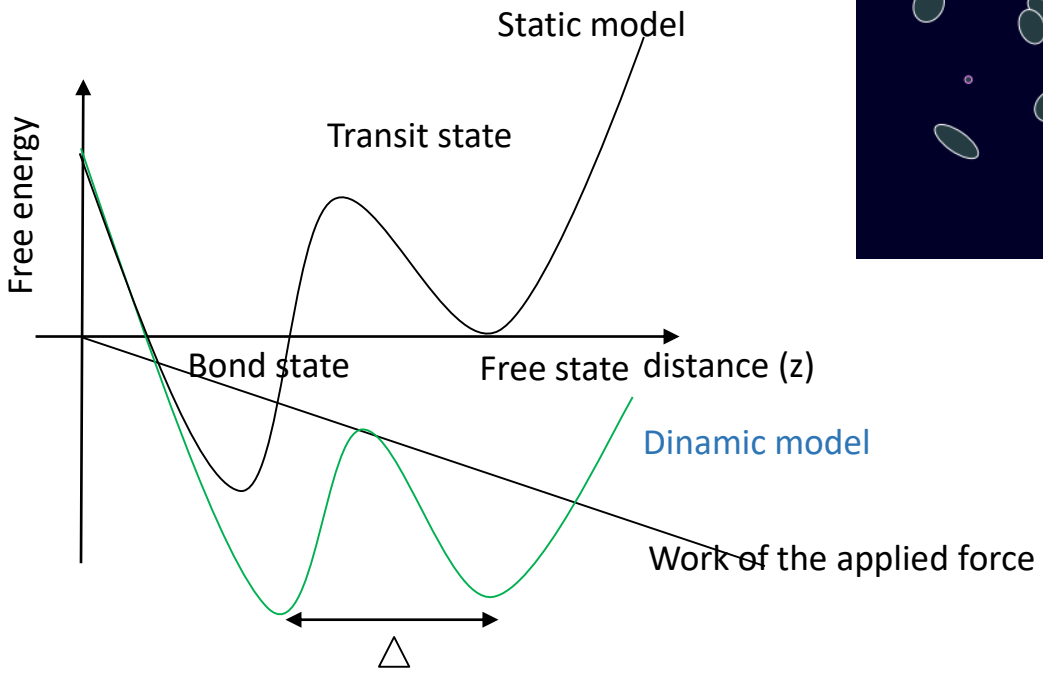


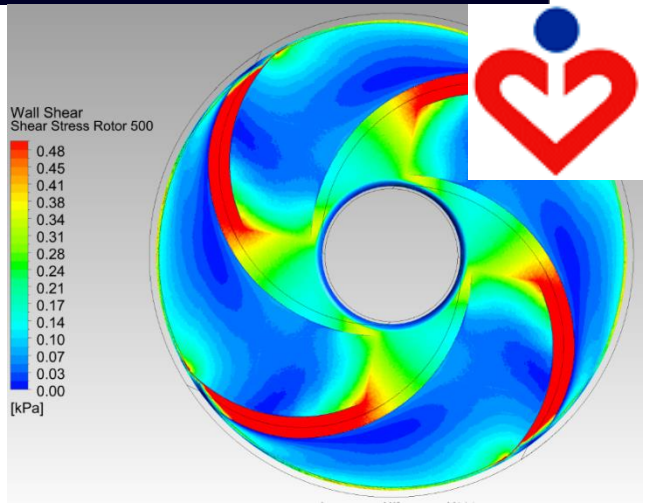
”A hemocompatibile material must not adversely interact with any blood component”

Bio-inspired materials

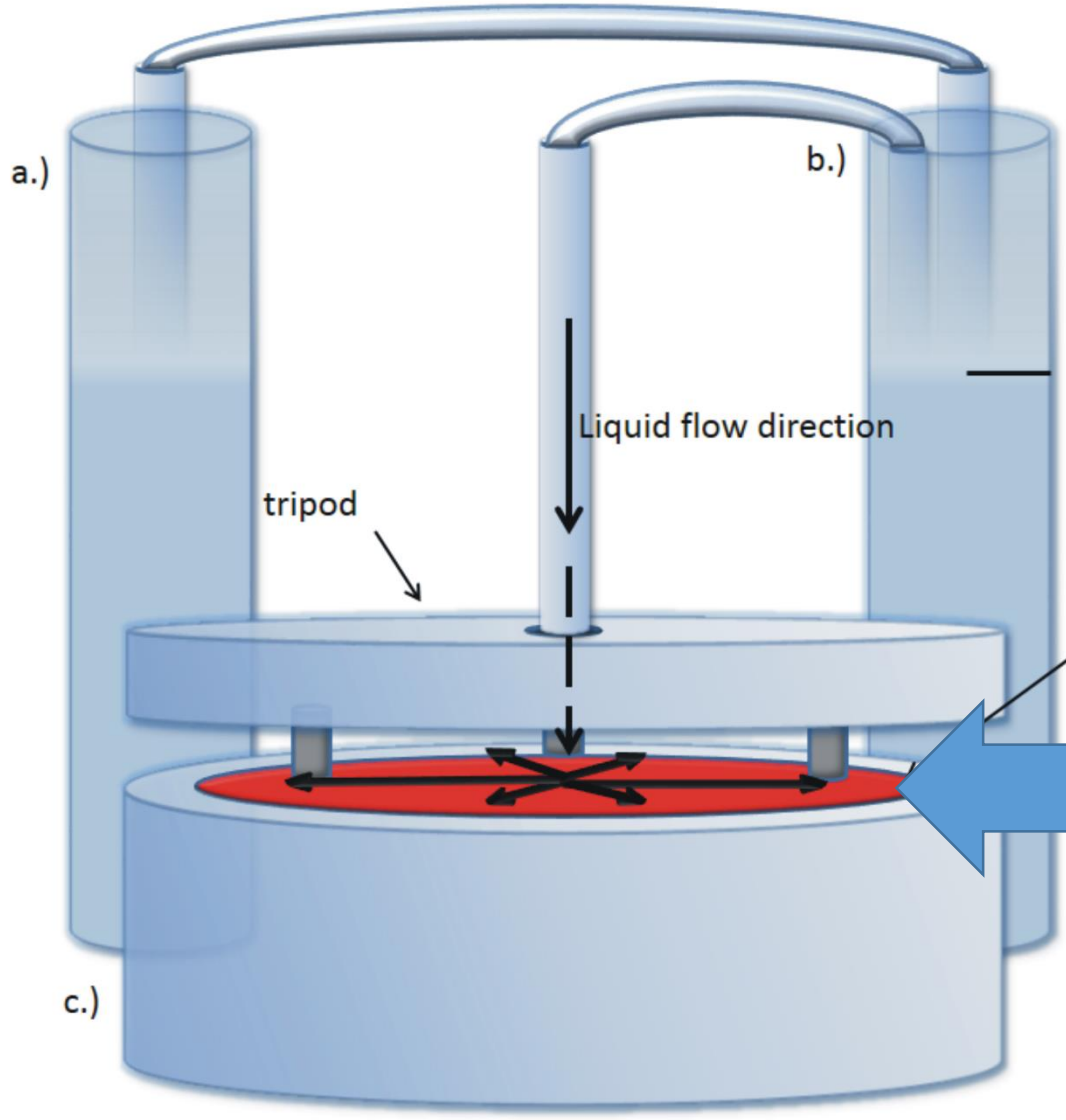
Material	Composition	Thickness
C170_2	a-C:H	100nm
C170_4	Si-DLC	500nm
C170_5	Si-DLC	300nm
C170_6	Si-DLC	200nm
C170_7	Si-DLC	125nm
C170_8	Si-DLC	15nm



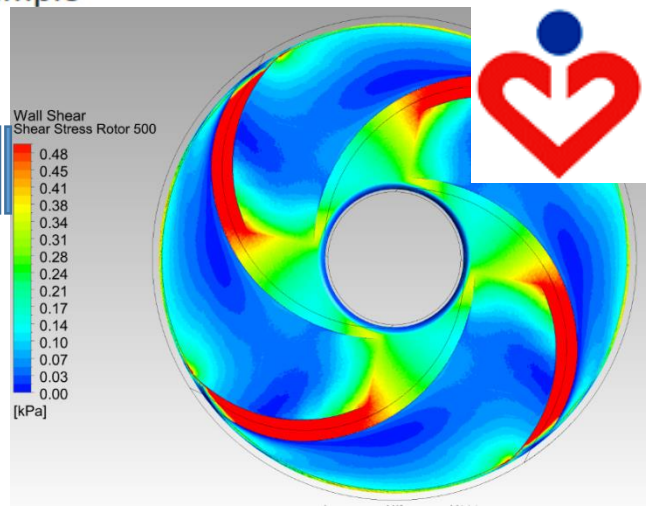




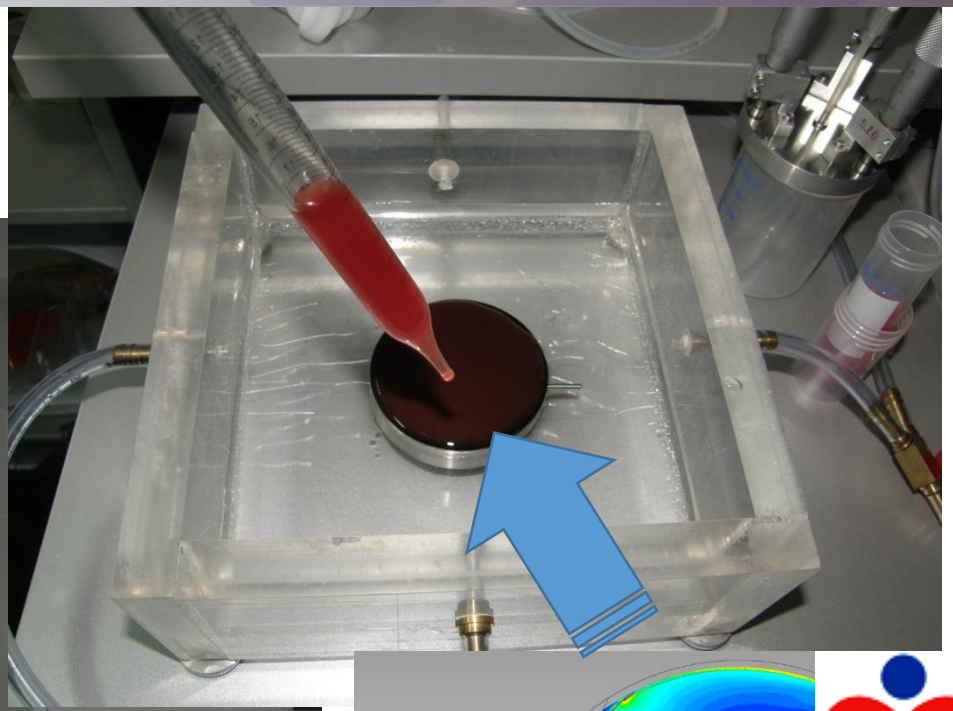
Bio-inspired materials

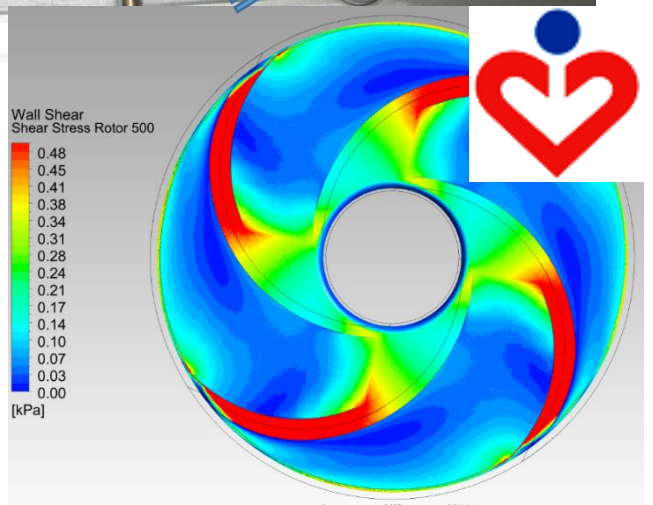


High shear stress simulation



Bio-inspired materials





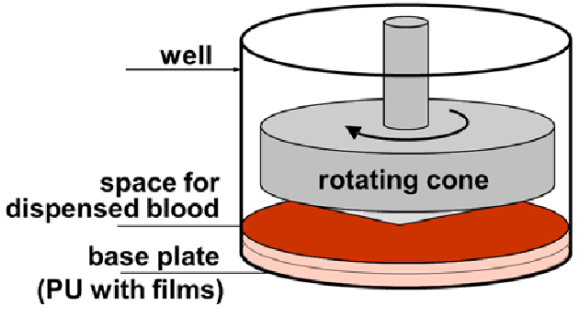
Bio-inspired materials

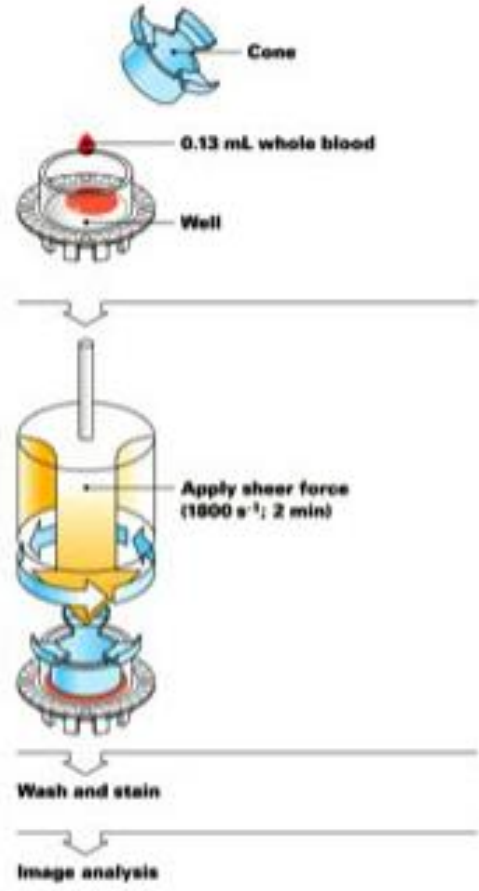
- Human blood (4 x 4.5 mL)
- ADP activation 5 min.
- Arterial flow conditio simulation.
- 130 μL after the test.

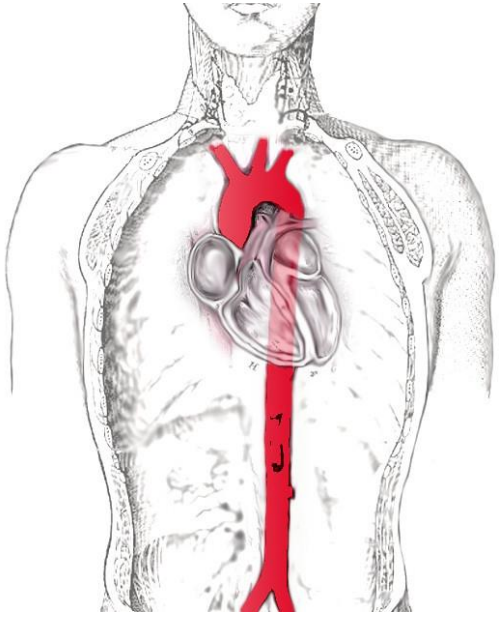
Analysis:

- Platelet marker expresion CD61
- PAC-1 glycoprotein IIb/IIIa
- CD62P for P-selectin
- Small and big PLT agregates

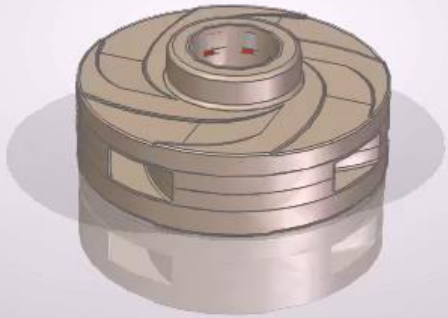
Arterial flow conditions





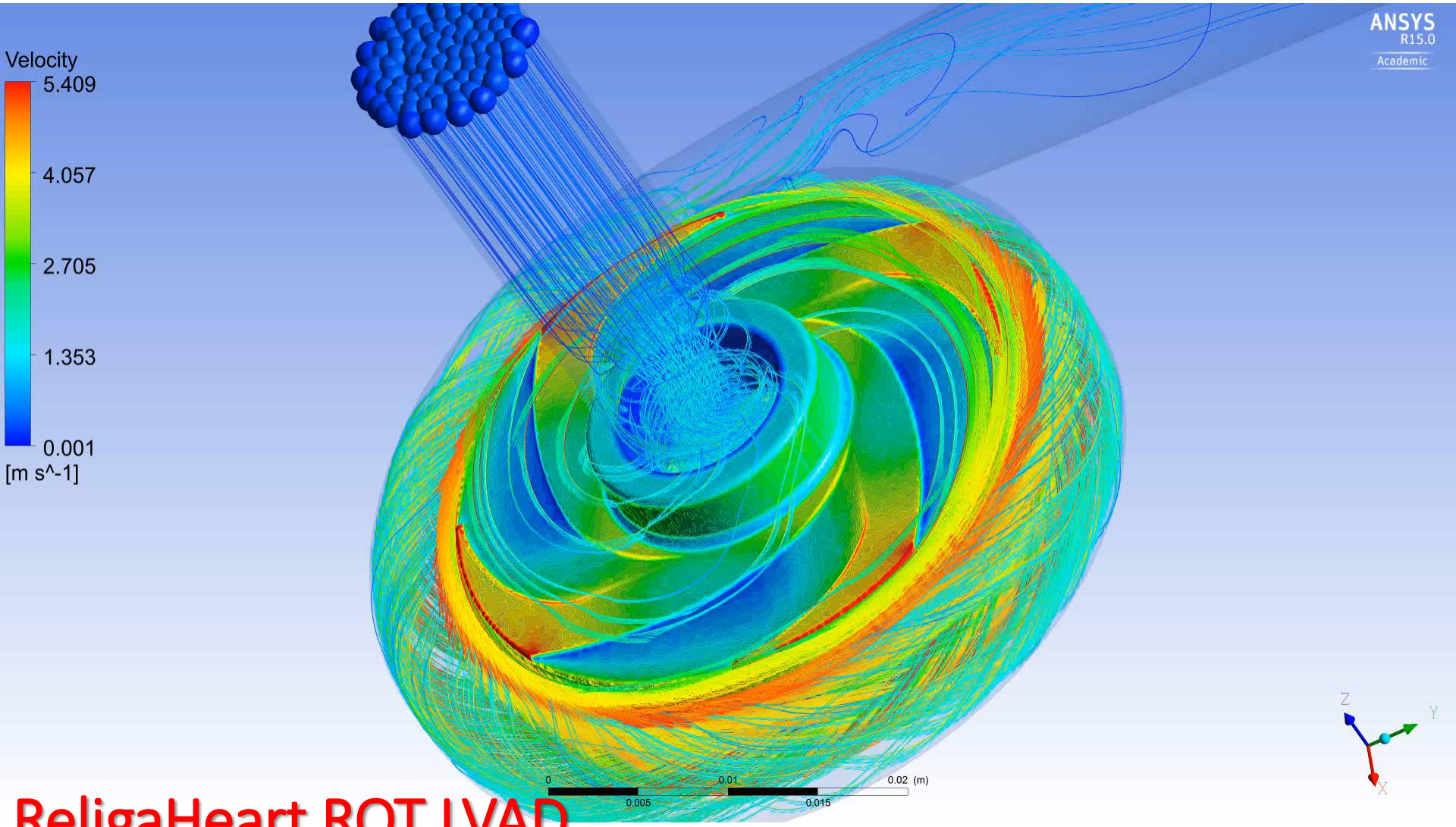


Bio-inspired materials



ReligaHeart ROT LVAD

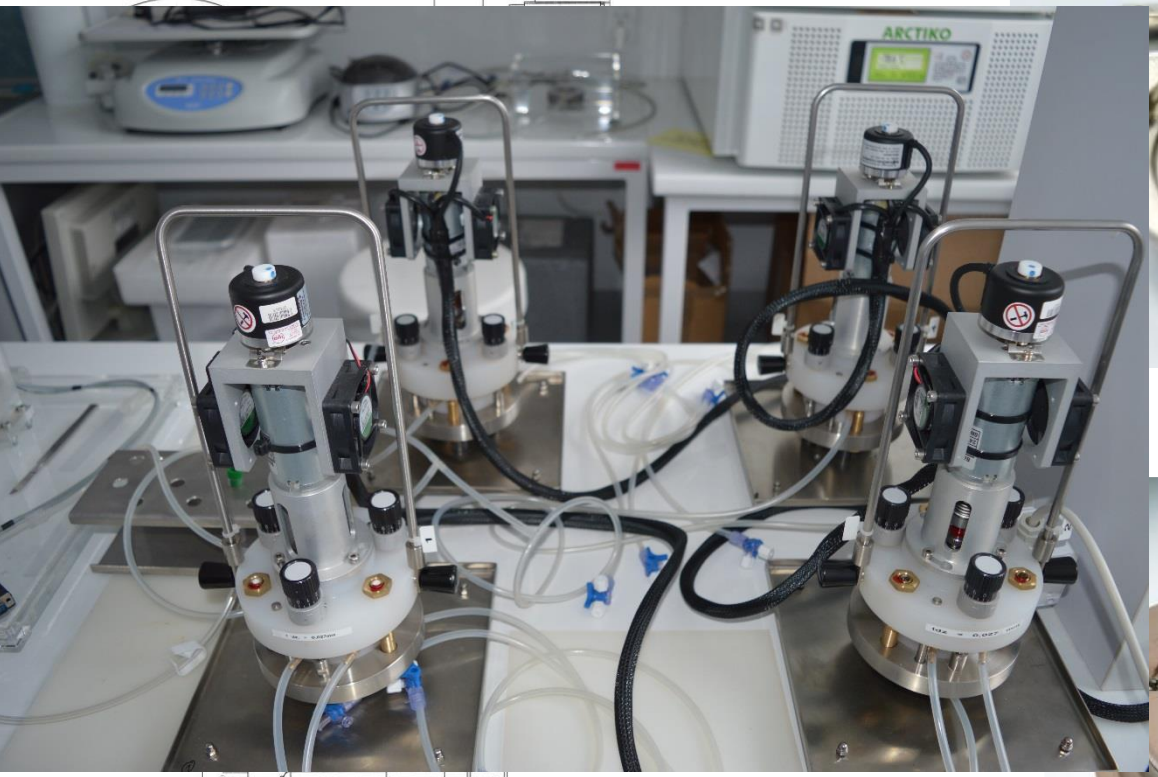
Bio-inspired materials

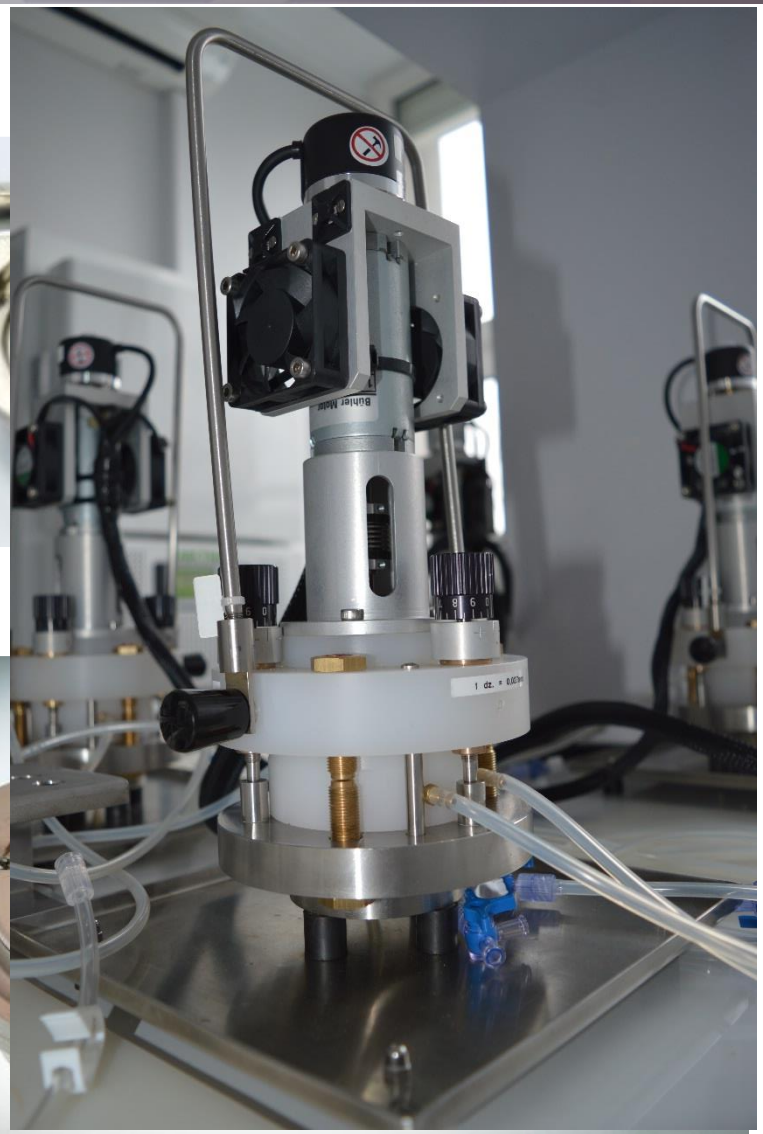


ReligaHeart ROT LVAD

Bio-inspired materials

Tester IMIM PAN



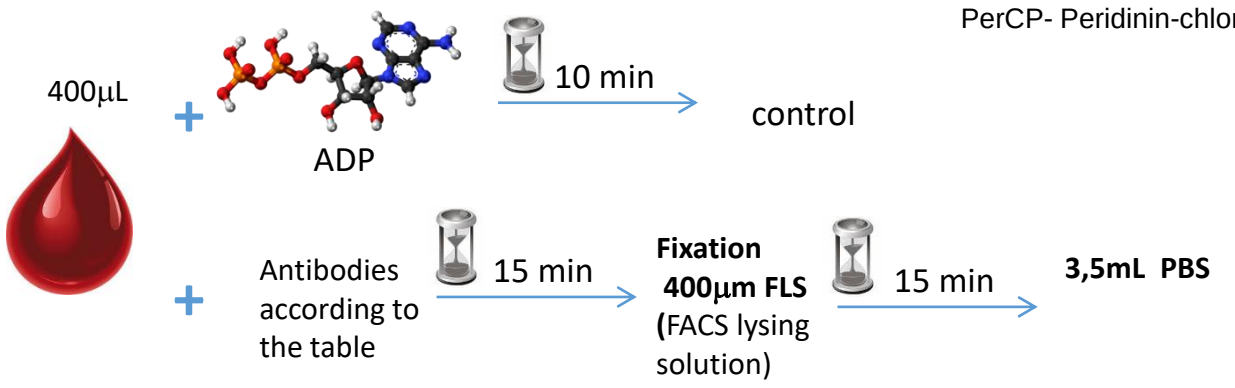


Blood-Material Interaction

Tester IMIM PAN

Name	Composition	Application
M	CD61-PerCP, CD62P, PAC-1	assessment of platelet activation under the influence of contact with the material (+ control)
A	CD61-PerCP, CD62P, PAC-1	assessment of platelet activation using ADP
ADP 1	ADP 0.4mM	Positive control
ADP	ADP 40mM	Kontrola pozytywna

PerCP- Peridinin-chlorophyll-protein

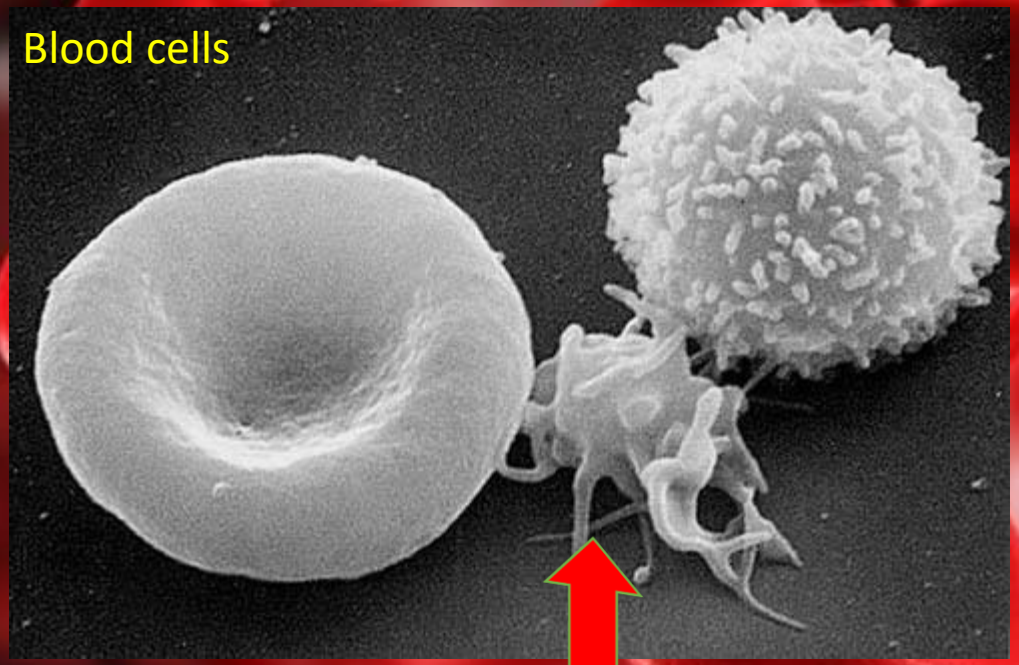


Bio-inspired materials

CLSM-

CONFOCAL LASER SCANNING MICROSCOPY

Anty- CD62

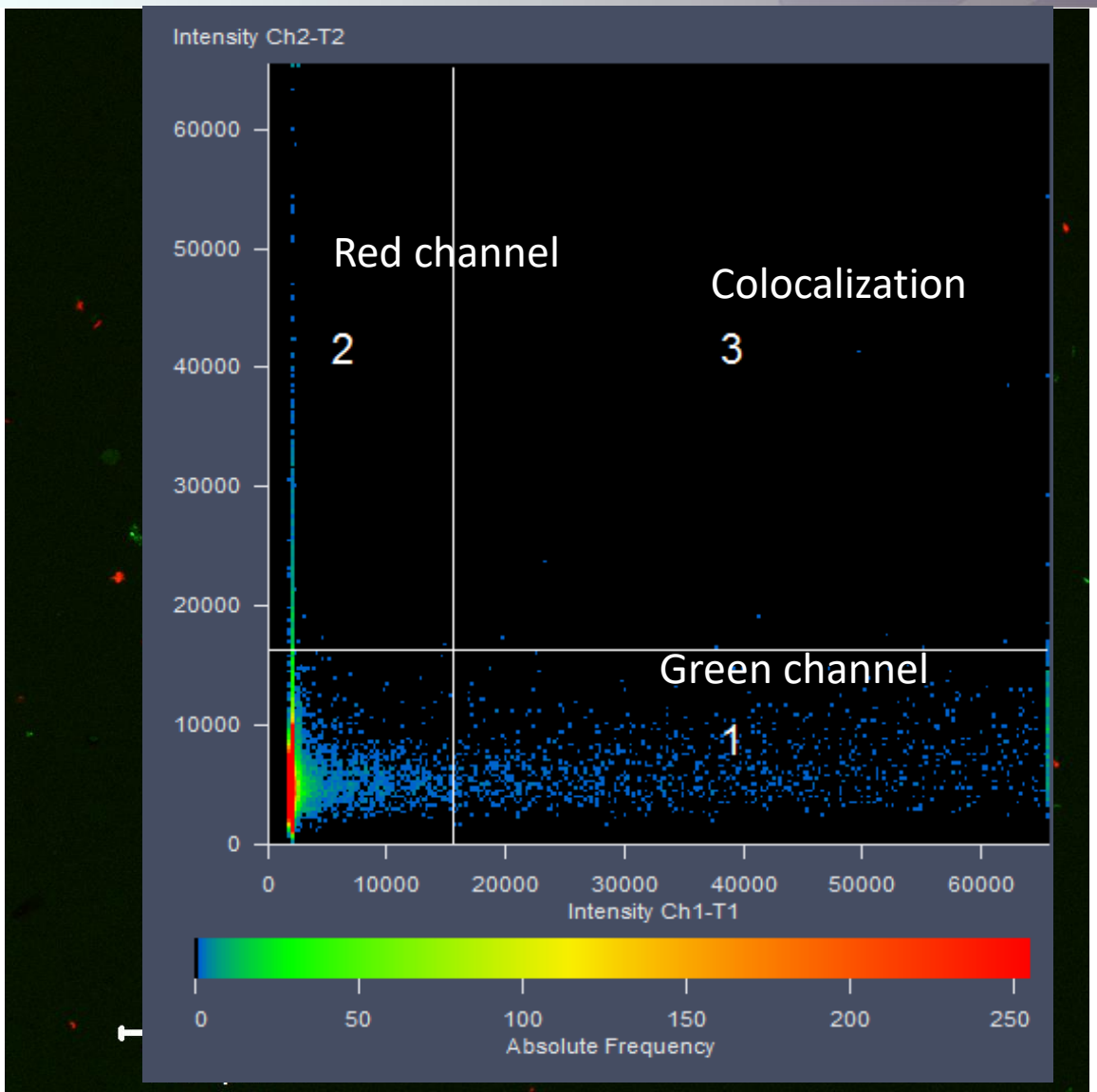


Bio-inspired materials

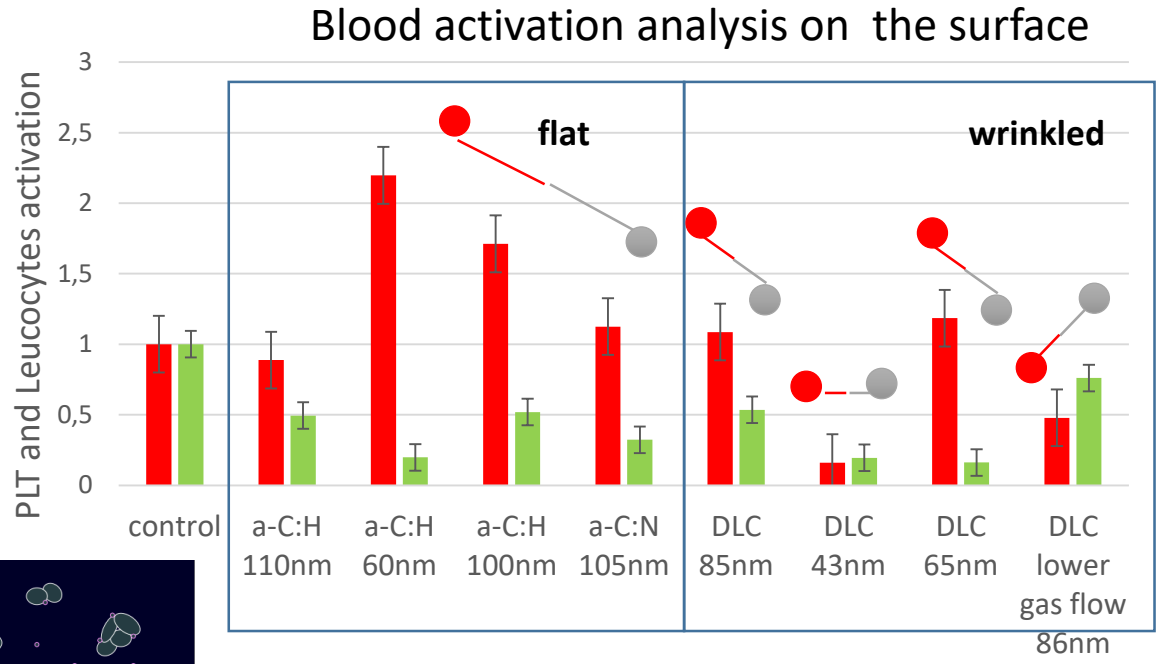
- Confokal Modul LSM 5 Exciter, two canals, RGB
- Laser HeNe 633nm 5mW
- Laser HeNe 543nm 1mW
- Laser argon 458/488/514nm, 25mW
- Laser diodowy V 405nm
- Main Beam Splitter turret PASCAL
- Software ZEN 2008 LSM 5 EXCITER
- Light division system (405, 458, 488, 514, 543 nm)
- Filtr BP 505-530
- Filtr BP 505-600
- Filtr BP 530-600
- Filtr BP 560-615
- Fitr LP 420
- Filtr BP 420-480
- System ECU LSM 5 EXCITER
- Moduł DIC I/0,9 z polaryzatorem
- Transmited light detectorT-PMT LSM 710

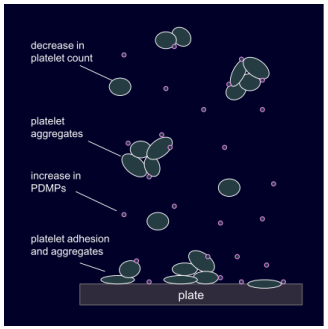
AB 120

Bio-inspired materials



Bio-inspired materials

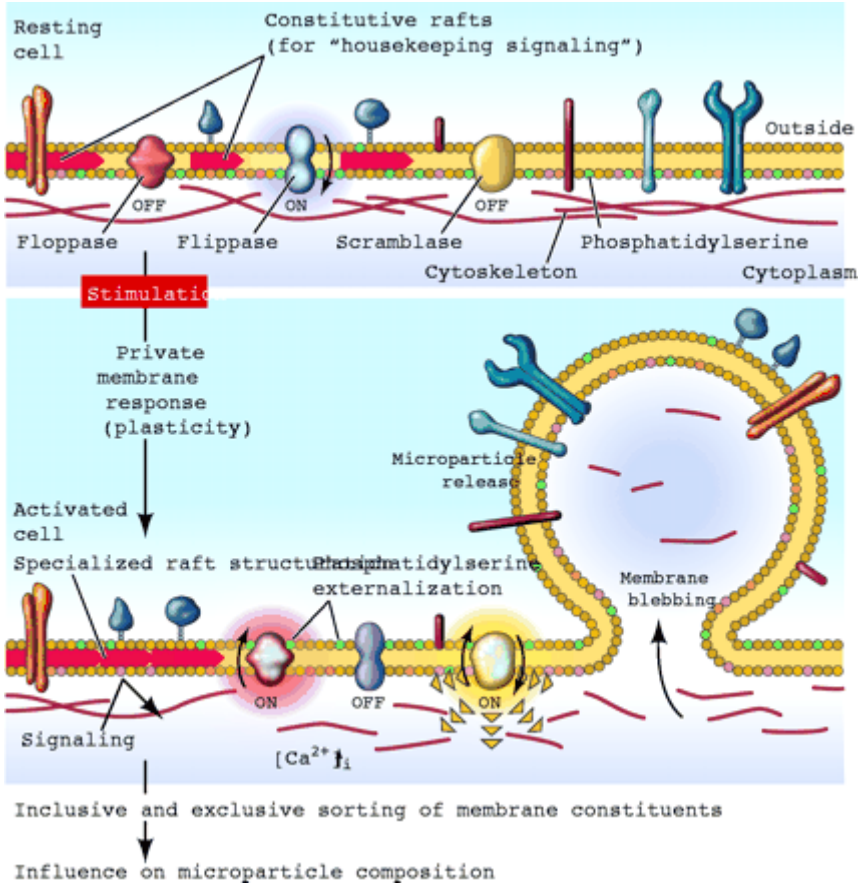


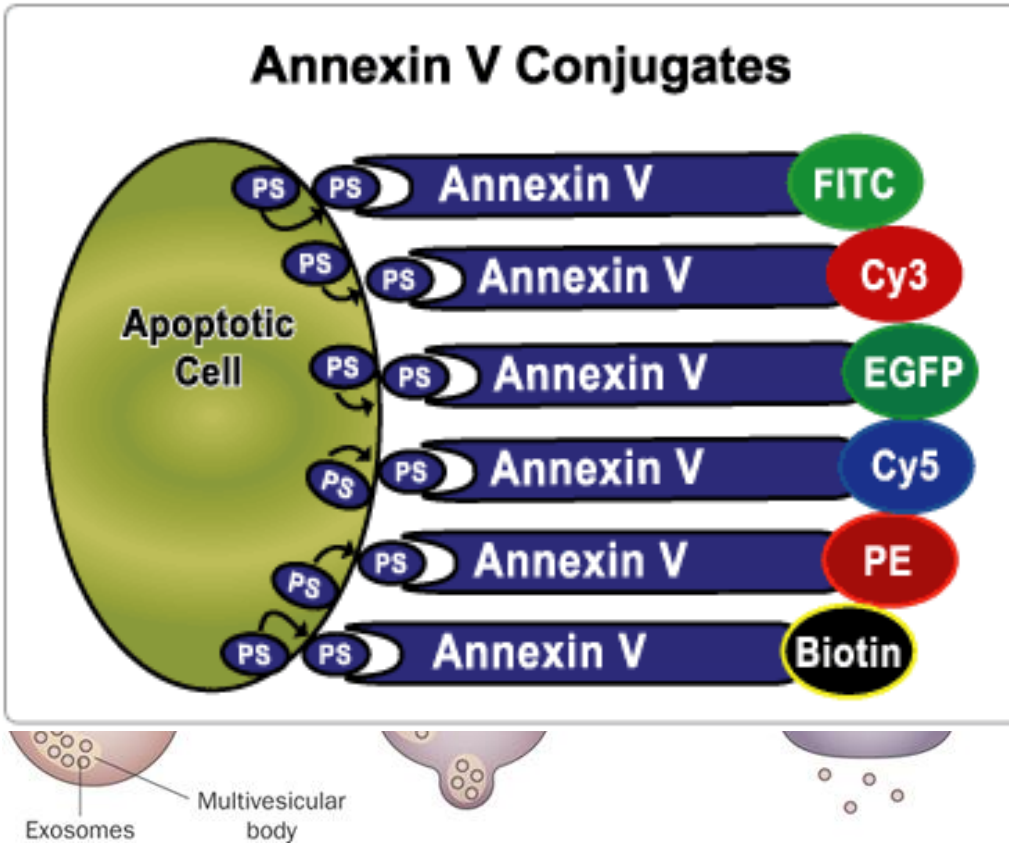


Confocal microscopy

Bio-inspired materials

Microparticles



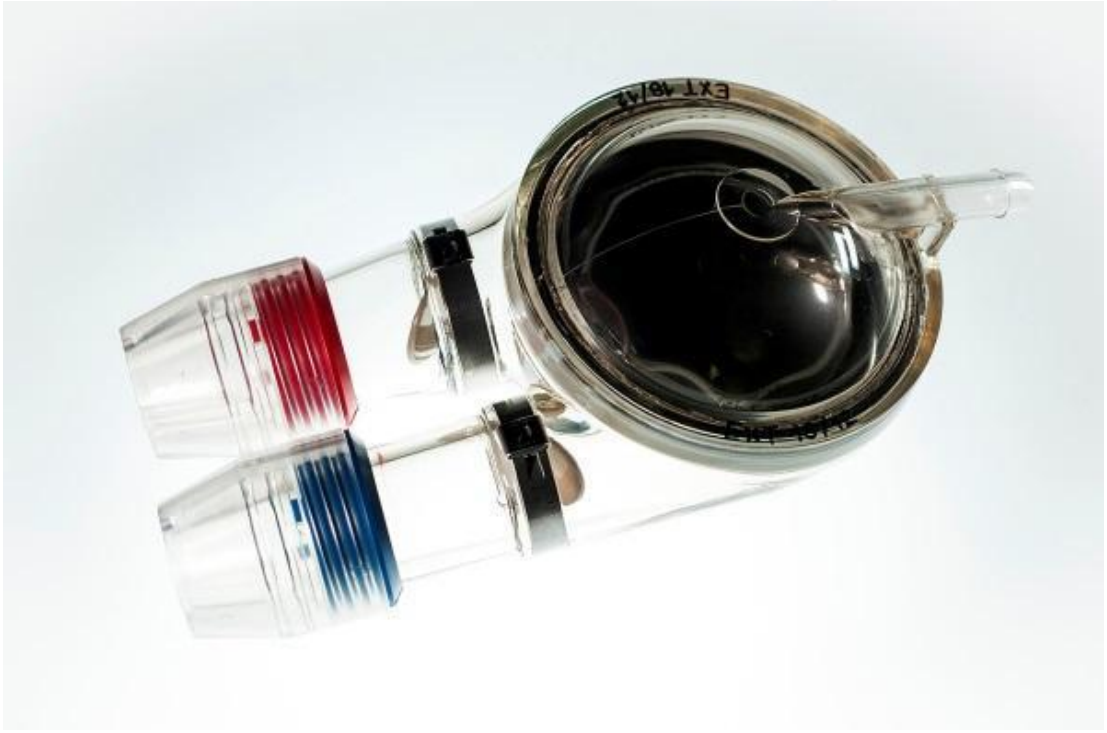


Part 2.3

Executed projects

Cardiovascular system

Artificial materials modification

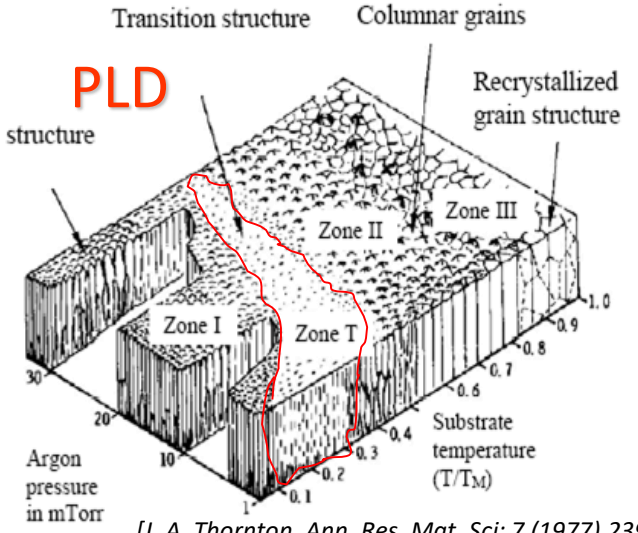


M-ERA.NET
Nonthrombogenic metal-polymer composites with adaptable micro and macro flexibility for next generation heart valves in artificial heart devices

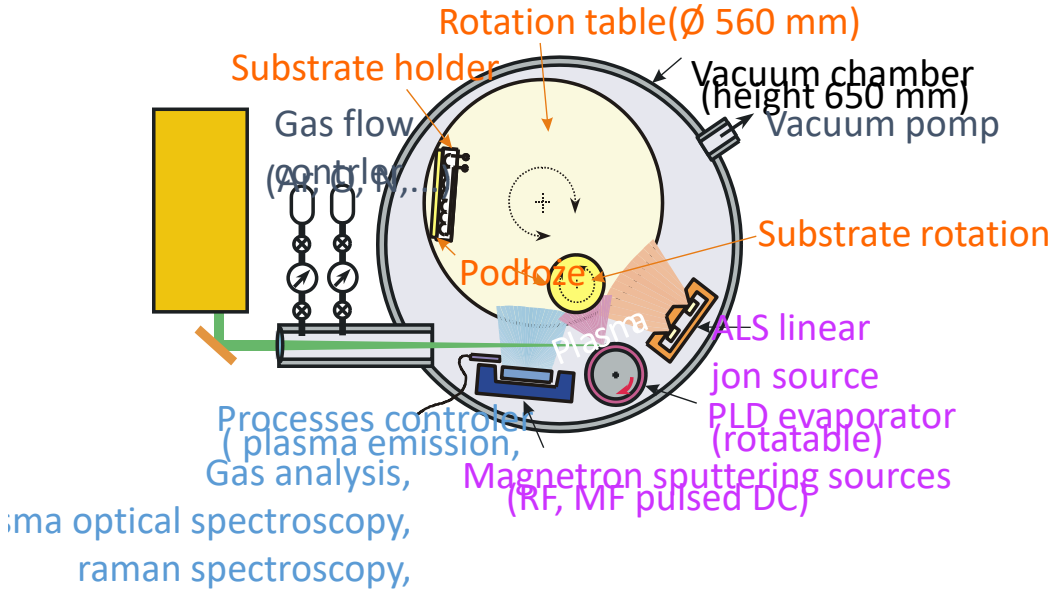
„Artificial Heart Laboratory”

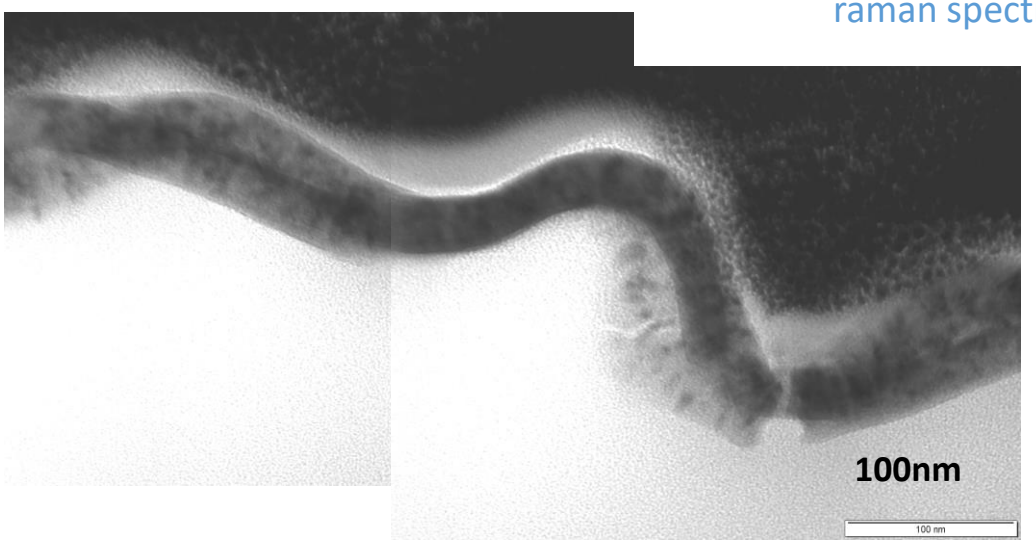
ReligaHeart EXT VAD:

Artificial materials modification



[J. A. Thornton, Ann. Res. Mat. Sci; 7 (1977) 239]





Main assumptions

Good adhesion

Biocompatibility

Lack of substrate degradation

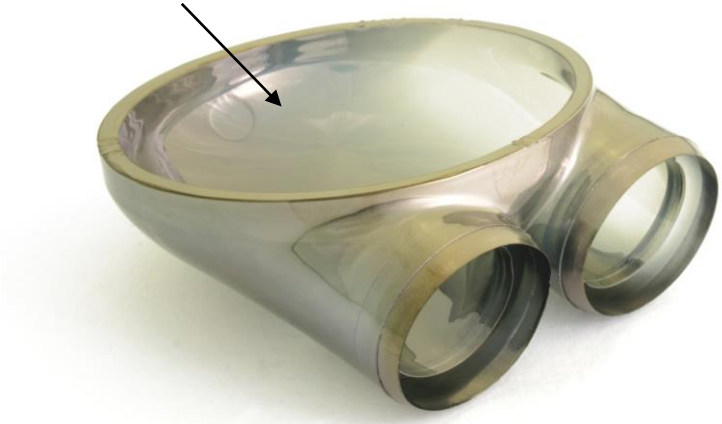
TiN, TiO, Si-DLC, Ti(C,N), DLC

Artificial materials modification

Membrane

The goal of the work is the reduction of life-threatening thrombo-emboli formation in heart assist systems by a new material design

Chamber



Valve

Material development for miniaturized blood rotor

Bogusław MAJOR

Polish Academy of Sciences Institute of Metallurgy and Materials Science

Narodowe Centrum Badań i Rozwoju

Projects:

M-ERA.NET Transnational Call 2014; Project Acronym: **bioVALVE**

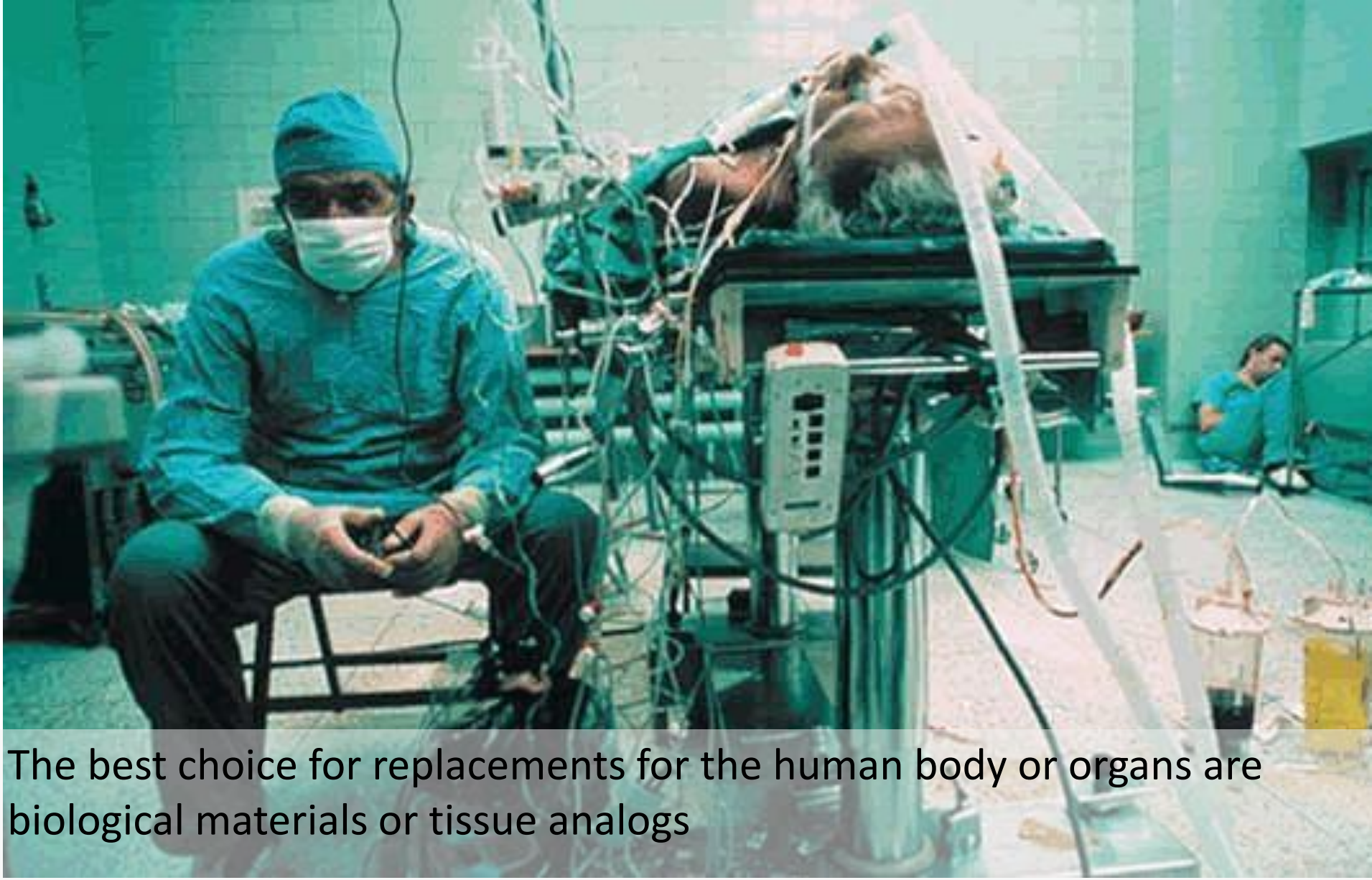
Partners:

JOANNEUM RESEARCH Forschungsges.m.b.H., Institute for Surface Technologies and Photonics, Austria;
Polish Academy of Sciences Institute of Metallurgy and Materials Science in Cracow, Poland;
Collegium Medicum of Jagiellonian University Cracow, Poland;
Foundation for Heart Surgery Development,Poland;
Chirmed Sp. z o.o. Poland

M-ERA.NET Transnational Call 2017; Project Acronym: **4DbloodROT**

Partners:

JOANNEUM RESEARCH Forschungsges.m.b.H., Institute for Surface Technologies and Photonics, Austria;
Polish Academy of Sciences Institute of Metallurgy and Materials Science in Cracow, Poland
Foundation for Heart Surgery Development,Poland;
In-Vision Digital Imaging Optics GhbH, Austria; Lithoz GmbH, Austria;
Polymer Competence Center Leoben GmbH, Austria;
University of Leoben, Institute of Chemistry of Polymeric Materials, Austria,
MESco Sp.z o.o. Poland



The best choice for replacements for the human body or organs are biological materials or tissue analogs

Prof. Zbigniew Religa *watches over the patient during heart transplant surgery*
Photo: Jim Stanfield (1987 National Georgraphic)

Heart failure (HF)-

complex clinical syndrome resulting from any structural or functional impairment of heart ventricular filling or ejection of blood

HF is anepidemic of the 21th century, occurrs in 33% of the population above 55 and 22% above 40 years; in Europe 15 milions patients with HF; responsible for up to 45% of deaths (more than concer)

Heart transplantation is a gold standard for end-stage heart insufficiency;

small numer of donort hearts (covering <0.5 % of the demand

Alternative – application of mechanical heart assist systems LVADs

Implantable rotary blood pumps for continuous flow - technology leader (61% in 2016; 98.3% in 2015)

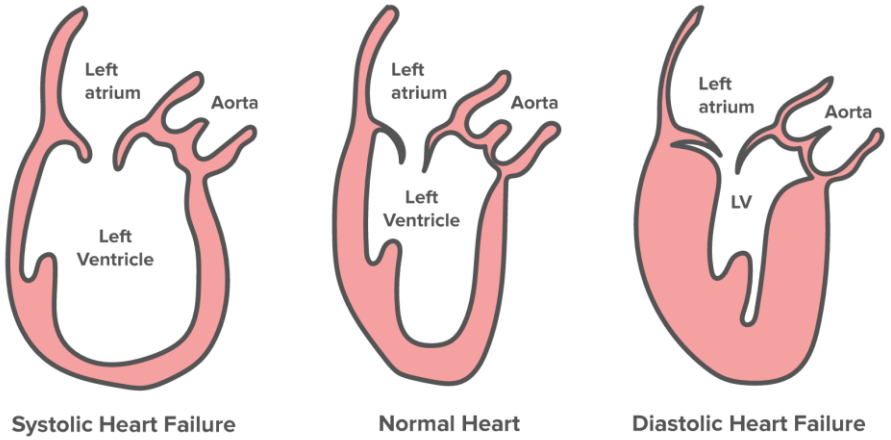
Rotary LVADs consist of a rotor equipped strong permanent magnet for rotor suspension and impulsion by coills in the stator.

LVADs indicate 2 problems: **they are too expensive and far to be free from adverse events**; mainly strokes (trombus formation in the pump and blocking of vessels in distance), bleedings, infections and malfunctions

Heart insuficiency

A set of symptoms caused by damage to the myocardium to an extent that prevents its normal operation.

Failure is defined when the cardiac output and arterial pressure are sufficient to cover the body's current metabolic needs.

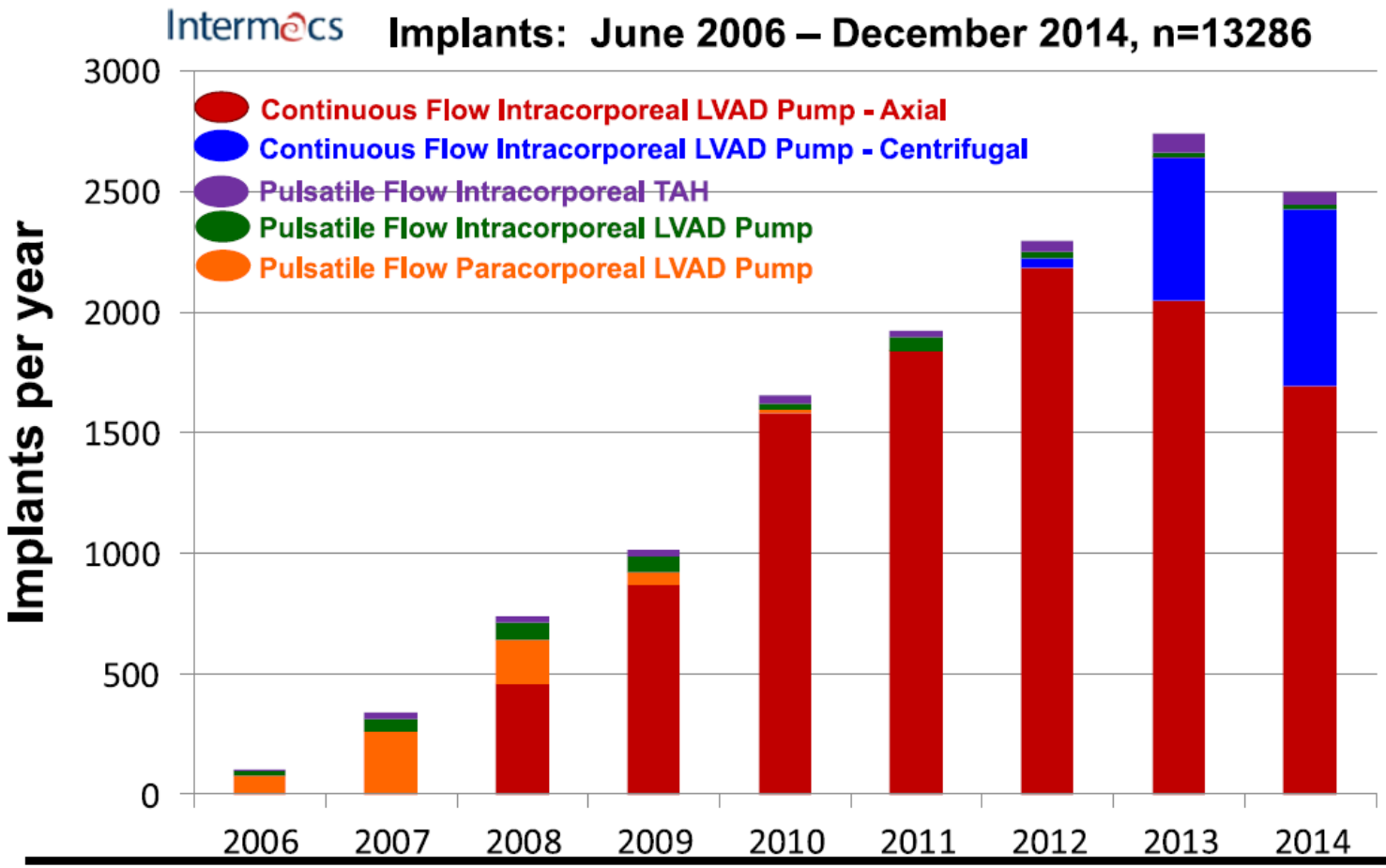


The problem usually affects the right or left ventricle, sometimes both chambers together.

The most common causes of chronic heart failure include:

- ischemic heart disease,
- heart pressure resulting from increased blood pressure,
- cardiomiopathy,
- complications of myocardial infarction
- valve injury
- pericardial disease.

Statistics



	2006	2007	2008	2009	2010	2011	2012	2013	2014
CF Intra Pump/Axial	0	0	459	867	1580	1838	2183	2044	1695
CF Intra Pump/Centrif	0	0	0	0	0	0	38	598	728
PF Intra TAH	1	22	30	24	29	26	41	74	54
PF Intra Pump	76	260	180	54	13	2	0	1	0
PF Para Pump	18	55	72	65	29	54	31	21	24

Heart insuficiency

Heart valve ***states a regulatory part of the circulatory system, responsible for the proper blood flow in the heart.***

The most natural solution in the case of valve pathology beyond standard drug therapy seems to be grafts from dead persons and animals;
it is limited and it is not able to meet the needs of the patiens.

Alternative solutions for transplantation are: artificial heart valves

Currently about 300 000 artificial heart valves are implanted annualy

Mechanical valves can be divided into: ball, single-petal and double-sided

Currently Medtronic or Moll type artificial mechanical artificial valves are in use

Narodowe Centrum Badań i Rozwoju

M-ERA.NET Transnational Call 2014
Project Acronym: bioVALVE

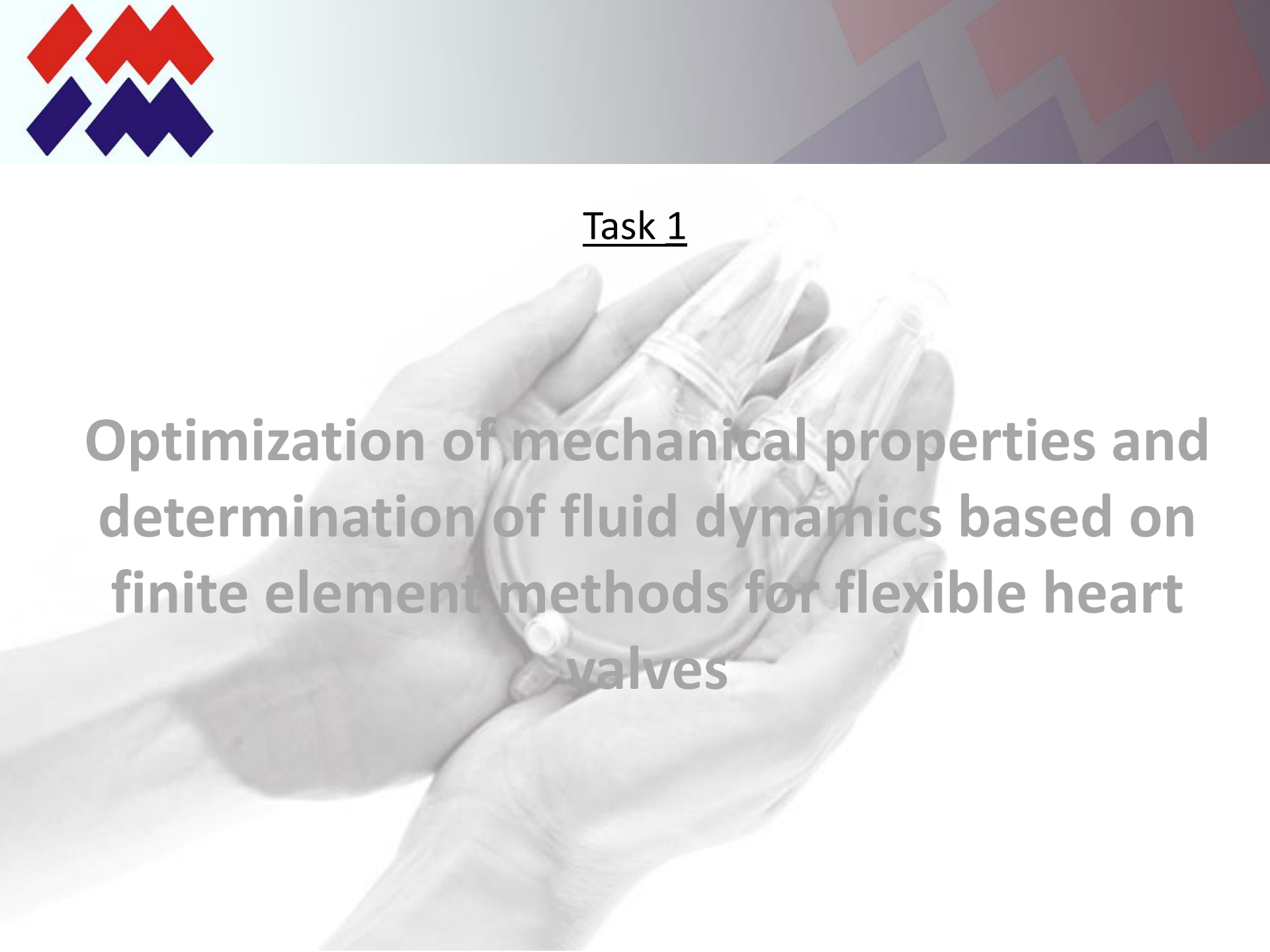
Partners:
JOANNEUM RESEARCH Forschungsges.m.b.H., Institute for Surface Technologies and Photonics, Austria;
Polish Academy of Sciences Institute of Metallurgy and Materials Science in Cracow, Poland;
Collegium Medicum of Jagiellonian University Cracow, Poland;
Foundation for Heart Surgery Development,Poland;
Chirmed Sp. z o.o. Poland

DZP/M-ERA.NET-2014/291/2015 “Nonthrombogenic metal-polymer composites with adaptable micro and macro flexibility for the next generation heart valves in artificial heart devices” of the Polish National Centre of Research and Development.

The aim of project bioVALVE was to life-treatening thrombo-embolii formation in pulsatile assist devices by ***a new biomimetic heart valve design***

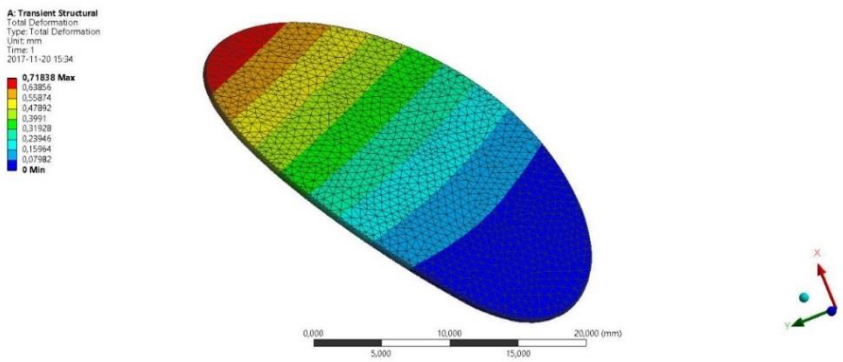
Task 1

Optimization of mechanical properties and determination of fluid dynamics based on finite element methods for flexible heart valves

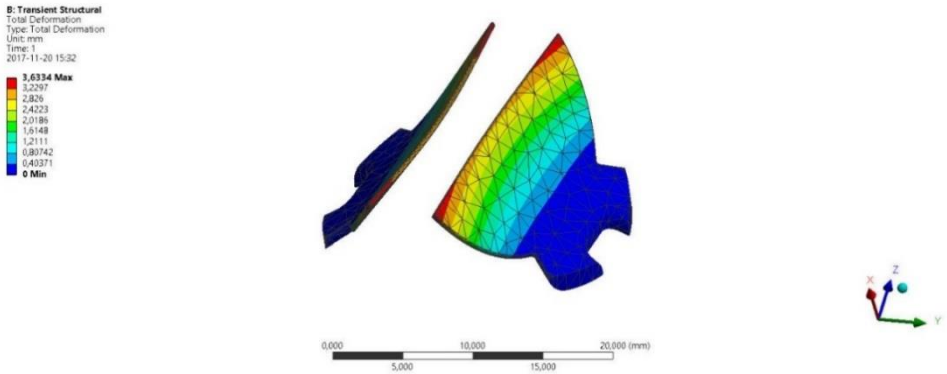


Optimisation

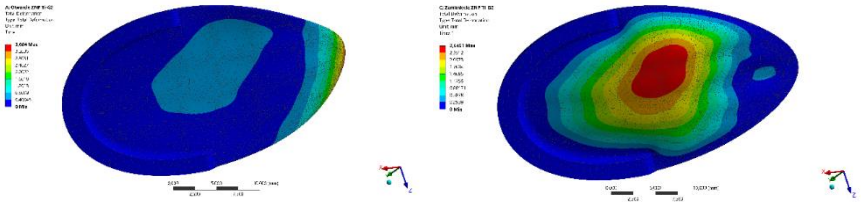
Optimization of mechanical properties and determination of fluid dynamics based on finite element methods for flexible heart valves



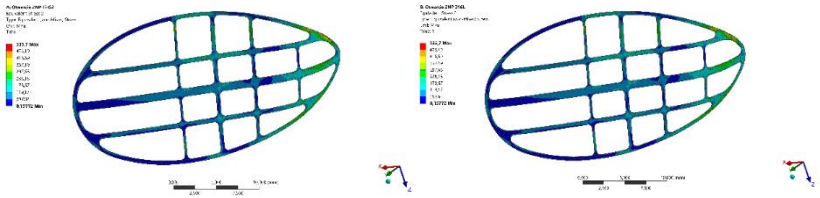
An example drawing of stress simulations in an inflow valve



An example drawing of stress simulations in an outflow valve



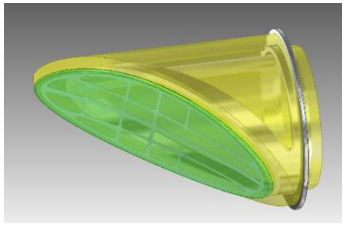
Strain of the inflow petal (Ti mesh) a- opened b- closed

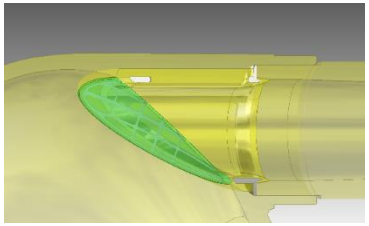


Stress in the inflow petal mesh a- Ti b- Steel

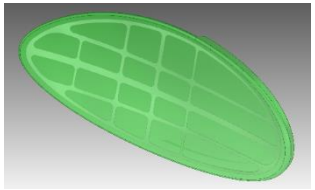
Optimisation

Optimization of mechanical properties and determination of fluid dynamics based on finite element methods for flexible heart valves

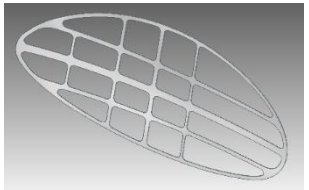




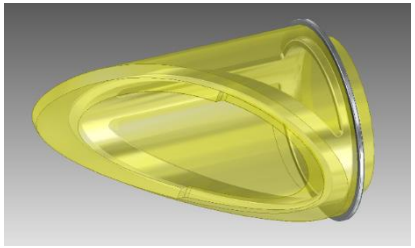
Inflow valve and cross-section through the area of the inflow channel.



a- Petal with embedded reinforcing mesh



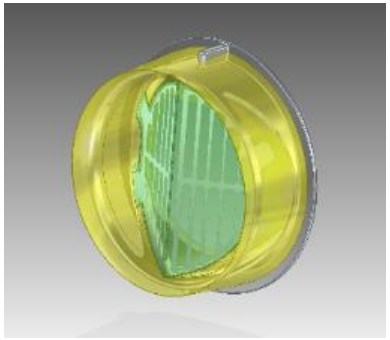
b – titanium reinforcing mesh

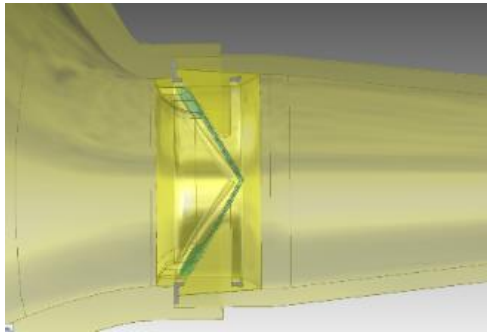


The carrier ring of the petal

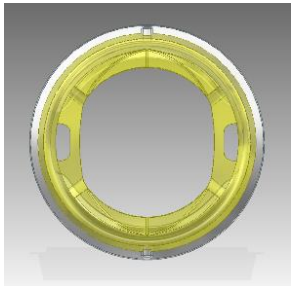
The frame of the ring

Optimisation

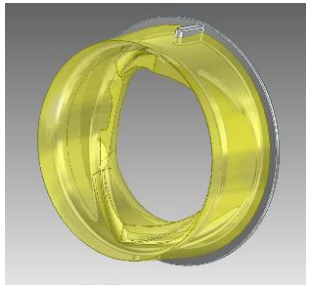




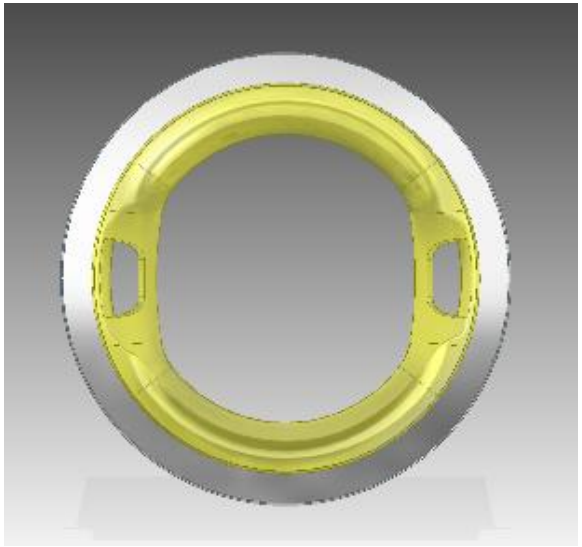
Polyurethane inflow valve and cross section through the outflow channel.



a - Valve ring: view from the outflow side

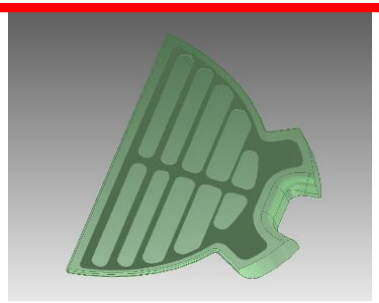


b - Valve ring: general view



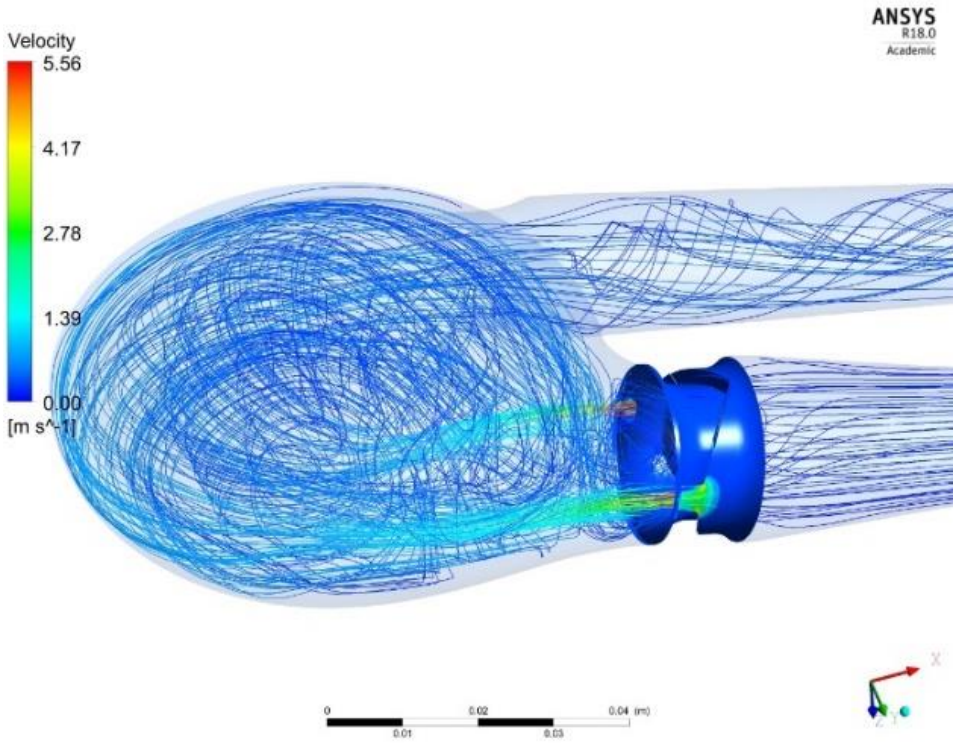
c - Valve ring; view from the inflow side

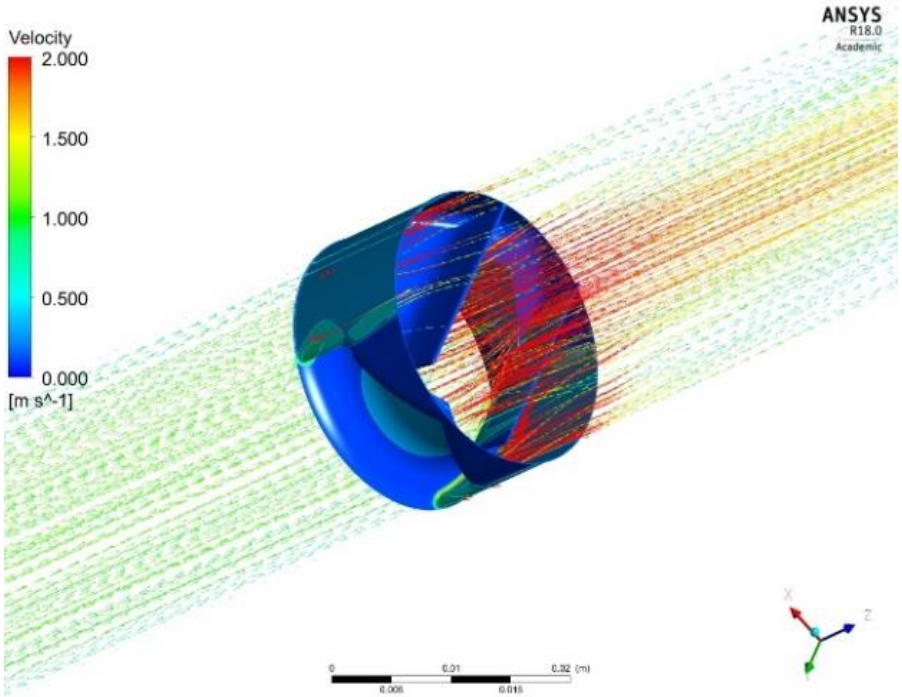
The frame of the ring



Polyurethane flake with covered frame

Optimisation



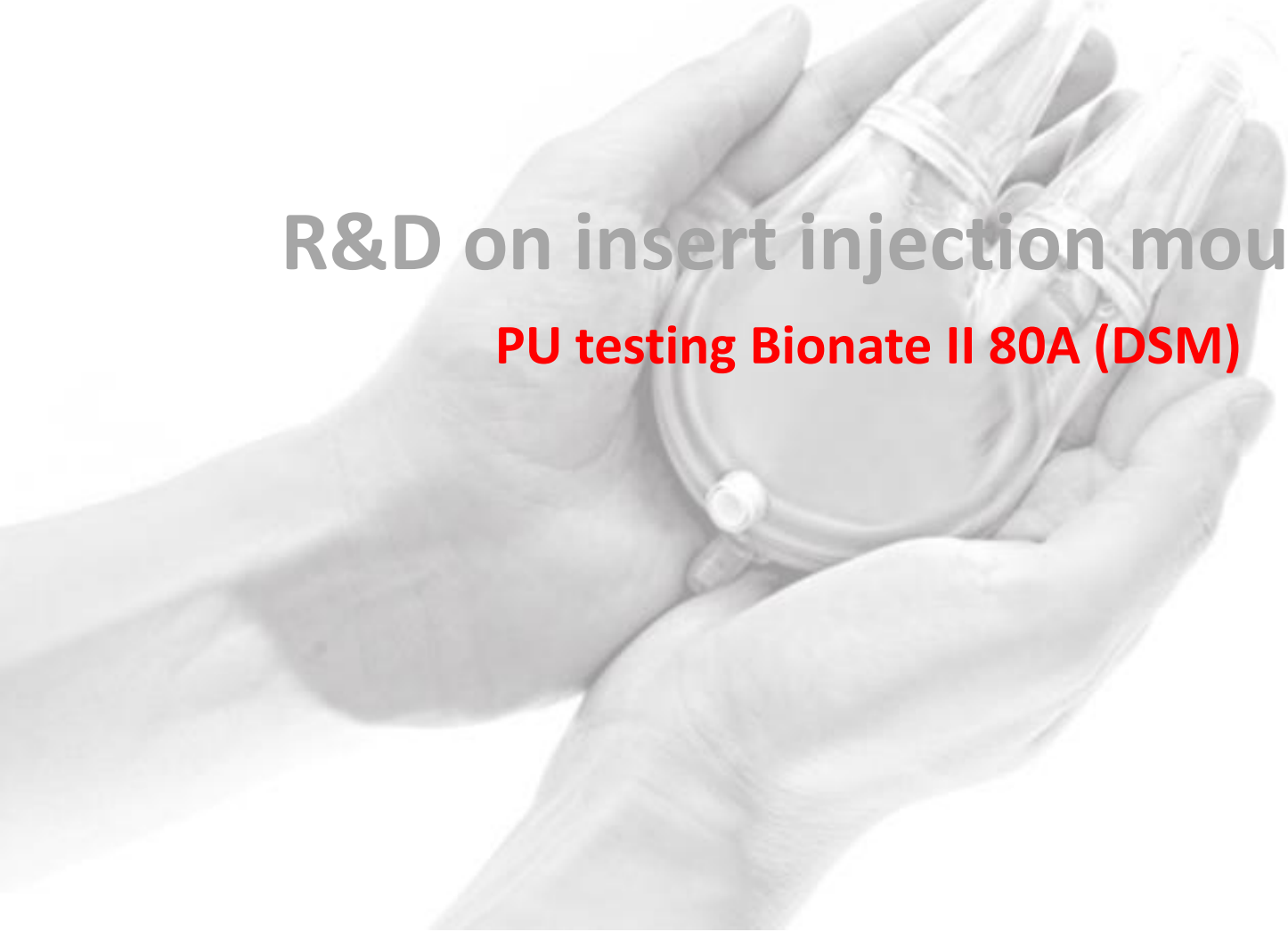


An example drawing of a flow velocity simulation (a) through a outflow valve in a ReligaHeart® heart assist pump equipped with new valve: (b) backflow flushing holes

Task 2

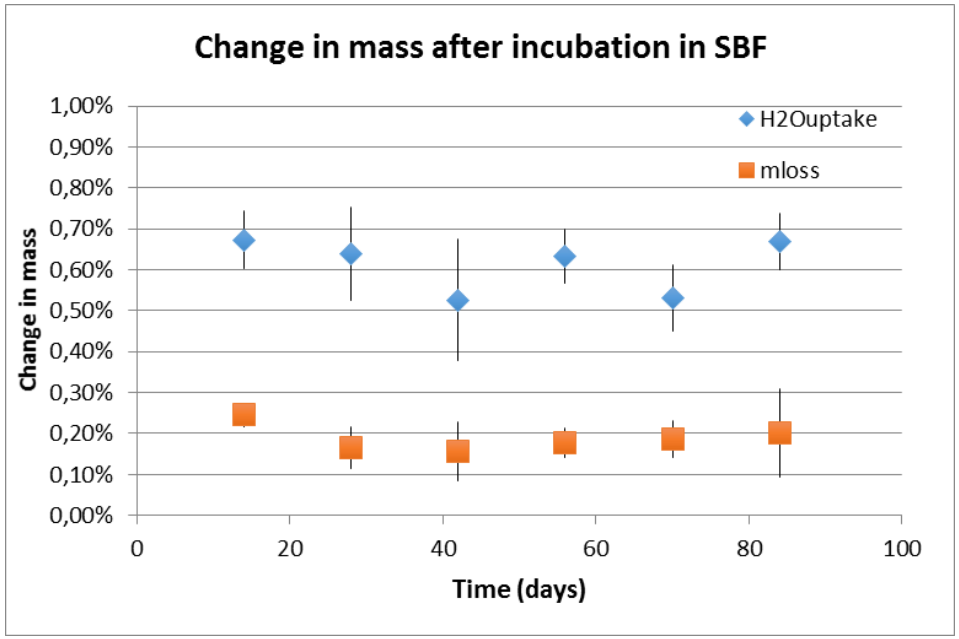
R&D on insert injection moulding

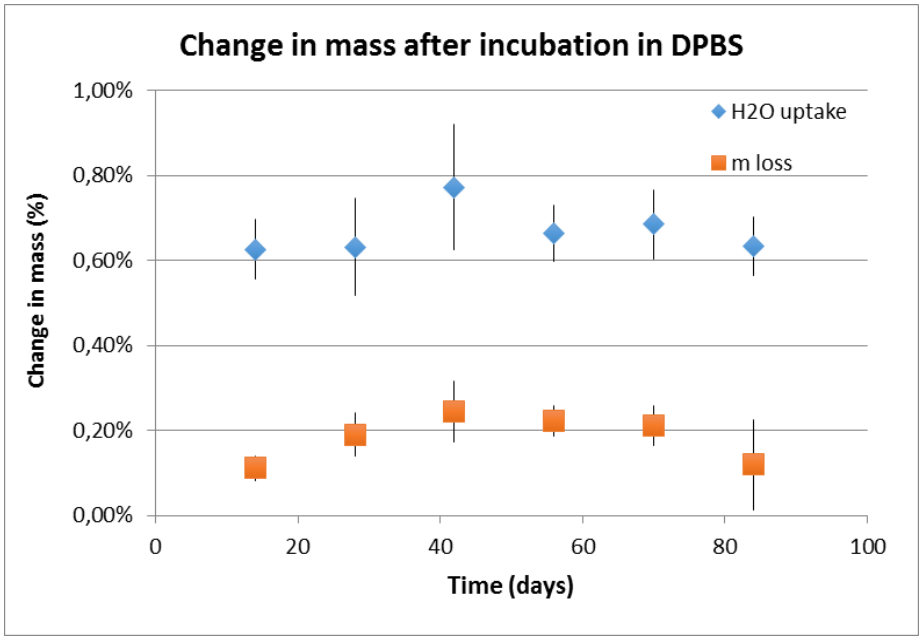
PU testing Bionate II 80A (DSM)



Bionate II 80A (DSM)

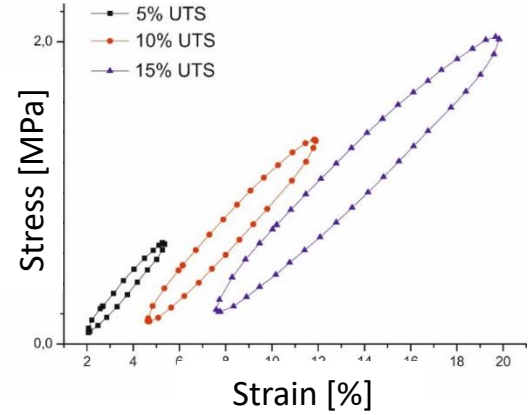
UPTAKE: Bionate II 80A, temperaturę 37 deg, 12 weeks
BIOEROSION: Bionate II 80A, temperaturę 37 deg, 72h



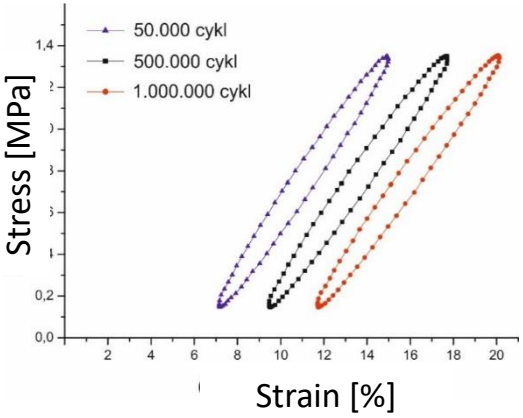


Changes in water absorption **values and mass loss during degradation. a) degradation in SBF; b) degradation in PBS**

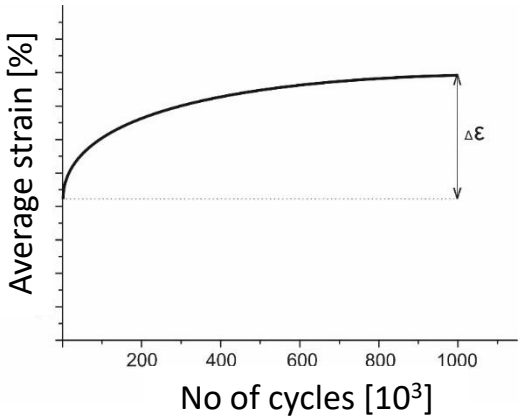
Mechanical testing: Bionate II 80A (DSM)



Hysteresis loops for individual load cycles



Hysteresis loops of the tested material for cycles of 50,000; 500 thousand and 1 million



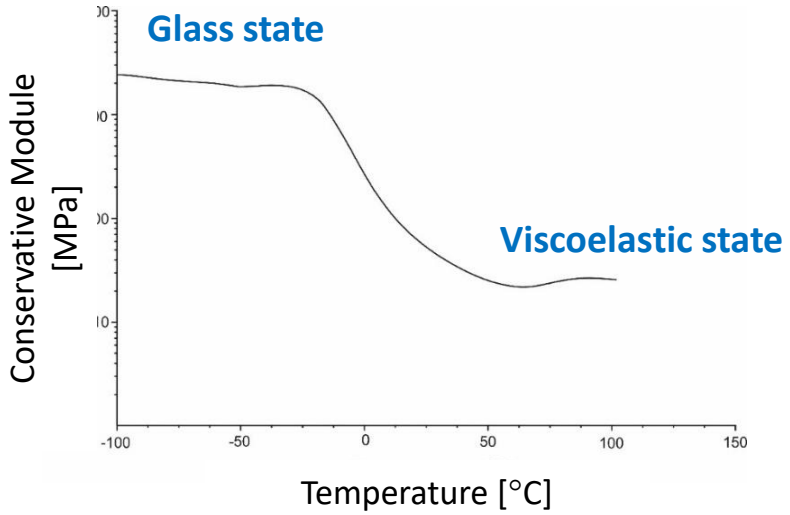
Creep curve of dynamic Bionate 90A polyurethane

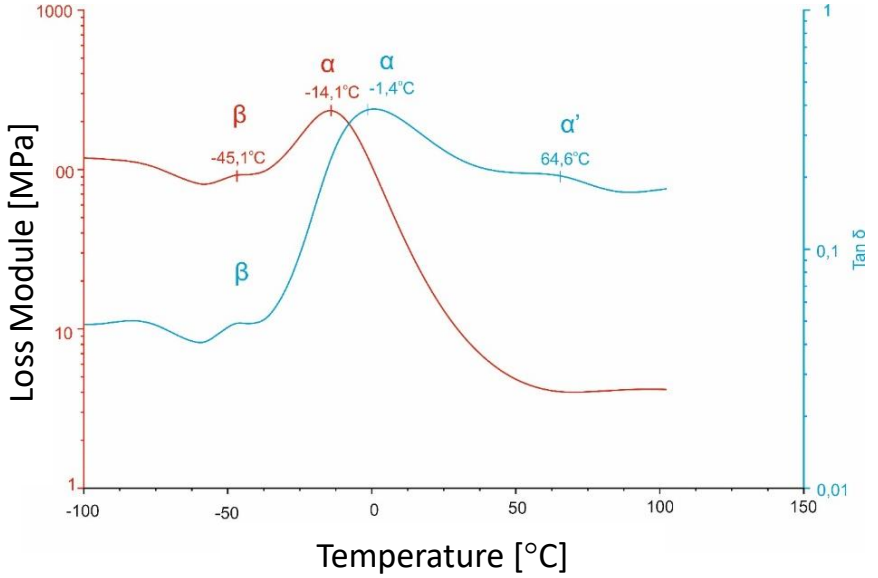
From the course of the hysteresis loop or individual load cycles it follows that as the stress on a particular step increases, the shape of the loop and their surface change significantly. The larger area of the loop indicates high damping.

Despite the low creep resistance, the tested polyurethane Bionate 80A exhibits similar hysteresis loop characteristics, regardless of the number of load cycles. Hysteresis loops are characterized by a small surface area, which indicates low attenuation and thus low dissipation energy (energy dissipation).

On the basis of the dynamic creep curve presented in it is stated that the total creep Δε, defined as the sum of delayed elastic strain εb and material flow εc, for the tested material was about 5.5 %. To the group of significant factors influencing the creep of the material should be not only the size of the load being applied and the duration of the test, but also the temperature and the environment in which the sample is located.

Thermodynamic testing: Bionate II 80A (DSM)





Thermomechanical properties of the Bionate 90A elastomer on the example of: a) a conserving module; b) loss modality and tan δ

As the temperature rises, the characteristic plateau of the conserving module is observed, which includes a third temperature range of +50 °C to +100 °C. On the loss modulus curve and tangent of the loss angle, the maximum associated with the glass transition temperature (-14 °C and -1.4 °C respectively) is visible. In addition, small additional relaxation maxima (β) can be observed in the vitreous state at a temperature of about -45 °C, and in the viscoelastic state (α ') at a temperature of about +65 °C, which corresponds to a slight decrease in the module.

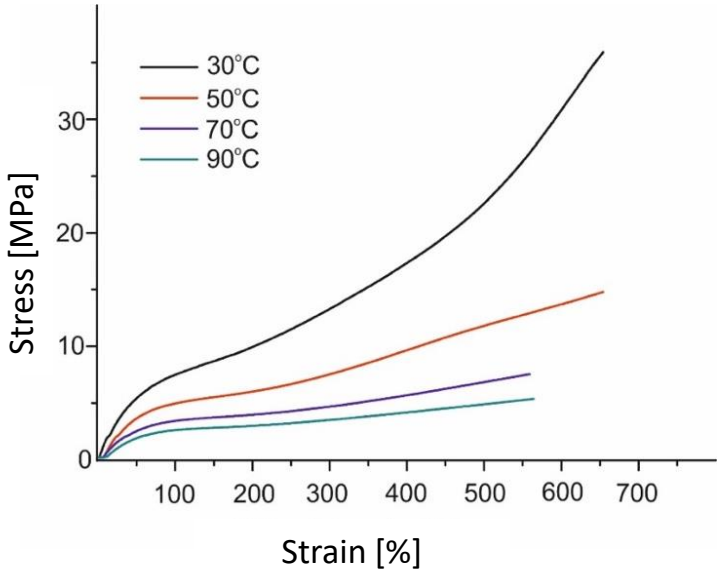
Thermomechanical testing: Bionate II 80A (DSM)

Mechanical properties of the investigated polymers

Temperature (°C)	Young's modulus (MPa)	Secant modulus at 50% strain (MPa)	Modulus at incisal at 100% strain (MPa)	Tensile stress (MPa)	Tensile strain (%)
30	16,6±2,1	10,6±1	7,2±0,5	36,2±1	>650
50	11,9±1,4	7,5±0,1	4,8±0,1	14,5±0,1	>650
70	7,2±0,3	5,1±0,2	3,3±0,2	7,3±0,1	>550
90	5,5±0,6	3,9±0,4	2,6±0,3	5,2±0,2	>550

Rheological research (MFI) of polymers:

The Bionate 90A polymer melt index was tested at two temperatures of +200 °C and +210 °C under a constant load of 2.16 kg. It was impossible to carry out the measurement at lower temperatures due to the very high melt viscosity of the tested elastomer. MFI (+200 °C, 2.16 kg) = 4.73 ± 0.35 [g/10min]; MFI (+210 °C, 2.16kg) = 6.22 ± 0.41 [g/10min]



Stress diagram as a function of deformation of Bionate 90A material for different measurement temperatures; (right) Universal testing machine Instron 3366

From the course of the hysteresis loop or individual load cycles it follows that as the stress on a particular step increases, the shape of the loop and their surface change significantly. **The larger area of the loop indicates high damping.**

The need for reinforcement of the valve through a metal scaffold

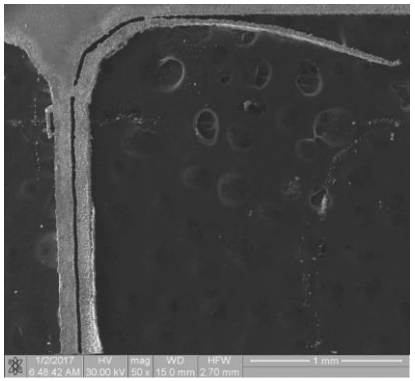
On the basis of the dynamic creep curve presented in it is stated that the total creep Δε, defined as the sum of delayed elastic strain εb and material flow εc, for the tested material was about 5.5 %. To the group of significant factors influencing the creep of the material should be not only the size of the load being applied and the duration of the test, but also the **temperature and the environment in which the sample is located.**

Task 3

R&D of mesh-like metal scaffold with smooth sealing surfaces

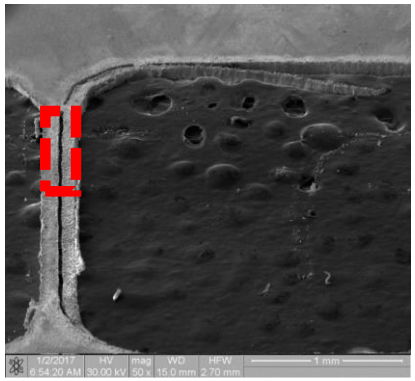
R&D mesh metal

Analysis of defects (SEM)

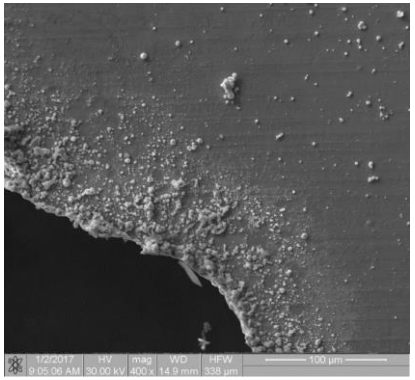


SE

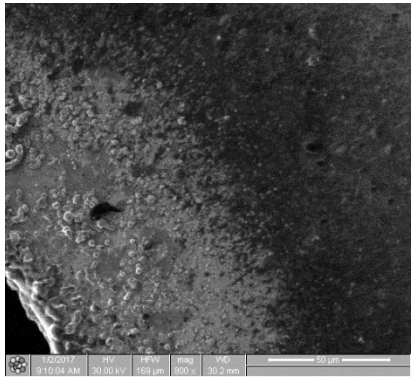
Secondary electrons



SE



SE

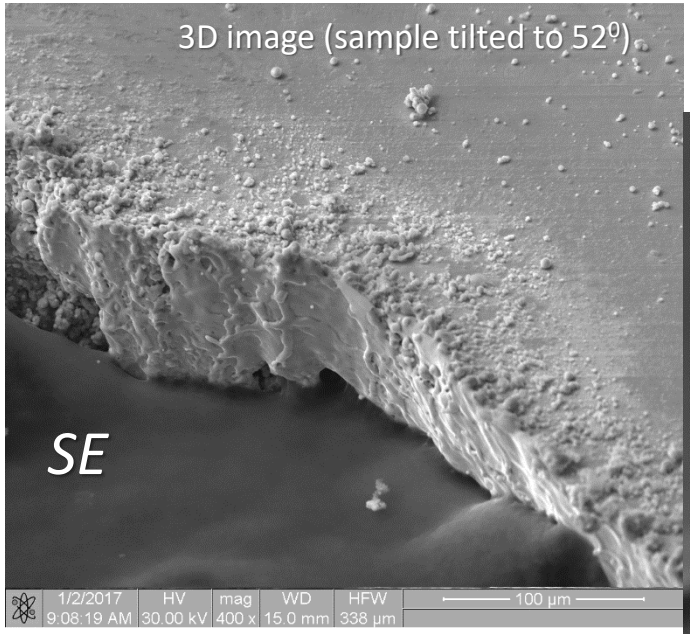


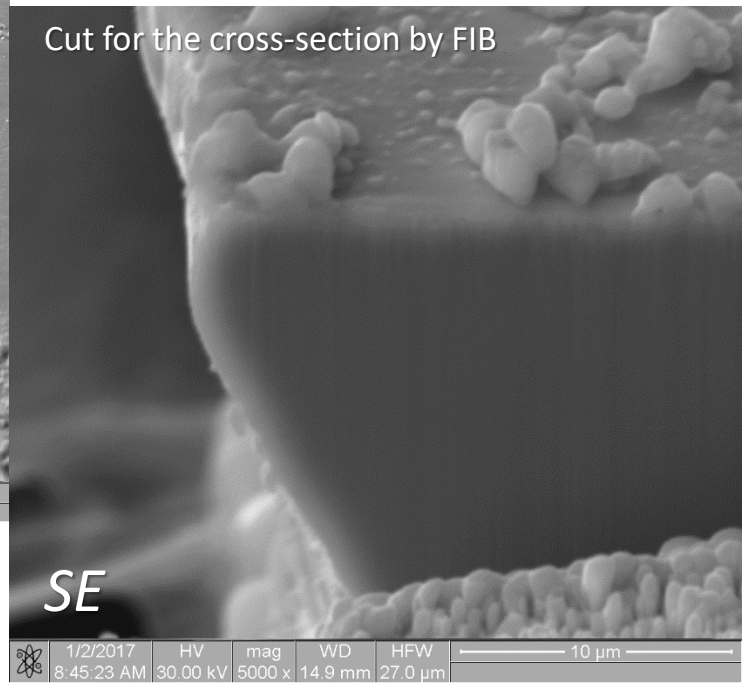
BSE

R&D of mesh-like metal scaffold with smooth sealing surfaces

R&D mesh metal

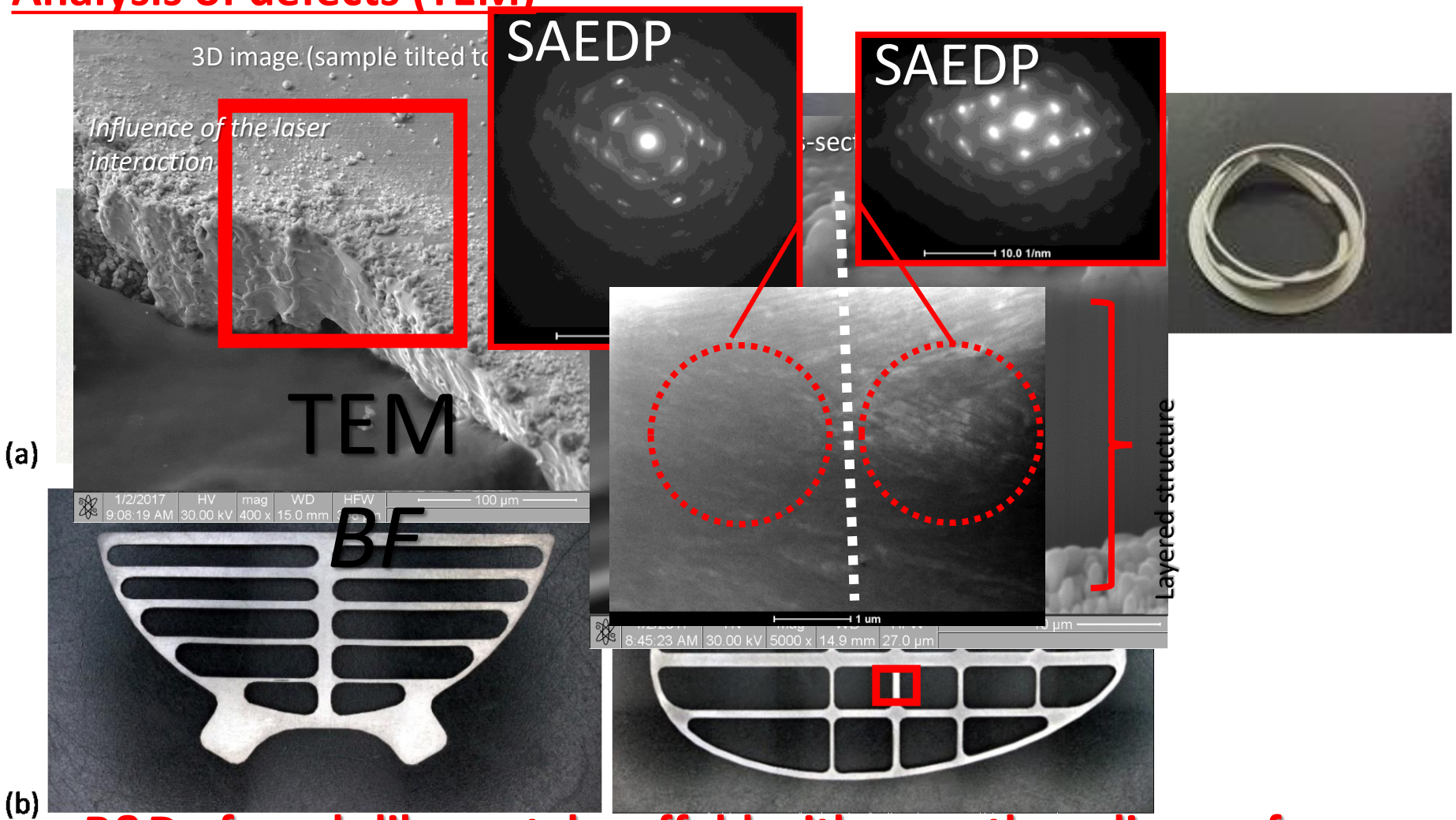
Analysis of defects (SEM)





bio-VALVE

Analysis of defects (TEM)

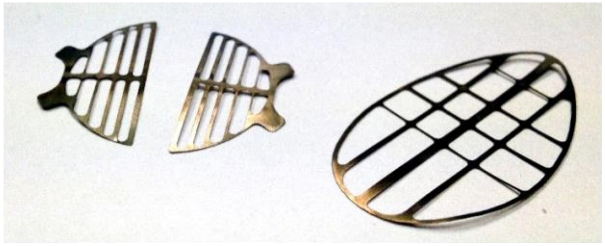


R&D of mesh-like metal scaffold with smooth sealing surfaces

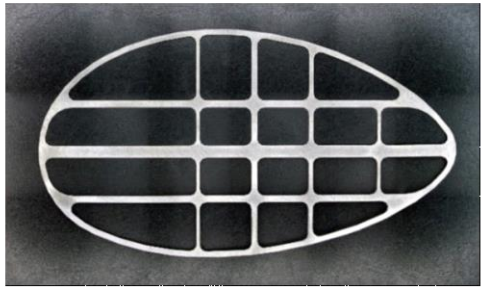
R&D mesh metal

Analysis of defects (adjucement- MESH WITHOUT DEFECTS)

(a)



(b)



R&D of mesh-like metal scaffold with smooth sealing surfaces

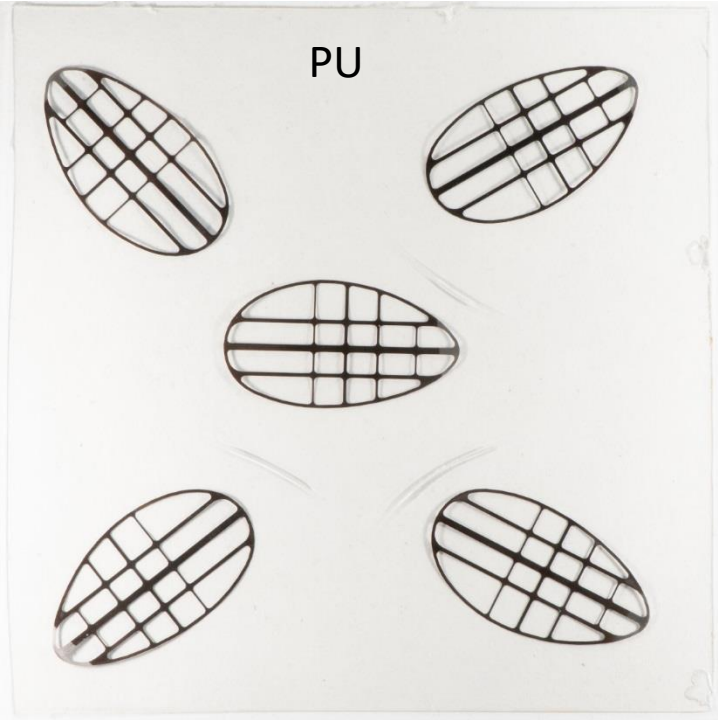
Task 4

R&D of mesh-like metal scaffold covered by polymer

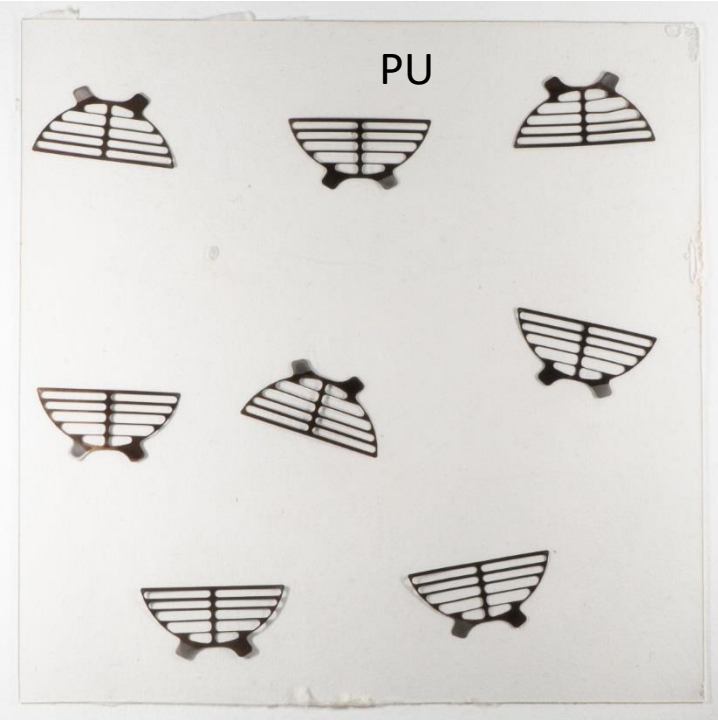
R&D mesh metal+PU

Metallic meshes pressed in PU

inflow valve



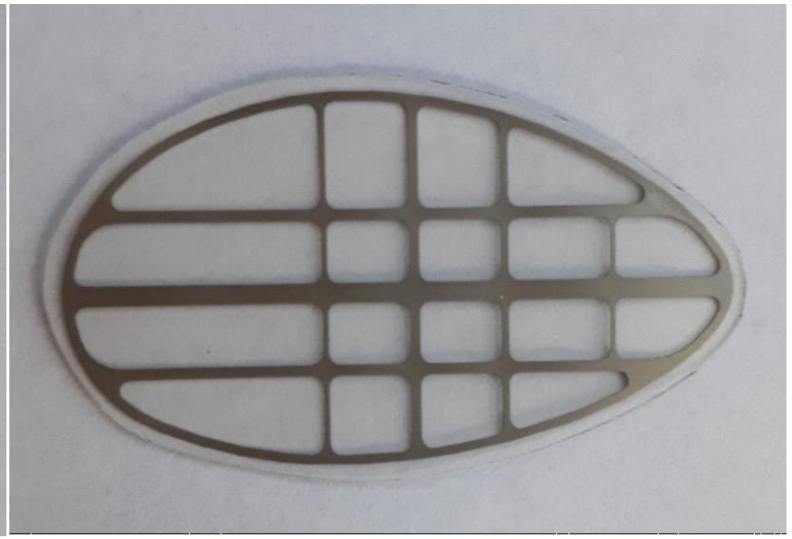
outflow valve



Heart valve testing

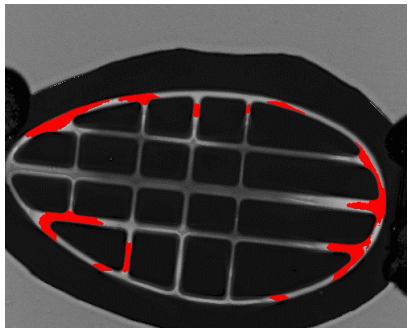
R&D mesh metal+PU

Valve flakes covered with polymer

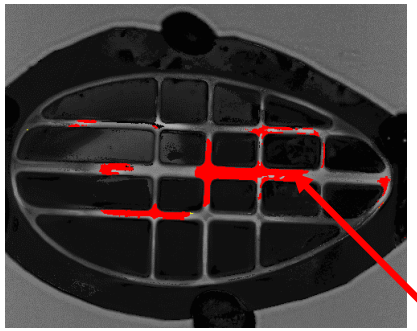


Polymer delamination analysis (SAM)

Tests of adhesion, *i.e. the risk of delamination of the polymer sheath from the metallic frame, were carried out using a scanning acoustic microscope. The acoustic microscope works in pulse reflection mode. The most important element in scanning acoustic microscopy is a high frequency sound wave and a piezoelectric transducer.*

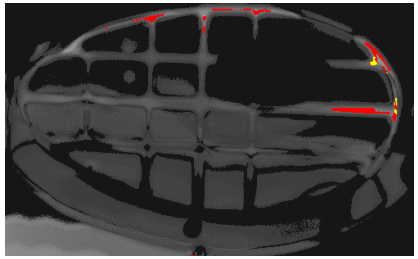


a.)



b.)

Delamination



c.)

Scanning acoustic microscopy (SAM) analysis after wear test

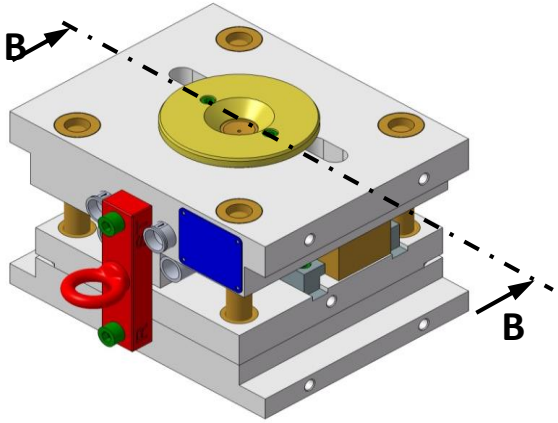
Rings dedicated for inflow and outflow valve- DRAWING

inflow valve

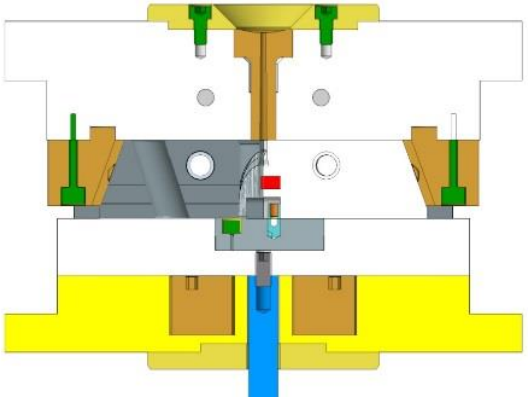
outflow valve

R&D injection moulding

Rings dedicated for inflow and outflow valve- IM PROCESS



Research Form INFLOW VALVE



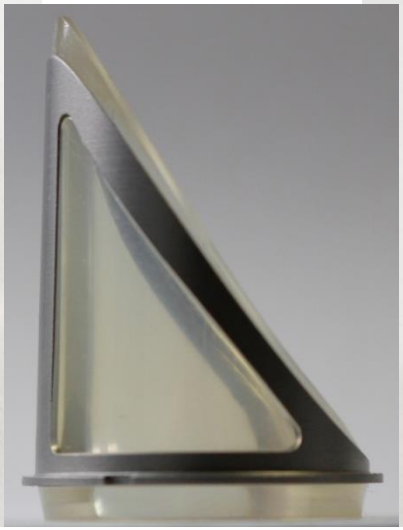
Shape concept of
component INFLOW VALVE

Research form OUTFLOW VALVE.

R&D injection moulding

Inflow valve- PRODUCT






R&D injection moulding

The need for improveent of the metall scaffold surface with hemocompatibile, coatings additionally improving METAL- PU adhesion!

Task 5

R&D on surface coating

R&D on surface coating deposition

Liferschein 2016096

Oznaczenie	Skład						Grubość	Przygotowanie	Atmosfera gazowa	Chropowatość	Kąt	Energia powierzchniowa		
	a-C:H	a-C:H:N	a-C:N	ta-C:H	ta-C:H:N	Si - a-C:H	[nm]		[sccm]	Ra [nm]	zwilżania	polar	dispers	Summe
B334_7				x			92,2	Ionenquelle	20,0 C_2H_2		79,9	3,5	40,0	43,4
B334_8				x			48,0	Ionenquelle	20,0 C_2H_2		78,3	3,5	42,4	45,9
B334_10				x			67,4	Ionenquelle	20,0 C_2H_2		79,7	3,3	41,4	44,6
B334_12				x			90,8	Ionenquelle	5,0 Ar + 15,0 C_2H_2		84,0	2,1	40,8	43,0
B372_6	x						94,7	sputtern	24,0 Ar + 6,0 C_2H_2	14,7	60,82	9,68	46,74	56,43
B372_7		x					103,8	sputtern	24,0 Ar + 4,5 C_2H_2 + 1,5 N_2	14,2	58,53	10,17	48,82	58,99
B372_8		x					103,1	sputtern	24,0 Ar + 3,0 C_2H_2 + 3,0 N_2	12,7	57,83	11,55	45,54	57,09
B372_9		x					95,2	sputtern	24,0 Ar + 1,5 C2H2 + 4,5 N2	24,0	53,03	13,70	46,55	60,25
B372_10			x				95,2	sputtern	24,0 Ar + 6,0 N_2	19,0	45,90	17,14	47,45	64,59
B372_15				x			94,7	Ionenquelle	20,0 C_2H_2	13,0	41,87	20,94	43,6	64,54
B372_16					x		110,2	Ionenquelle	17,5 C_2H_2 + 2,5 N_2	23,3	25,37	30,09	41,61	71,70
B372_17					x		100,3	Ionenquelle	15,0 C_2H_2 + 5,0 N_2	12,7	32,63	24,61	45,87	70,49
B372_18					x		82,7	Ionenquelle	12,5 C_2H_2 + 7,5 N_2	14,3	38,80	25,99	36,90	62,89
B372_23						x	124,3	sputtern	28,2 Ar + 1,8 C_2H_2	16,3	27,80	27,07	45,35	72,42
B372_24						x	15,0	sputtern	28,2 Ar + 1,8 C_2H_2	41,3	22,93	29,66	44,17	73,,83
B372_25						x	127,6	sputtern	27,0 Ar + 3,0 C_2H_2	19,2	19,37	29,47	46,89	76,36
B372_26						x	15,0	sputtern	27,0 Ar + 3,0 C_2H_2	38,3	25,97	29,98	41,36	71,33
B372_27						x	123,5	sputtern	24,0 Ar + 6,0 C_2H_2	26,2	24,35	27,62	47,07	74,69
B372_28						x	15,0	sputtern	24,0 Ar + 6,0 C_2H_2	27,7	23,33	30,11	43,05	73,16
B372_29						x	118,5	sputtern	18,0 Ar + 12,0 C_2H_2	14,0	54,33	11,98	49,66	61,64
B372_30						x	15,0	sputtern	18,0 Ar + 12,0 C_2H_2	52,7	51,17	14,28	47,68	61,96

Group	Symbol	Layer	Thickness [nm]
1	B372_6	a-C:H	94,7
	B372_7	a-C:H,N	103,8
	B372_8	a-C:H,N	103,1
	B372_9	a-C:H,N	95,2
	B372_10	a-C:N	95,1
2	B372_15	a-C:H	94,7
	B372_15+E059	a-C:H+SF6 (fluoridation)	94,7
	B372_16	a-C:H,N	110,3
	B372_17	a-C:H,N	100,3
	B372_17+E059	a-C:H,N+SF6 (fluoridation)	100,3
	B372_18	a-C:H,N	82,7
3a	B372_23	Si/a-C:H	124,3
	B372_25	Si/a-C:H	127,6
	B372_27	Si/a-C:H	123,5
	B372_29	Si/a-C:H	118,5
3b	B372_24	Si/a-C:H	15
	B372_26	Si/a-C:H	15
	B372_28	Si/a-C:H	15
	B372_30	Si/a-C:H	15

R&D on surface coating

R&D on surface coating deposition

Blood-Material Interaction

Whole human blood dynamic tests

ELSEVIER

Biomolecular Engineering 19 (2002) 91–96

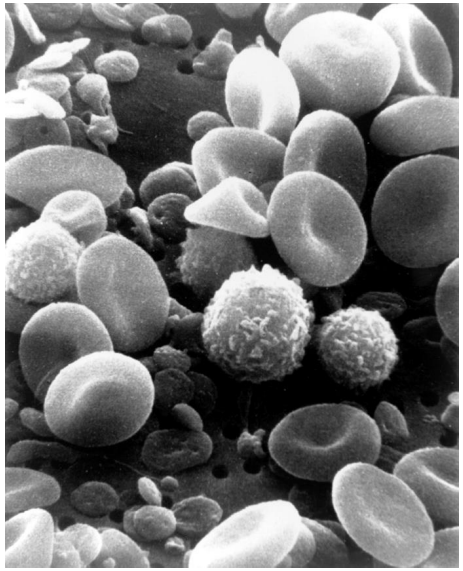
Biomolecular Engineering

www.elsevier.com/locate/geneanbioeng

In vitro hemocompatibility testing of biomaterials according to the ISO 10993-4

Ulrich Theo Seyfert [a,*], Volker Biehl [b], Joachim Schenk [a]

[a] *Abteilung Klinische Hämostaseologie und Transfusionsmedizin, Haus 75, Universitätskliniken, D-66421 Homburg, Germany*
[b] *Universität des Saarlandes, Lehrstuhl für Metallische Werkstoffe, Im Stadtwald, D-66123 Saarbrucken, Germany*



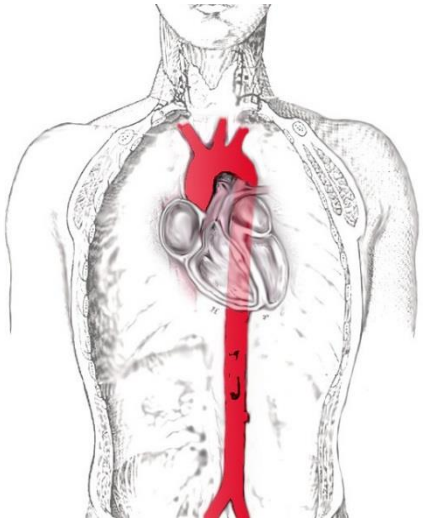
”A hemocompatibile material must not adversely interact with any blood component”

R&D on surface coating

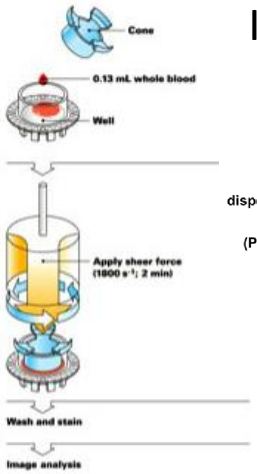
R&D on surface coating deposition

Blood-Material Interaction

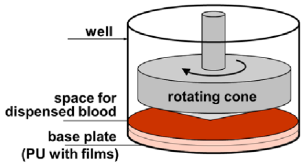
- Human blood (4 x 4.5 mL)
- ADP activation 5 min.
- Arterial flow condition simulation.
- 130 μL after the test.

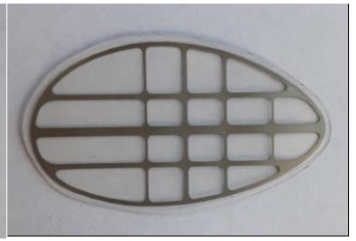


Arterial flow conditions on the flat surfaces



Impact R test



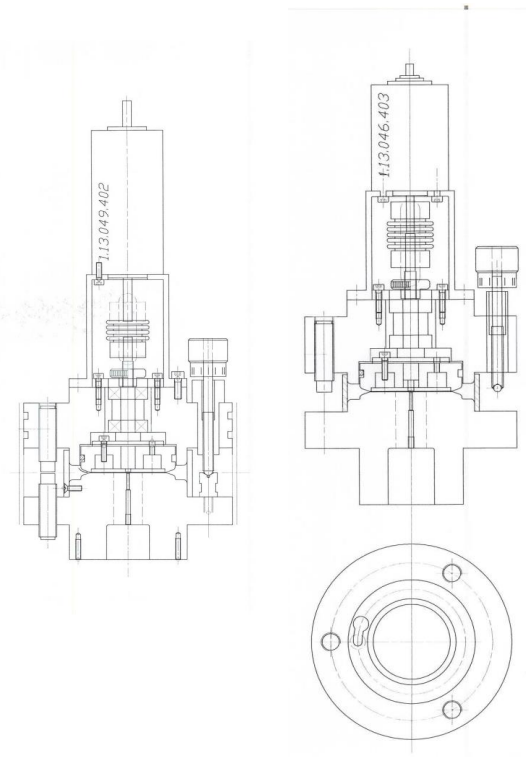


The blood-material interaction performed under the high shear stress conditions

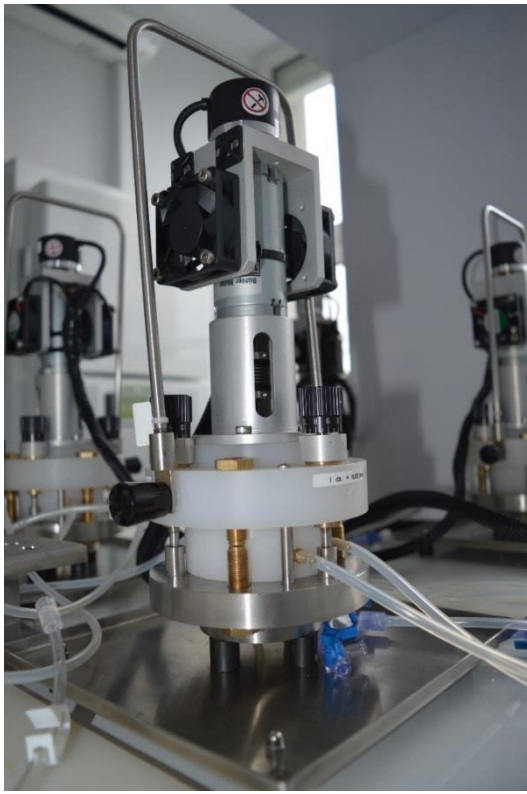
R&D on surface coating

R&D on surface coating deposition

Haemocompatibility in dynamic conditions



High speed rotor 3000-5000 rpm



R&D on surface coating

deposition: P-selectin activation

Blood-Material Interaction

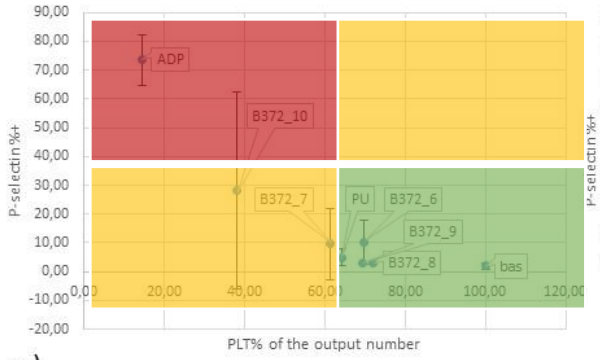
Hemocompatibility in dynamic conditions

P-selectin is protein that in humans is encoded by the SELP gene. P-selectin functions as a cel adhesive molecule
P-selectin- anti-selectin-P antibody

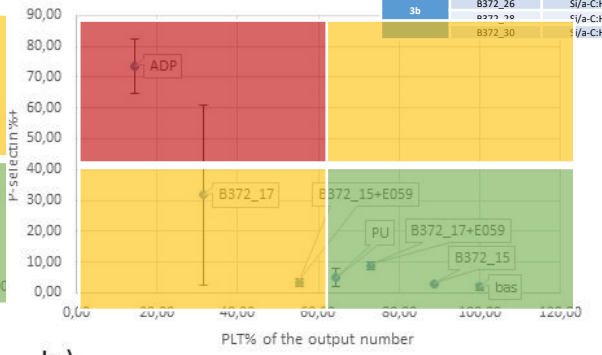
Integrin beta-3 (β3) encoded by the ITGB3 gene. CD61 is a cluster of differentiation found on thrombocytes.

negative	neutral
neutral	positive

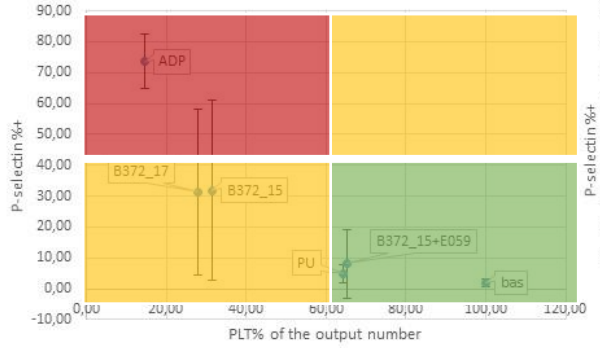
Group	Symbol	Layer	Thickness [nm]
1	B372_6	a-C:H	94,7
	B372_7	a-C:H,N	103,8
	B372_8	a-C:H,N	103,1
	B372_9	a-C:H,N	95,2
	B372_10	a-C:N	95,1
2	B372_15	a-C:H	94,7
	B372_15+E059	a-C:H+SF6 (fluoridation)	94,7
	B372_16	a-C:H,N	110,3
	B372_17	a-C:H,N	100,3
	B372_17+E059	a-C:H,N+SF6 (fluoridation)	100,3
3a	B372_18	a-C:H,N	82,7
	B372_23	Si/a-C:H	124,3
	B372_25	Si/a-C:H	127,6
	B372_27	Si/a-C:H	123,5
	B372_29	Si/a-C:H	118,5
3b	B372_24	Si/a-C:H	15
	B372_26	Si/a-C:H	15
	B372_28	Si/a-C:H	15
	B372_30	Si/a-C:H	15



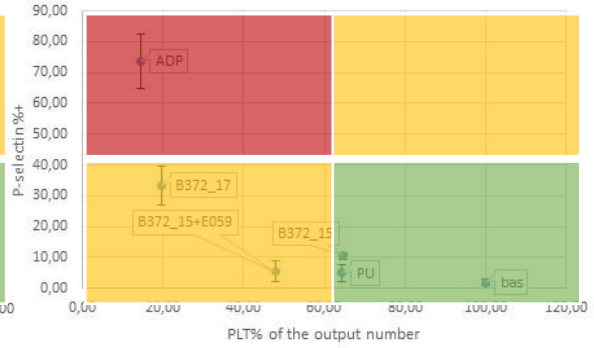
a.)



b.)



c.)



d.)

R&D on surface coating

R&D on surface coating deposition : IIb/IIIa activation

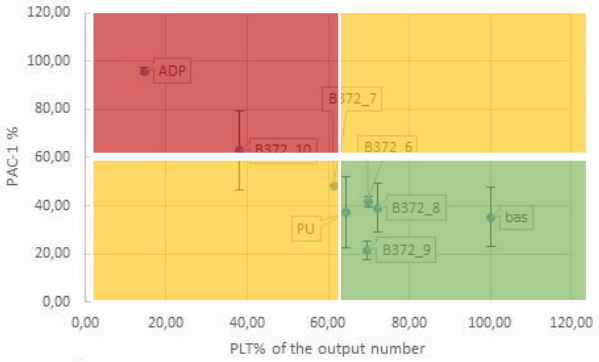
negative	neutral
neutral	positive

Group	Symbol	Layer	Thickness [nm]
1	B372_6	a-C:H	94,7
	B372_7	a-C:H,N	103,8
	B372_8	a-C:H,N	103,1
	B372_9	a-C:H,N	95,2
	B372_10	a-C:N	95,1
2	B372_15	a-C:H	94,7
	B372_15+E059	a-C:H+SF6 (fluoridation)	94,7
	B372_16	a-C:H,N	110,3
	B372_17	a-C:H,N	100,3
	B372_17+E059	a-C:H,N+SF6 (fluoridation)	100,3
	B372_18	a-C:H,N	82,7
3a	B372_23	Si/a-C:H	124,3
	B372_25	Si/a-C:H	127,6
	B372_27	Si/a-C:H	123,5
	B372_29	Si/a-C:H	118,5
3b	B372_26	Si/a-C:H	15
	B372_28	Si/a-C:H	15
	B372_30	Si/a-C:H	15

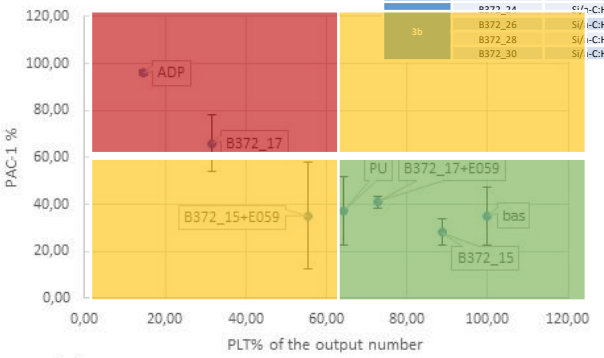
Haemocompatibility in dynamic conditions

Blood-Material Interaction

GP IIb/IIIa is an integrilin complex found on platelets. It is a receptor for fibrynogen and von Willebrand factor and aids platelet activation

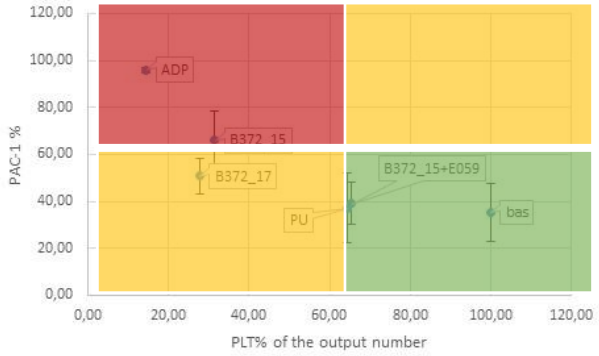


a.)



b.)

Integrin beta-3 (β3) encoded by the ITGB3 gene. CD61 is a cluster of differentiation found on thrombocytes.



c.)

d.)

R&D on surface coating

R&D on surface coating deposition

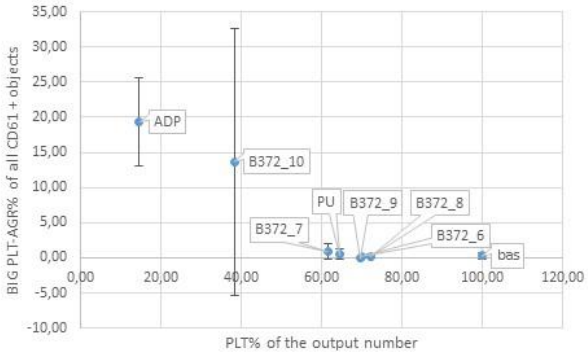
negative	neutral
neutral	positive

Group	Symbol	Layer	Thickness [nm]
1	B372_6	a-C:H	94,7
	B372_7	a-C:H,N	103,8
	B372_8	a-C:H,N	103,1
	B372_9	a-C:H,N	95,2
	B372_10	a-C:N	95,1
2	B372_15	a-C:H	94,7
	B372_15+E059	a-C:H+SF6 (fluoridation)	94,7
	B372_16	a-C:H,N	110,3
	B372_17	a-C:H,N	100,3
	B372_17+E059	a-C:H,N+SF6 (fluoridation)	100,3
	B372_18	a-C:H,N	82,7
3a	B372_23	Si/a-C:H	124,3
	B372_25	Si/a-C:H	127,6
	B372_27	Si/a-C:H	123,5
	B372_29	Si/a-C:H	118,5
3b	B372_24	Si/a-C:H	15
	B372_26	Si/a-C:H	15
	B372_28	Si/a-C:H	15
	B372_30	Si/a-C:H	15

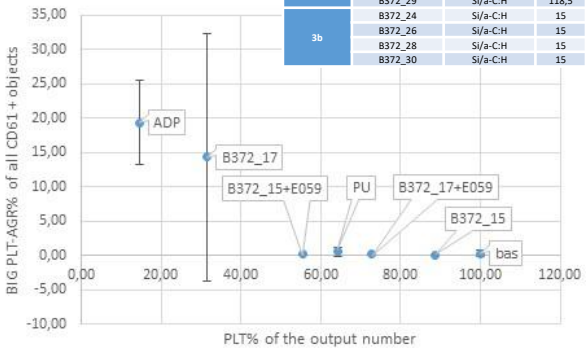
Haemocompatibility in dynamic conditions

Blood-Material Interaction

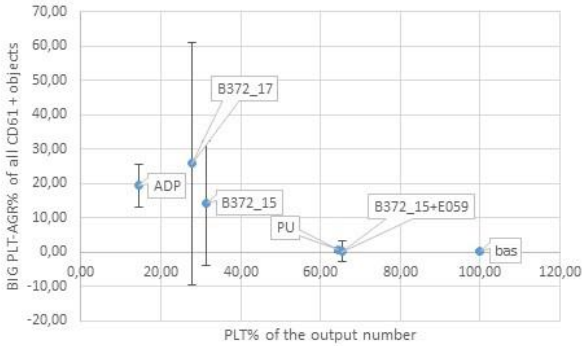
AGREGATION PLT [%]



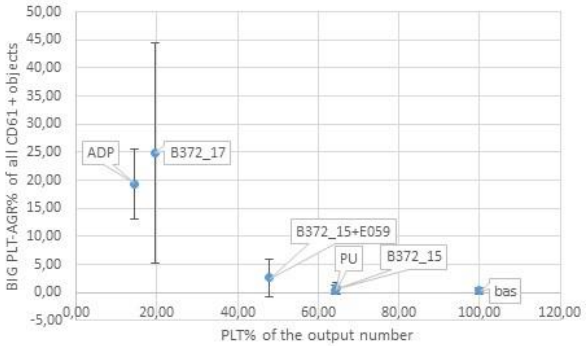
a.)



b.)



c.)



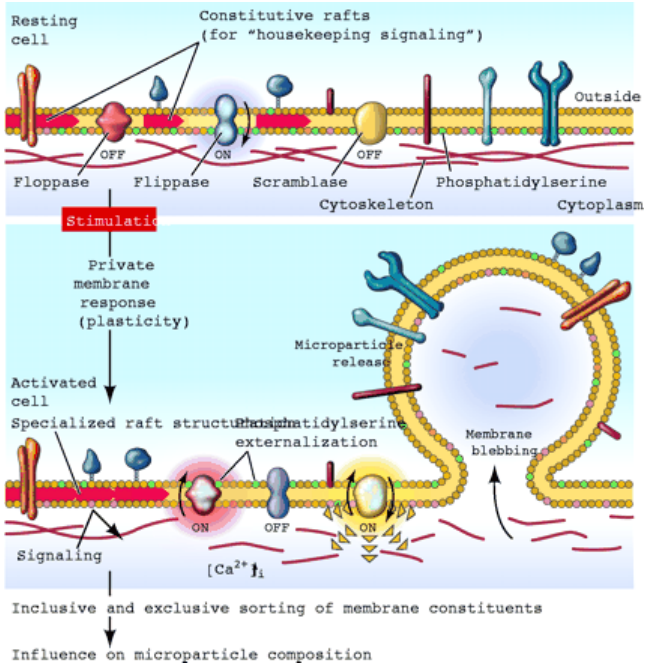
d.)

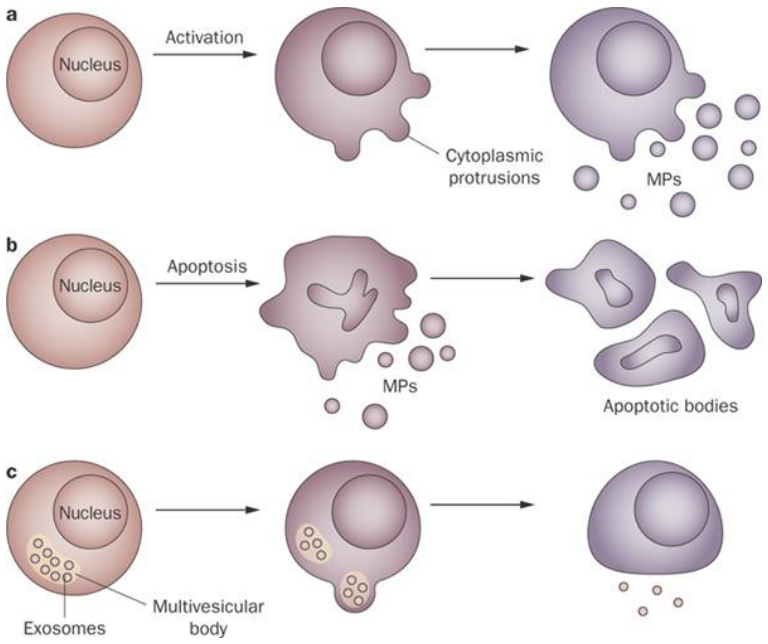
R&D on surface coating

R&D on surface coating deposition

Blood-Material Interaction

Microparticles (MPs) are small membrane vesicles that are released by activated or apoptotic cells.





R&D on surface coating

R&D on surface coating deposition

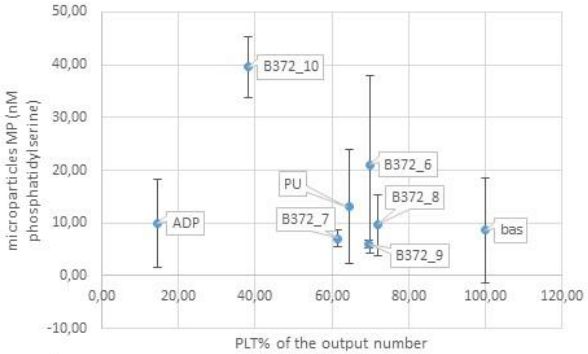
negative	neutral
neutral	positive

Group	Symbol	Layer	Thickness [nm]
1	B372_6	a-C:H	94,7
	B372_7	a-C:H,N	103,8
	B372_8	a-C:H,N	103,1
	B372_9	a-C:H,N	95,2
	B372_10	a-C:N	95,1
2	B372_15	a-C:H	94,7
	B372_15+E059	a-C:H+SF6 (fluoridation)	94,7
	B372_16	a-C:H,N	110,3
	B372_17	a-C:H,N	100,3
	B372_17+E059	a-C:H,N+SF6 (fluoridation)	100,3
	B372_18	a-C:H,N	82,7
3a	B372_23	Si/a-C:H	124,3
	B372_25	Si/a-C:H	127,6
	B372_27	Si/a-C:H	123,5
	B372_29	Si/a-C:H	118,5
3b	B372_24	Si/a-C:H	15
	B372_26	Si/a-C:H	15
	B372_28	Si/a-C:H	15
	B372_30	Si/a-C:H	15

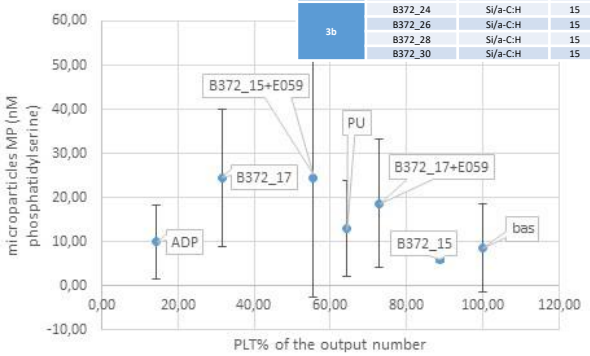
Haemocompatibility in dynamic conditions

Microparticles MP
[nm fosfatydyloserin]

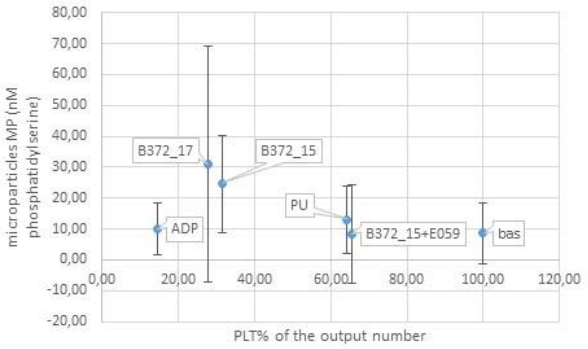
Blood-Material Interaction



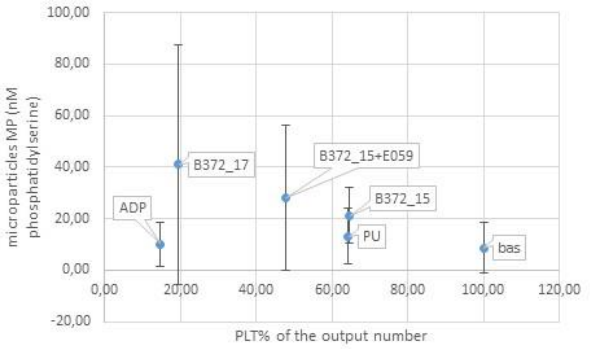
a.)



b.)



c.)

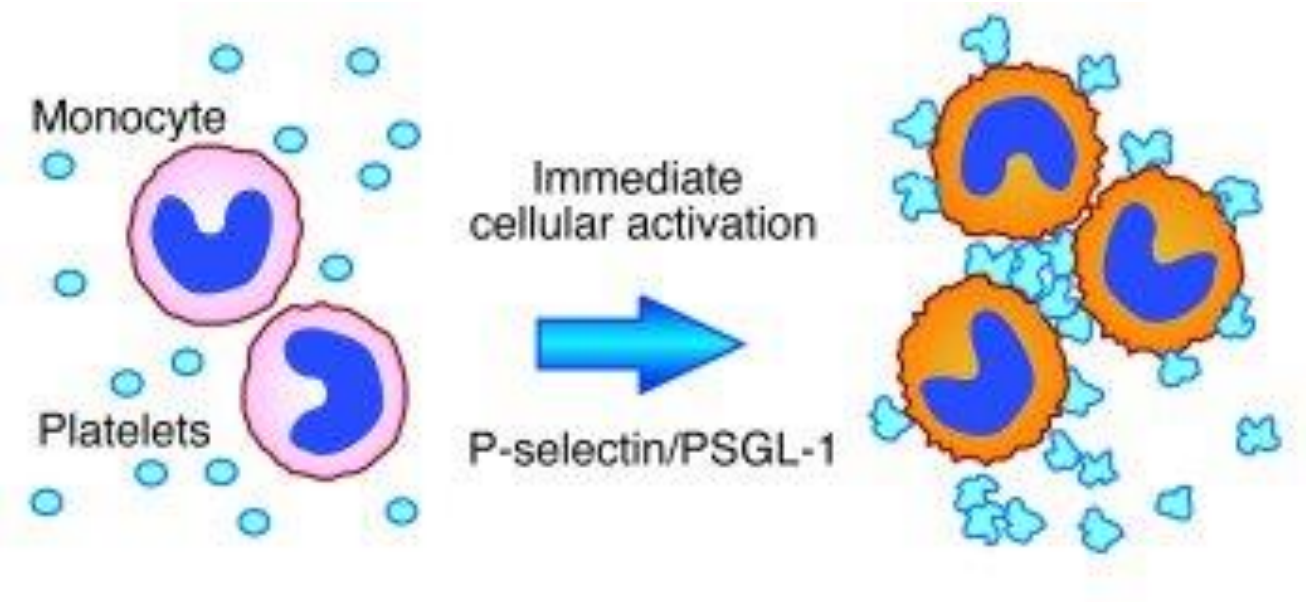


d.)

R&D on surface coating deposition

Haemocompatibility in dynamic conditions

Aggregation-mon-PLT



R&D on surface coating

R&D on surface coating deposition

negative	neutral
neutral	positive

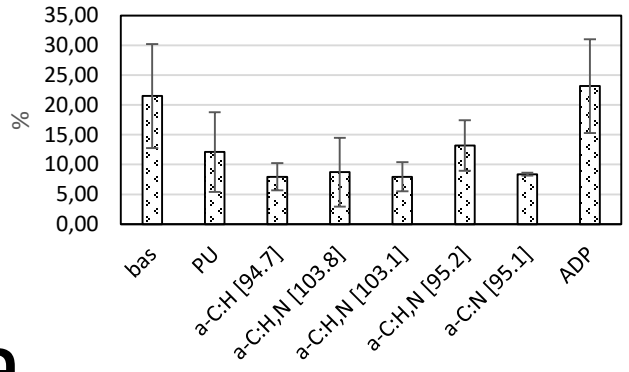
Group	Symbol	Layer	Thickness [nm]
1	B372_6	a-C:H	94,7
	B372_7	a-C:H,N	103,8
	B372_8	a-C:H,N	103,1
	B372_9	a-C:H,N	95,2
	B372_10	a-C:N	95,1
2	B372_15	a-C:H	94,7
	B372_15+E059	a-C:H+SF6 (fluoridation)	94,7
	B372_16	a-C:H,N	110,3
	B372_17	a-C:H,N	100,3
	B372_17+E059	a-C:H,N+SF6 (fluoridation)	100,3
	B372_18	a-C:H,N	82,7
3a	B372_23	Si/a-C:H	124,3
	B372_25	Si/a-C:H	127,6
	B372_27	Si/a-C:H	123,5
	B372_29	Si/a-C:H	118,5
3b	B372_24	Si/a-C:H	15
	B372_26	Si/a-C:H	15
	B372_28	Si/a-C:H	15
	B372_30	Si/a-C:H	15

Haemocompatibility in dynamic conditions

Blood-Material Interaction

Mon-PLT P-SEL G1

1



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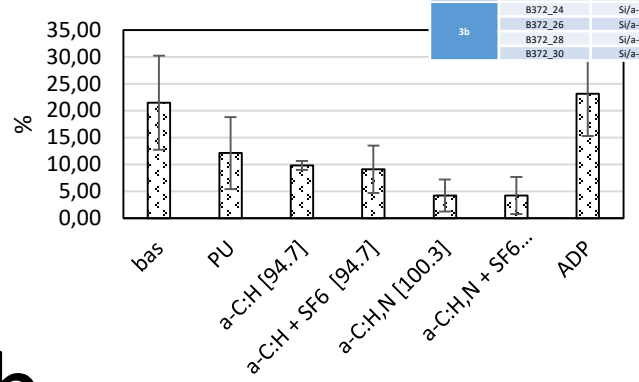
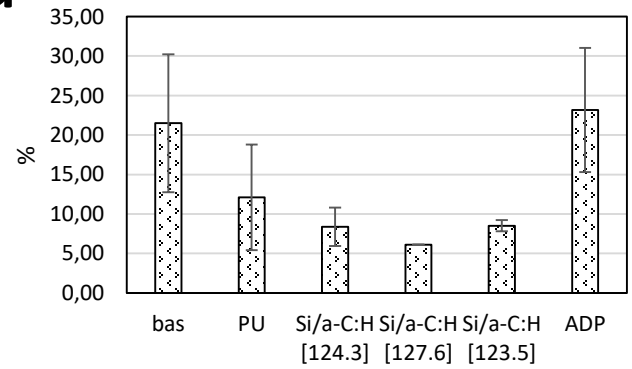
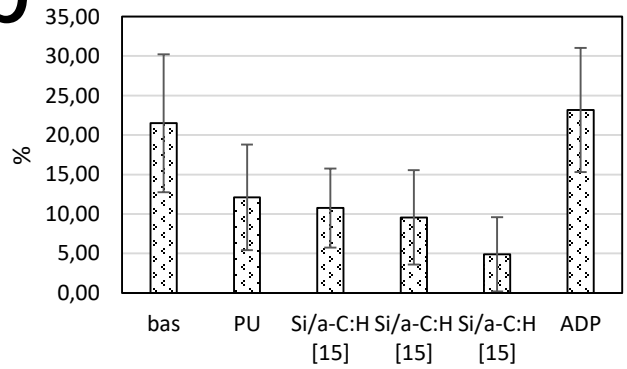


3a



3b



Task 6

Complete valve testing

Studies of local reactions after implantation

Complete valve testing

In vivo tests of „simple” samples

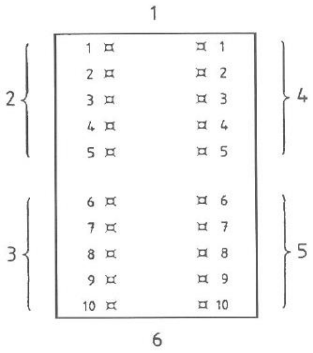
Complete valve testing

Reaction	Scoring irritation
Erythema formation	
No erythema	0
Very slight erythema	1
Well defined erythema	2
Moderate erythema	3
Severe erythema (dark red)	4
Edema formation	
Lack of edema	0
Very weak swelling (barely perceptible)	1
Well defined erythema (edges of the area well defined by elevation)	2
Moderate edema (elevation of about 1 mm)	3
Painful swelling (elevation above 1 mm goes beyond the area of exposure)	4
Maximum possible score	8

The scale of evaluation of responses in intradermal reactivity tests

An exemplary image of the animal's skin 72h after injection.

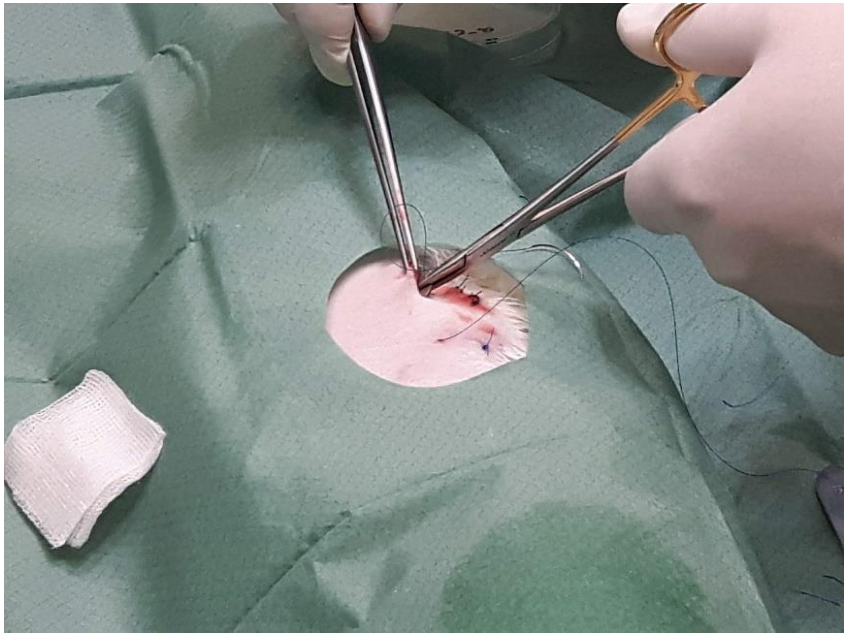
1- side of the animal's head, 2 injections of extract in polar solvent, 3 injections of pure polar solvent, 4 injections of the extract in a non-polar solvent, 5 injections of a pure non-polar solvent, 6 parts of the tail of the animal



Intradermal reactivity - location of injection points (according to ISO 10993-10) Intradermal reactivity - location of injection points (according to ISO 10993-10)

The test results showed that the tested material extracted with apolar and polar extract did not irritate the skin of rabbits during the 72 h observation period. The tested material met the test requirements because the difference between the average test score and the average control score was less than 1.0 (= 0.13) and in accordance with the normative guidelines ISO 10993-10 was considered non-irritating. It can be concluded that the tested material does not induce intradermal reaction.

Studies of local reactions

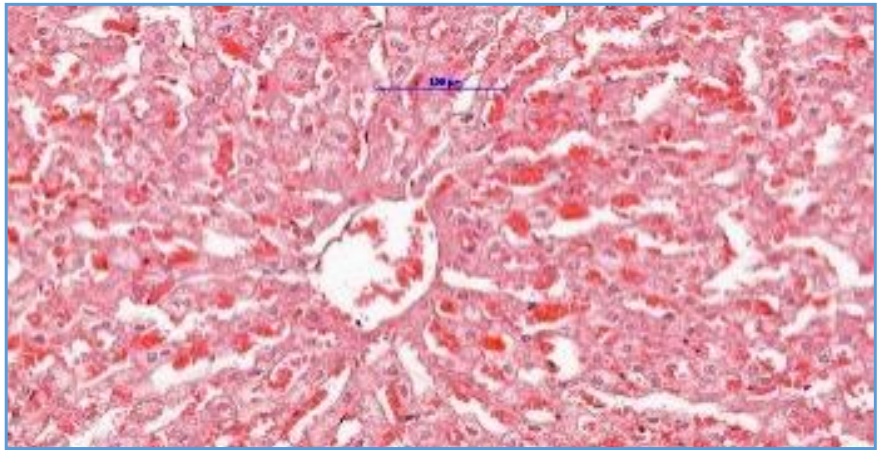


Placing the disc of the tested material under the skin on the back of the animal.

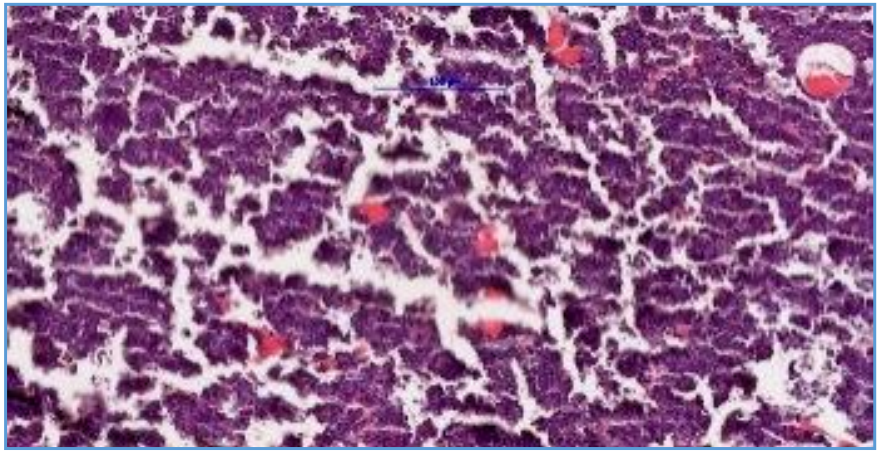
Study group, rabbit No. 2. Implanted site of implantation after 4 weeks, visible petechiae.

Studies of local reactions

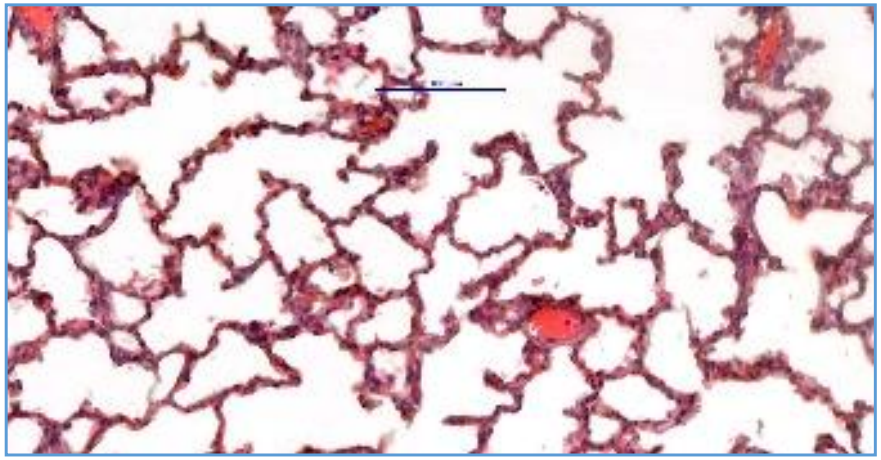
HISTOLOGY (liver, thymus, lung, myocardium)



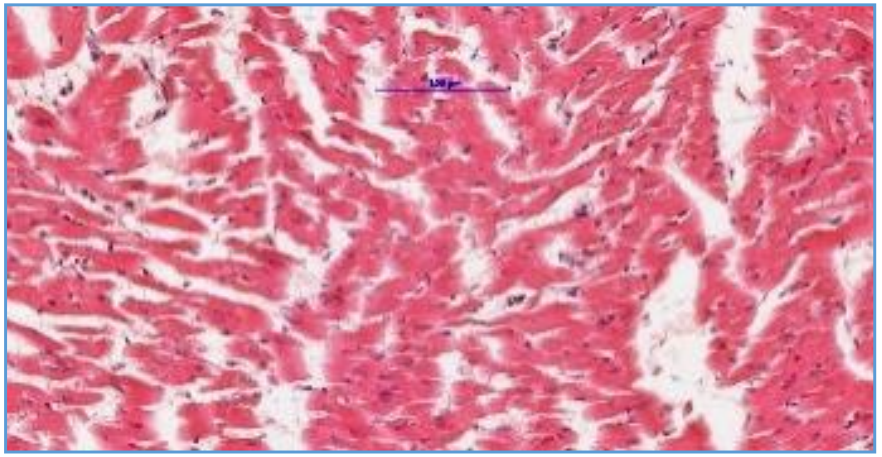
Correct liver lacunae. Mag. 200x



Proper thymic lacunae with Hassal's tiny pink bodies. Mag. 200x



Properly aerated lung parenchyma. Mag. 200x

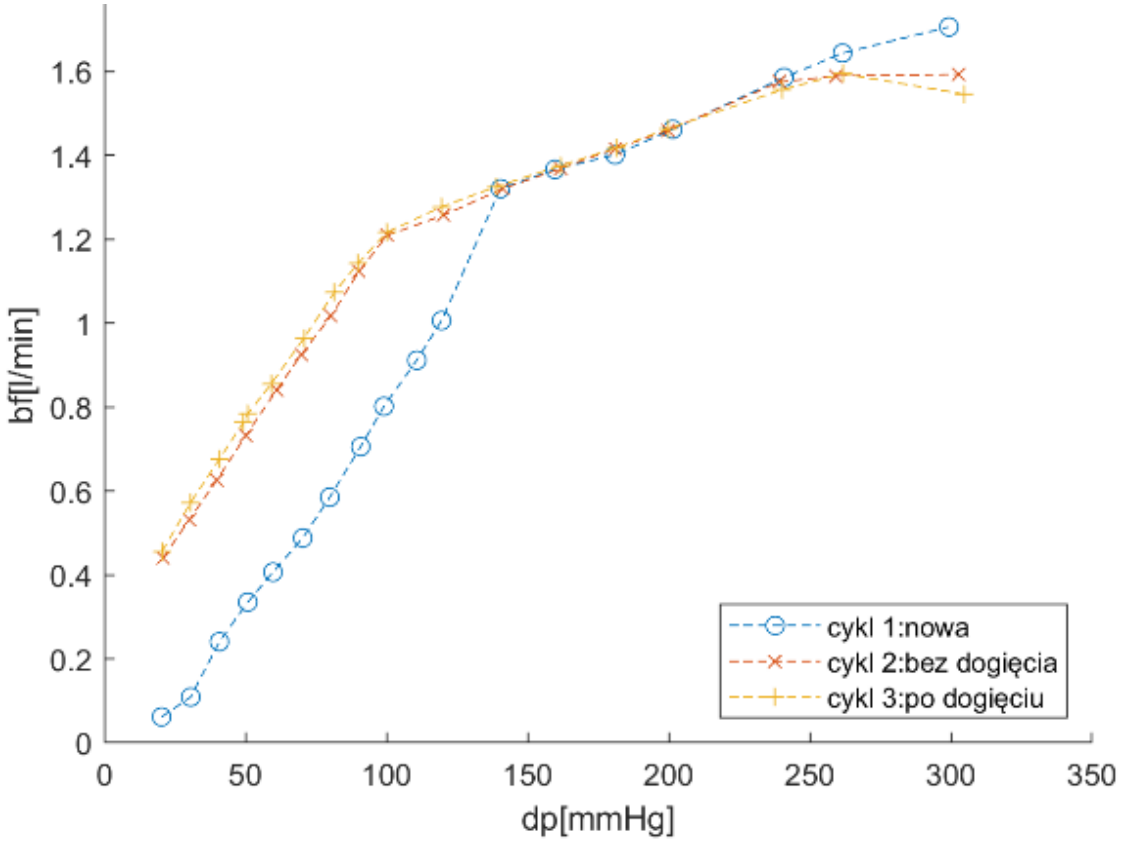


Correct binding of the myocardium. Mag. 200x

Technology readiness level

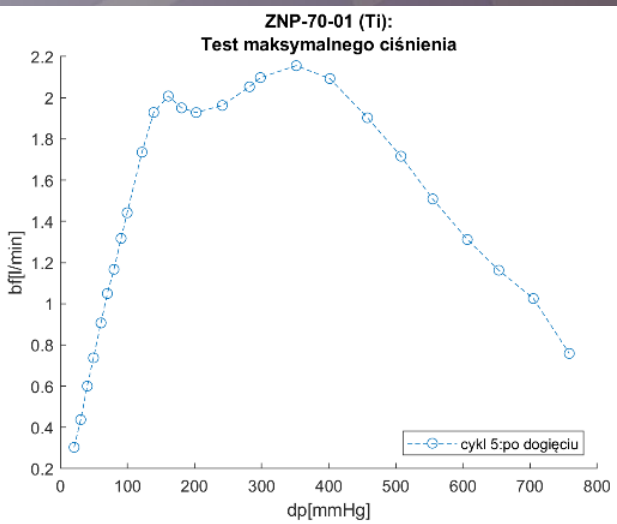
Technology readiness level

Technology readiness level

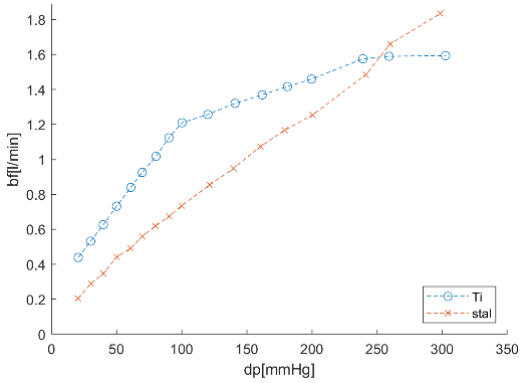


Hydrodynamic analysis in an artificial patient system using a blood simulating medium (glycerin solution) and determining the risk of turbulence and wear

Technology readiness level



ZNP-Ti: flake deformation for: (1) dp = 100mmHg, (2) dp = 760mmHg, (3) after removal from the system. On the right: sealing of the valve under the deformation of the flap with pressure of> 400mmHg.



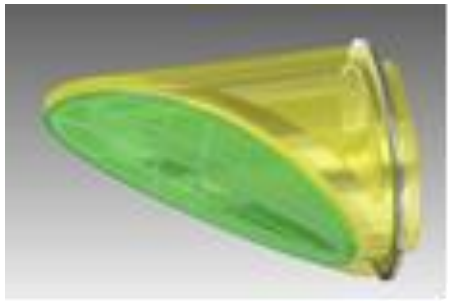
ZNP-S: (1) initial geometry, (2) deformation for dp = 300mmHg, (3) ditch is dp = 0mmHg. On the right: comparison of backflow of valves with a titanium and steel frame

Technology readiness level

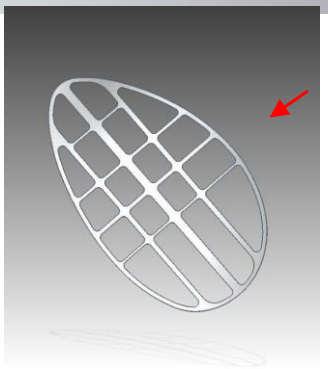
ZWP-Ti: breakthrough of the valve leaf at
a pressure of dp = 45mmHg.


Improvement
Design

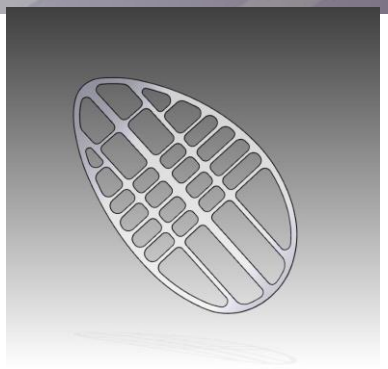
Further steps



a)



b)



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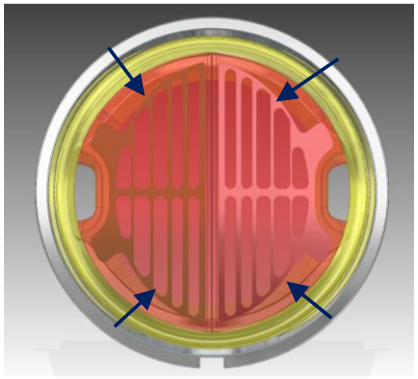
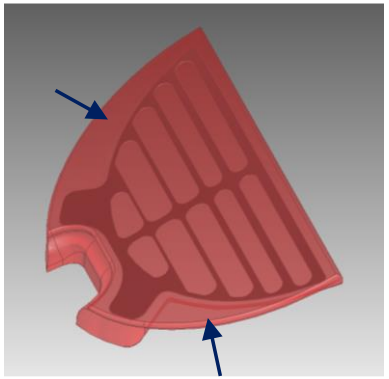
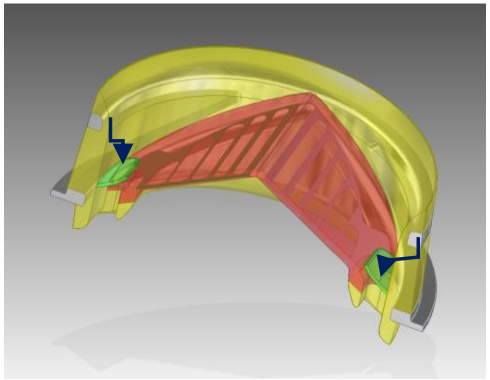
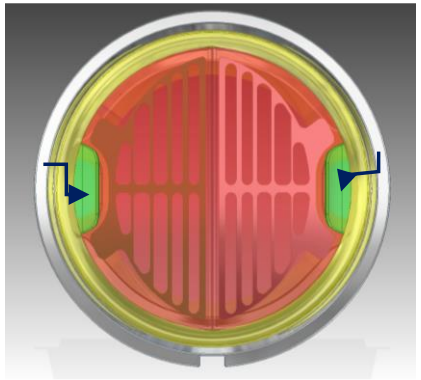








Narodowe Centrum
Badań i Rozwoju

M-ERA.NET Transnational Call 2017
Project Acronym: 4DbloodROT

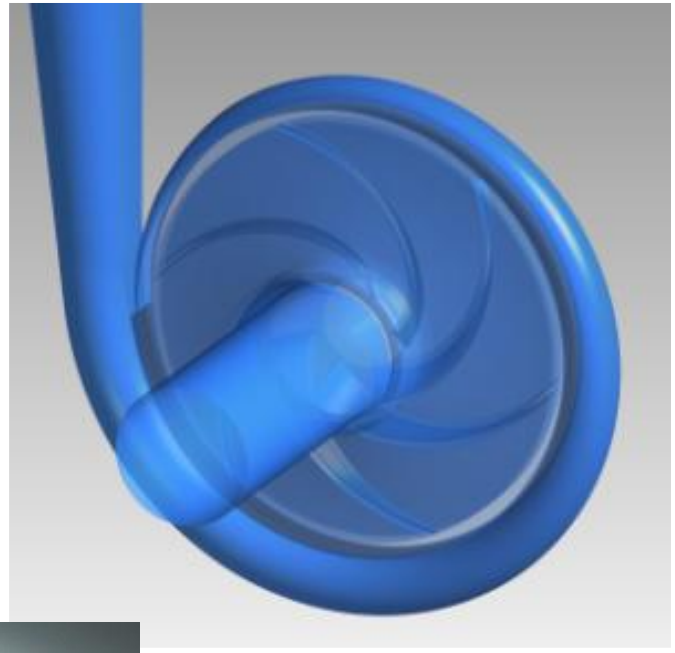
Partners:
JOANNEUM RESEARCH Forschungsges.m.b.H., Institute for Surface Technologies and Photonics, Austria;
Polish Academy of Sciences Institute of Metallurgy and Materiala Science in Cracow, Poland
Foundation for Heart Surgery Development,Poland;
In-Vision Digital Imaging Optics GhbH, Austria; Lithoz GmbH, Austria;
Polymer Competence Center Leoben GmbH, Austria;
University of Leoben, Institute of Chemistry of Polymeric Materials, Austria,
MESco Sp.z o.o. Poland

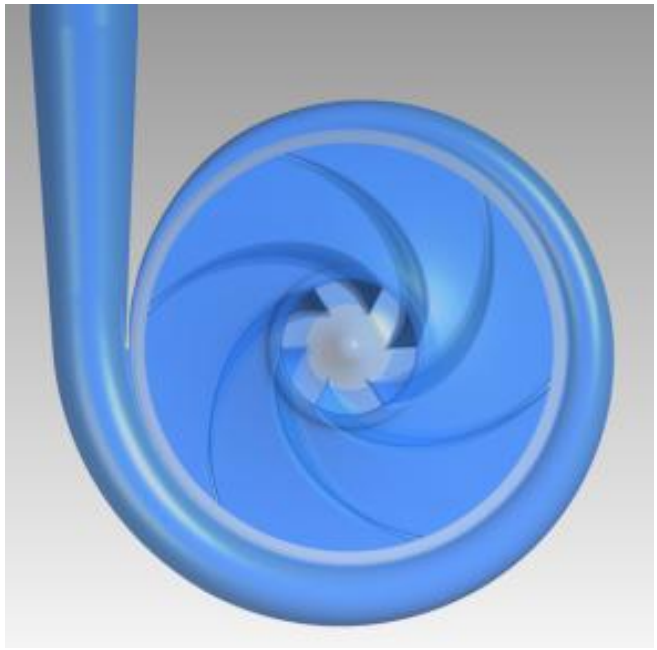
The research was financially supported by the Project no. DWM/M-ERA.NET-2017/16/2018 "4 Dimensional Single Piece Miniaturized Blood Rotor" of the Polish National Centre of Research and Development.

Aim

1.) Modification of the rotor pump

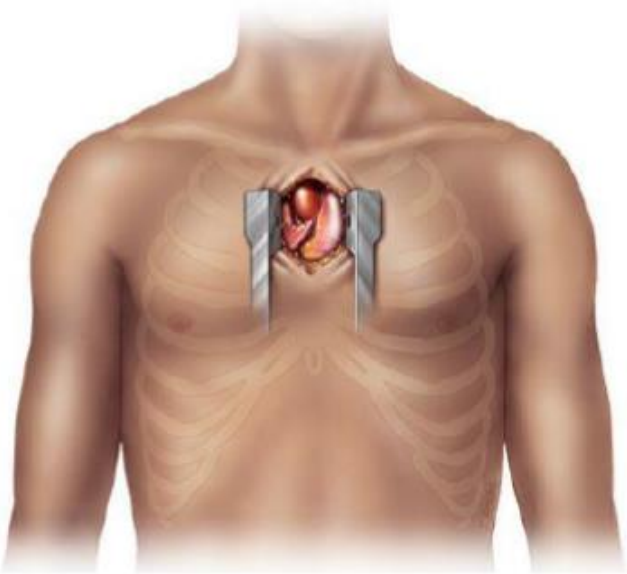
ReligaHeart ROT

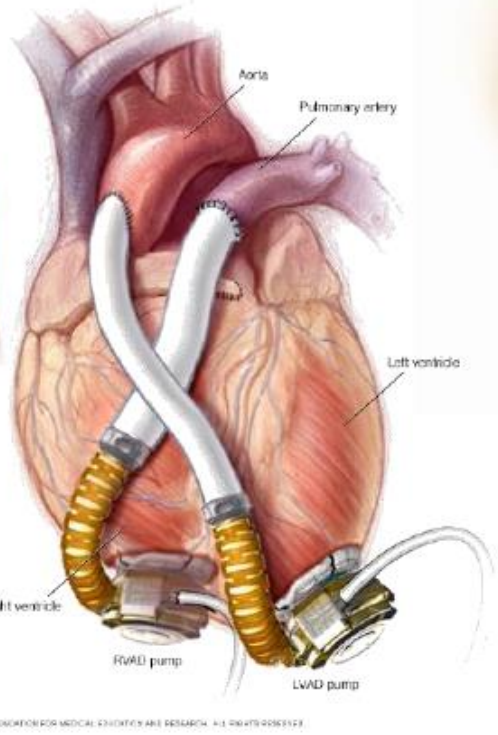


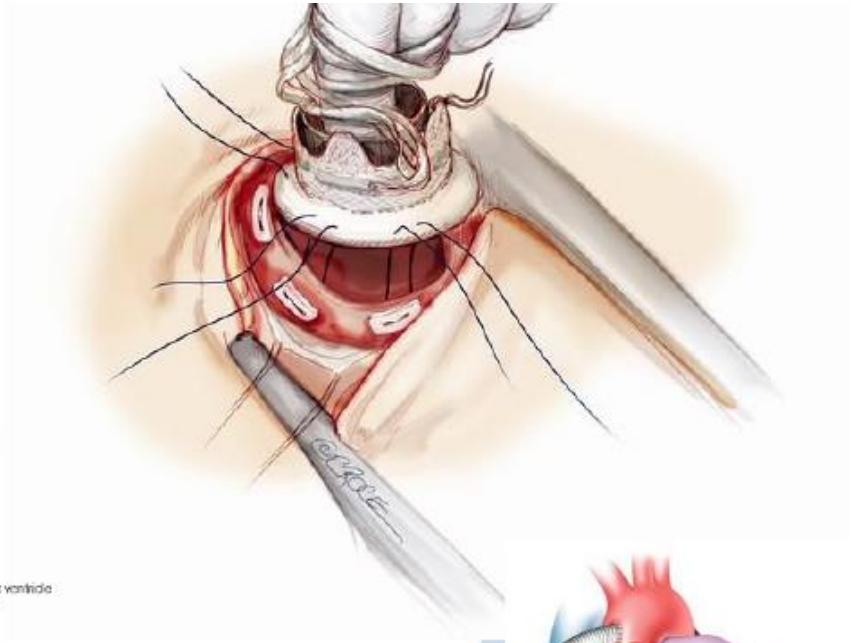


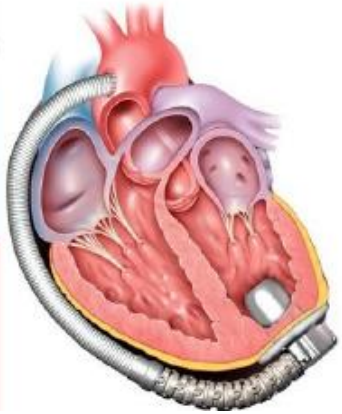
HeartWareInternational - Medtronic

4DbloodROT

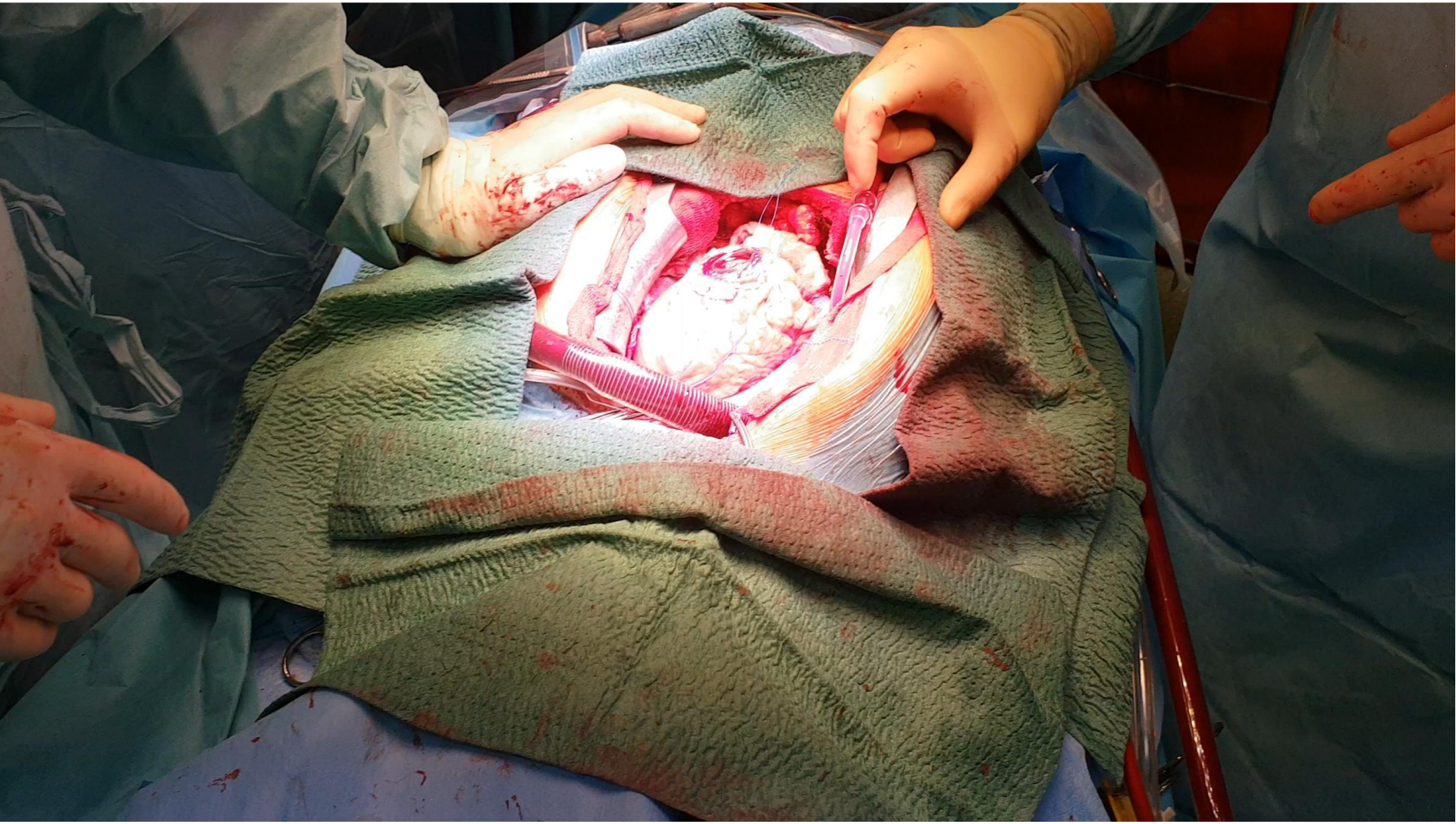



Aorta
Pulmonary artery
Left ventricle
Right ventricle
RVAD pump
LVAD pump

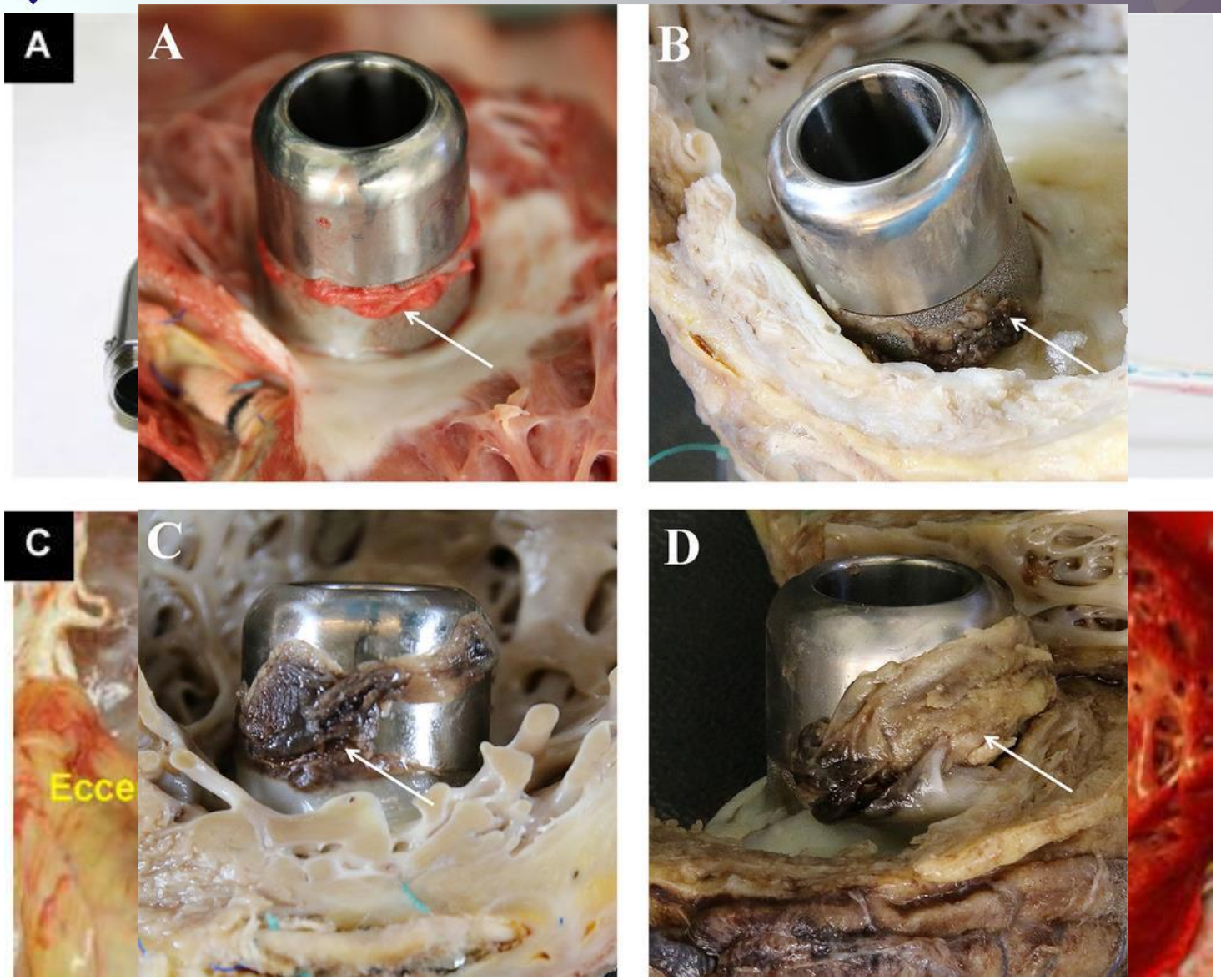




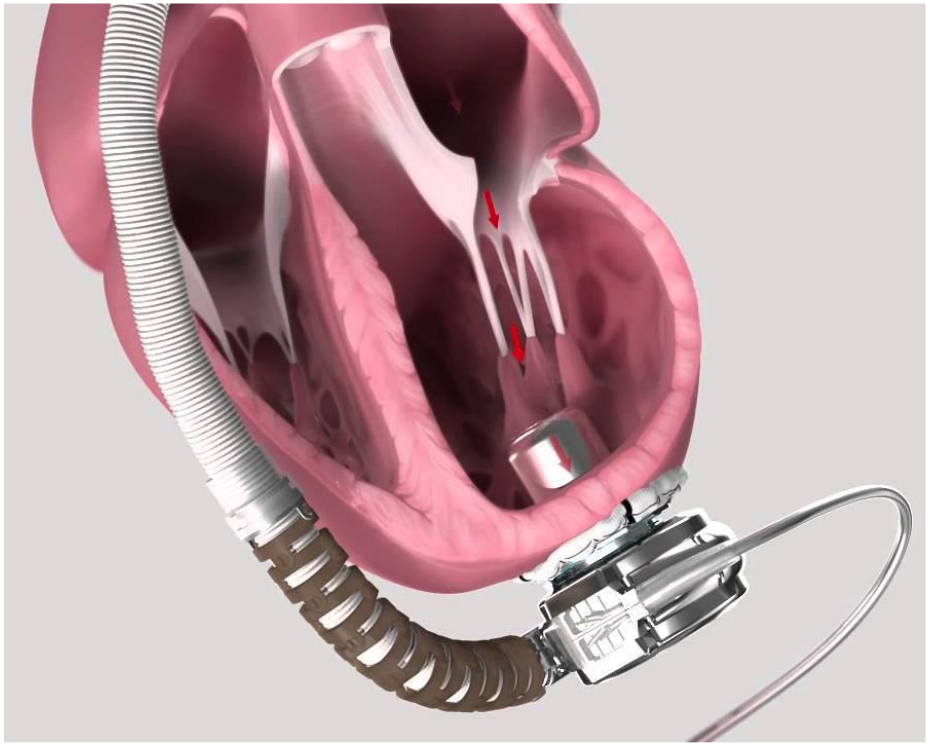
4DbloodROT

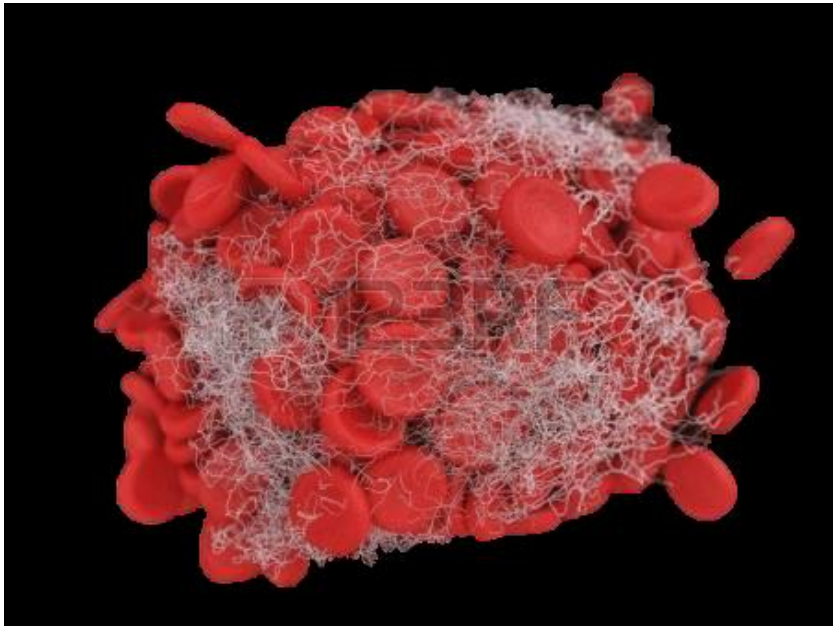


4DbloodROT



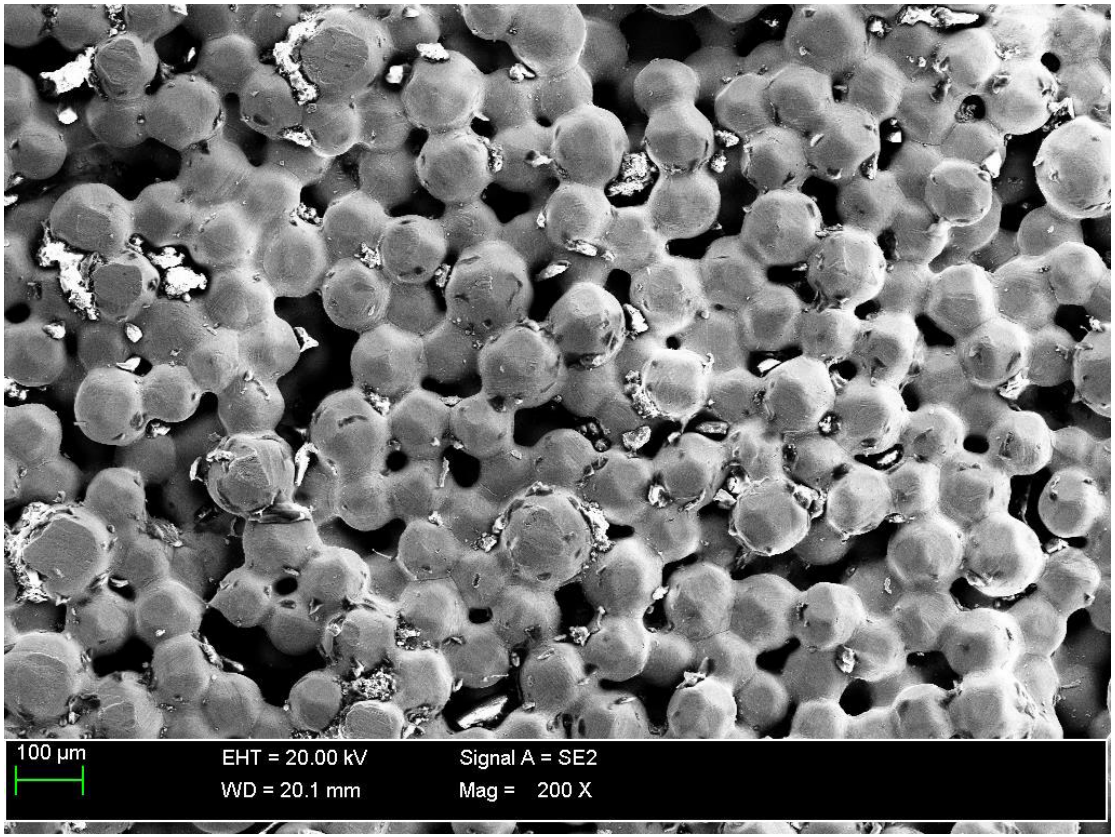
4DbloodROT



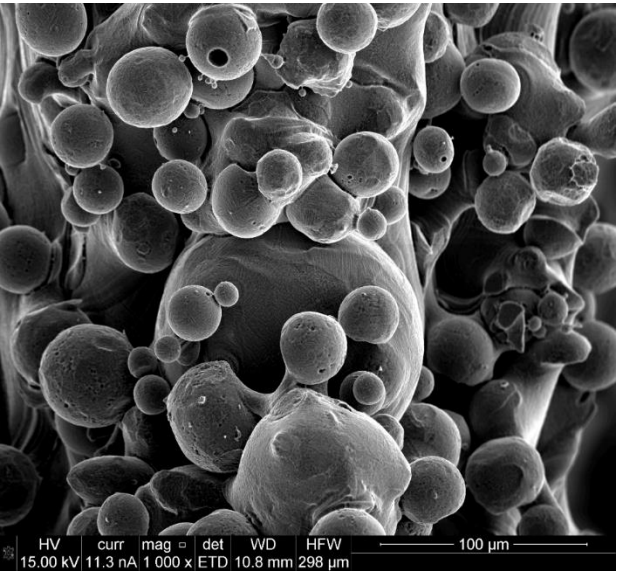


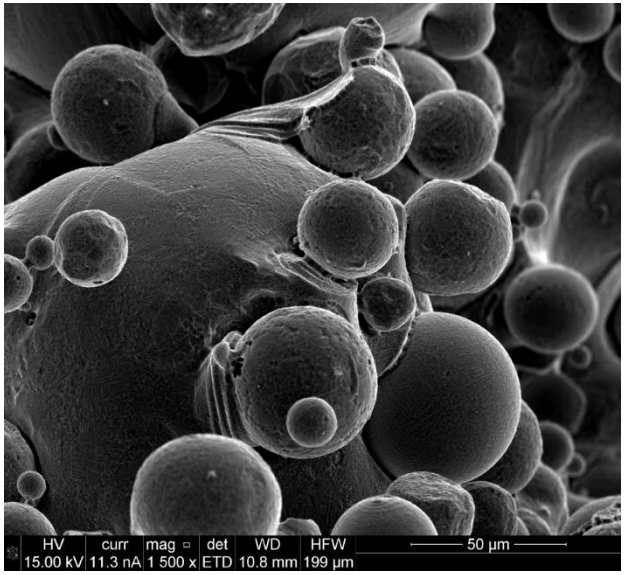
Thrombotic events are associated with:
the pump surface,
a drainage duct,
the bearing arrangement of the pump
the surface of the inflow cannula inserted into the apex of the left ventricle

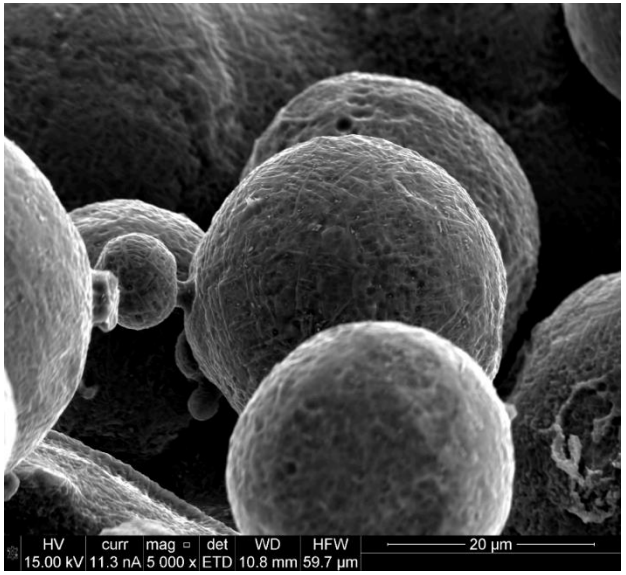
4DbloodROT



Scanning Electron Microscopy



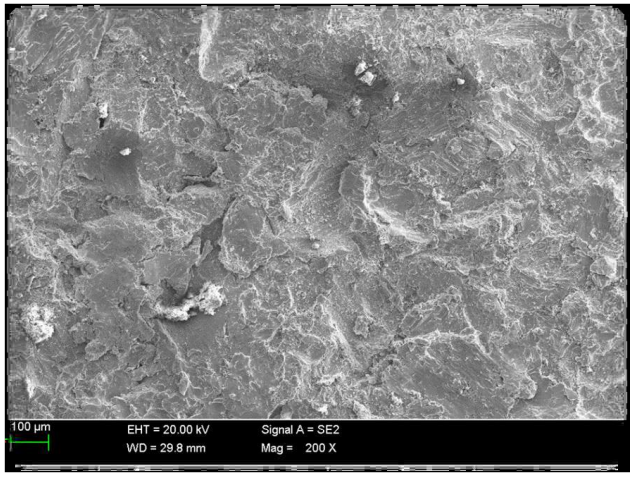


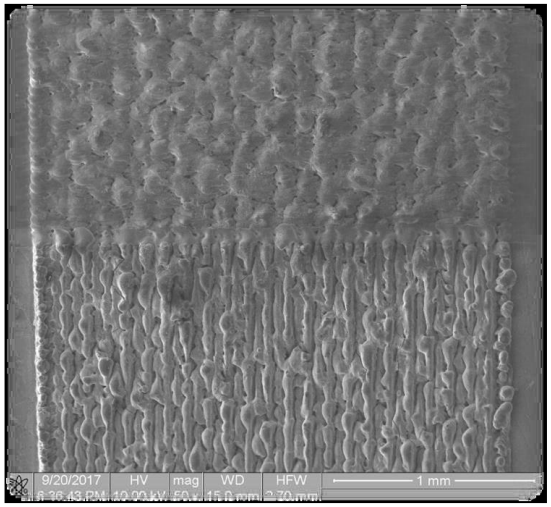


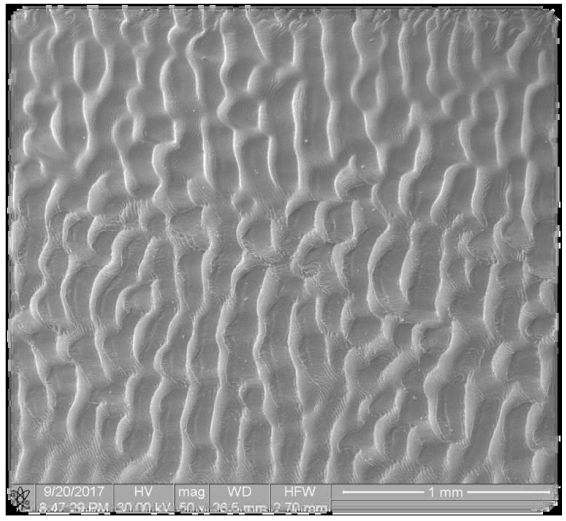
Coating with TiAl6Nb4 alloy

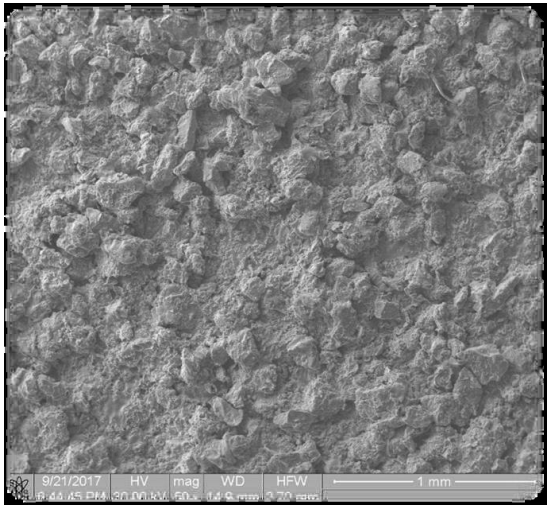
4DbloodROT

Institute of Opotoelectronics Military University of Technology Warsaw, Poland









2.) New design of the miniaturized pump

HeartMate PHP

High flow, low profile

HeartMate PHP is an acute percutaneous heart pump that disrupts the traditional relationship between size and flow to provide care without compromise.
It is designed to deliver the blood flow needed to quickly stabilise haemodynamically compromised patients and provide optimal haemocompatibility.
The catheter-based pump is designed for percutaneous entry through the femoral artery. Upon insertion via a low-profile introducer sheath, the catheter is advanced into the left ventricle where the cannula is unsheathed and fully expands to allowing for near–full physiological mean blood flow rates.

View full HeartMate PHP System

The covered nitinol cannula and integrated impeller expand from a low 13F insertion profile to 24F once unsheathed across the aortic valve for near–physiological mean blood flow of over 4 L/min

B3L

The interaction of ionizing radiation with tissue in the aspect of generating the process of acellularisation in the preparation of animal origin autologous tissue

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Purpose

The research was financially supported by the Project no. 2016/23/B/ST8/01481 Interdisciplinary methods of creating and functioning of biomimetic materials based on Animal origin extracellular matrix of the Polish National Center of Science. Part of the work was co-financed by the European Union from resources of the European Social Fund (Project No.WND-POWR.03.02.00-00-I043/16). The research was co-financed by the European Union from resources of the European Social Fund (Project No.WNDPOWR. 03.02.00-00-I043/16).

Motivation

"*My great desire to create a Polish artificial heart is finally being fully realized. Efforts to surround this undertaking to take care of the state, crowned with the adoption of the Multi-Year Program "Polish Artificial Heart", I consider a huge success. I am so happy and I wish you all the best for all those involved in the work*".

prof. Zbigniew Religa

Motivation

Transcatheter Aortic Valve Implantation (TAVI)

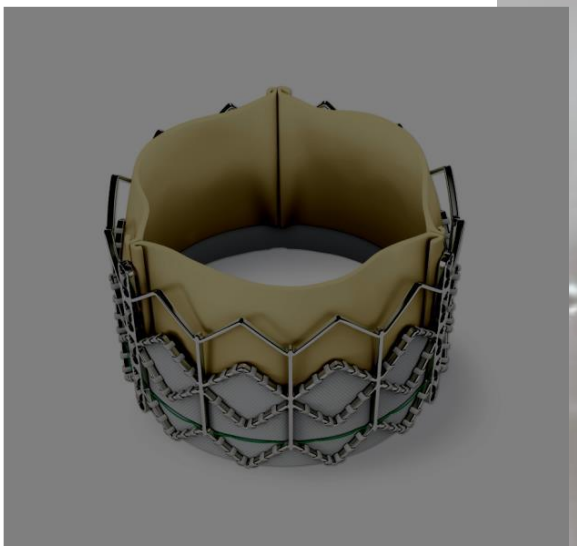
From the beginning of the twenty-first century, the method of percutaneous aortic valve implantation has been developing.

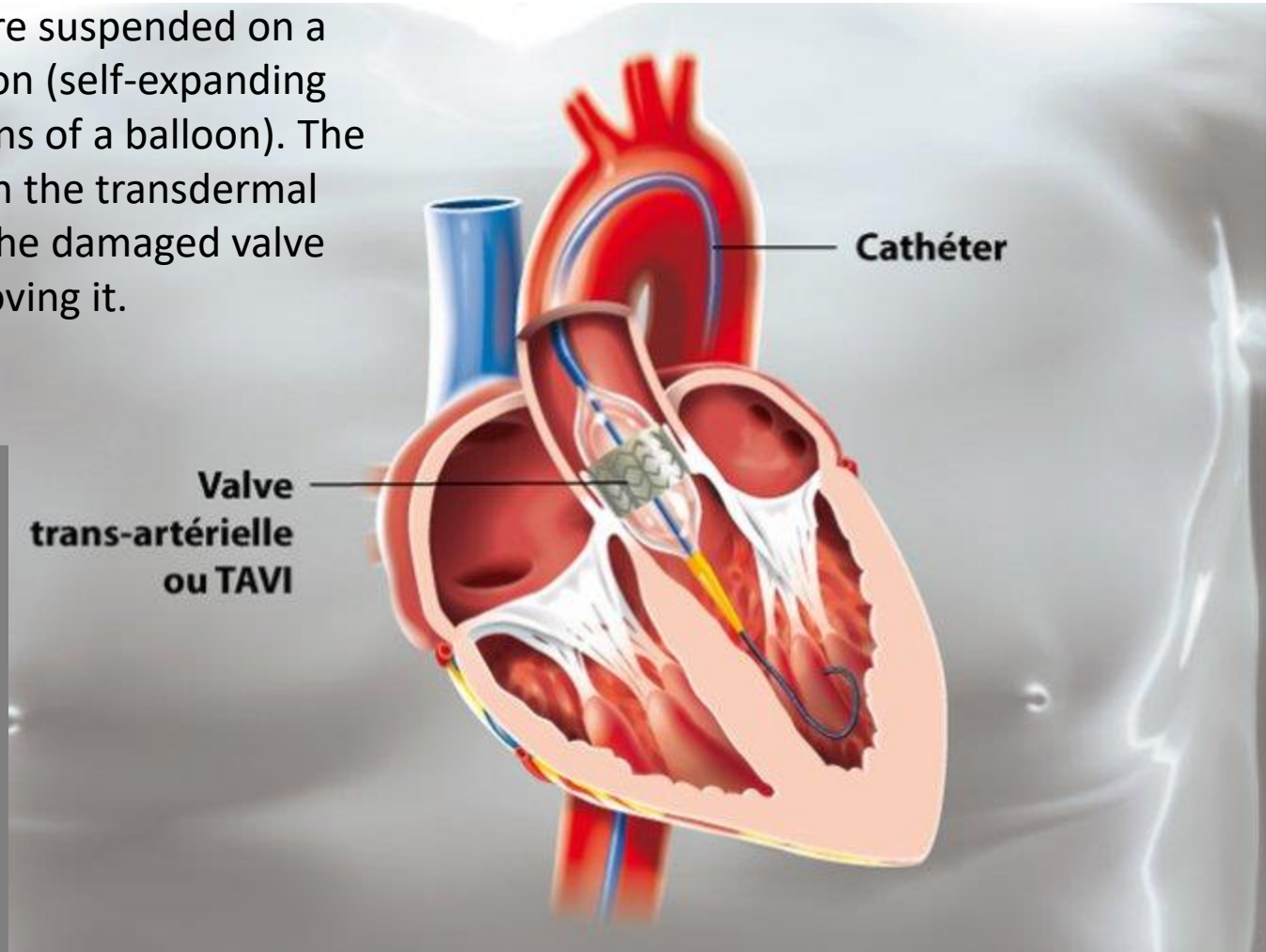
The valve for this procedure has a completely different structure from the classic one.

Motivation

TAVI

Prosthetic valve flakes are suspended on a special metal construction (self-expanding stent or expanded by means of a balloon). The valve is inserted through the transdermal system and expands in the damaged valve without removing it.





Motivation

TAVI indications

This is a method for a group of patients who according to experts (heart team) are not eligible for the classic AVR cardiac surgery (aortic valve replacement) due to the severe general condition, the severity of the underlying disease and associated conditions.

Motivation

TAVI - clinical contraindications

- Expected life expectancy <1 year
- A small chance to improve the quality of life due to the presence of comorbidities
- Severe primary related disease of another valve that causes symptoms and can only be treated surgically

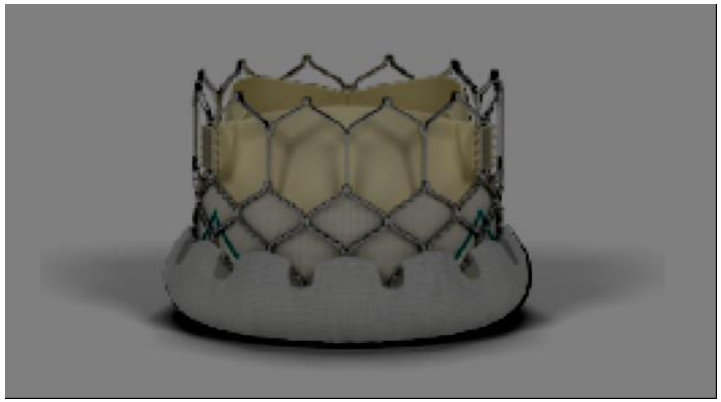
Motivation

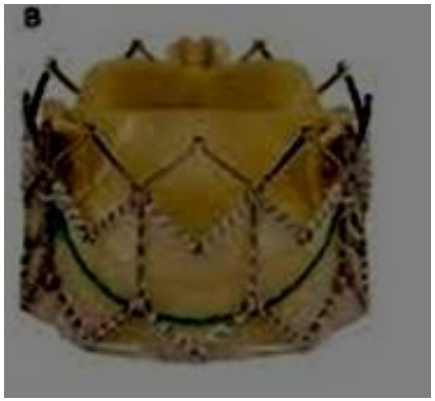
TAVI - anatomical contraindications

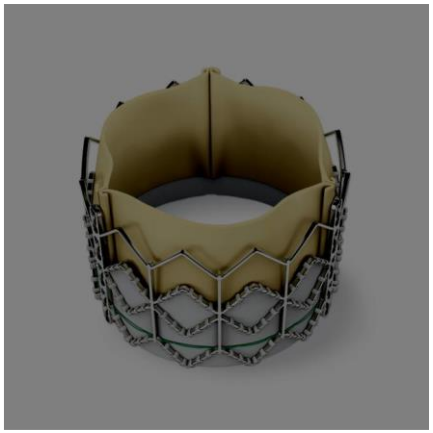
- Incorrect size of the aortic valve ring (<18 mm,> 29 mm)
- Thrombus in the left ventricle
- Active endocarditis
- Increased risk of coronary artery blockage
(asymmetric calcification of the valve, short distance between the ring and the onset of coronary arteries, small aortic sinuses)
- Lamellae with moving blood clots in the ascending aorta or in the aortic arch
- For access via femoral and subclavian arteries: inappropriate vascular access
(vessel size, calcification, tortuosity)

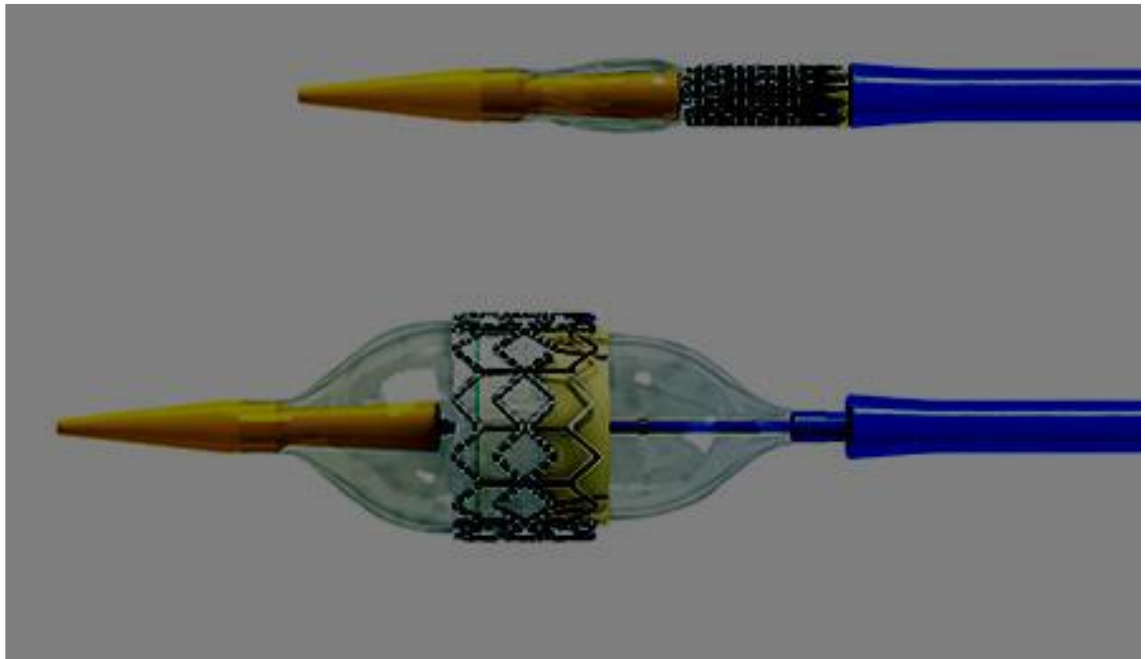
Motivation

Extended by baloon





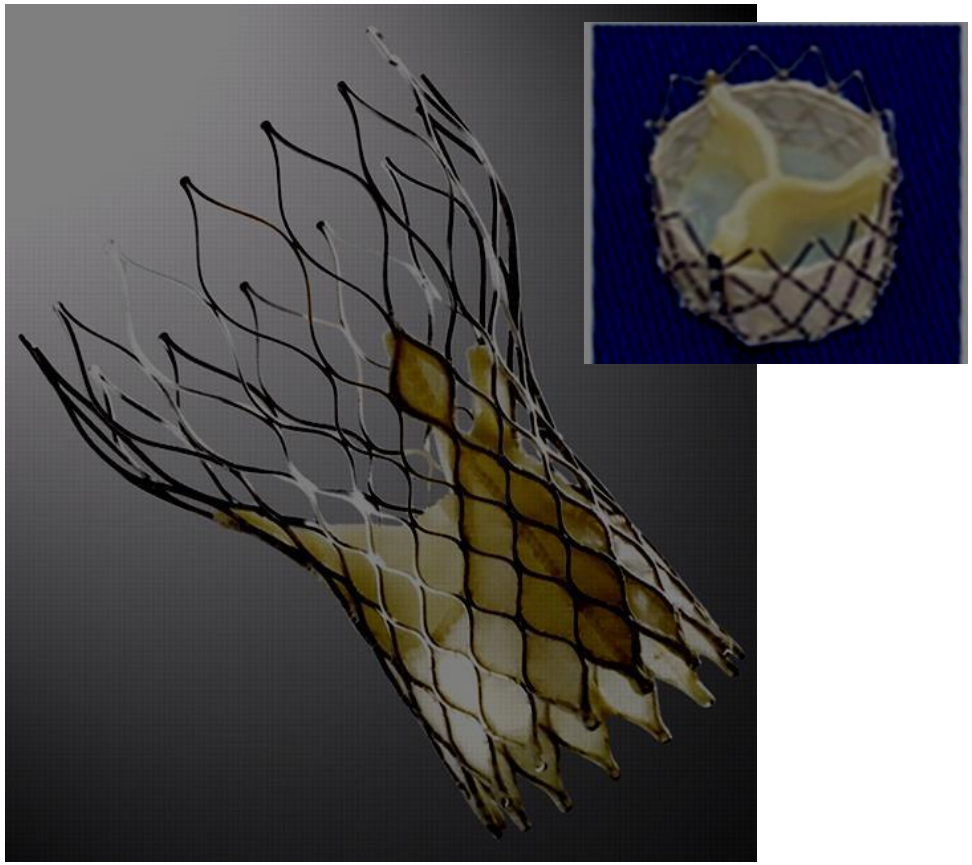




TAVI –types of valves

Motivation

TAVI –self extended



Motivation

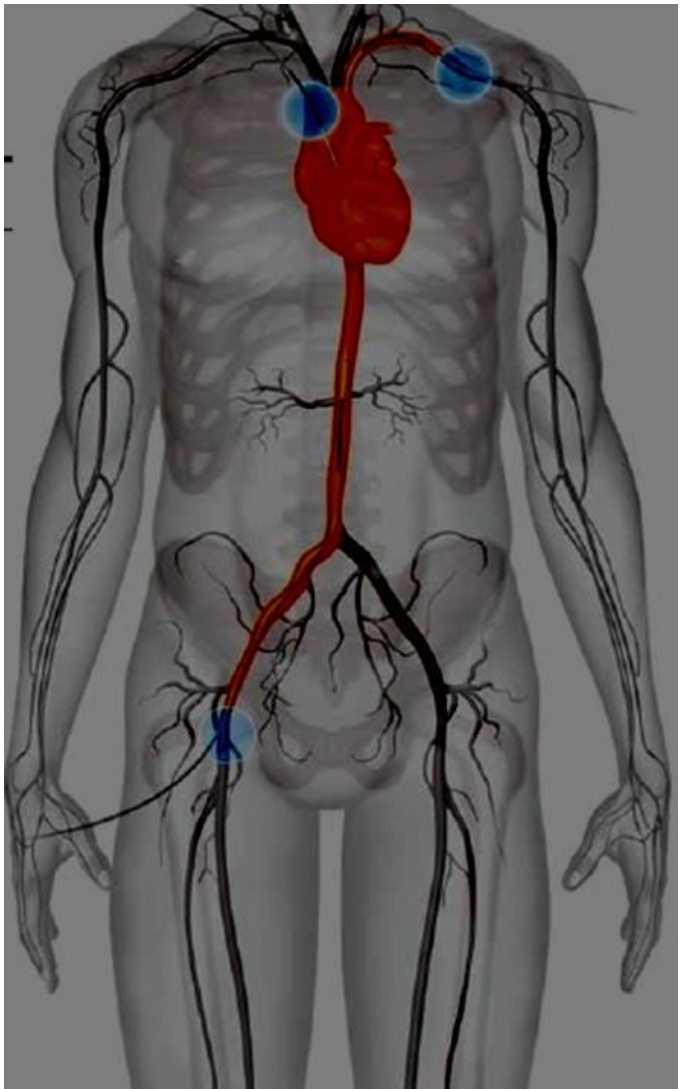
TAVI –curently applied access

•Basic access:

- Through the femoral artery

•Alternative access:

- Through the apex of the heart
- Through the ascending aorta
- Arteria subclavia



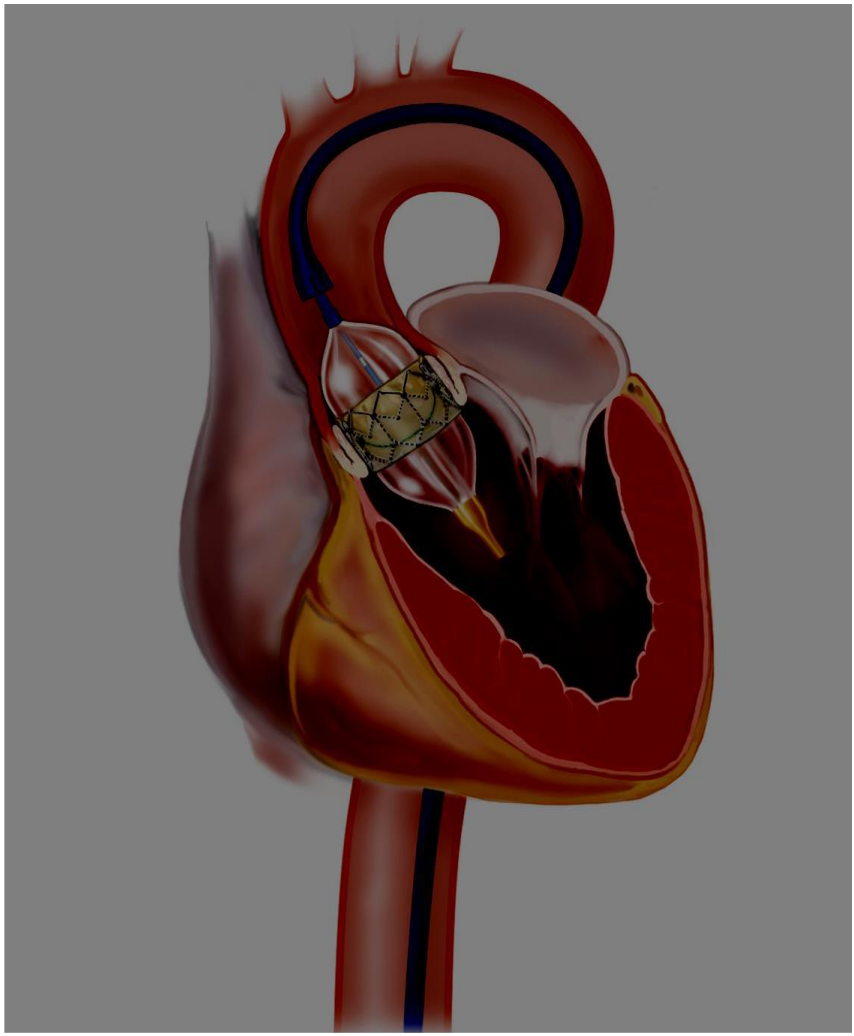
Motivation

TAVI –curently applied access

Access through the femoral artery

Disadvantages:

- Risk of damage to the femoral and iliac arteries
- Difficult passage through the catheter through the aortic stenosis



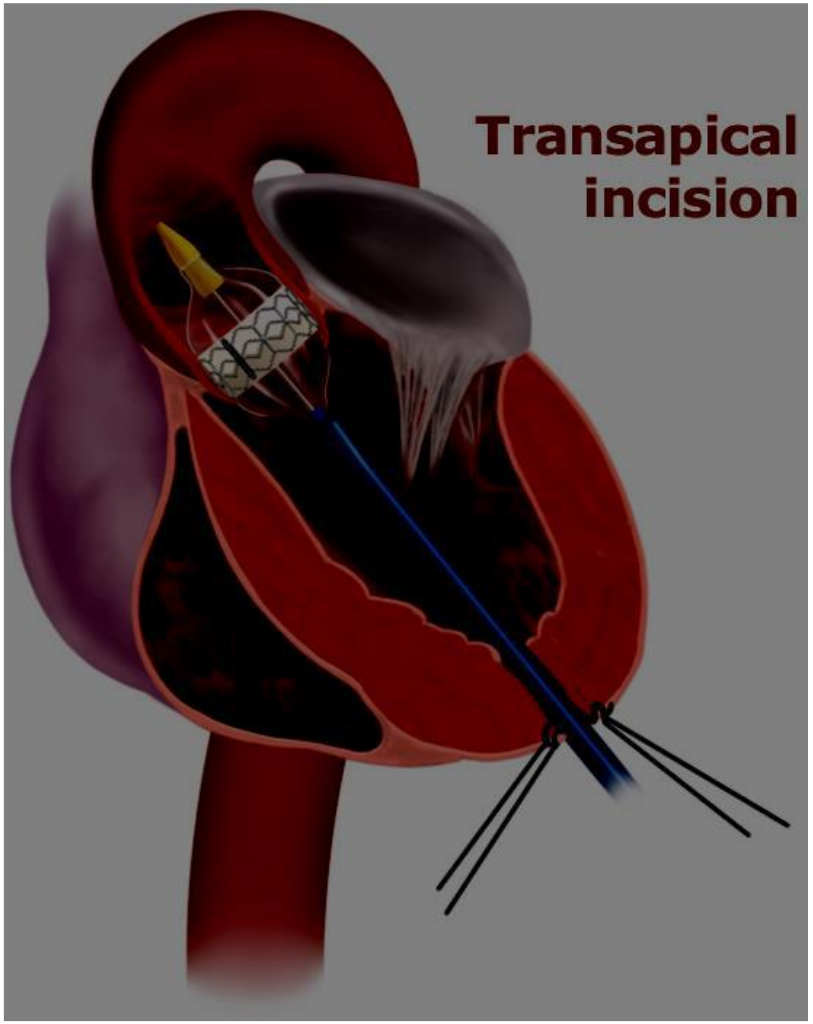
Motivation

TAVI –curently applied access

Access through apex. The catheter is introduced by a small thoracotomy and then by a puncture of the apex.

Advantages: the most direct access to the valve. The risk of damaging the femoral and iliac artery as well as the passage through the atherosclerotic changed valve.

The disadvantage of this method is the potential complication of apex puncture, requires general anesthesia and pleural drainage.



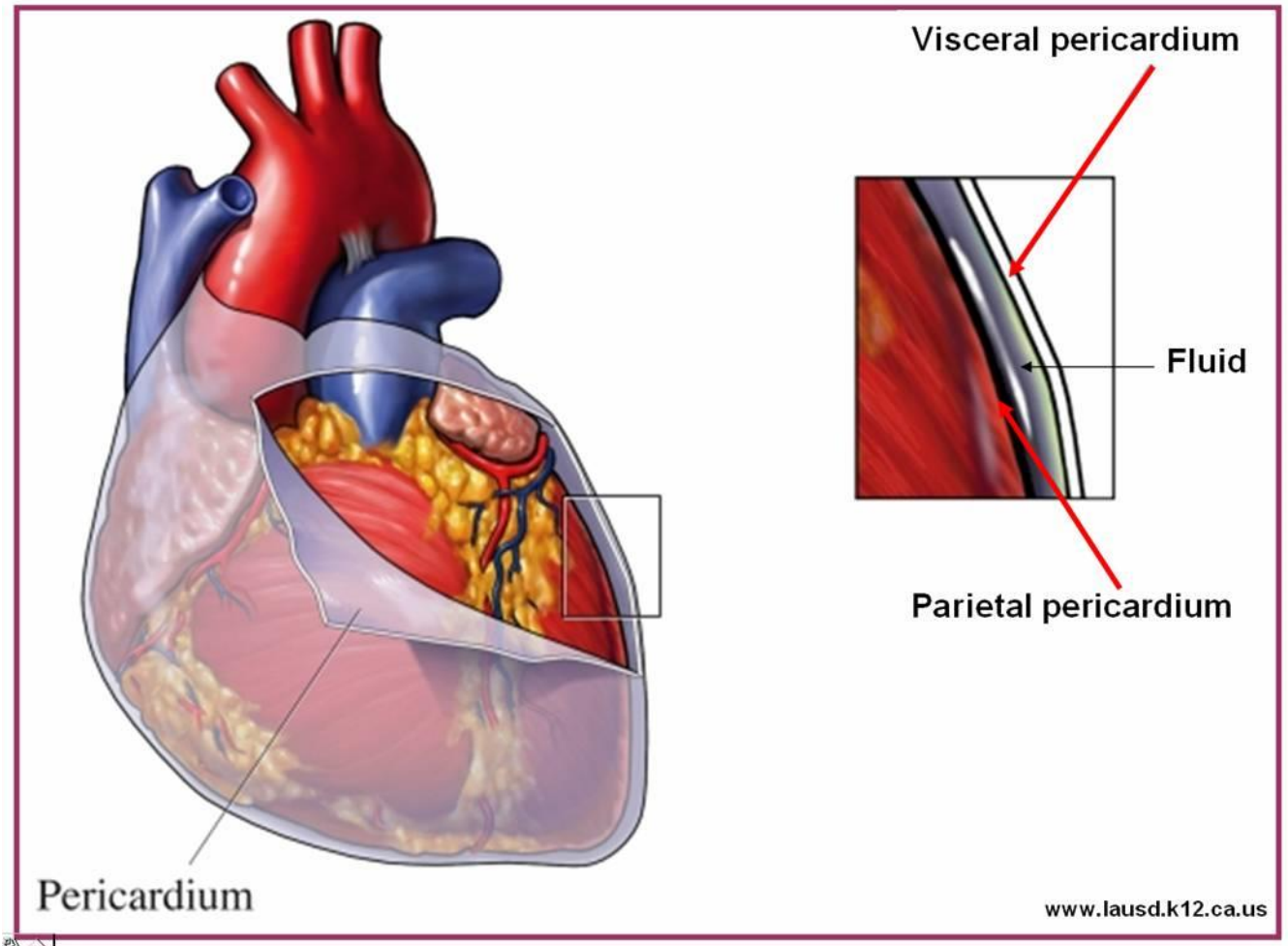
Substrate

Acknowlegement

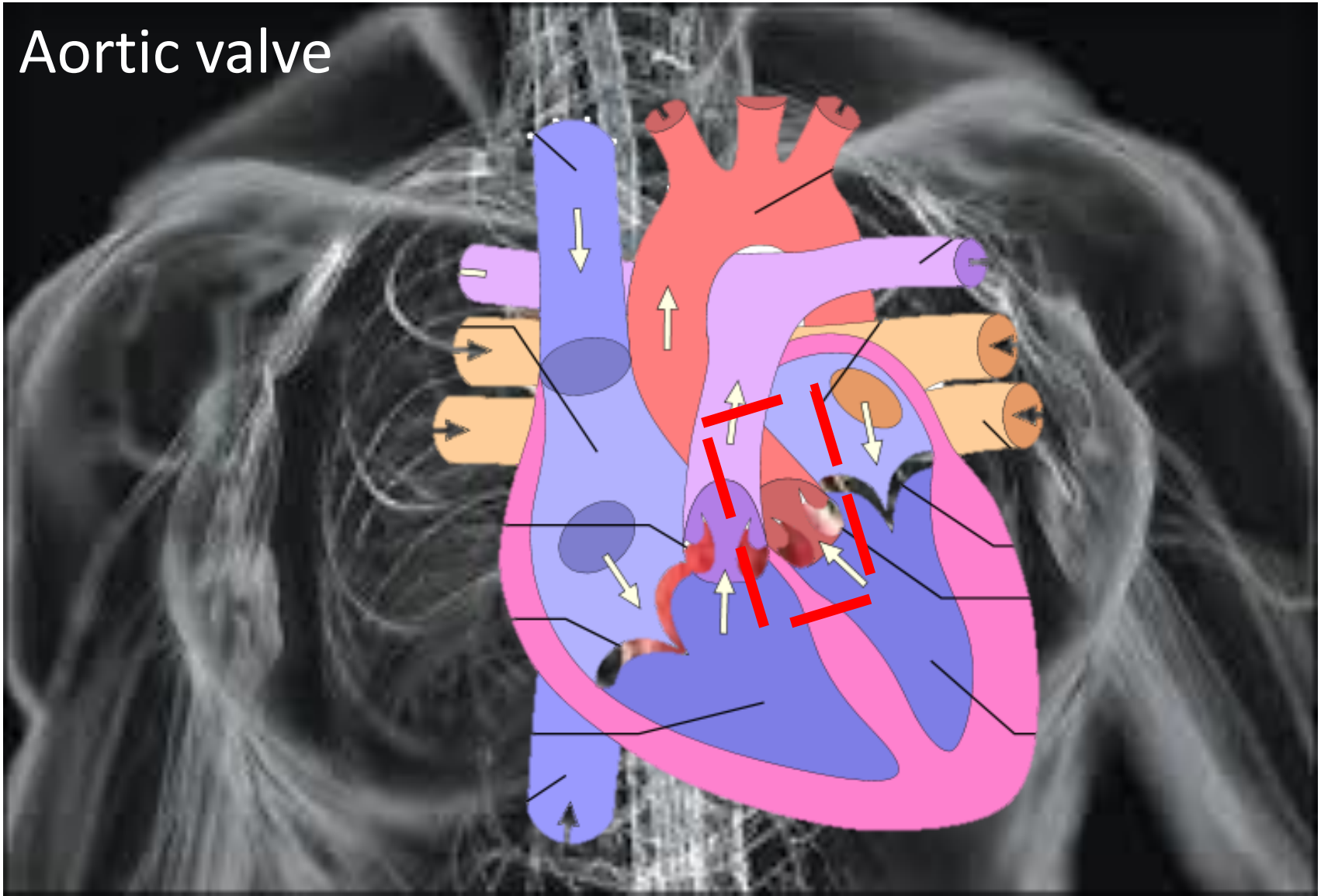
The research was financially supported by the **Project no. 2016/23/B/ST8/01481 "Interdisciplinary methods of creating and functioning of biomimetic materials based on Animal origin extracellular matrix" of the Polish National Center of Science.** Part of the work was co-financed by the European Union from resources of the European Social Fund (Project No.WND-POWR.03.02.00-00-I043/16).

Substrate

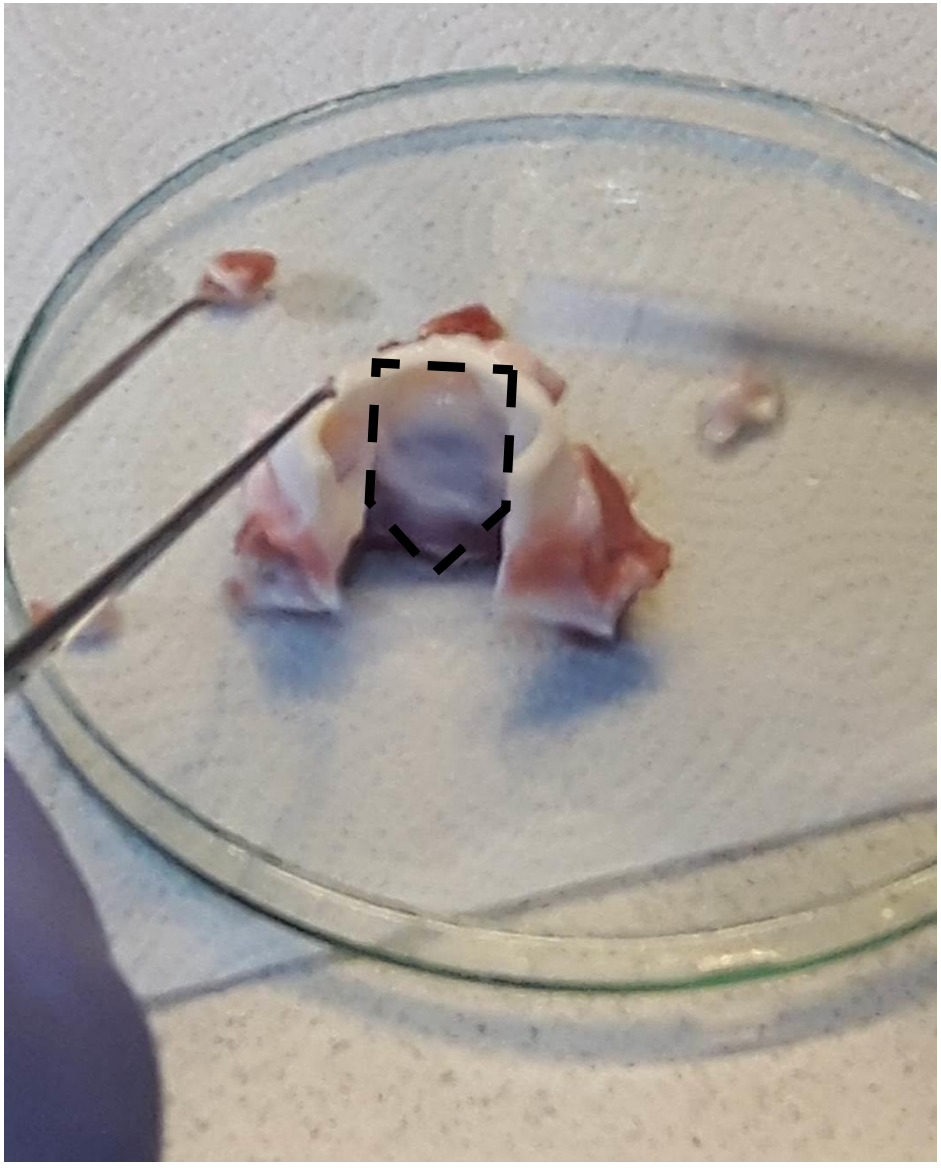
Pericardium

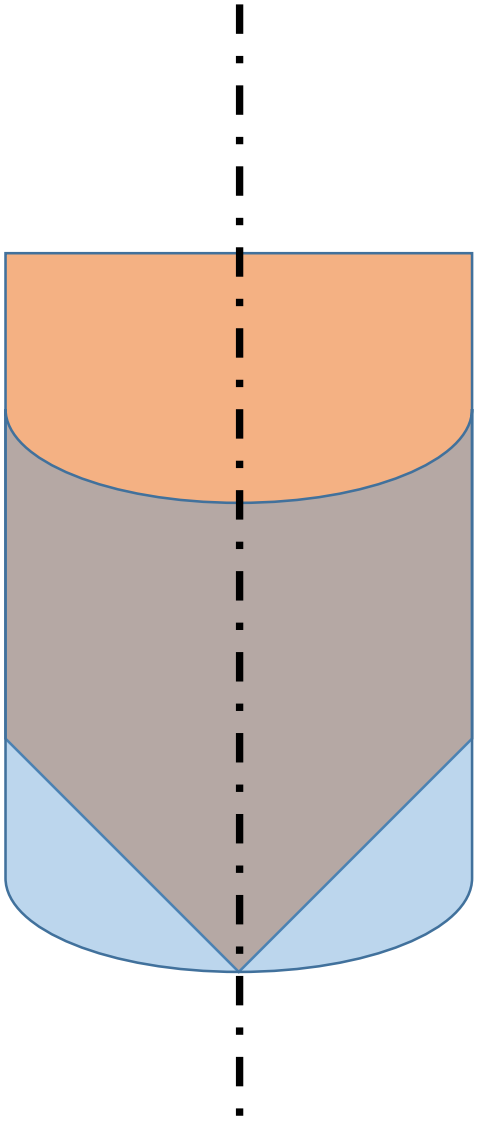


Substrate



Materials&Methods

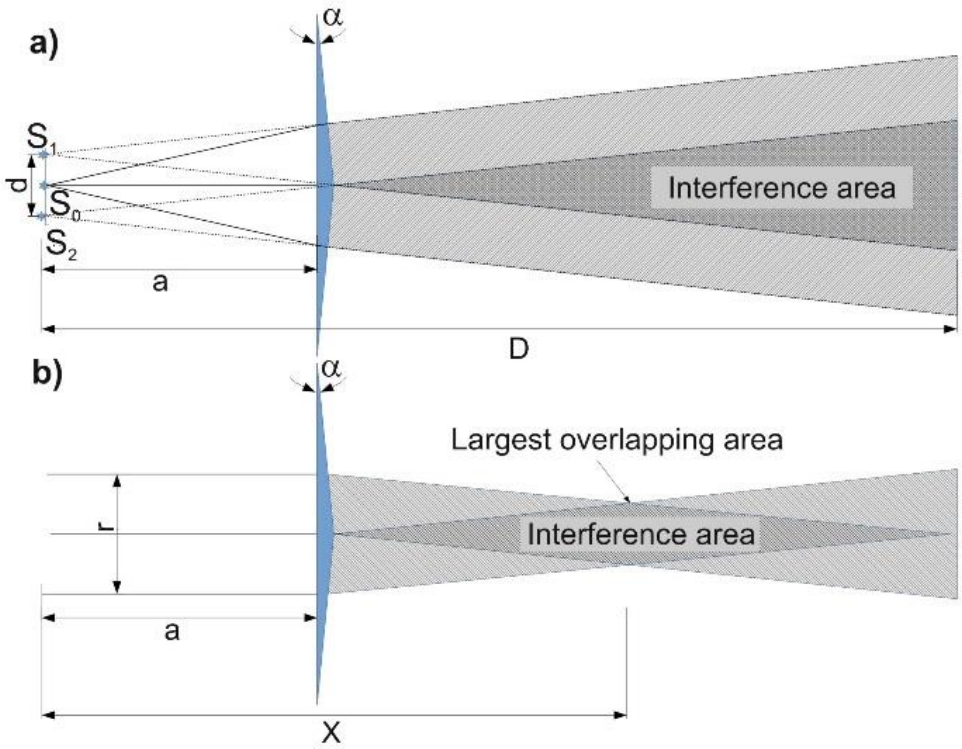


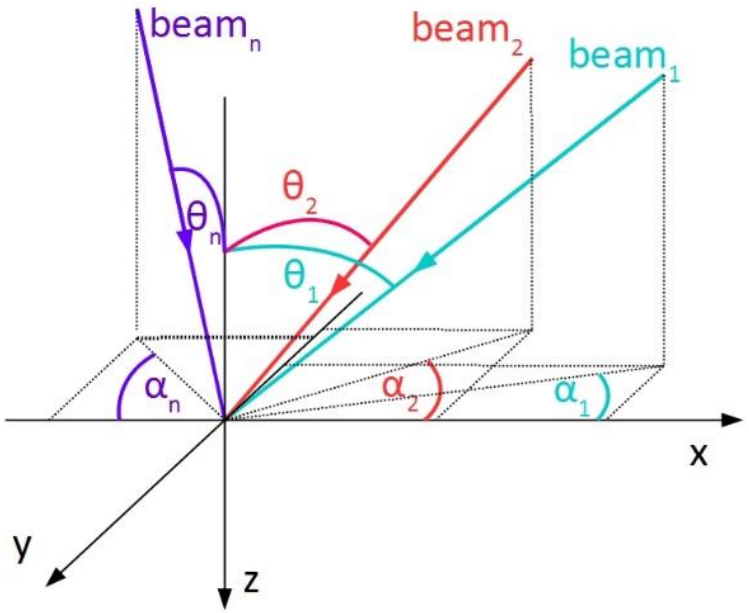


Materials&Methods

Radiation incidence on bi-prism

DLIL

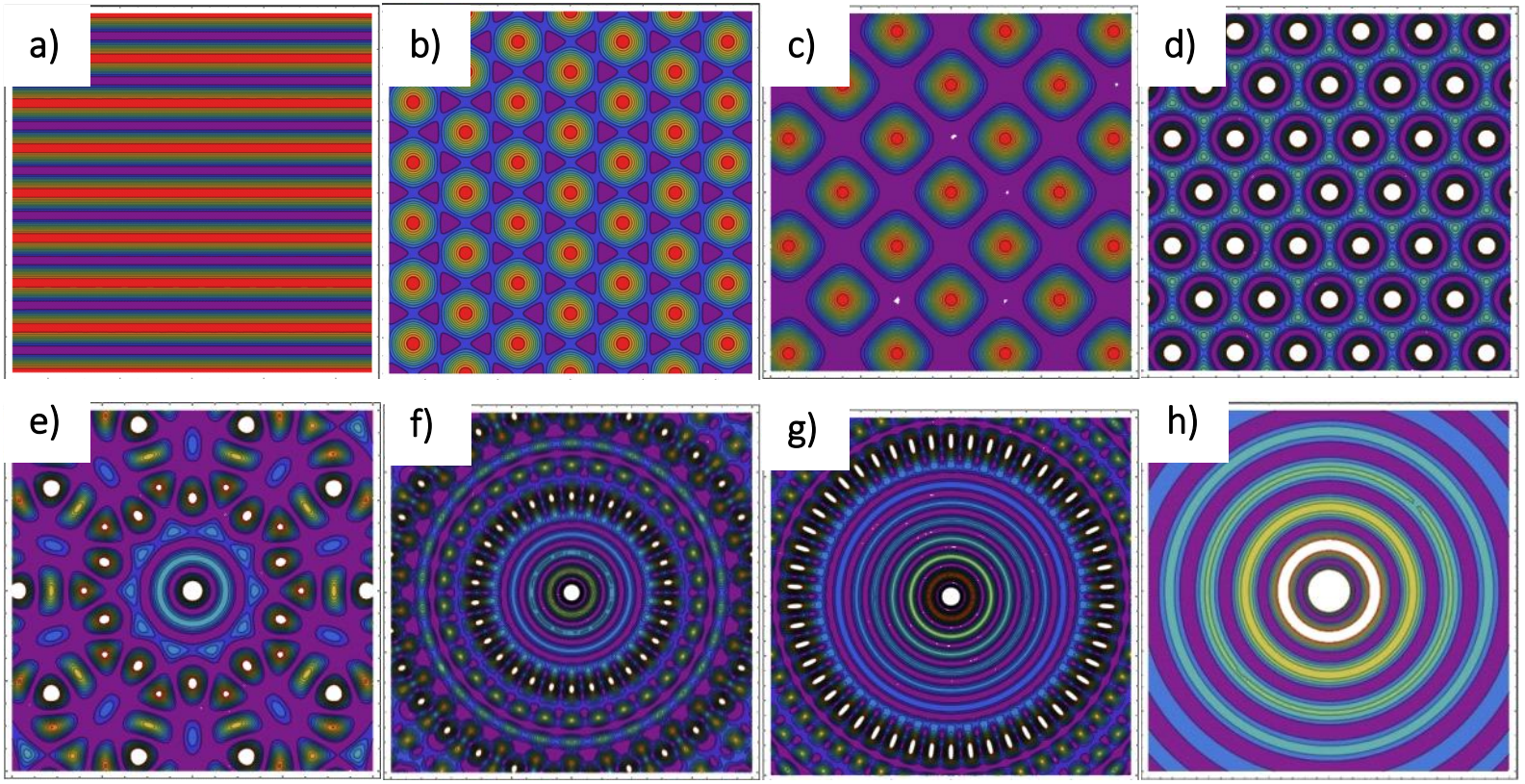




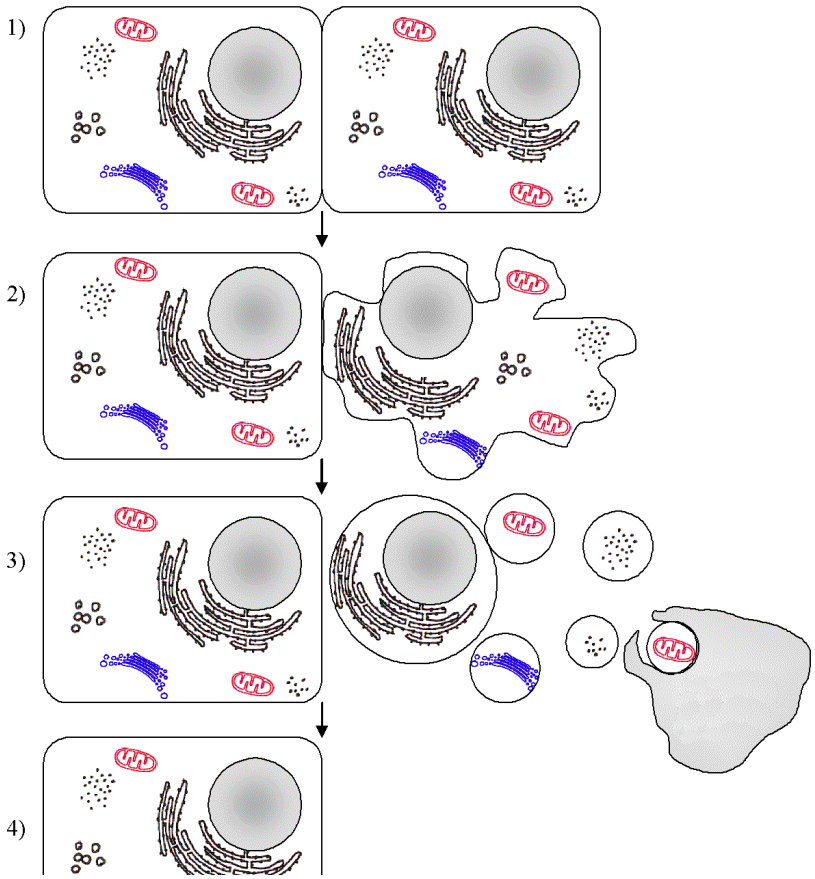
$$\Lambda = \frac{\lambda}{2\sin\left(\arcsin(n\sin\alpha) - \alpha\right.}$$

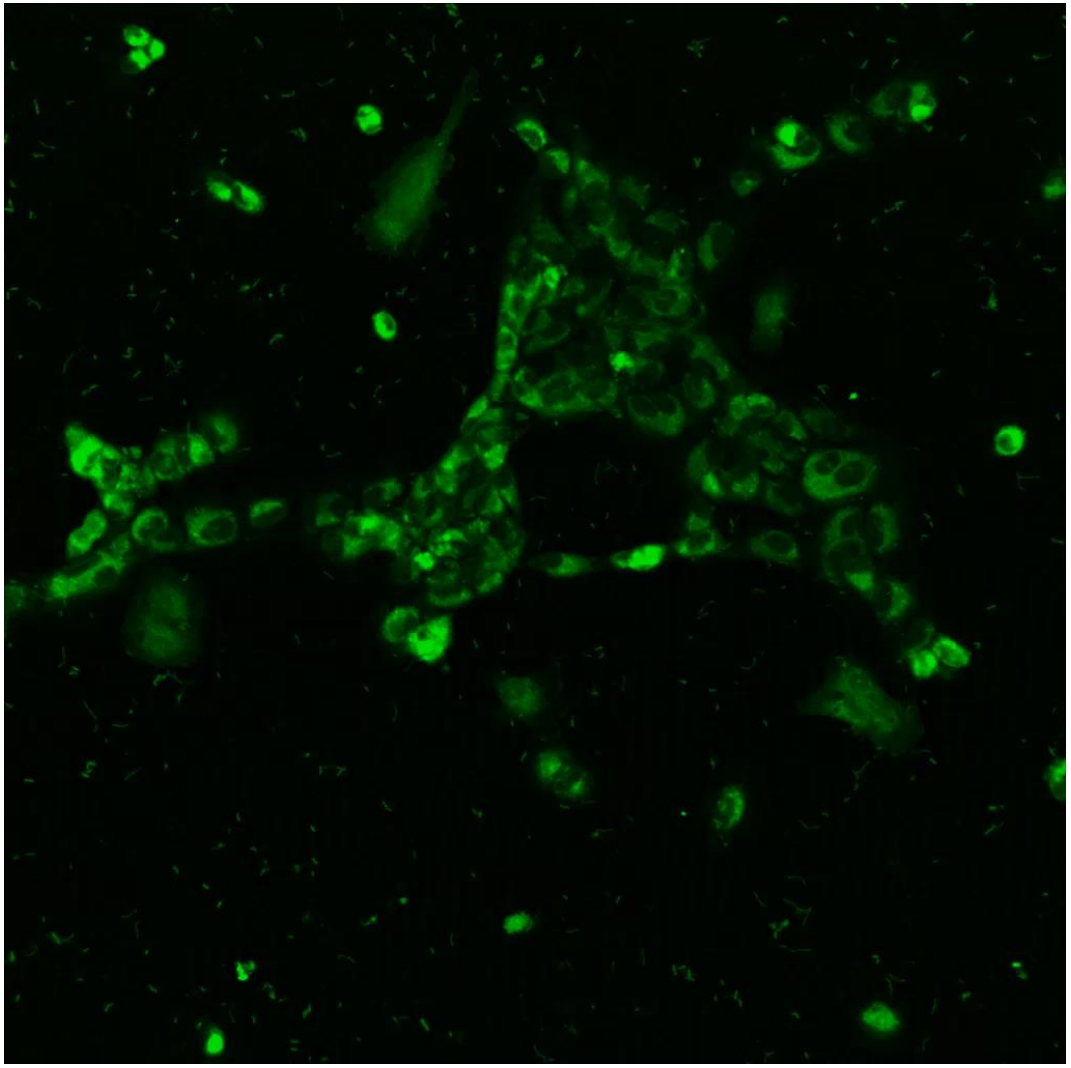
Materials&Methods

Radiation incidence on bi-prism

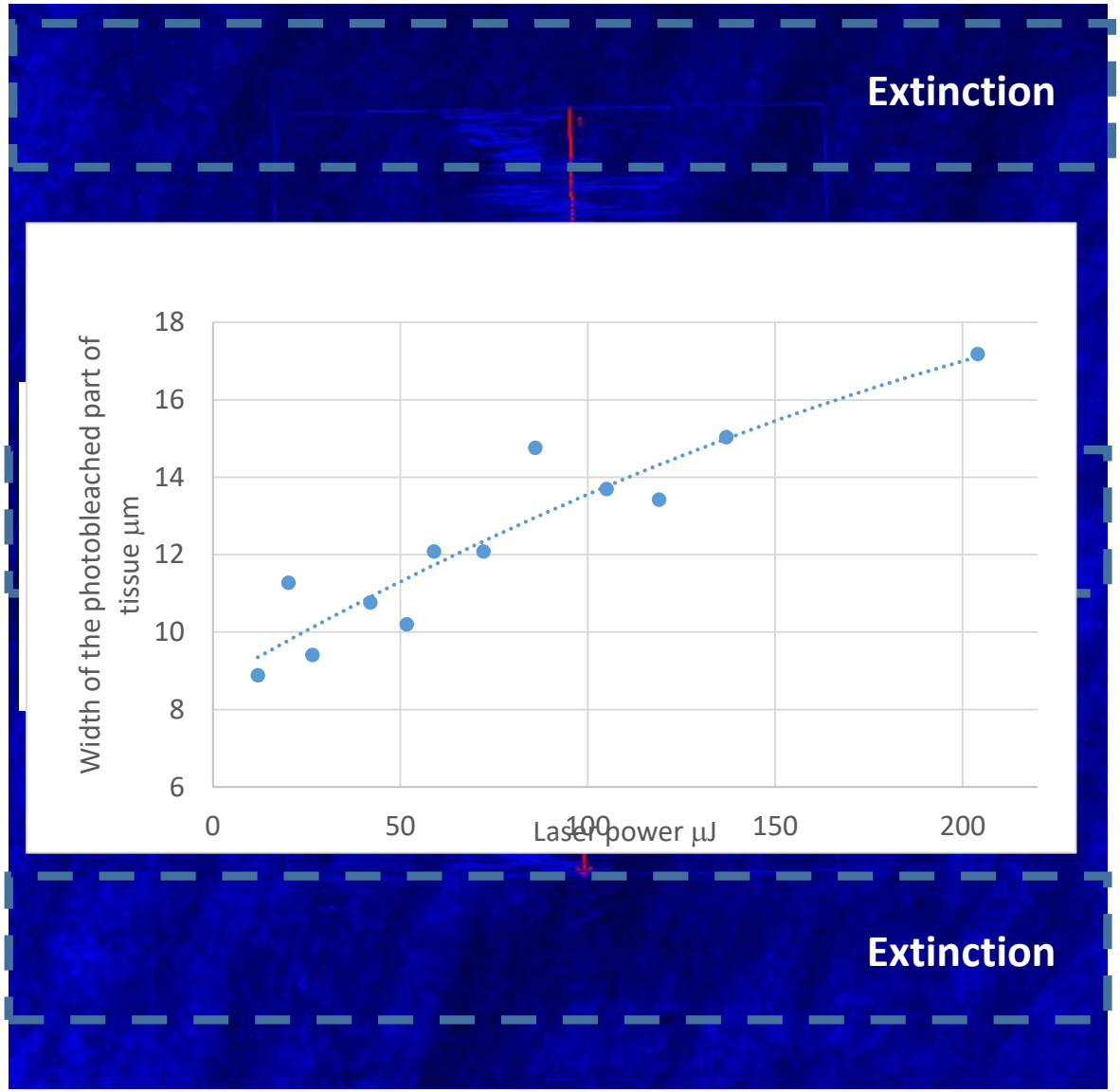


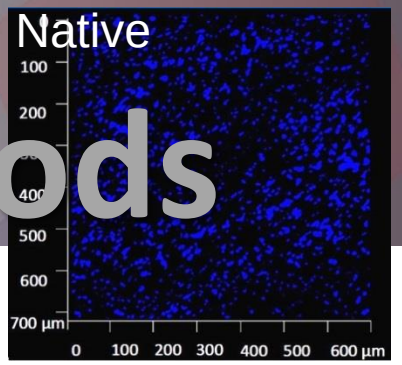
Materials&Methods

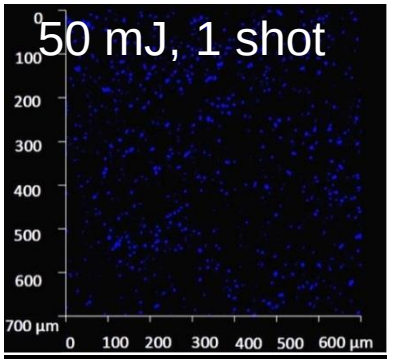


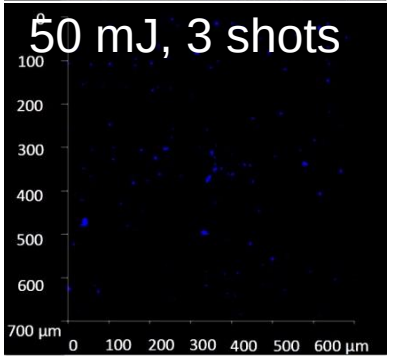


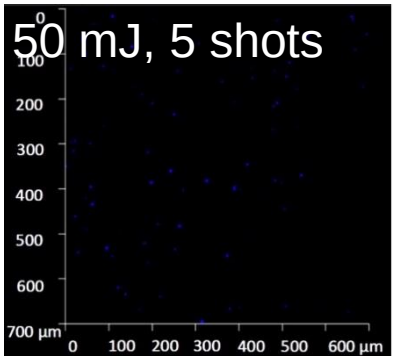
Materials&Methods



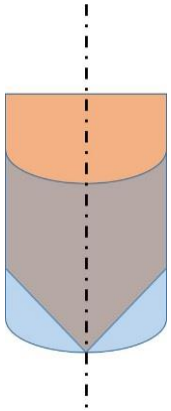








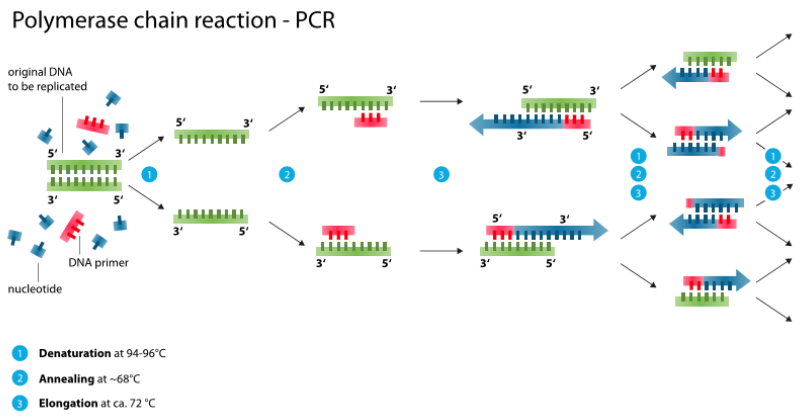
Real Time PCR



Low laser energy

Medium laser energy

High laser energy



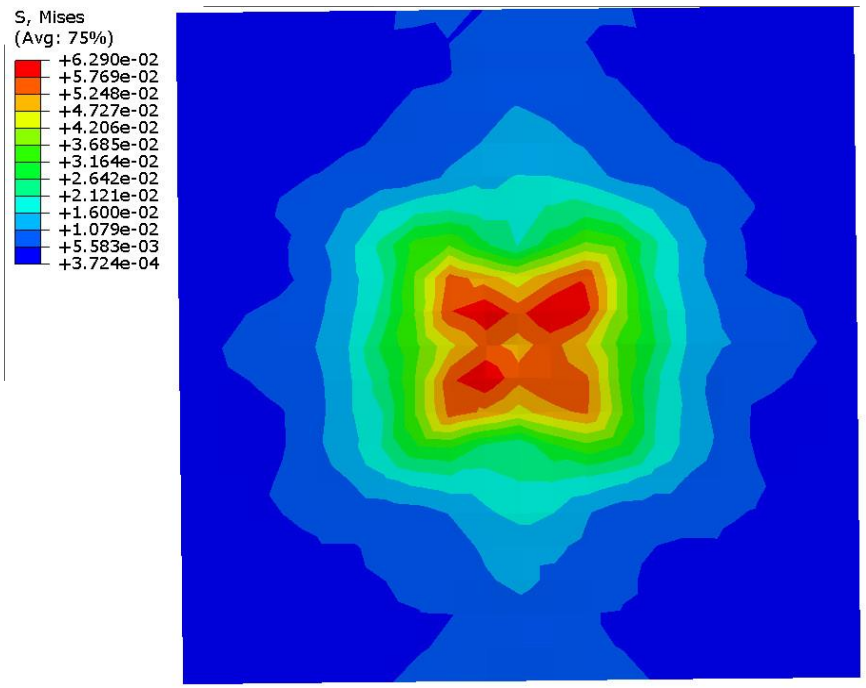
Kary Mullisa

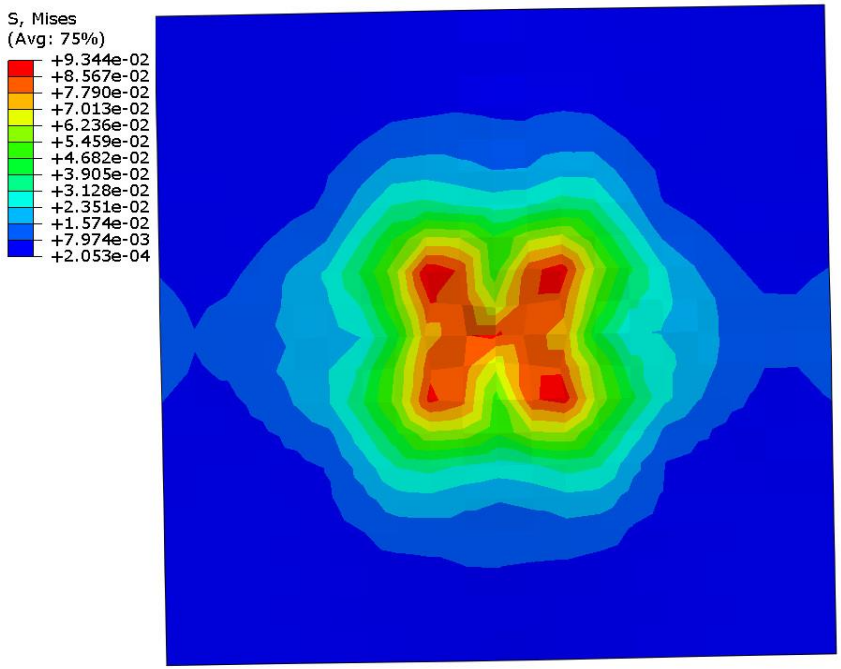
Sample	Energy	Energy density	Diameter of the beam	Repetition	No of impulses	Wavelength
AA1	65mJ	0.169 J/cm^2	7 mm	5 Hz	100	1064 nm
AA2				5 Hz	50	
AP3				5 Hz	10	
AP4				5 Hz	200	
AP5				5 Hz	500	
AP6				1 Hz	100	
XZ7	65mJ	0.169 J/cm^2	7 mm	5 Hz	100	1064 nm
XZ8	32 mJ	0.083 J/cm^2		5 Hz	100	
AA9	32 mJ	0.083 J/cm^2	7 mm	5 Hz	100	1064 nm
AA10				1 Hz	100	
AA11				1 Hz	200	
AA12				5 Hz	200	
AA13				5 Hz	500	
AA14				5 Hz	50	
AA15				5 Hz	10	
AA16	62 mJ	0.109 J/cm^2	8.5 mm	5 Hz	500	532 nm
AA17				5 Hz	200	
AA18				5 Hz	100	
AA19				1 Hz	100	
AA20				5 Hz	50	
AA21				5 Hz	10	
XZ22				5 Hz	100	
AP23	62 mJ	0.109 J/cm^2	8.5 mm	5 Hz	500	532 nm
AP24				5 Hz	200	
AP25				5 Hz	100	
AP26				1 Hz	100	
AP27				5 Hz	50	
AP28				5 Hz	10	
AP29	32 mJ	0.056 J/cm^2	8.5 mm	5 Hz	500	532 nm
AP30				5 Hz	200	
AP31				5 Hz	100	
AP32				1 Hz	100	
AP33				5 Hz	50	
AP34				5 Hz	10	
AA35	32 mJ	0.056 J/cm^2	8.5 mm	5 Hz	500	532 nm
AA36				5 Hz	200	
AA37				5 Hz	100	
AA38				1 Hz	100	
AA39				5 Hz	50	
AA40				5 Hz	10	

FE- Simulation

Stress

$$I(r) = \frac{2E}{\pi w^2} \exp[-2\,r^2/w^2]$$

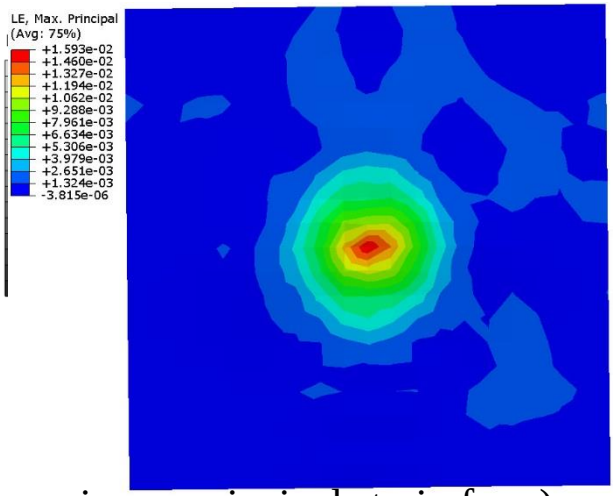


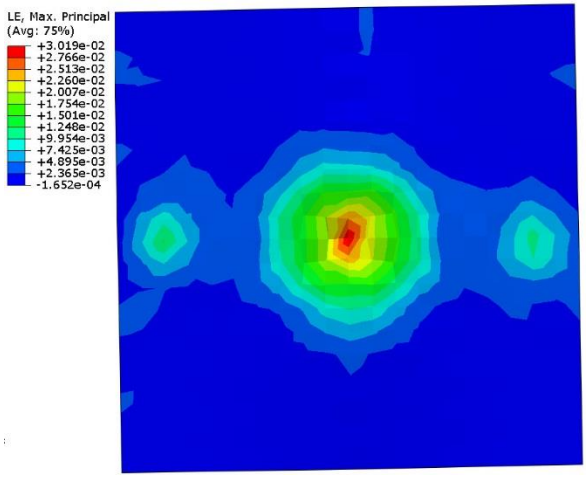


Distributions of von Mises stress, MPa for a) aortic and b) pulmonary valve sample models (top view).

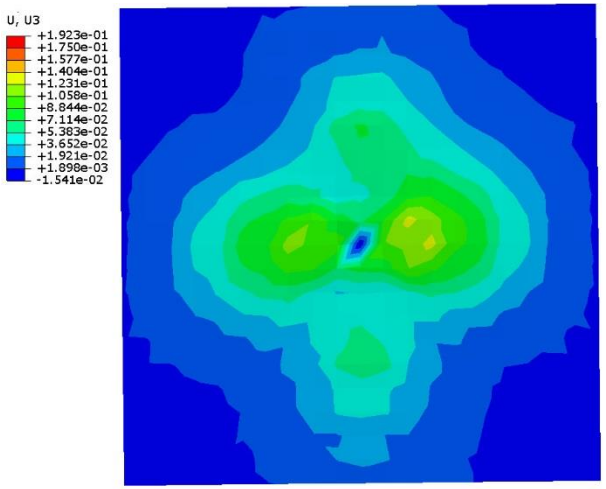
FE- Simulation

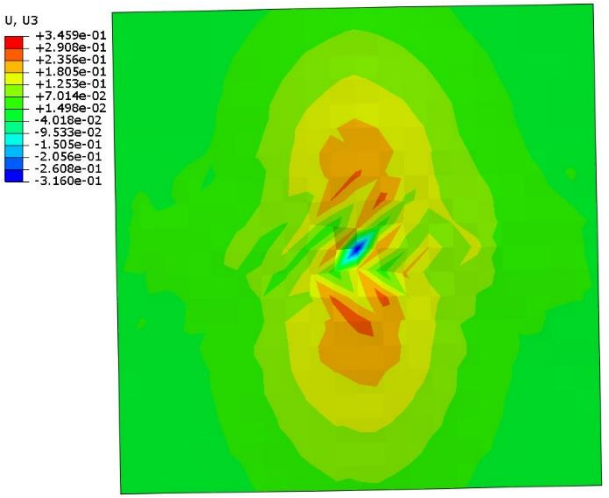
Strain





Distributions of maximum principal strain for a) aortic and b) pulmonary valve sample models (top view).





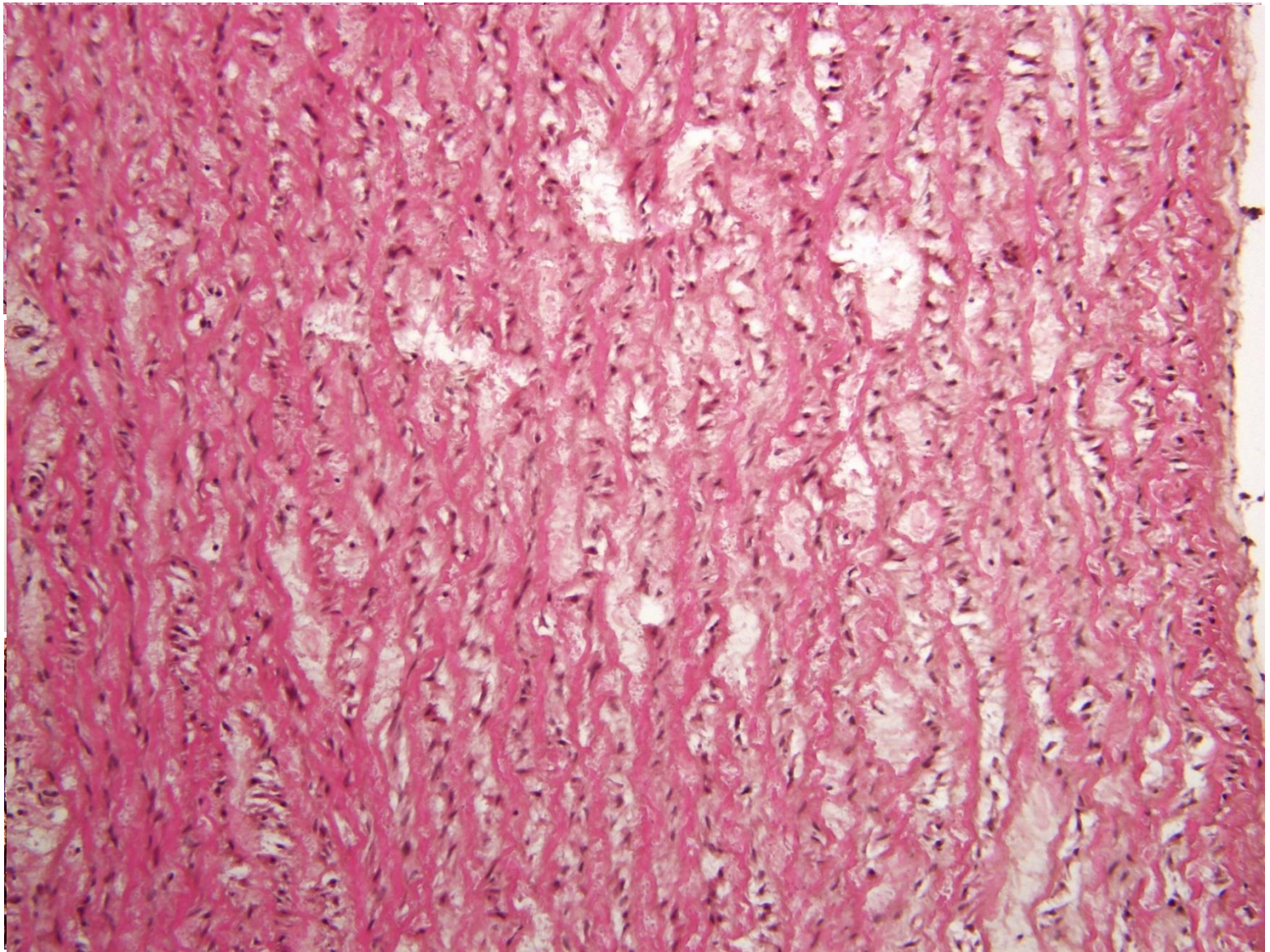
Distributions of Z - displacement, mm for a) aortic and b) pulmonary valve sample models (top view).

Tissue Preparation

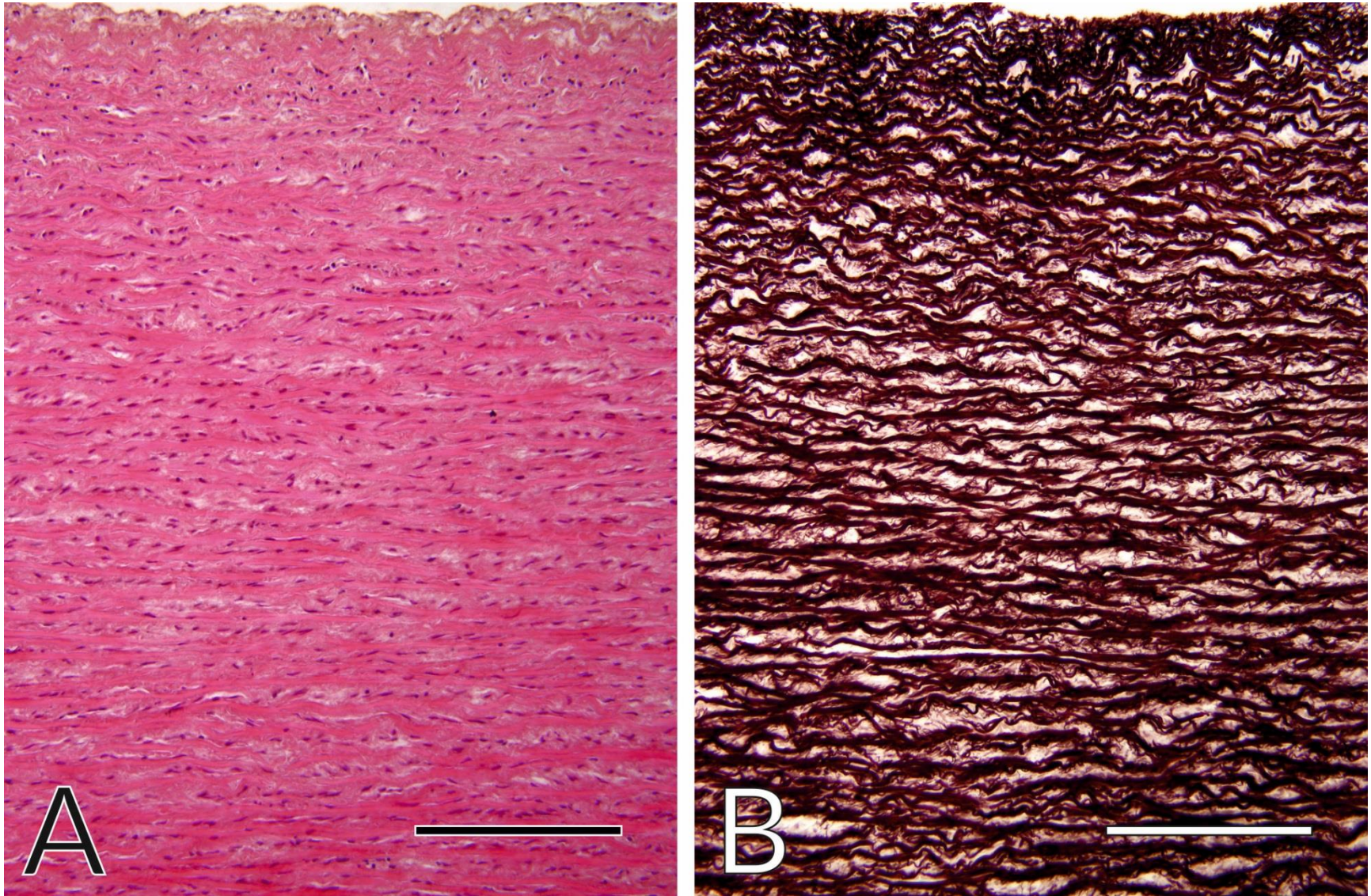
- Orcein for visualization of elastic fibers,
- Red syrium to visualize collagen fibers,
- Alcian blue for the visualization of the proteoglycans of the basic substance,
- Routine staining with hematoxylin / eosine and three- and multi-colored staining to visualize the overall morphology and evaluate the effectiveness of acellularisation,
- Hoechst 33342 / DAPI fluorescence staining reveal cell nuclei to assess the effectiveness of acellularisation.

Histology

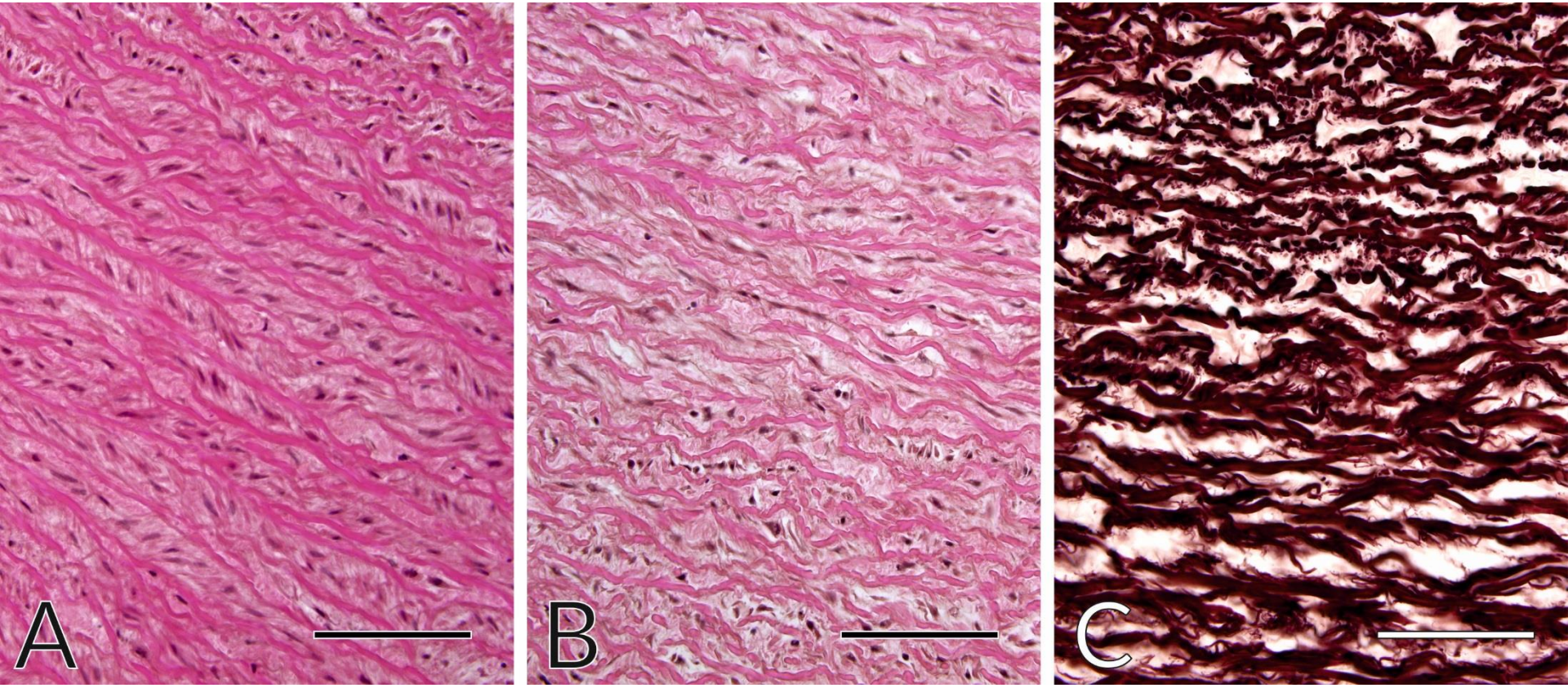
Histology



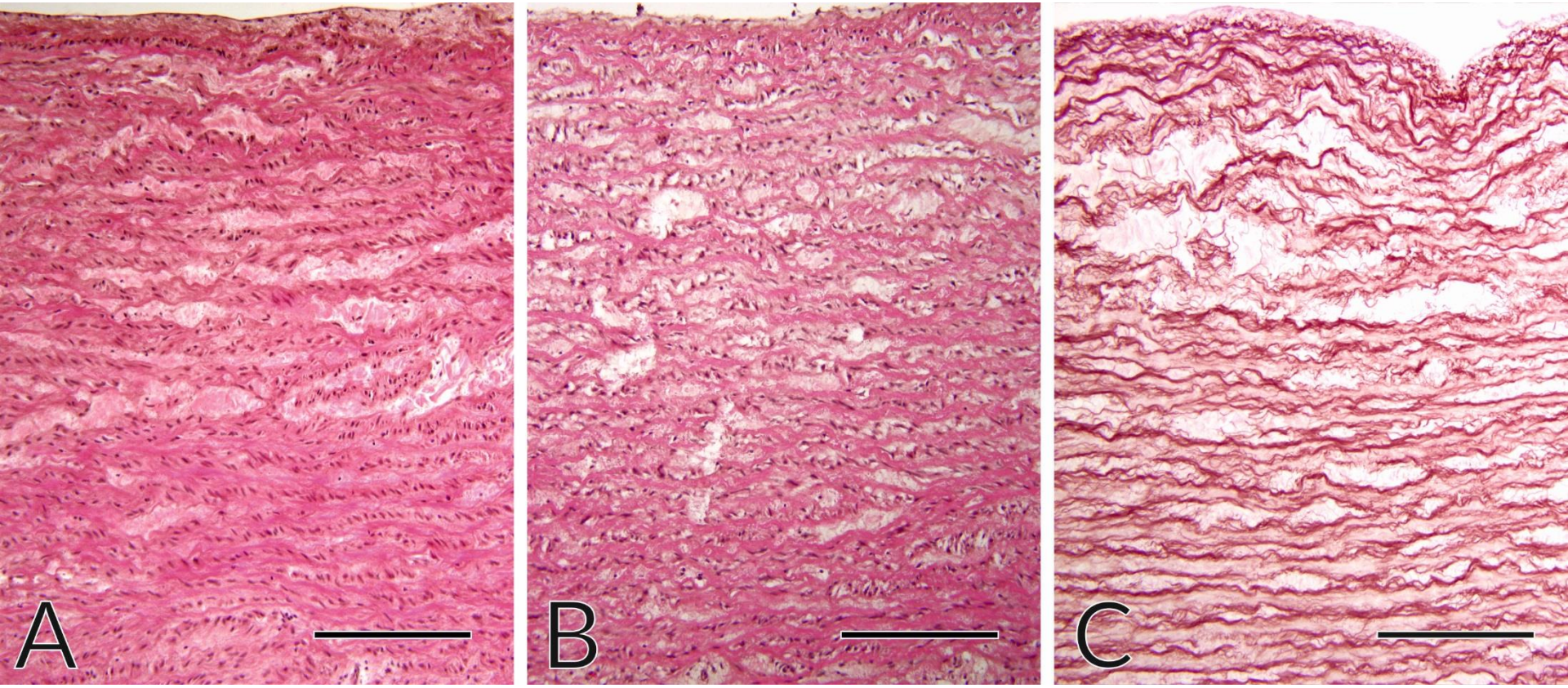
Histology



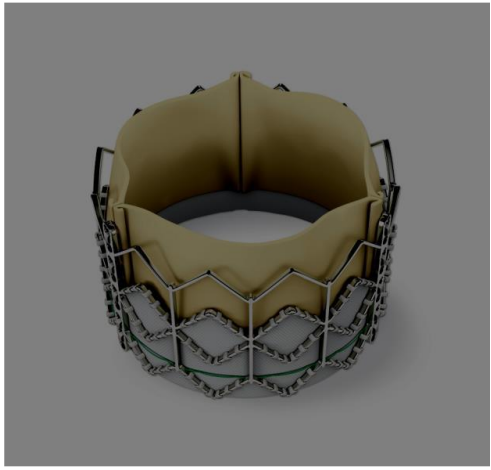
Histology



Histology



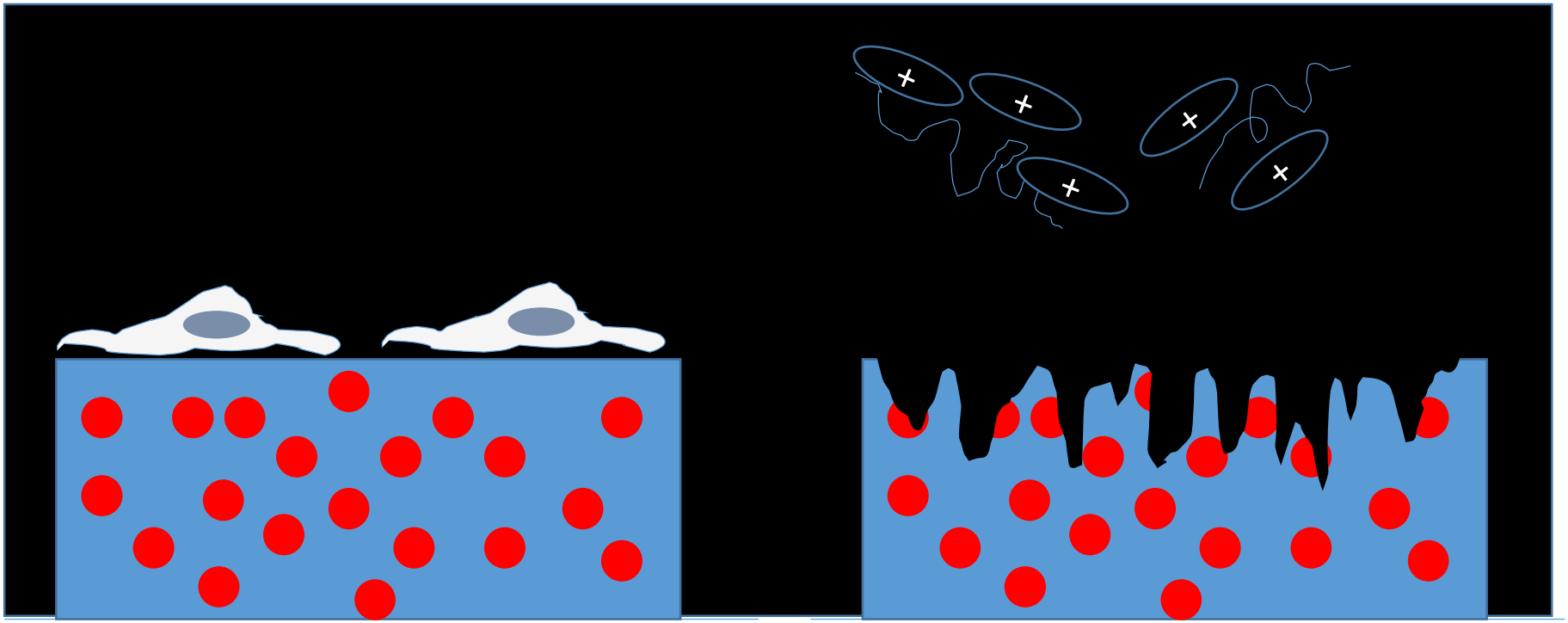
Coating



Acknowlegement

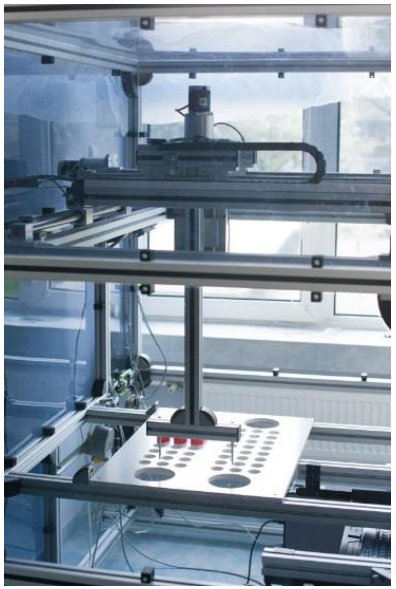
The research was financially supported by the Project no. 2016/23/B/ST8/01481 "Interdisciplinary methods of creating and functioning of biomimetic materials based on Animal origin extracellular matrix" of the Polish National Center of Science. Part of the work was co-financed by the **European Union from resources of the European Social Fund (Project No.WND-POWR.03.02.00-00-I043/16).**

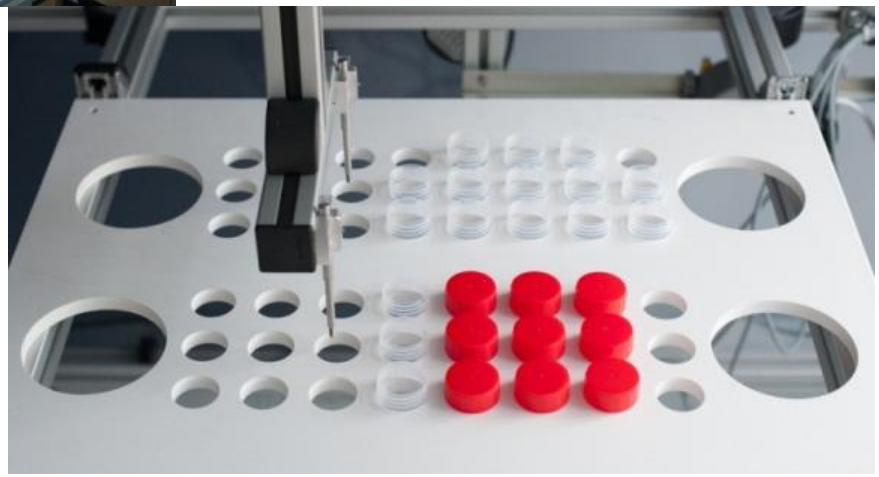
Analogi tkanki



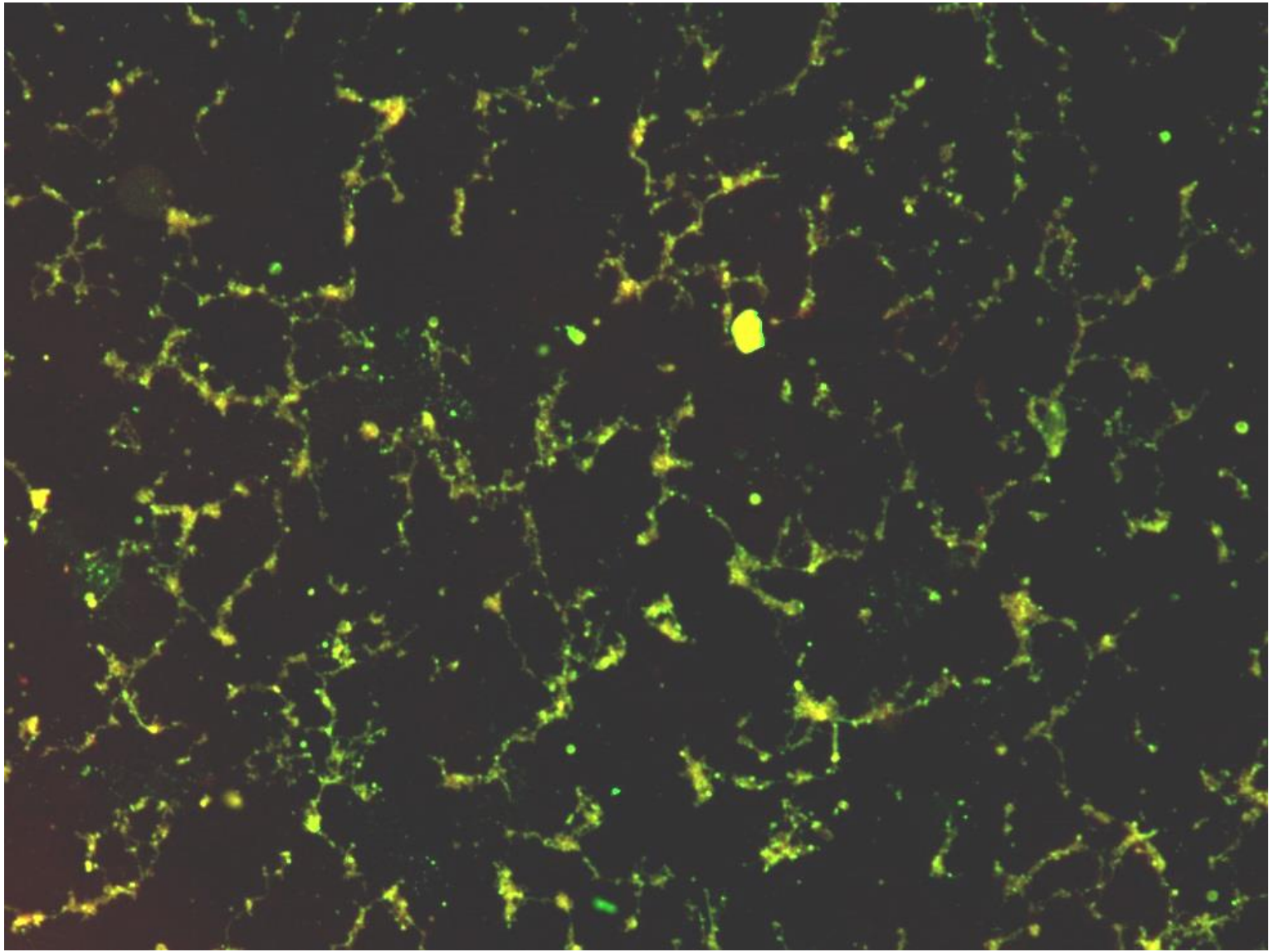
Bioaktywność poprzez kontakt

Bioaktywność poprzez degradację



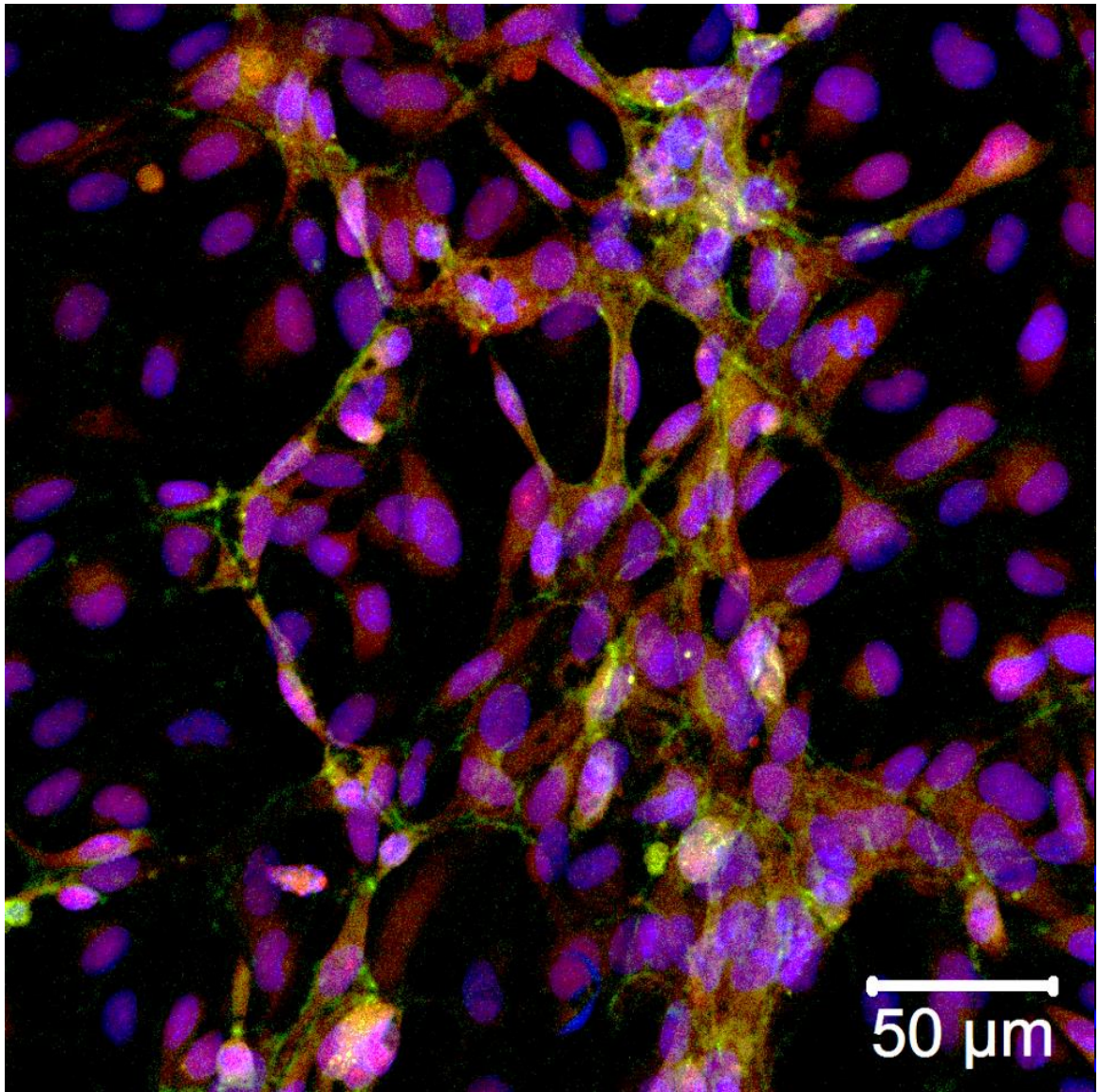


Polyelectrolytes



Cells

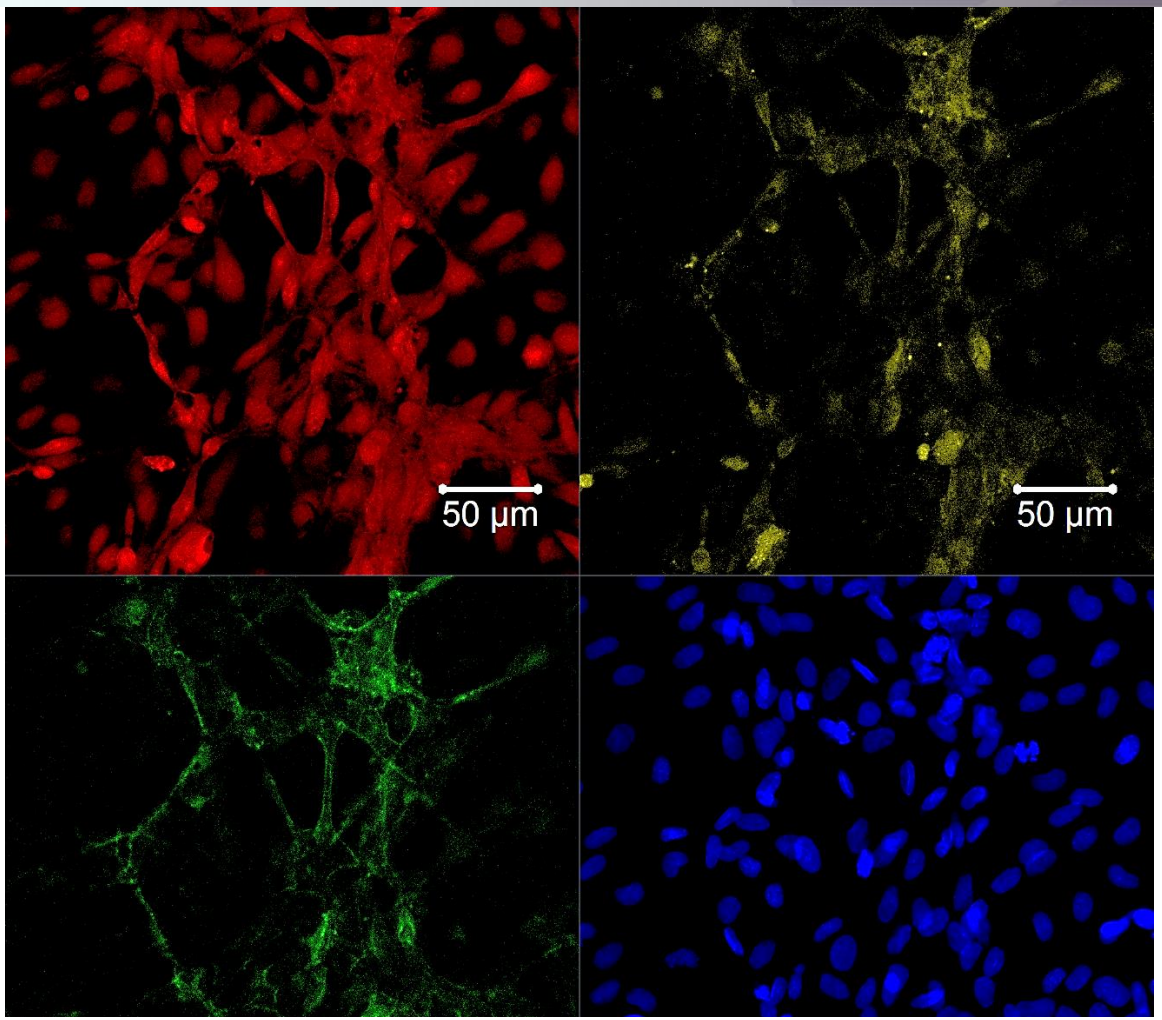
Differentiation



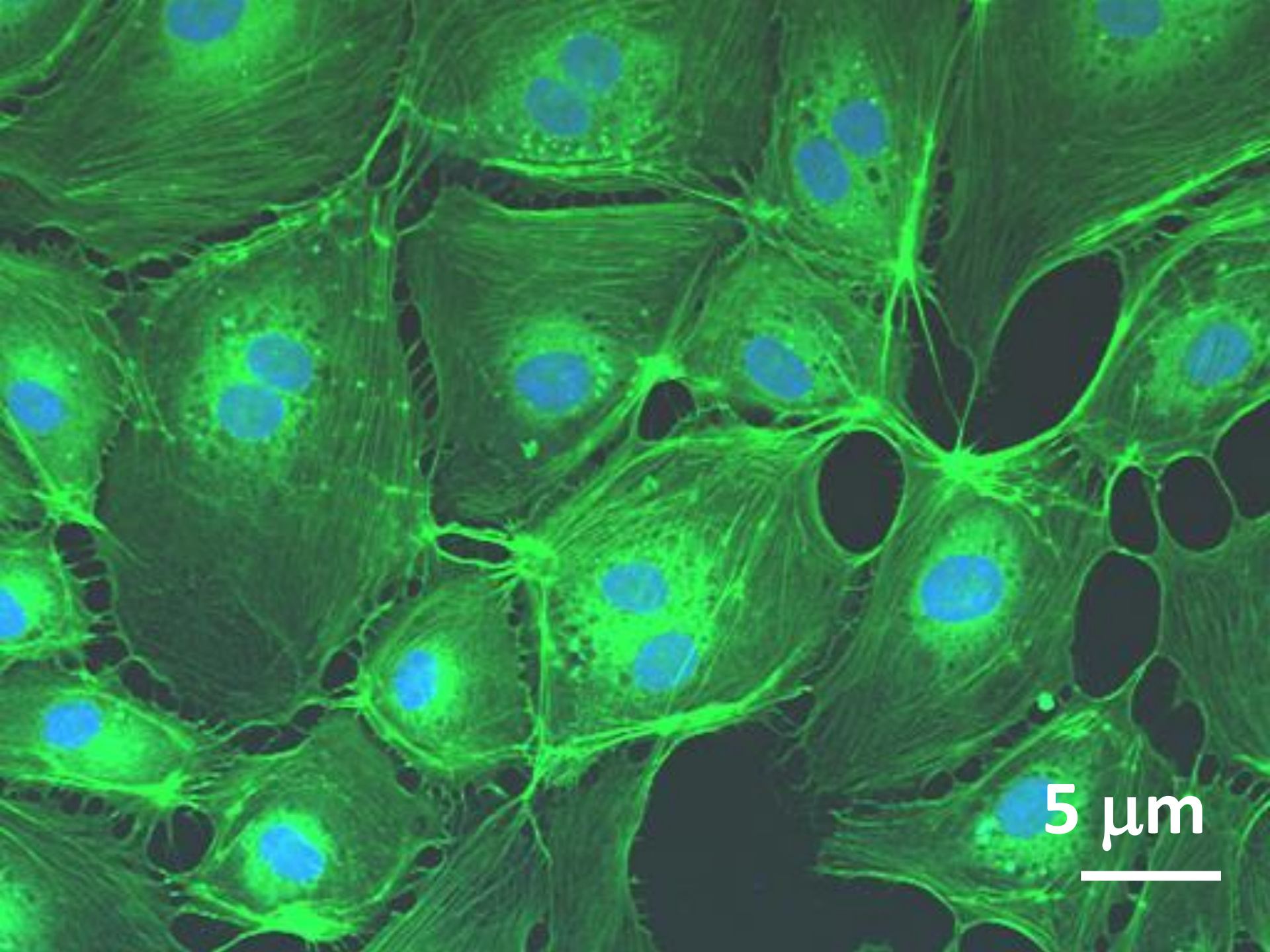
Red – PECAM-1
Yellow- selektin E
Green – tight junctions
Blue – nuclei

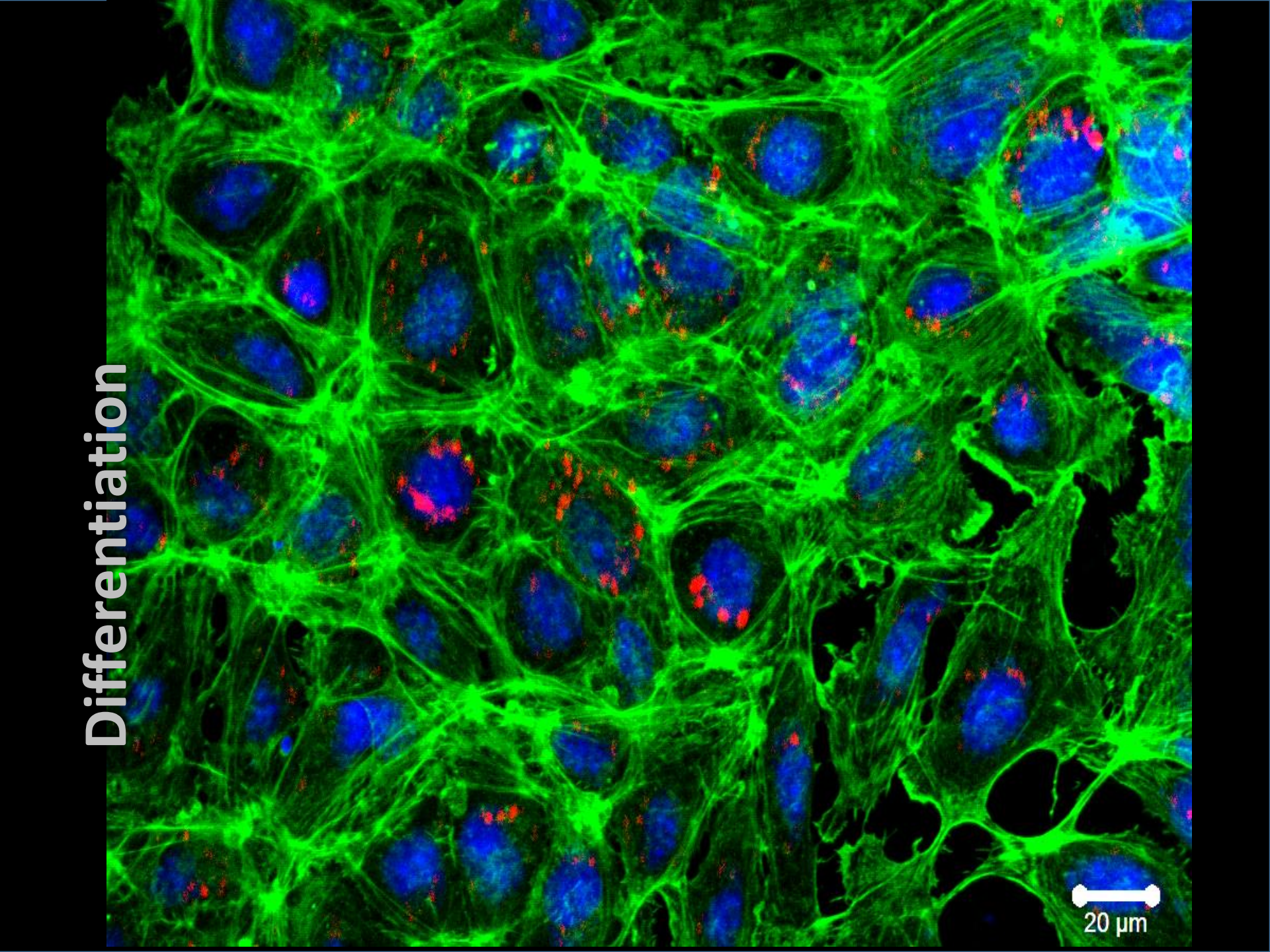
Cells

Differentiation

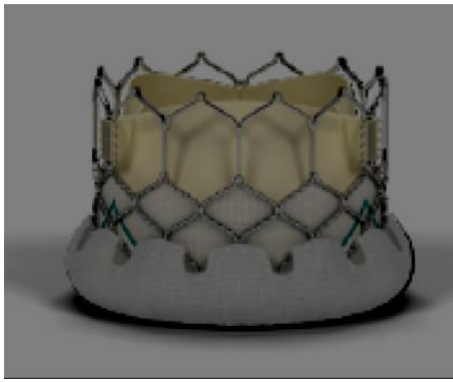


Red – PECAM-1
Yellow- selektin E
Green – tight junctions
Blue – nuclei


5 µm


Differentiation
20 µm

Coating

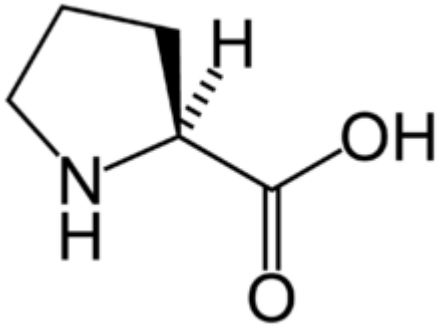


New cooperation and tasks

ProSAM- novel oligopropylene

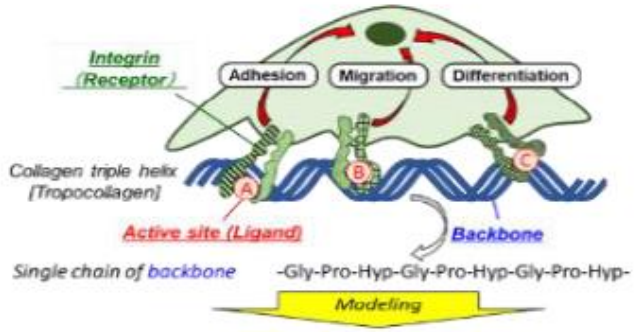
Pro6 (Ac-Cys-$(Pro)_6$-$CONH_2$)

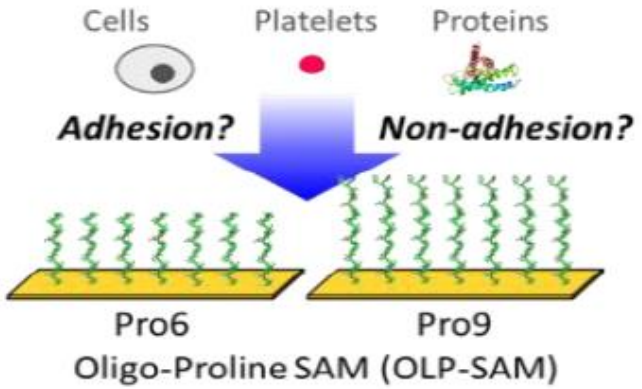
Pro9 (Ac-Cys-$(Pro)_9$-$CONH_2$)



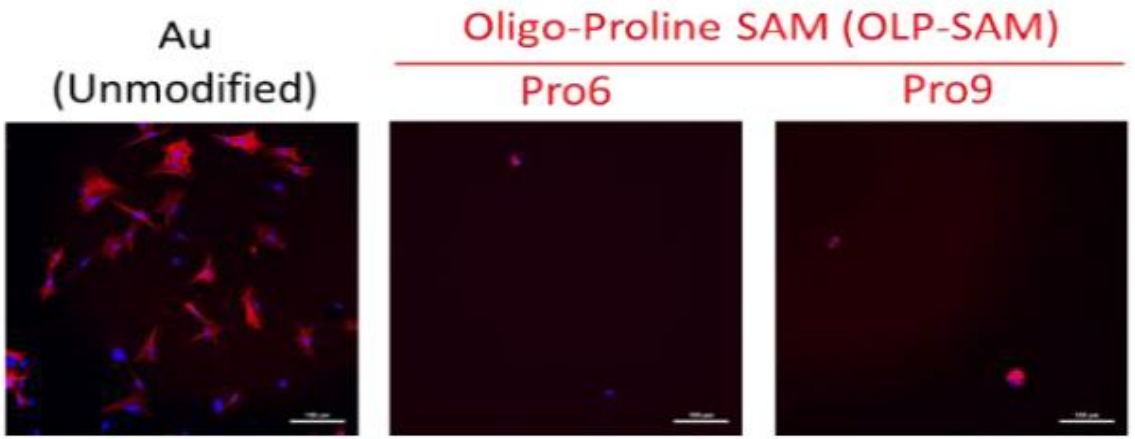
ProSAM

(A)


Integrin
(Receptor)
Adhesion
Migration
Differentiation
Collagen triple helix
[Tropocollagen]
A
B
C
Active site (Ligand)
Backbone
Single chain of backbone
-Gly-Pro-Hyp-Gly-Pro-Hyp-Gly-Pro-Hyp-
Modeling
Oligo-prolines
Pro6: Ac-Cys-Pro-Pro-Pro-Pro-Pro-Pro-CONH2
Pro9: Ac-Cys-Pro-Pro-Pro-Pro-Pro-Pro-Pro-Pro-Pro-CONH2

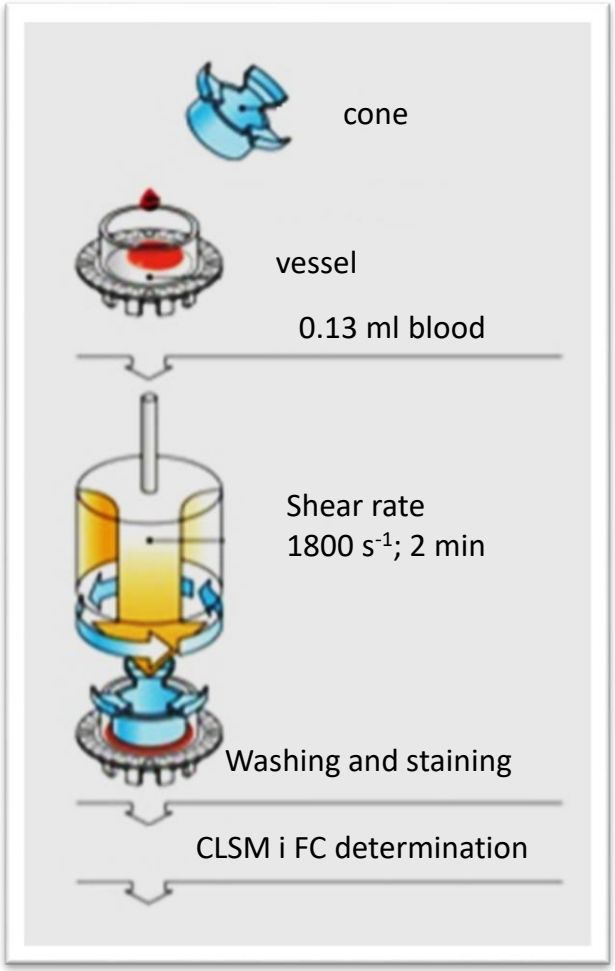

Cells
Platelets
Proteins
Adhesion?
Non-adhesion?
Pro6
Pro9
Oligo-Proline SAM (OLP-SAM)

(B)

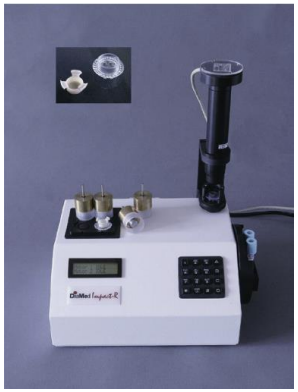

Au
(Unmodified)
Oligo-Proline SAM (OLP-SAM)
Pro6
Pro9

ProSAM

ProSAM interaction with blood



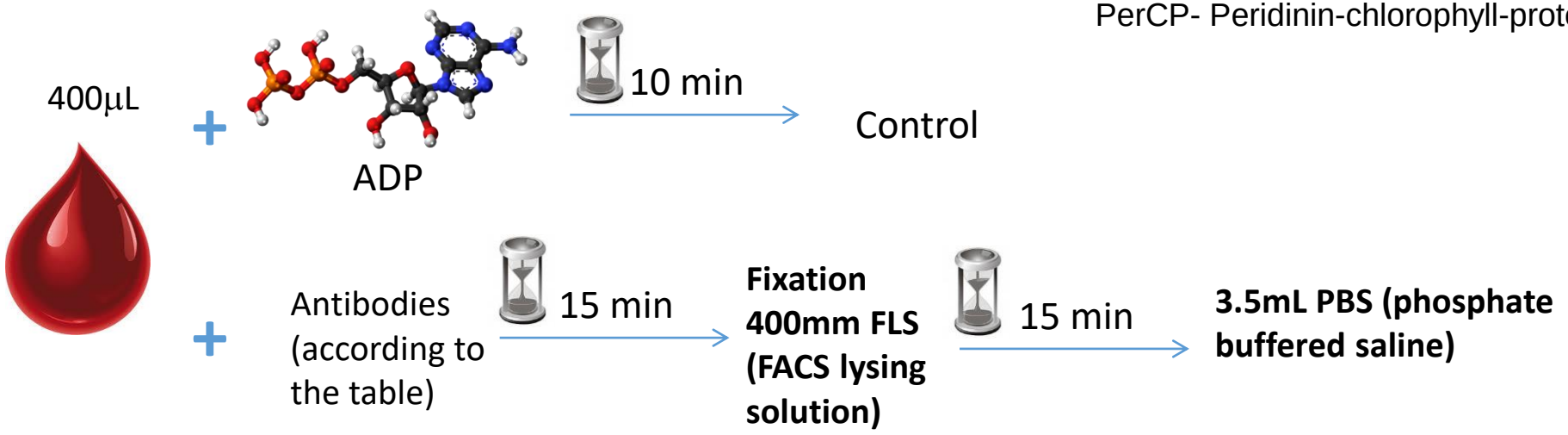
Impact R test



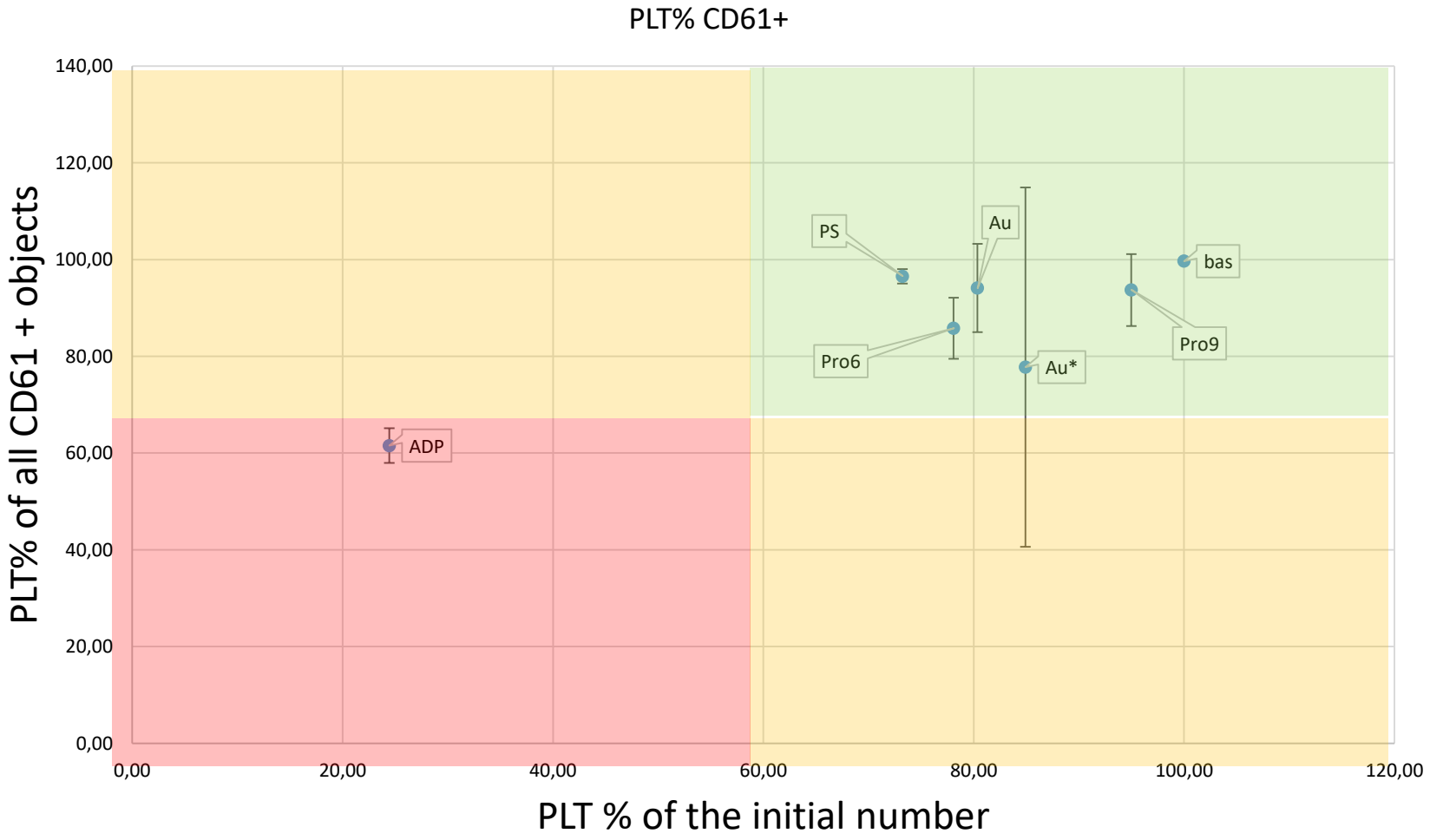
ProSAM

Name	Composition	Application
M	CD61-PerCP, CD62P, PAC-1	evaluation of activation in terms of contact with the material (+ control)
A	CD61-PerCP, CD62P, PAC-1	assessment of platelet activation using ADP
ADP 1	ADP 0.4mM	Positive control

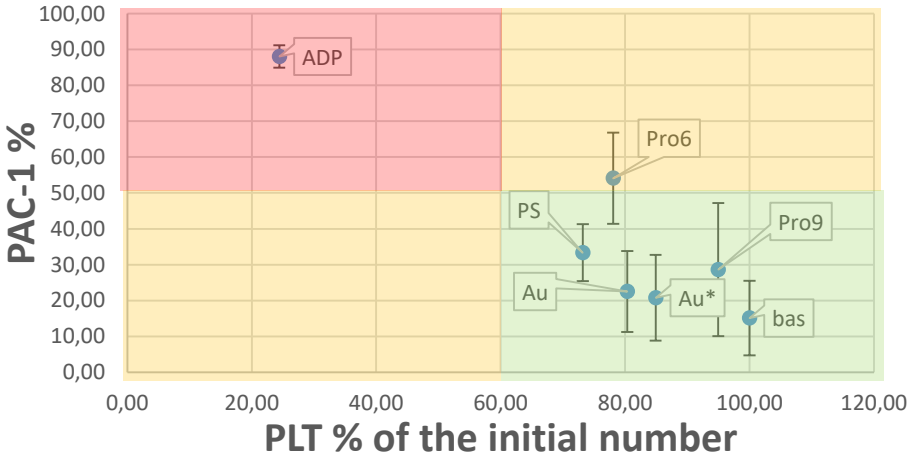
PerCP- Peridinin-chlorophyll-protein

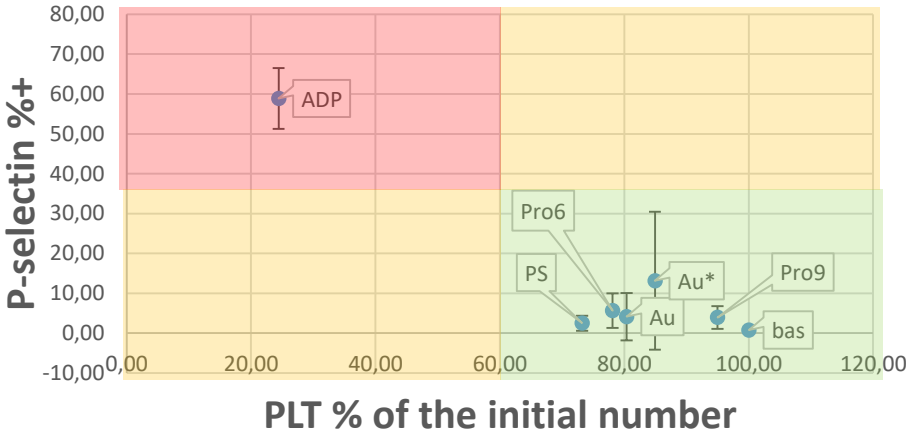


Impact-R test- PLT consumtion



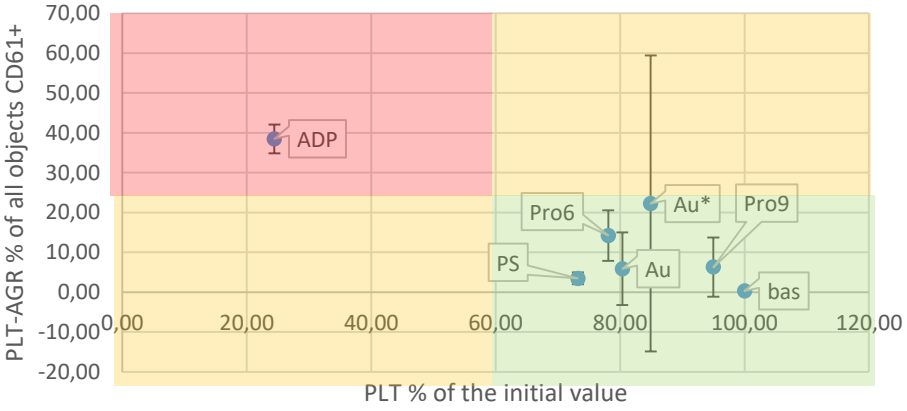
Impact-R test- PLT activation

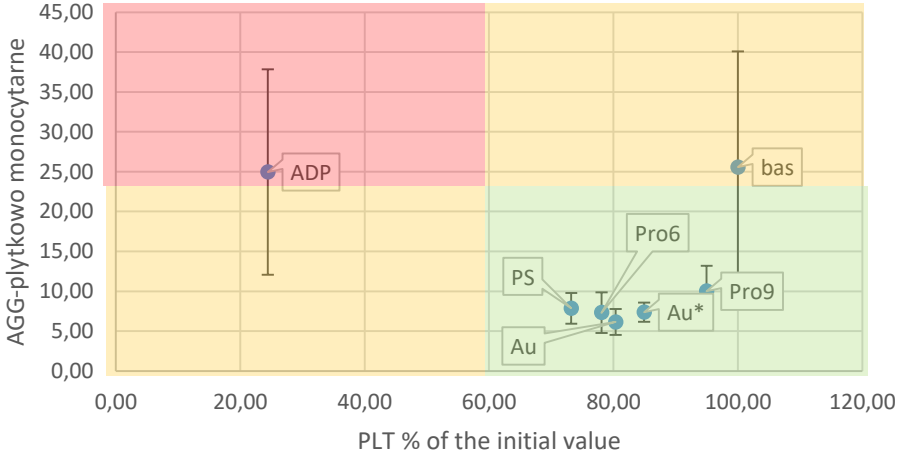


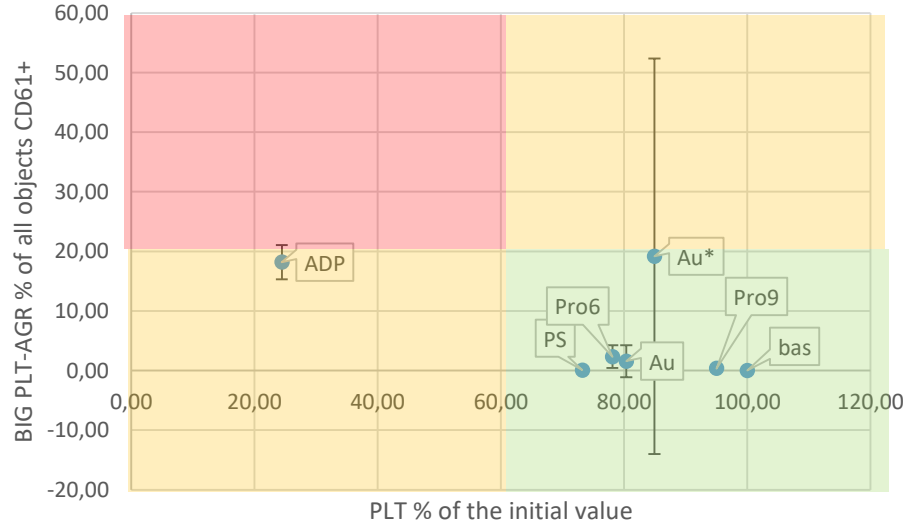


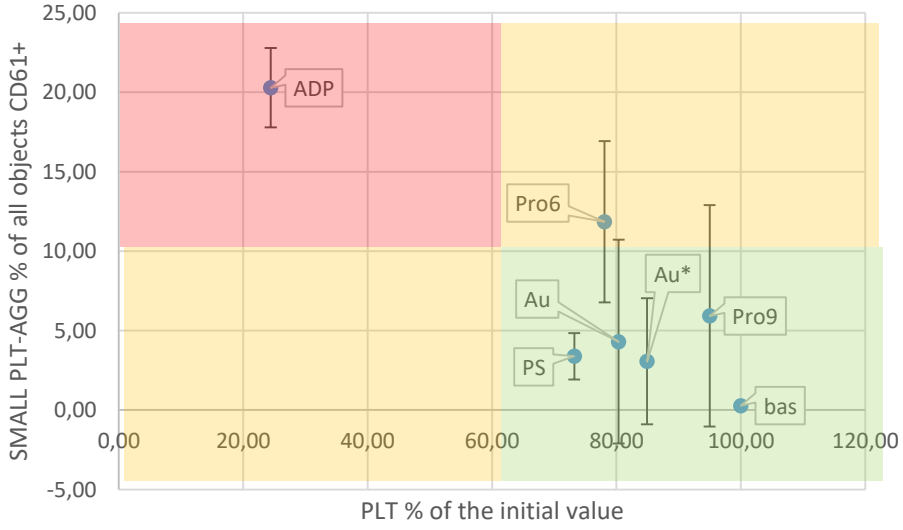
ProSAM

Impact-R test- PLT activation



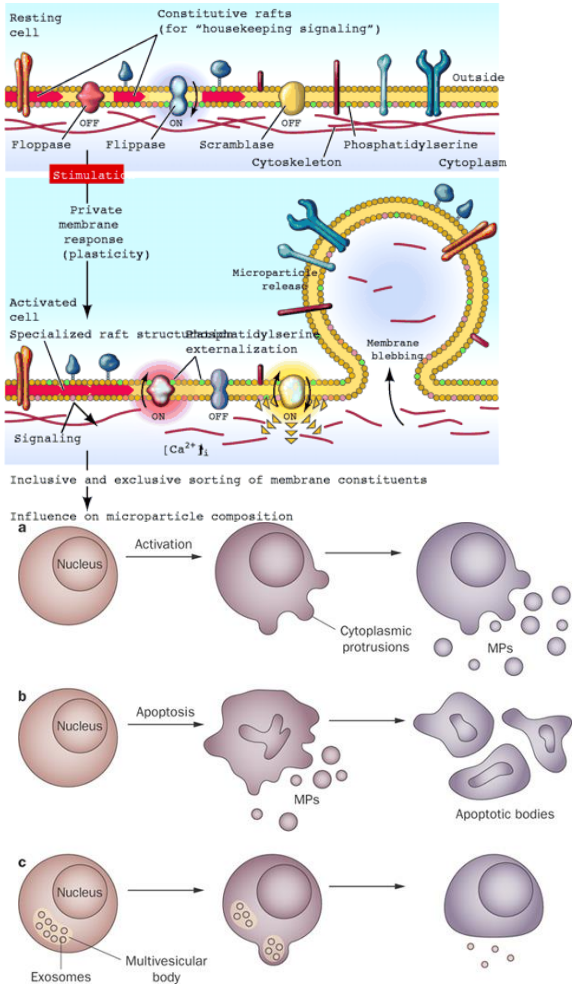


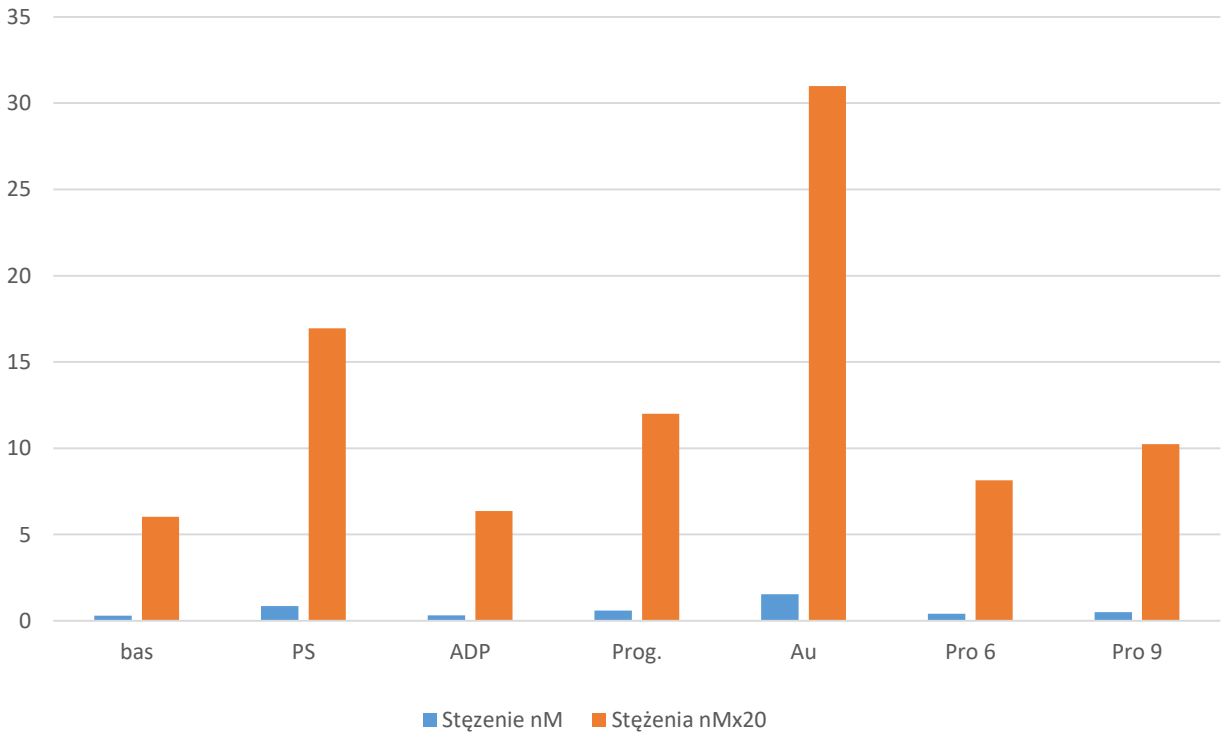




ProSAM

Impact-R test- PLT microparticles





The first challenge of the research will concern synthesis of oligopeptides library that contains sequences without specific functionality and with variable chain length and secondary structure (especially polyproline-II helix) depends on the single substitutions in the amino acid sequence preserved among all library variants.

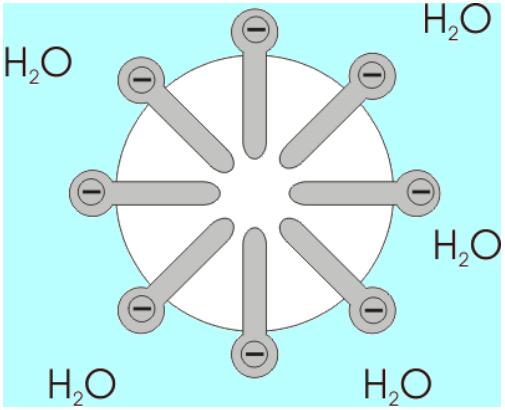
In the next step, all oligopeptide variants will be immobilized on the surface of polyurethane materials previously modified with thin carbon based ceramic coating in order to correlate oligopeptides secondary structure, chain length and spatial orientation with number, type, aggregation range and lateral interactions of proteins adsorbed from blood serum.

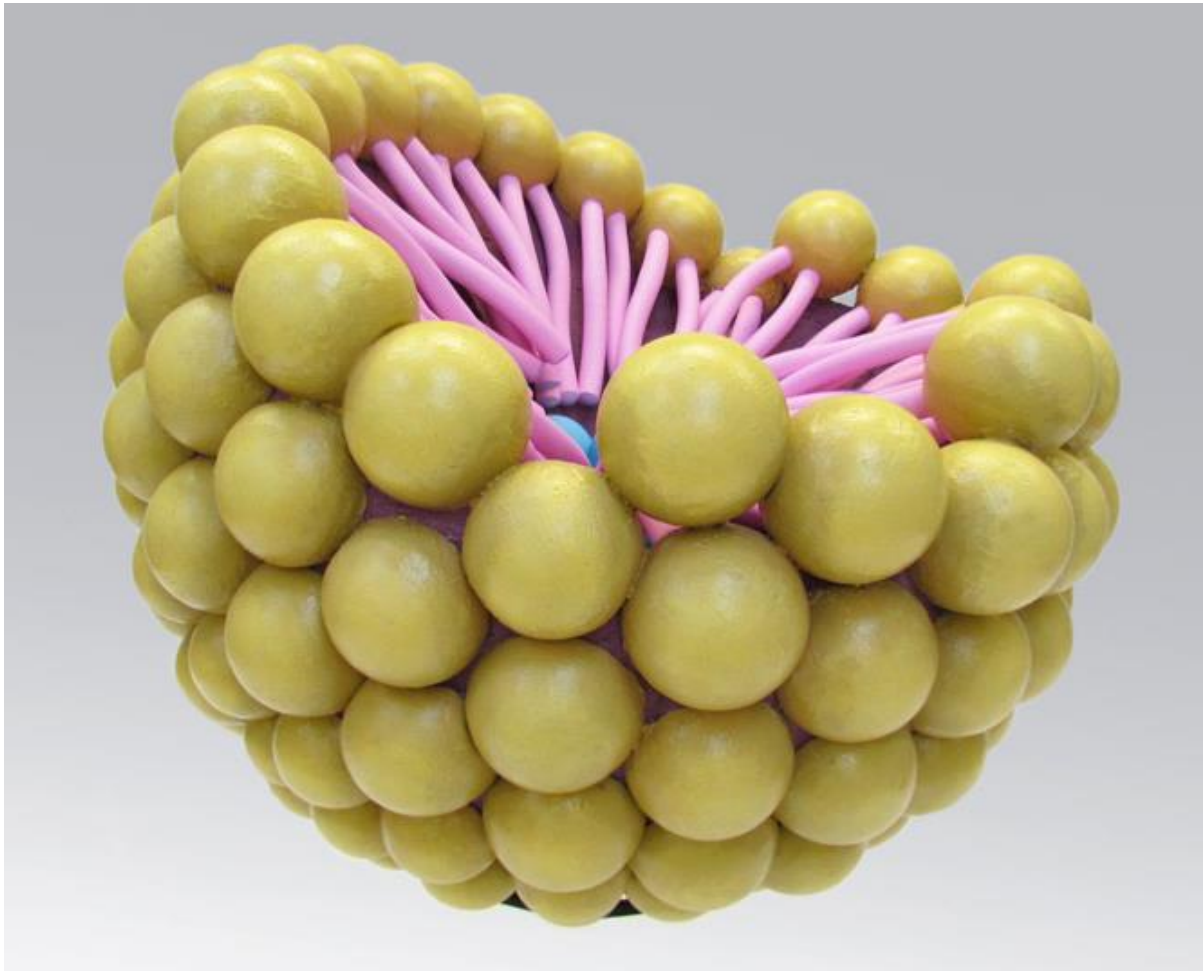
The oligopeptides will be deposited and characterized regarding to the physicochemical properties, the endothelial cells response as well as the *in vitro* hemocompatibility under dynamic conditions.

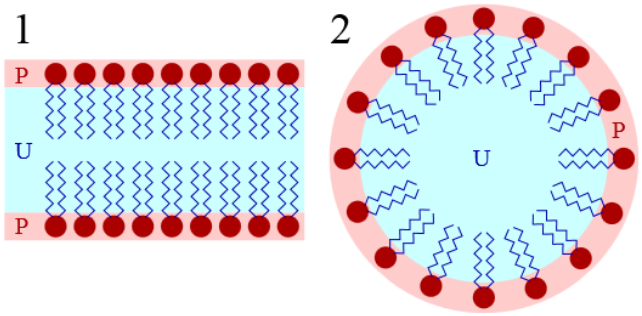
The project will result in phenomenological description of the influence of surface-immobilized oligopeptides secondary structure, mobility, backbone stiffness on their biological activity evaluated based on interaction with serum proteins, blood morphotic elements and endothelial cells under flow conditions.

This will decide about final design of peptide-based SAM coating that may be successfully applied to functionalize surface of the heart assist device.

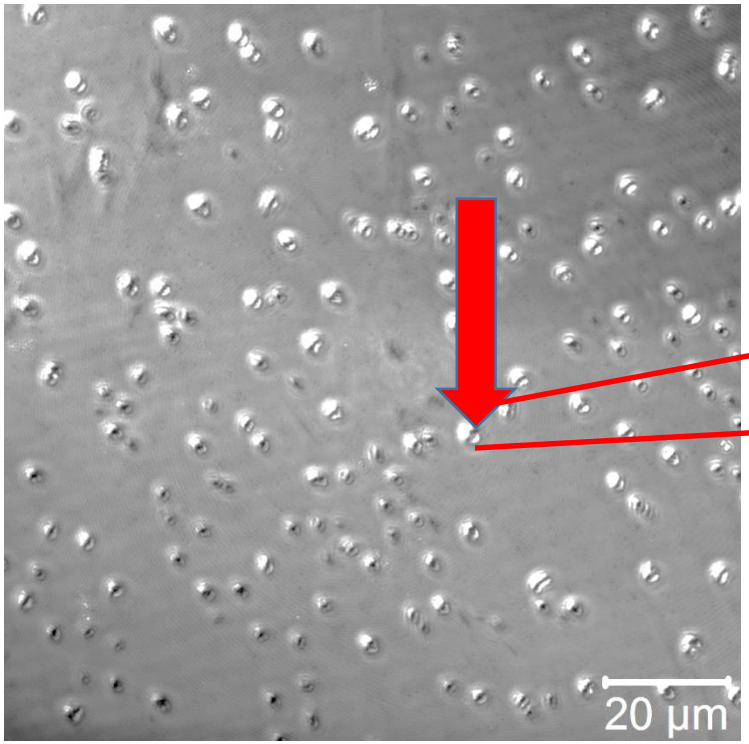
Micelles

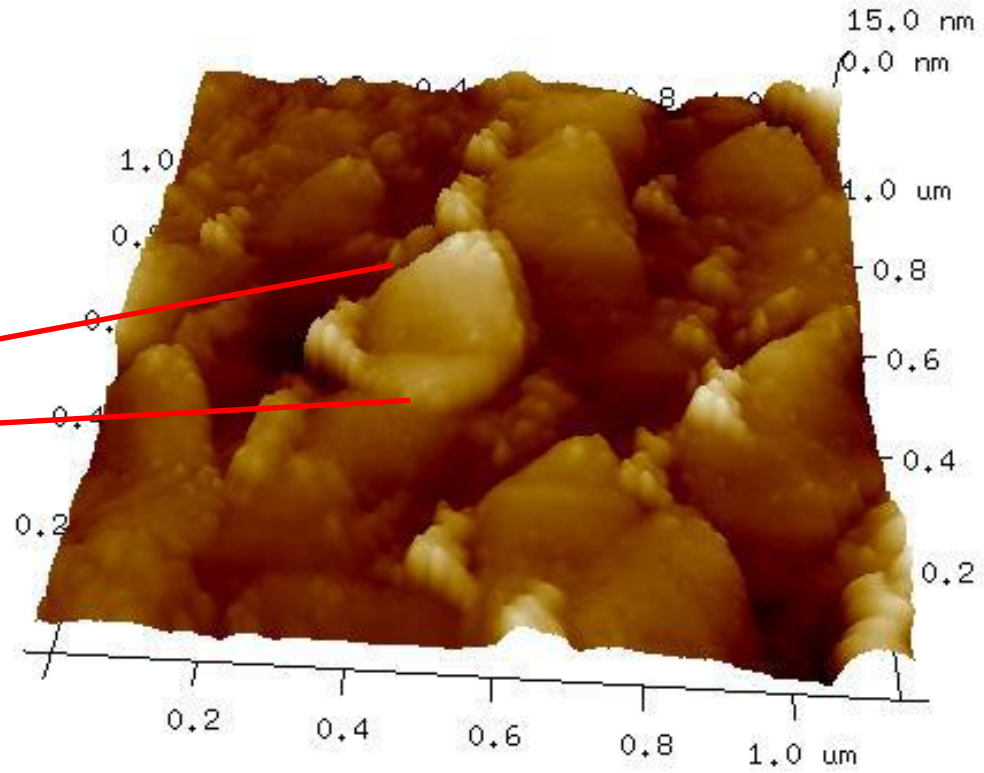




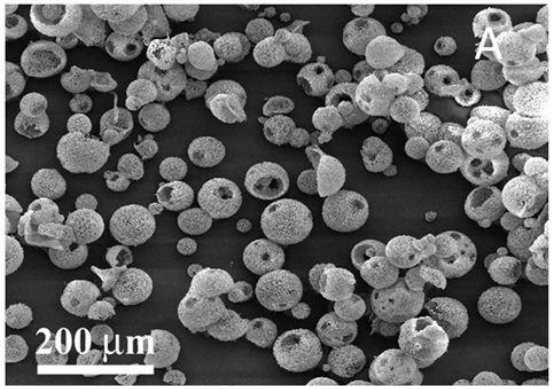


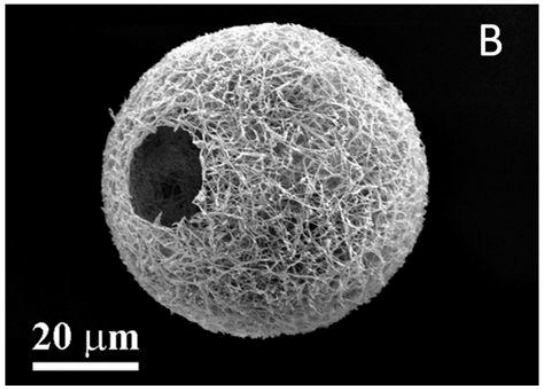
Micelles

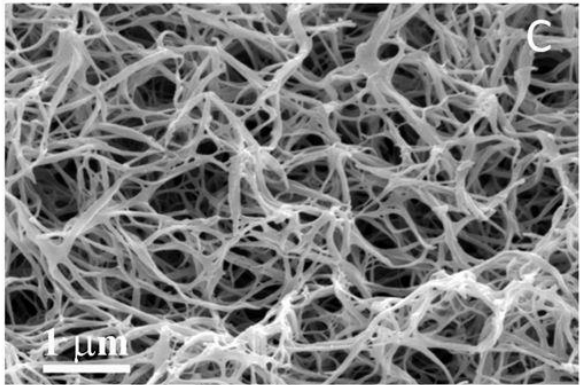


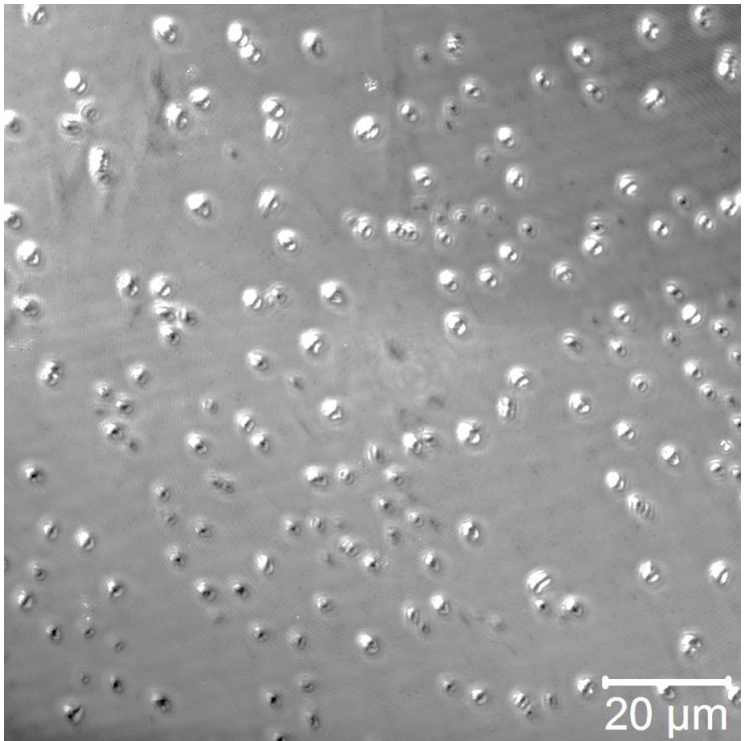


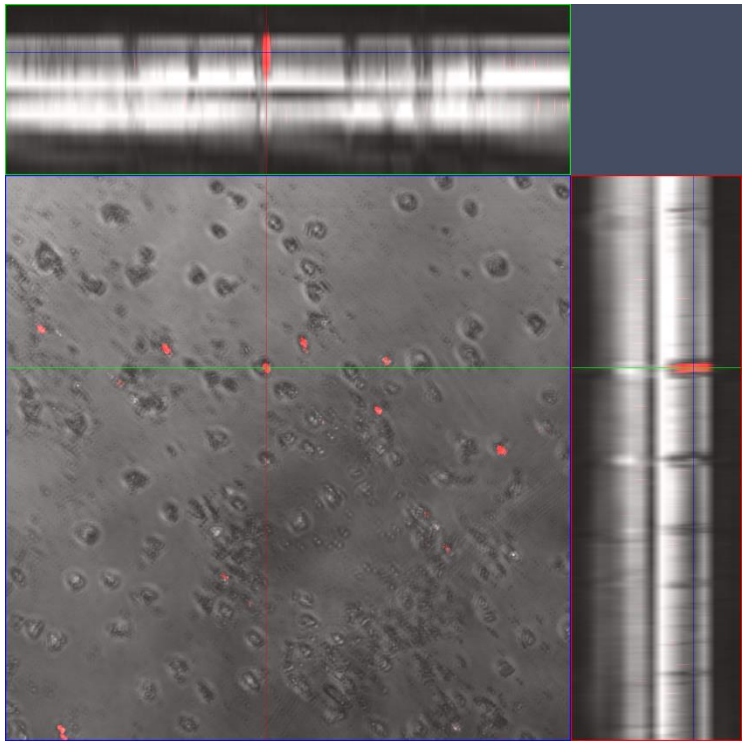
Micelles





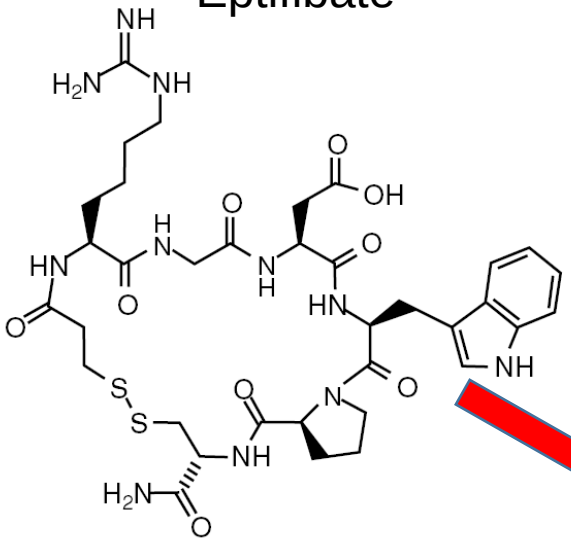




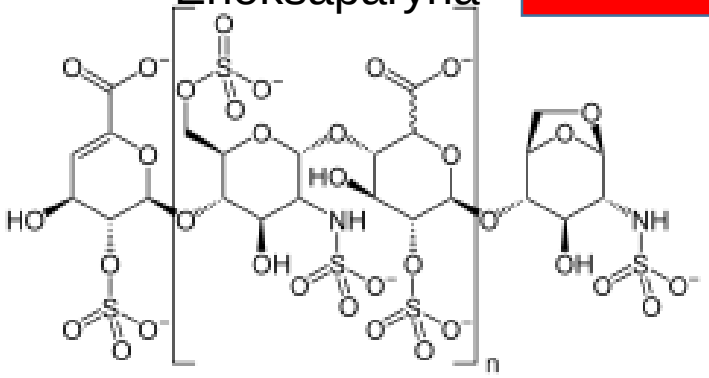


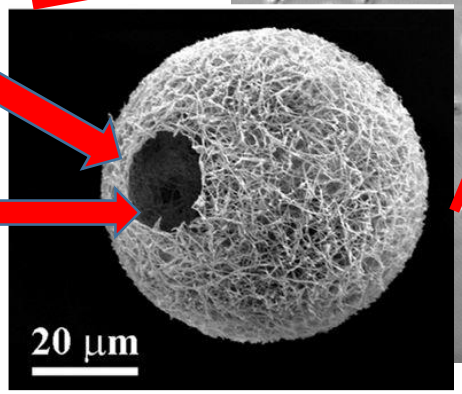
Micelles

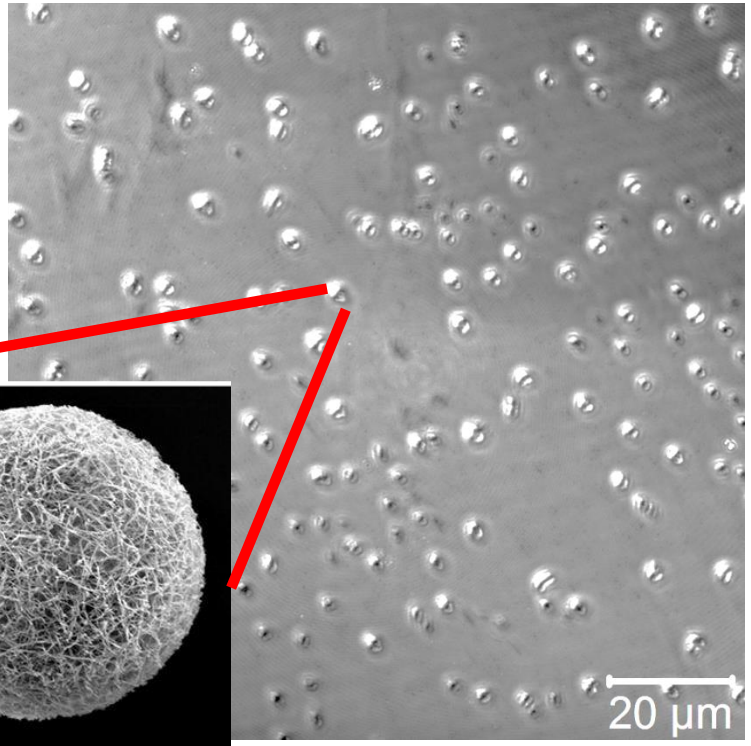
Eptifibate



Enoksaparyna







Micelles

Chitosan (CS)

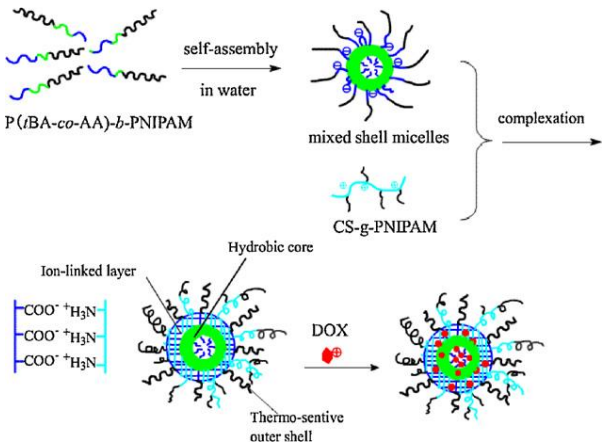
Carboxymethyl cellulose (CMC)

N-Isopropylacrylamide (NIPAM)

Cerium ammonium nitrate (CAN) (ini)

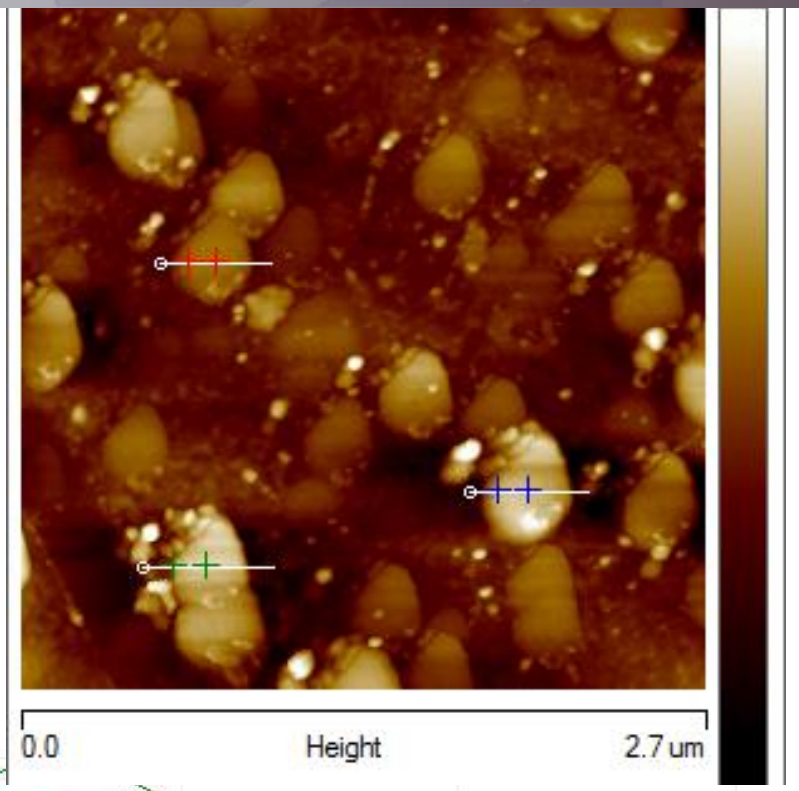
glutaraldehyde (GA)

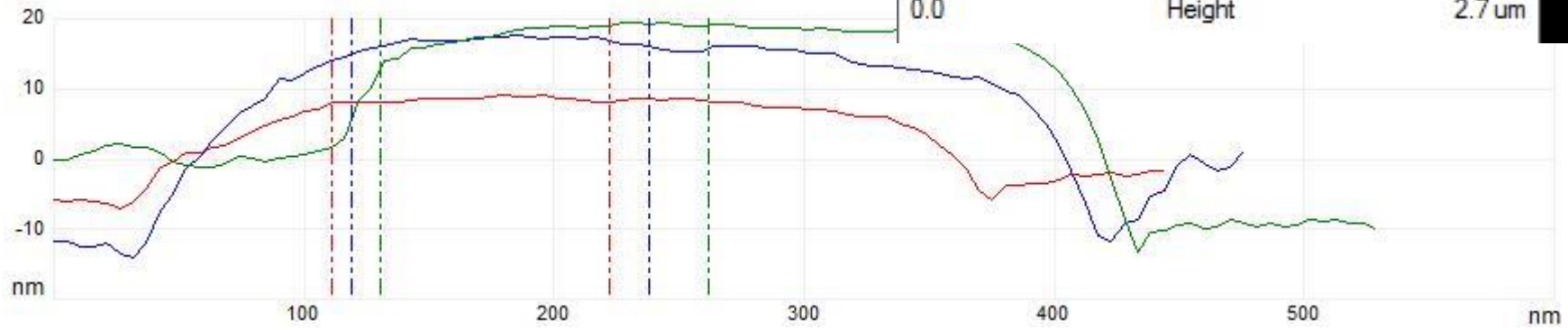
1. Chitozan m.cz. 100,000-300,000 /ACROS 349051000
2. ACRS34905-030-100
3. Karboksymetyloceluloza sól sodowa śr. M.W. 90000 (DS=0.7) /ACROS 332601000/
4. 4. N-Izopropyloakrylamid stab., czysty, 99% /ACROS 412780250/ ACRS41278-030-25
5. Amonu ceru(IV) azotan do analizy, 99.5% /ACROS 201441000/
6. Glutarowy aldehyd , roztwór 50 % /ACROS 410960
7. Kwas octowy 99,5%--99,9% CZDA
8. Aceton CZDA, ODCZ. FP
9. Metanol CZDA, ODCZ. FP



Micelles

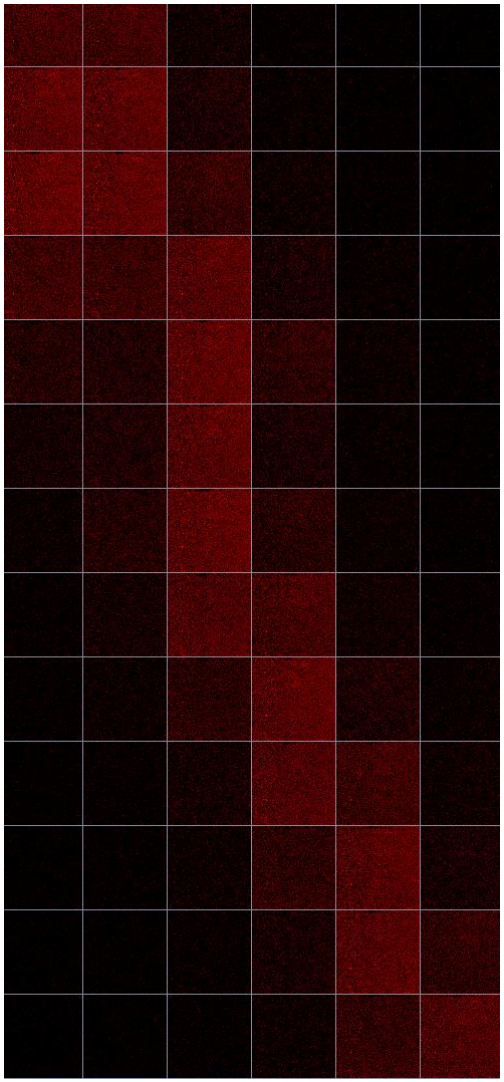
d= 327,9 ± 45 nm
h= 31 ± 9 nm



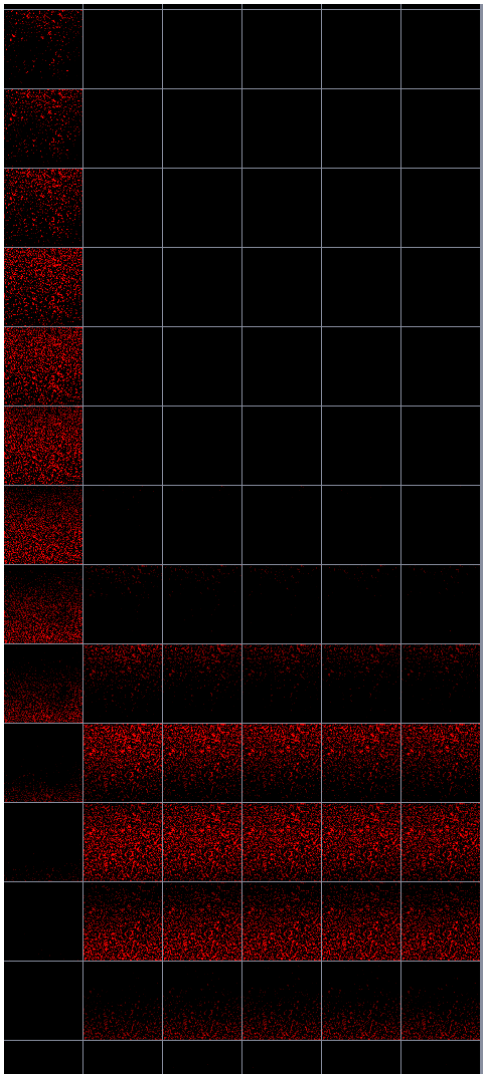


Micelles

Non cross linked

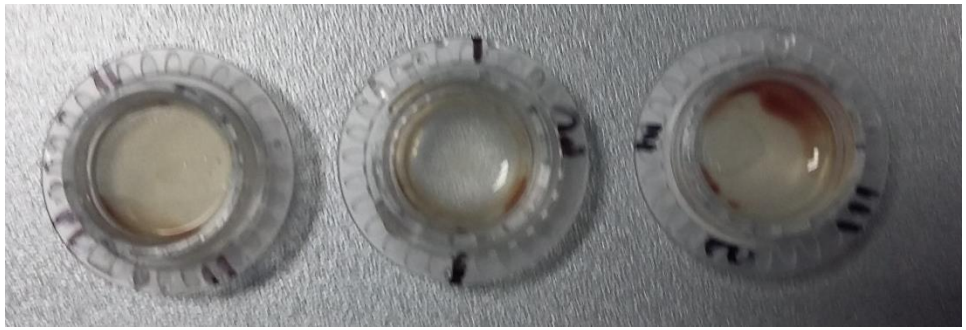


Cross linked



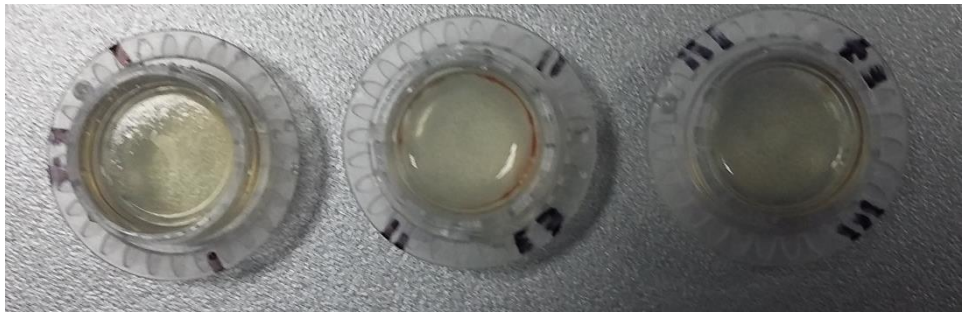
Micelles

Poliuretan

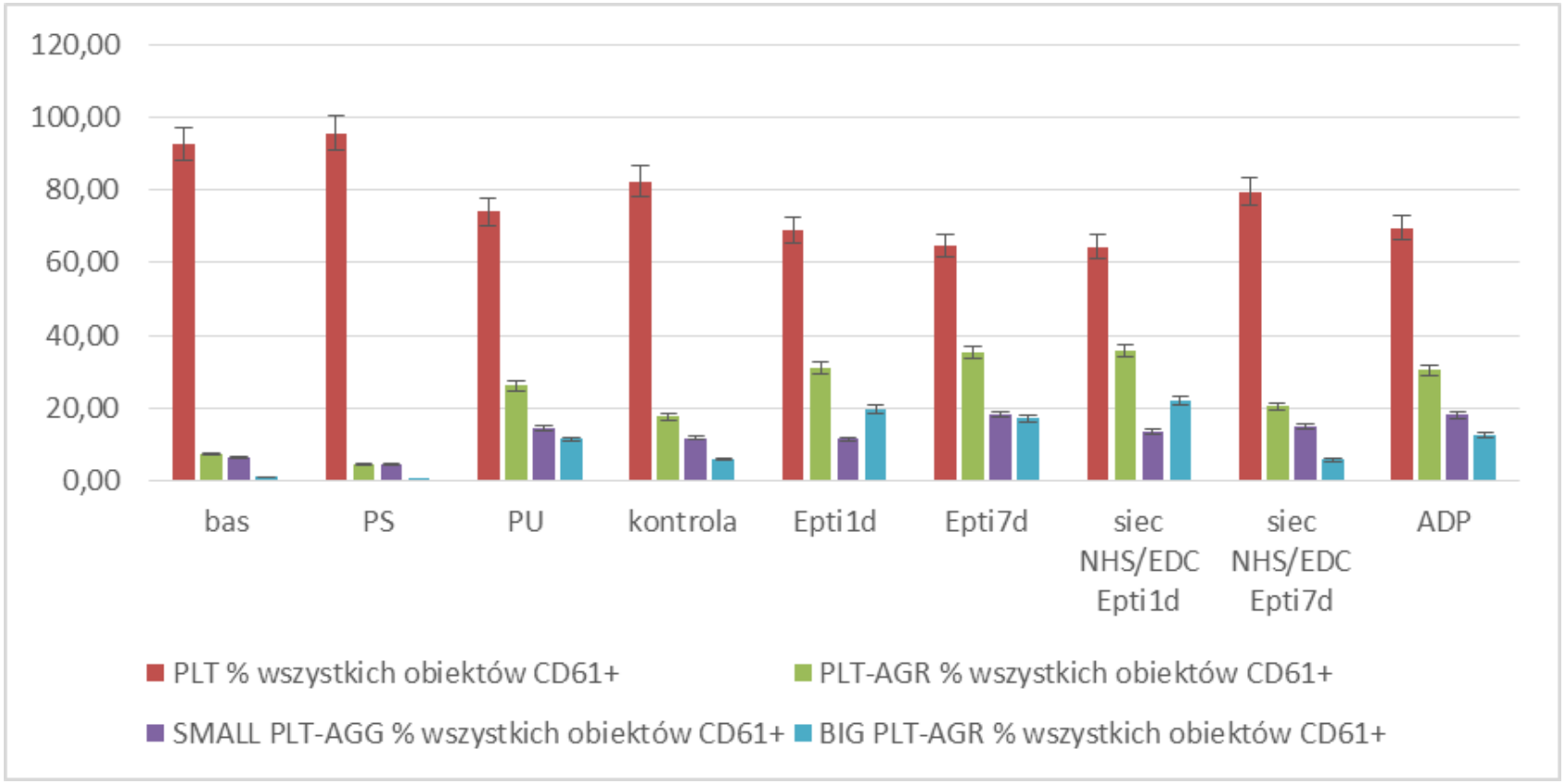


Integrilin 1 day

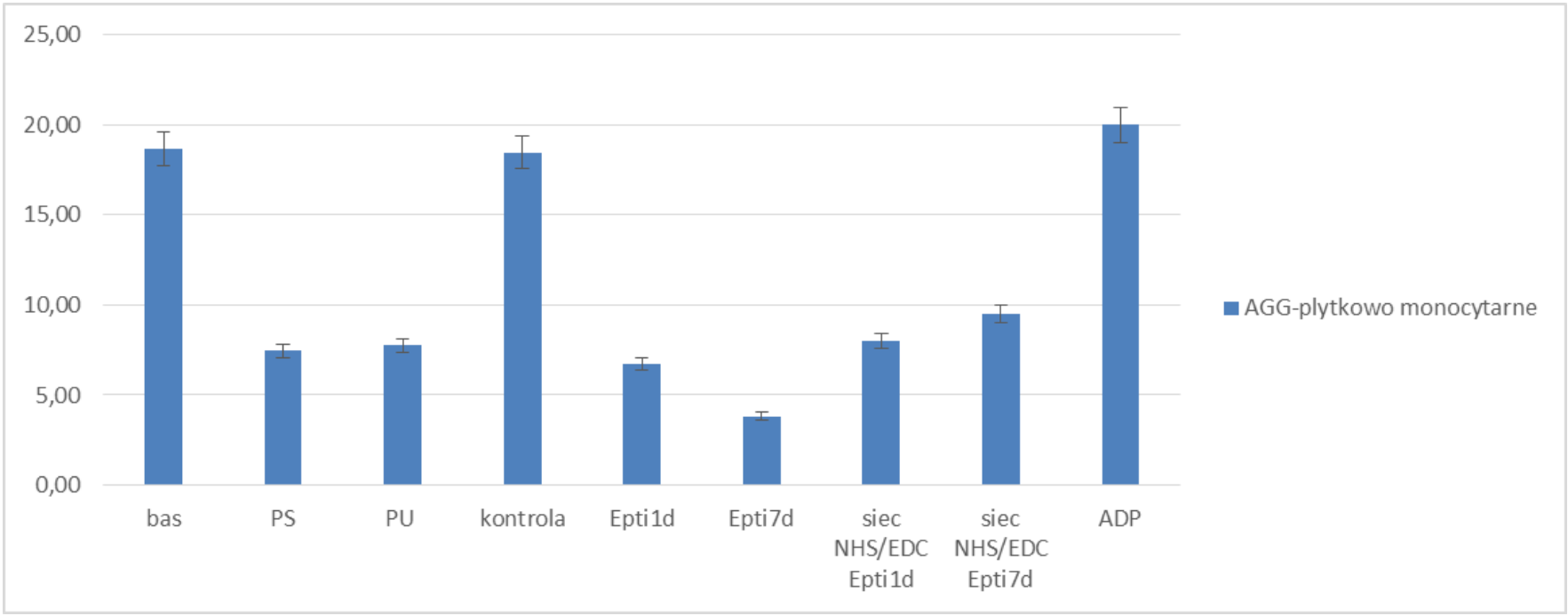
Integrilin 7 days



Micelles


120,00
100,00
80,00
60,00
40,00
20,00
0,00
bas
PS
PU
kontrola
Epti1d
Epti7d
siec NHS/EDC Epti1d
siec NHS/EDC Epti7d
ADP
PLT % wszystkich obiektów CD61+
PLT-AGR % wszystkich obiektów CD61+
SMALL PLT-AGG % wszystkich obiektów CD61+
BIG PLT-AGR % wszystkich obiektów CD61+

Micelles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kontrola
Epti1d
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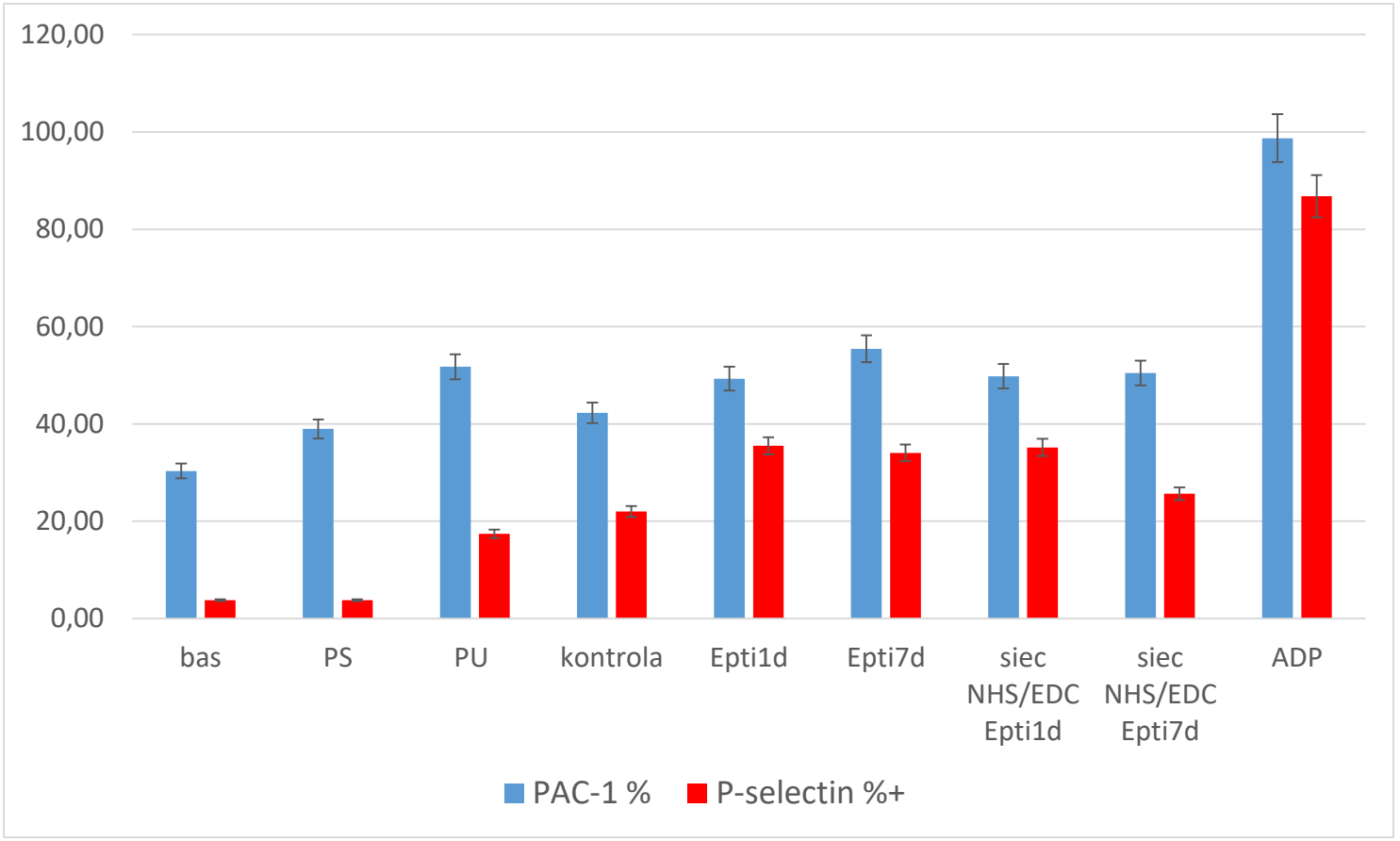
siec NHS/EDC Epti1d
siec NHS/EDC Epti7d
ADP
AGG-plytkowo monocytarne

Micelles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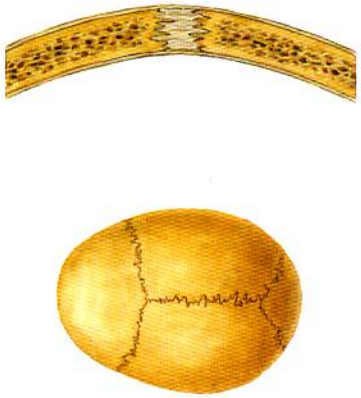
Epti7d
siec NHS/EDC Epti1d
siec NHS/EDC Epti7d
ADP
PAC-1 %
P-selectin %+

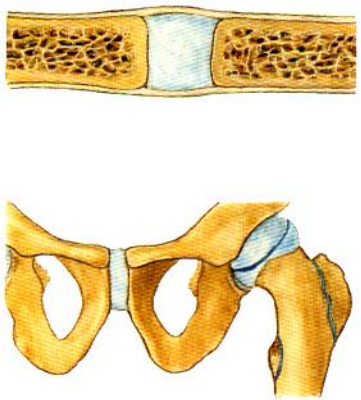
Part 3

Regeneration of the bone and joint system

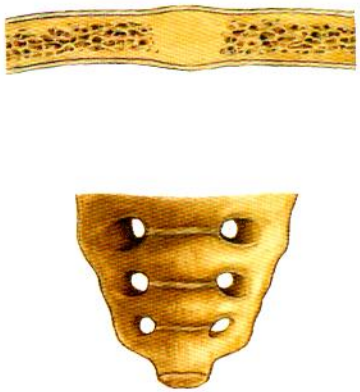
The bone-joint system



Ryc. 15 Więzozrost, *articulatio fibrosa*, na przykładzie szwów czaszki.



Ryc. 16 Chrząstkozrost, *articulatio cartilaginea*, na przykładzie spojenia łonowego.



Ryc. 17 Kościozrost, *articulatio ossea*, na przykładzie kości krzyżowej.

Strict (immovable)- Ścisłe (nieruchome)

Więzozrosty (syndesmosis)

Chrząstkozrosty (synchondrosis)

Kościozrosty (synostosis)

Free (mobile) or joints (articulatio)- Wolne (ruchome) czyli stawy (articulatio)

Joints

Selection

Zawiasowy (ginglymus)

Obrotowy (trochoidea)

Śrubowy (cochlearis)

Elipsoidalny (elipsoidea)

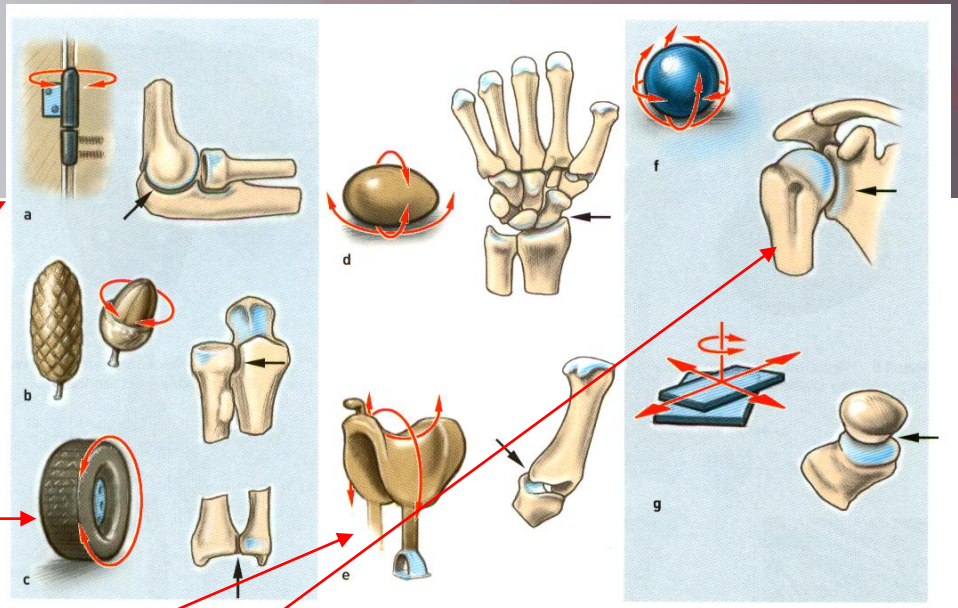
Siodełkowaty (sellaris)

Kulisty wolny (spheroidea)

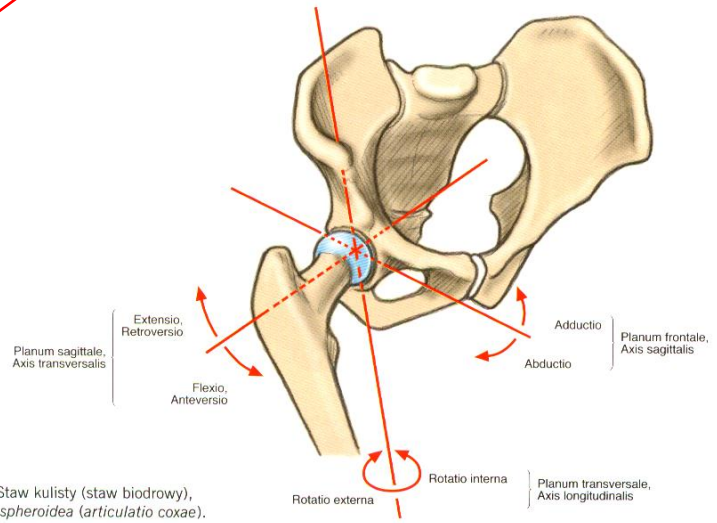
Kulisty panewkowy (cotylica)

Nieregularny

Płaski



Ryc. 19 a–g Stawy, *junctiurae synoviales*.
a staw zawiasowy, *articulatio cylindrica*
b staw stożkowaty, *articulatio conoidea*
c staw obrotowy, *articulatio trochoidea*
d staw owalny, *articulatio ovoidea*
e staw siodełkowy, *articulatio sellaris*
f staw kulisty, *articulatio spheroidea*
g staw płaski, *articulatio plana*



Ryc. 20 Staw kulisty (staw biodrowy), *articulatio spheroidea* (*articulatio coxae*).

Skull

Kość czołowa (os frontale)

Kość klinowa (os sphenoidale)

Kość ciemieniowa (os parietale)

Kość skroniowa (os temporale)

Kość sitowa (os ethmoidale)

Kość potyliczna (os occipitale)

Żuchwa (os mandibule)

Szczęka (os maxillare)

Kość jarzmowa (os zygomaticum)

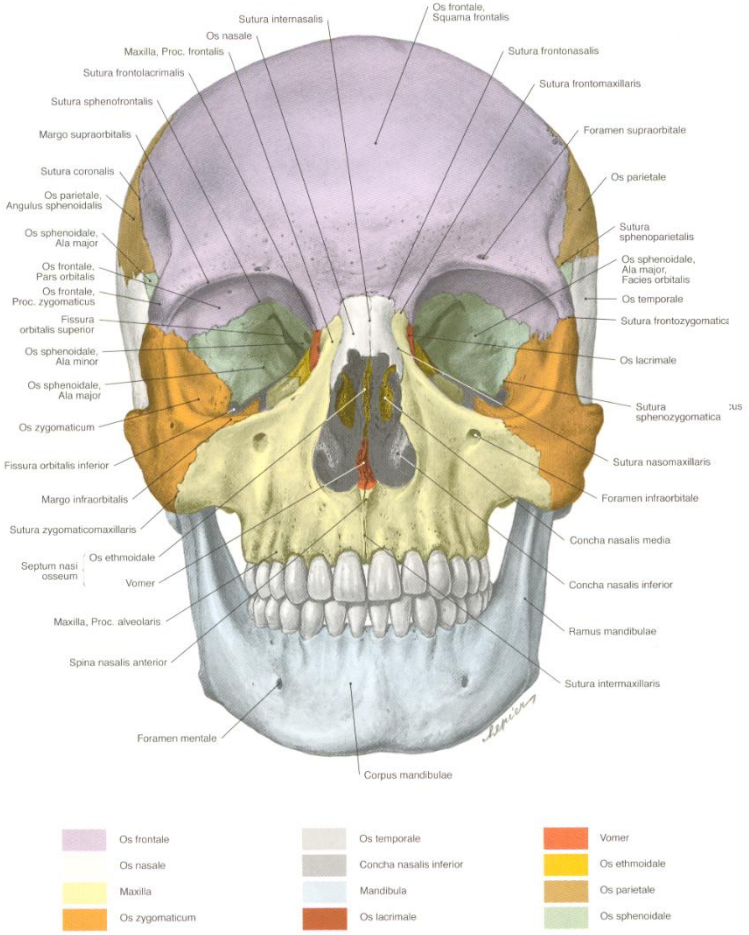
Kość nosowa (os nasale)

Kość łzowa (os lacrimale)

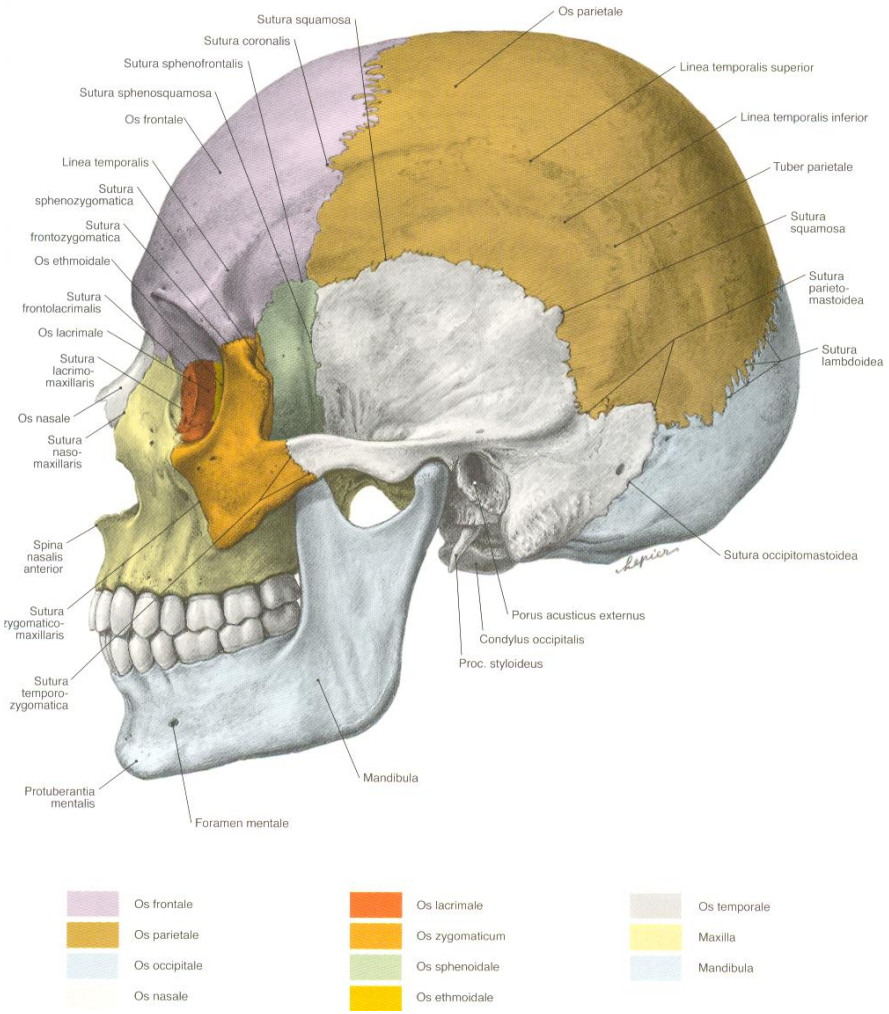
Lemiesz (vomer)

Kość podniebienna (os palatinum)

Małżowina nosowa dolna (concha nasalis inferior)



Skull


Sutura squamosa
Sutura coronalis
Sutura sphenofrontalis
Sutura sphenosquamosa
Os frontale
Linea temporalis
Sutura sphenozygomatica
Sutura frontozygomatica
Os ethmoidale
Sutura frontolacrimalis
Os lacrimale
Sutura lacrimo-maxillaris
Os nasale
Sutura naso-maxillaris
Spina nasalis anterior
Sutura zygomatico-maxillaris
Sutura temporo-zygomatica
Protuberantia mentalis
Foramen mentale
Mandibula
Os parietale
Linea temporalis superior
Linea temporalis inferior
Tuber parietale
Sutura squamosa
Sutura parieto-mastoidea
Sutura lambdoidea
Sutura occipitomastoidea
Porus acusticus externus
Condylus occipitalis
Proc. styloideus
Os frontale
Os parietale
Os occipitale
Os nasale
Os lacrimale
Os zygomaticum
Os sphenoidale
Os ethmoidale
Os temporale
Maxilla
Mandibula

M-ERA.NET Transnational Call 2016

Full Proposal

Project Acronym:

jawMPLANT

Project Coordinator:

(Organisation and country):

JOANNEUM RESEARCH Forschungsges.m.b.H., Institute for Surface Technologies and Photonics

Part 3.1

Executed projects

The bone-joint system

M-ERA.NET Transnational Call 2016

Full Proposal

Project Acronym:

jawMPLANT

Project Coordinator:

(Organisation and country):

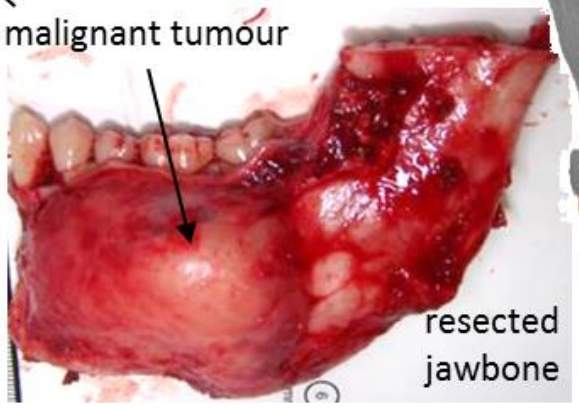
JOANNEUM RESEARCH Forschungsges.m.b.H., Institute for Surface Technologies and Photonics

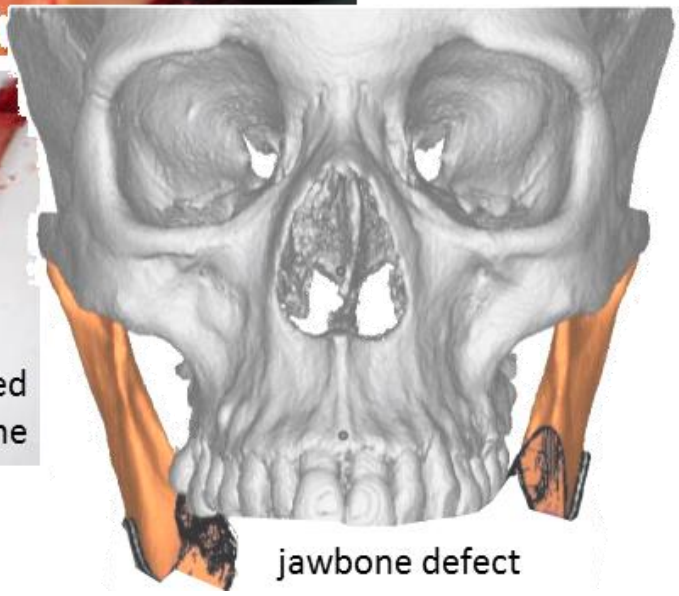
Bioactive, antimicrobial 3D printed implants for large jawbone defects after tumour resection

Roman Major[1], Marcin Surmiak[2], Juergen M. Lackner[3], Michal Charkiewicz[5]

1 Institute of Metallurgy and Materials Science, Polish Academy of Sciences, Reymonta St. 25, Crakow, PL.
2 Department of Medicine, Jagiellonian University Medical College, 8 Skawinska Street, 31-066 Cracow, PL.
3 JOANNEUM RESEARCH Forschungsgesellschaft mbH MATERIALS - Institute of Surface Technologies and Photonics Functional Surfaces Leobner Straße 94 A-8712 Niklasdorf A
4 ChM sp. z.o.o. Lewickie 3B 16-061 Juchnowiec Koscielny PL

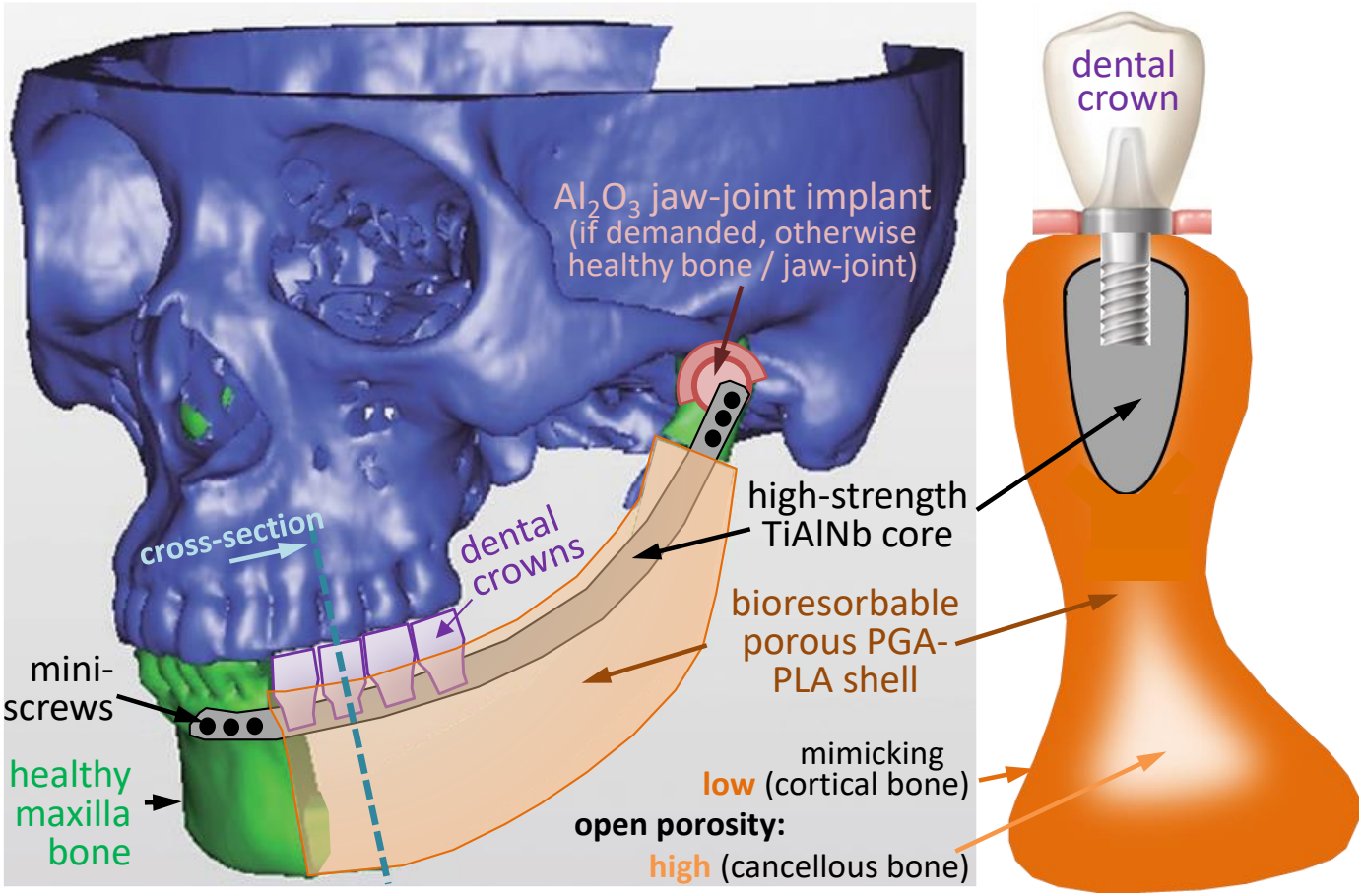
Introduction





Introduction

Concept of the patient-specific metal-core, polymer-shell maxillofacial implant with artificial jaw-joint



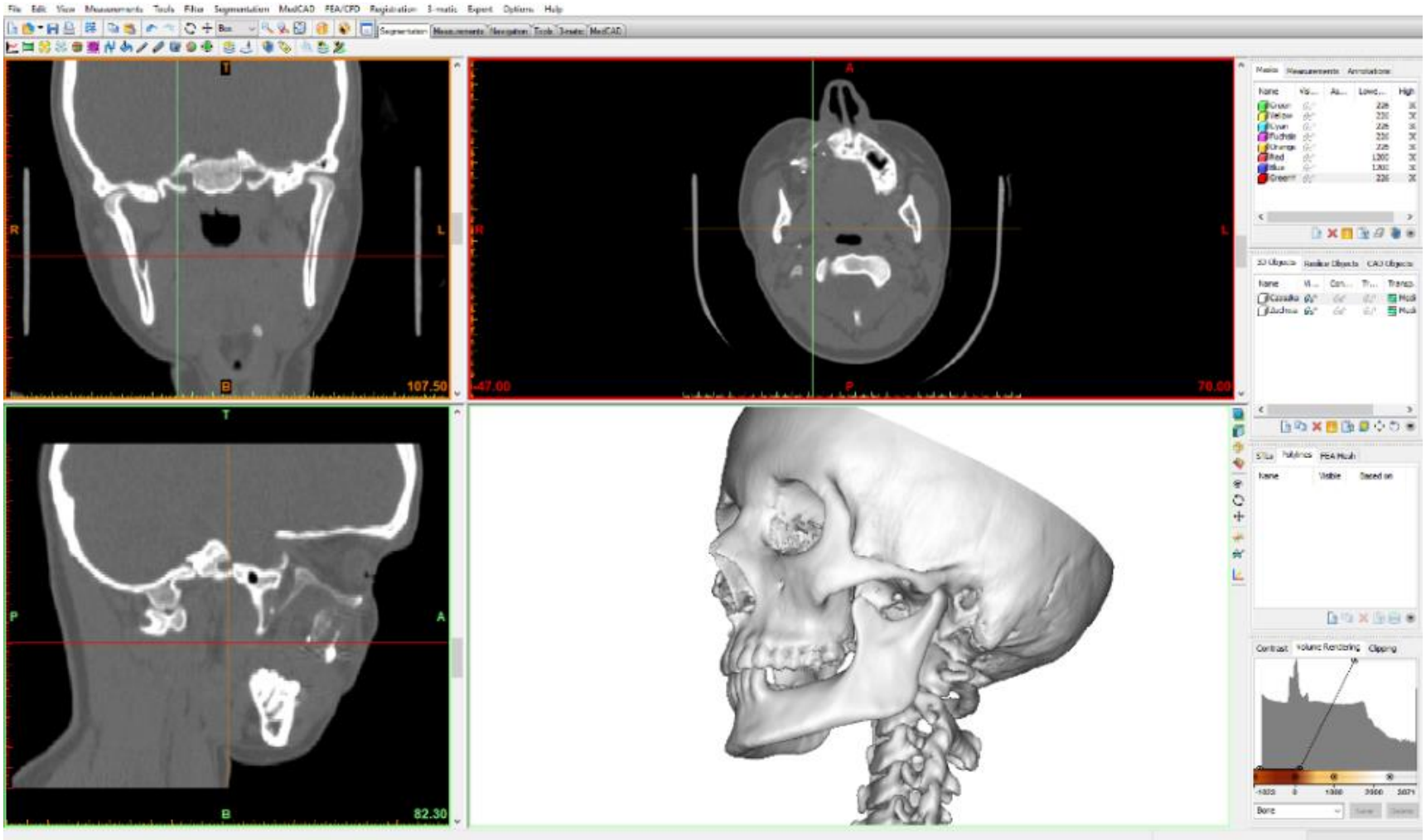
Project partners

- JOANNEUM Research Forschungsgesellschaft m.b.H., Materials, Institute for Surface Technologies and Photonics (JR)
- ChM Sp. z.o.o. (ChM)
- Lithoz GmbH (Lithoz)
- Haratech GmbH (HT)
- Alphacam Austria GmbH (aC)
- Medical University of Vienna, Department of Cranio-, Maxillofacial and Oral Surgery (VMU)
- Warsaw University of Technology, Faculty of Chemical and Process Engineering (WUT)
- Polish Academy of Sciences, Institute of Metallurgy and Material Sciences (IMIM)

Materials

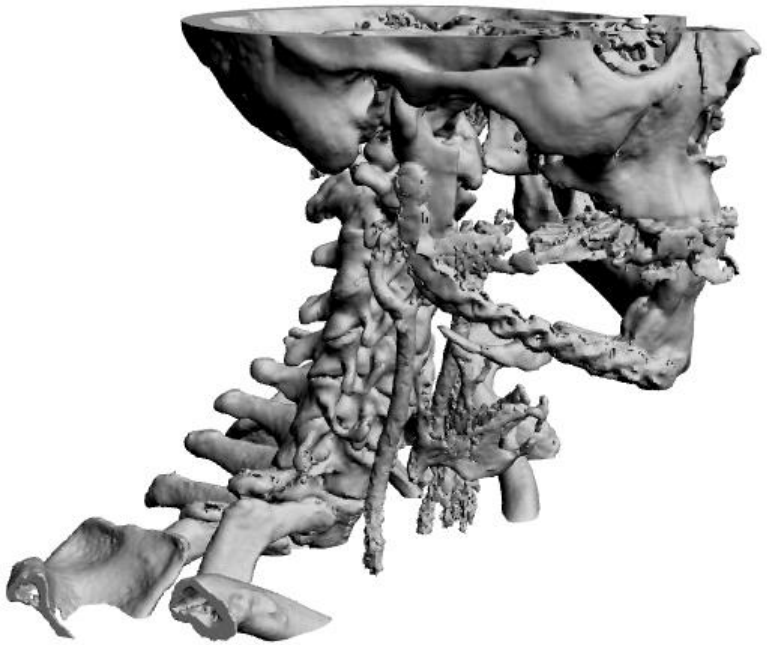
Med. 610 meshes 1mm
Med. 610 meshes 2mm
Med. 610
Zirconia LithaCon 3Y230 ZTA10 (Zirconia)
Alumina LithaLox 350D
ChM nonporous ChM 0.5 porosity

Introduction



Cases

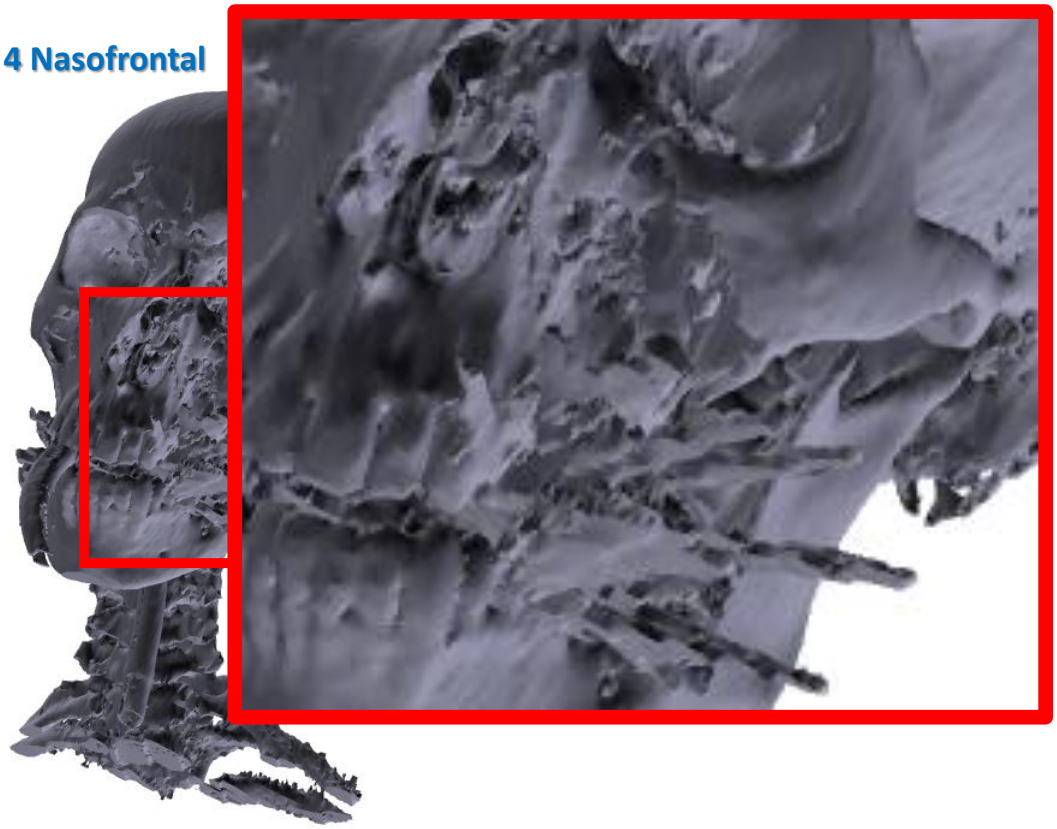
Case 1: Mandibula



Case 3: Skullcup (perietalis)

Cases

Case 4 Nasofrontal



Case 5: Upper anterior tooth

Case 1

CASE 01 MANDIBULA

LITHOZ

Original data

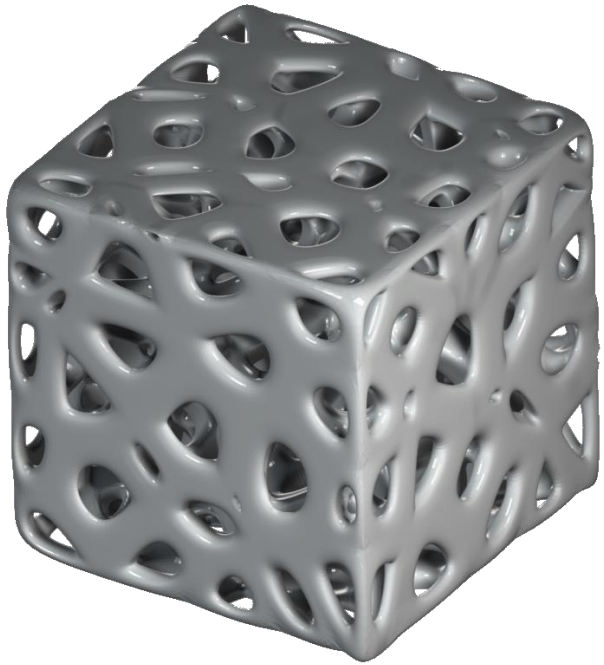
Software: InVesalius 3.1

The idea

Report by Guillaume CLÉMENT, Haratech GmbH
T +43 (0) 732 287070-65
guillaume.clement@haratech.at
www.haratech.at

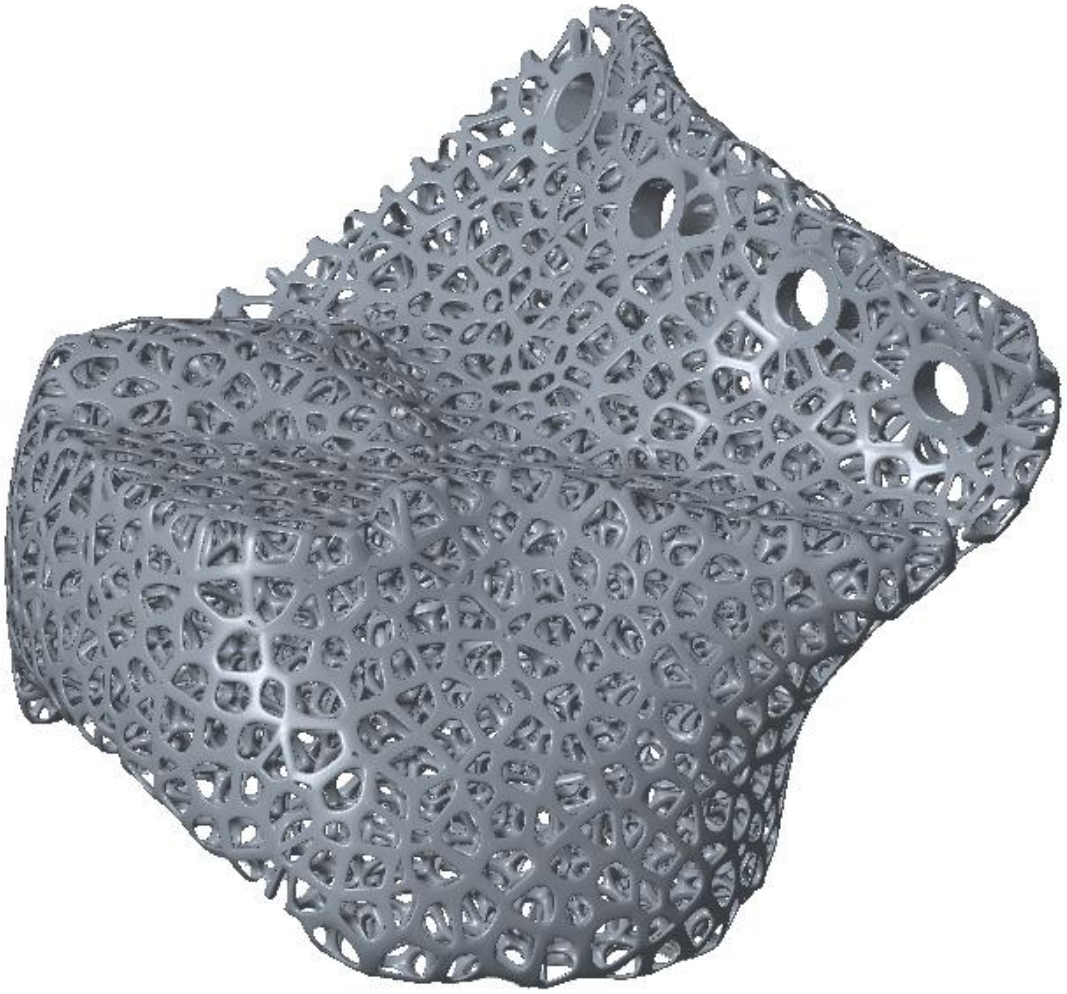
grasshopper

Rhinoceros



The idea

Grasshopper



The idea

Grasshopper

The idea

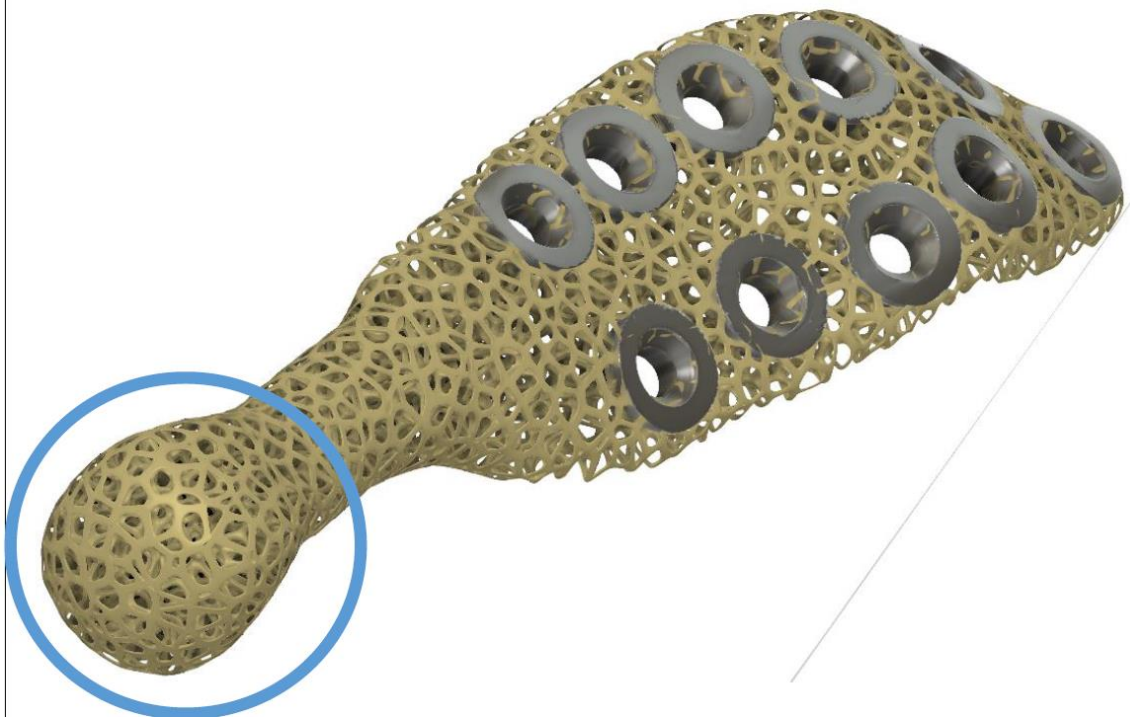
Grasshopper

Constituent materials – slender bone

The ball joint (blue circle on the next image) shall be made of ceramics.

The rest of the implant should be composed of (sorted by decreasing priority):

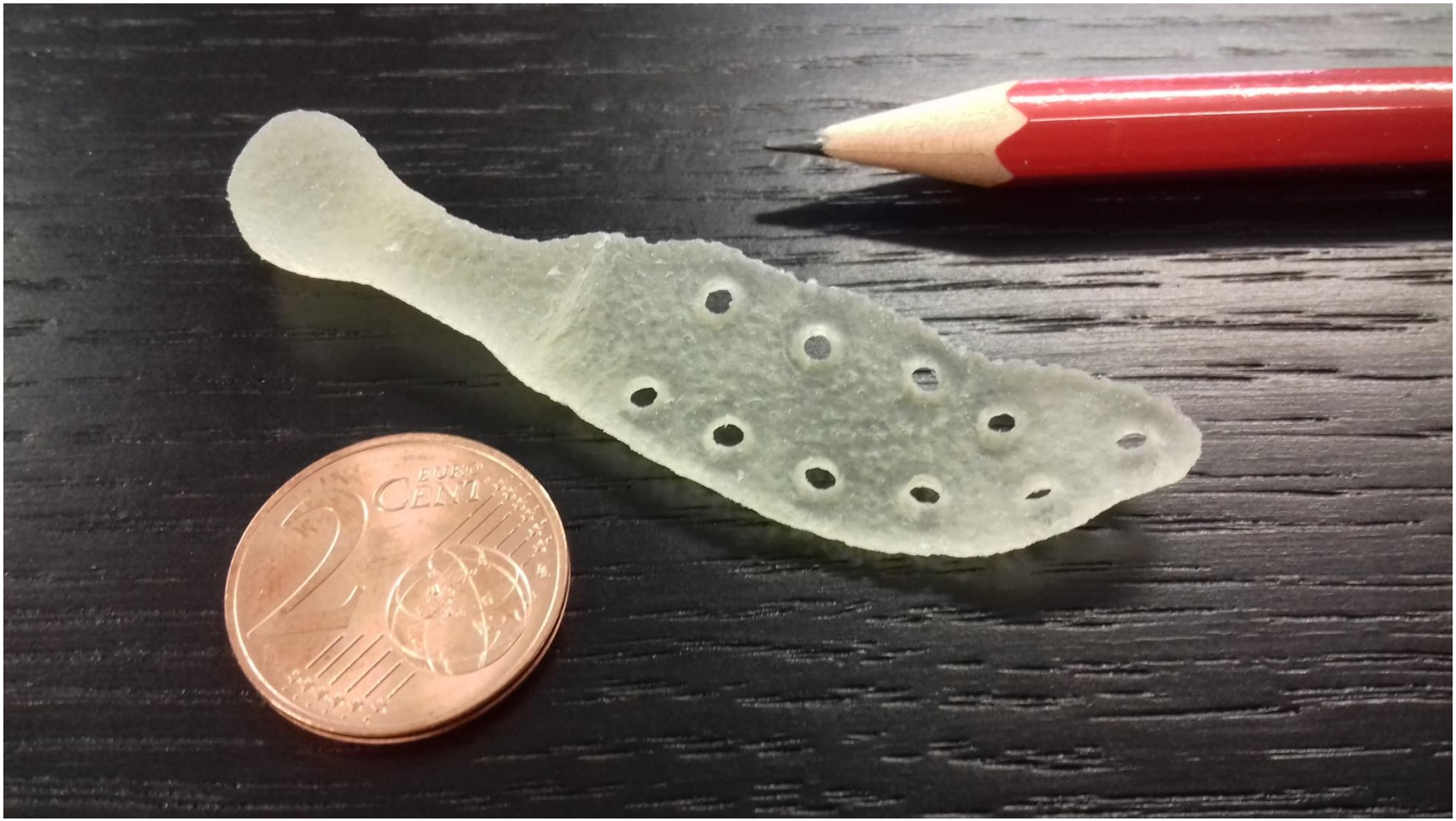
1. Non-resorbable polymer only
1. Titanium alloy only (equal priority)
2. A titanium alloy core (incl. the screw threads), incased in non-resorbable polymer
3. A resorbable polymer core, incased in titanium alloy (incl. the screw threads)
4. One half made of titanium alloy (on the exterior side) and one half made of resorbable/non-resorbable polymer (on the interior side)



The idea

Grasshopper

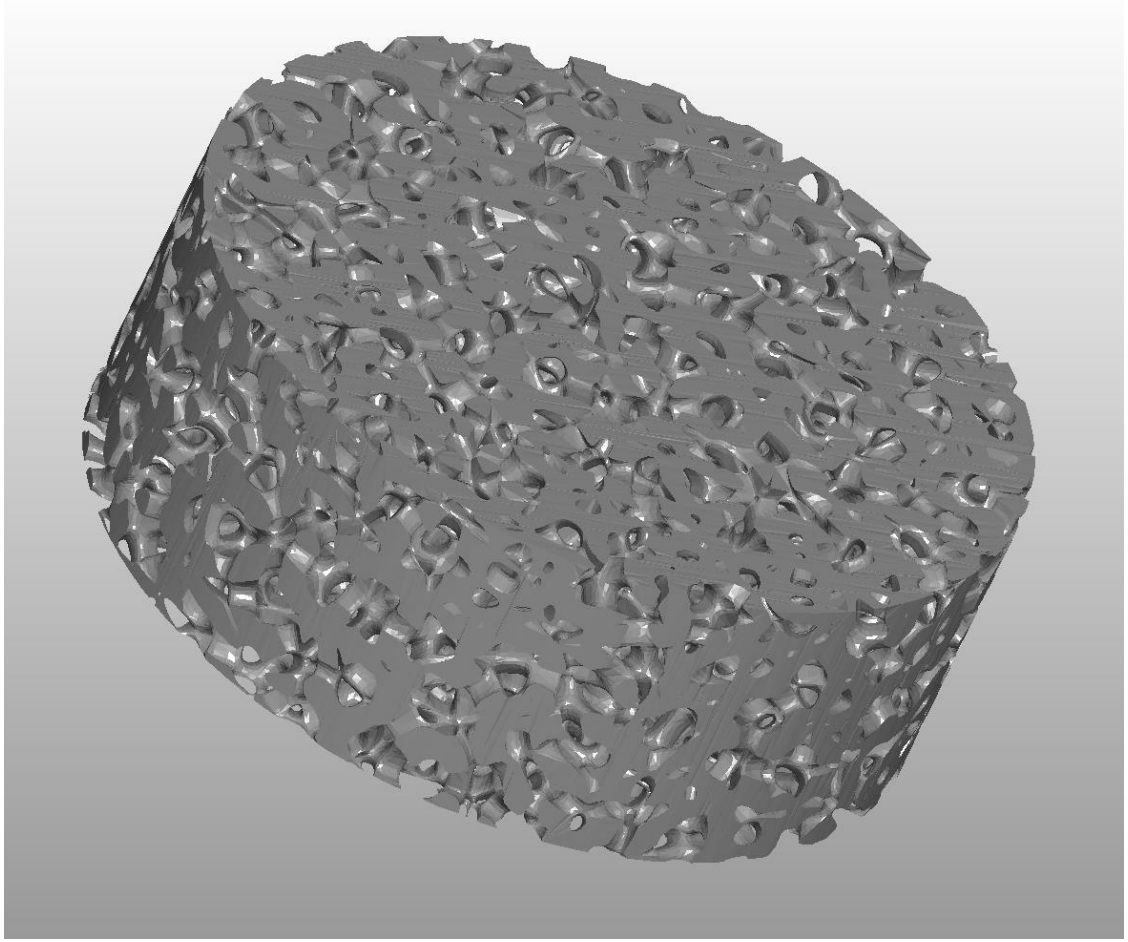
Quick print trial with a Objet Eden 350V
Half scale!



The idea

Grasshopper

Sample stl-file: diameter 13.7mm, height 5mm, main pore diameter 1mm

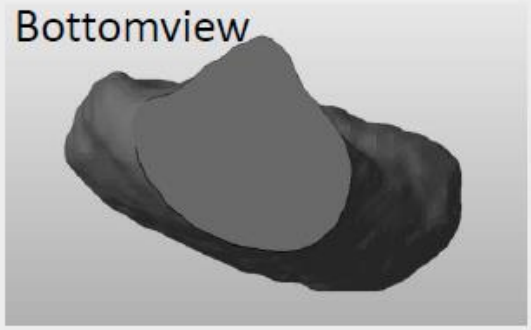


Case 1

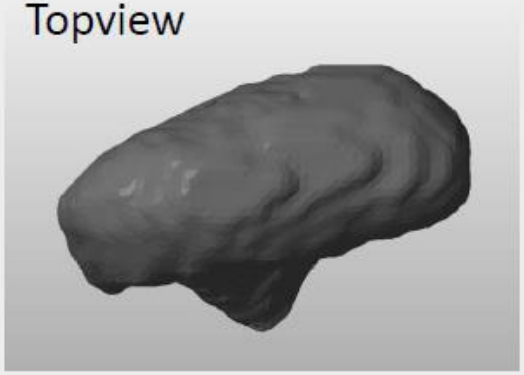
CASE 01 MANDIBULA

LITHOZ

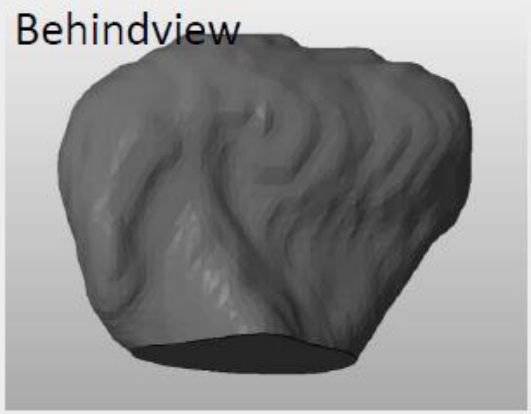
Bottomview



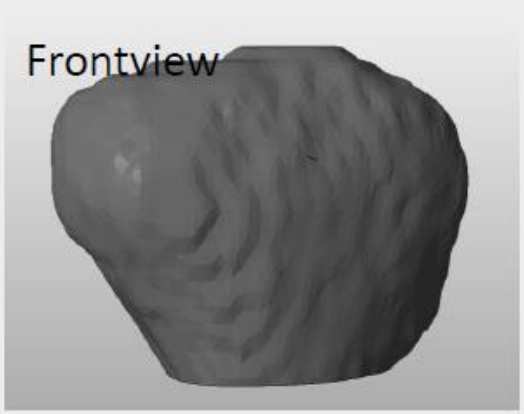
Topview



Behindview



Frontview

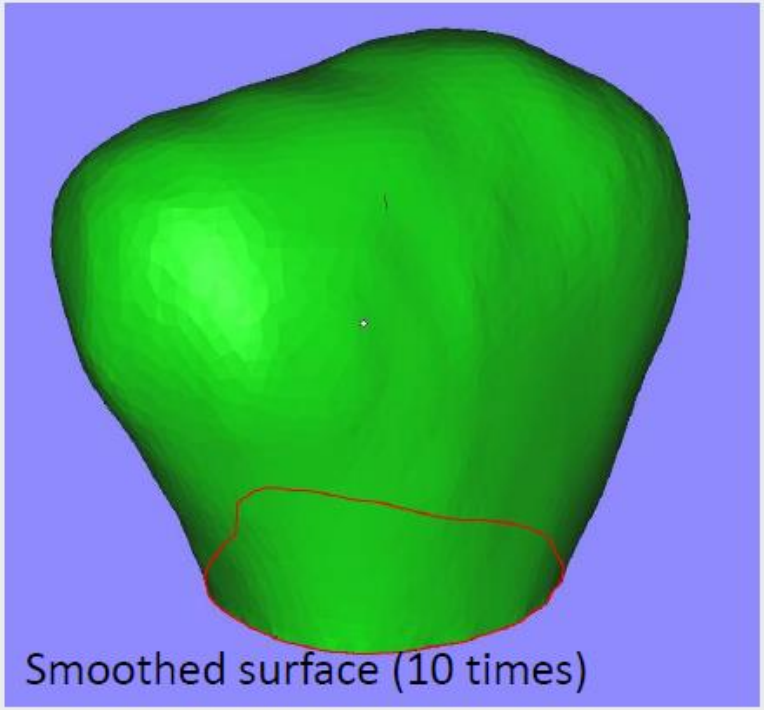


Case 1

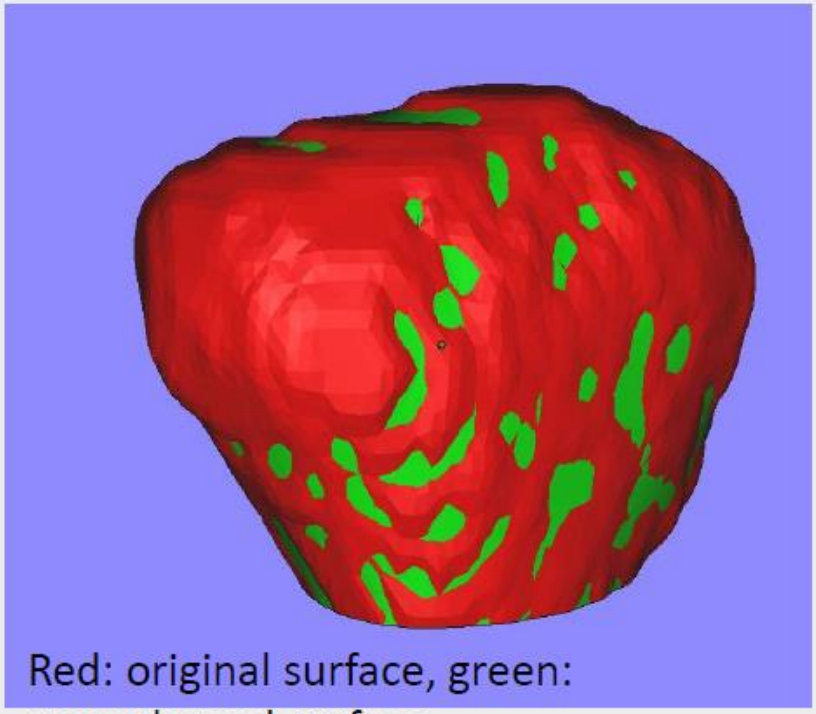
Mandibula

Smoothening

LITHOZ



Smoothed surface (10 times)



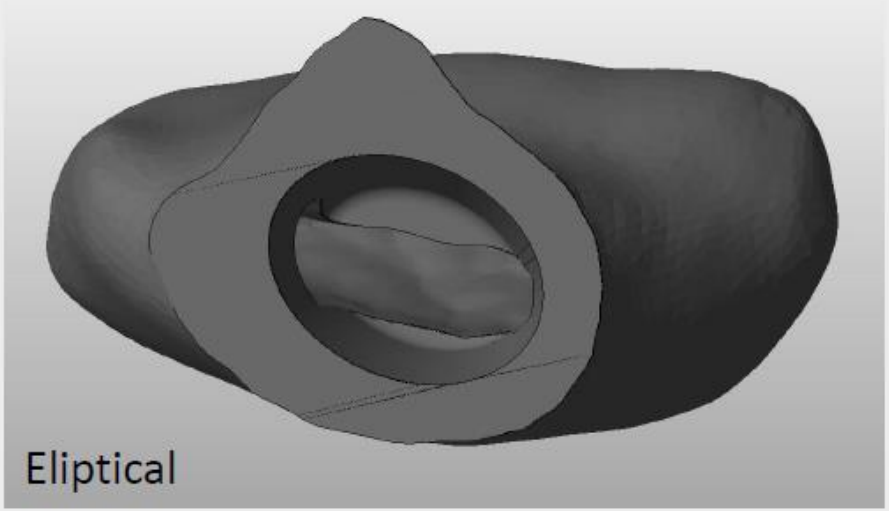
Red: original surface, green: smoothened surface

Case 1

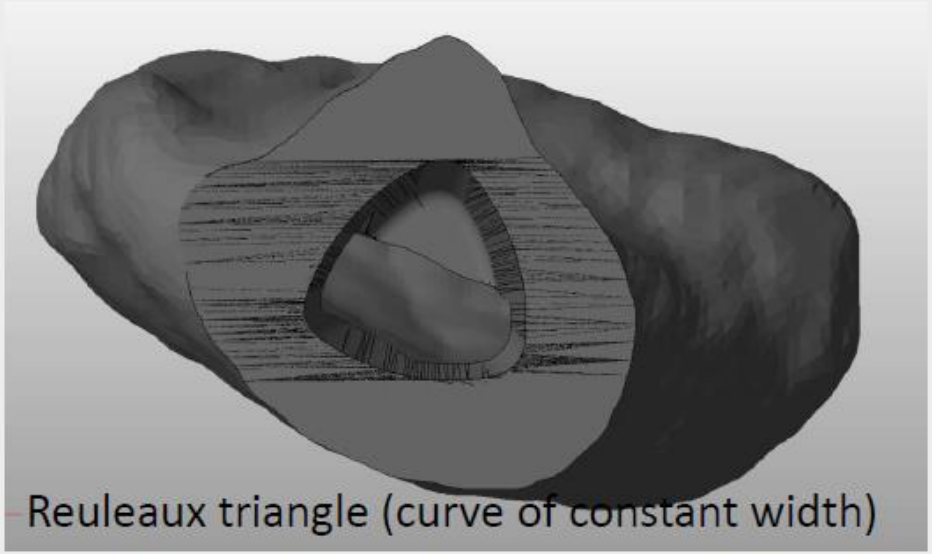
Mandibula

Connections – 2 options

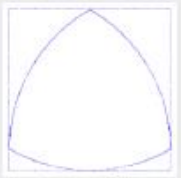
LITHOZ



Eliptical



Reuleaux triangle (curve of constant width)

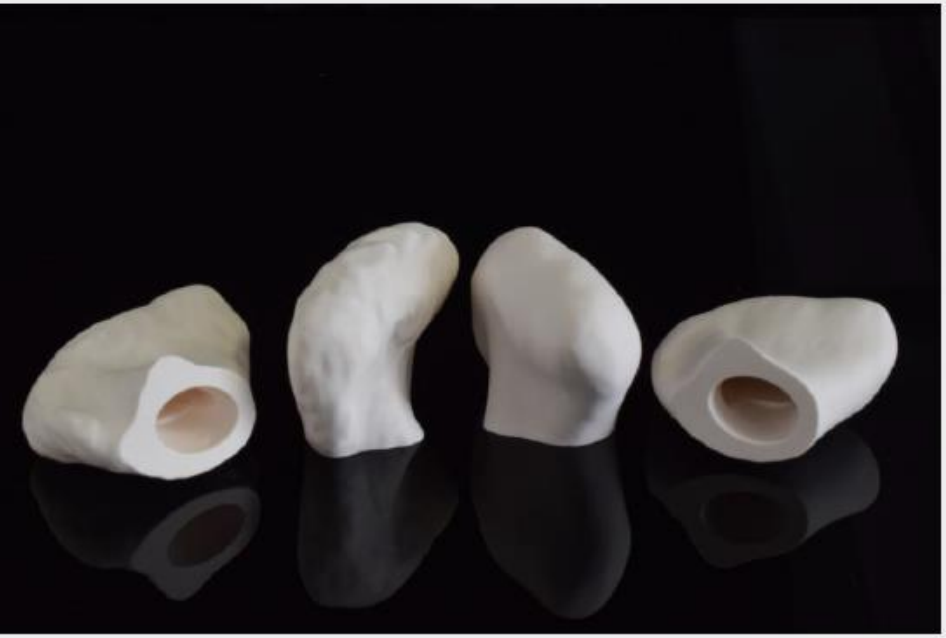


Case 1

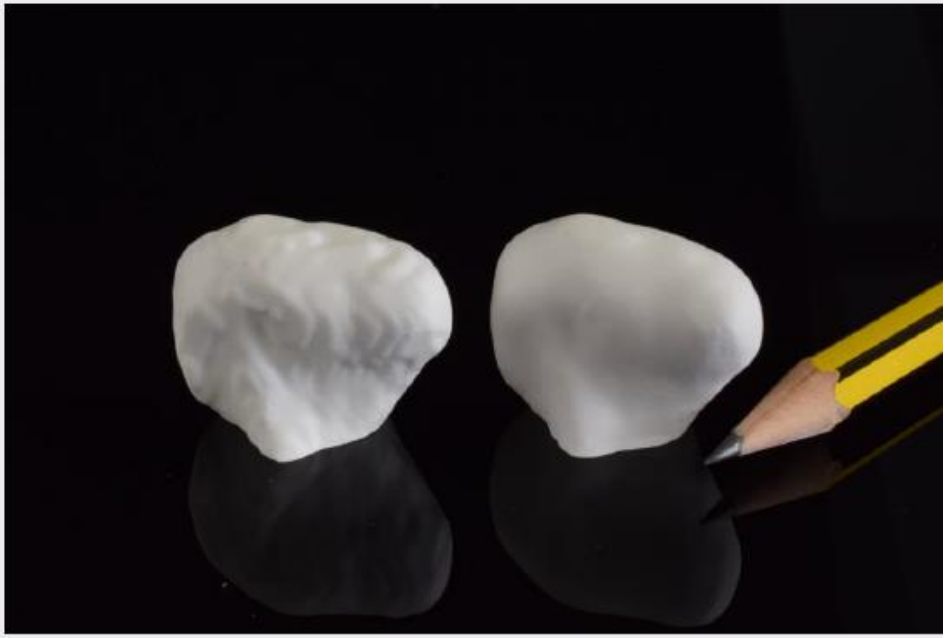
Mandibula

Pictures of green parts

LITHOZ



Overview



Surface comparison

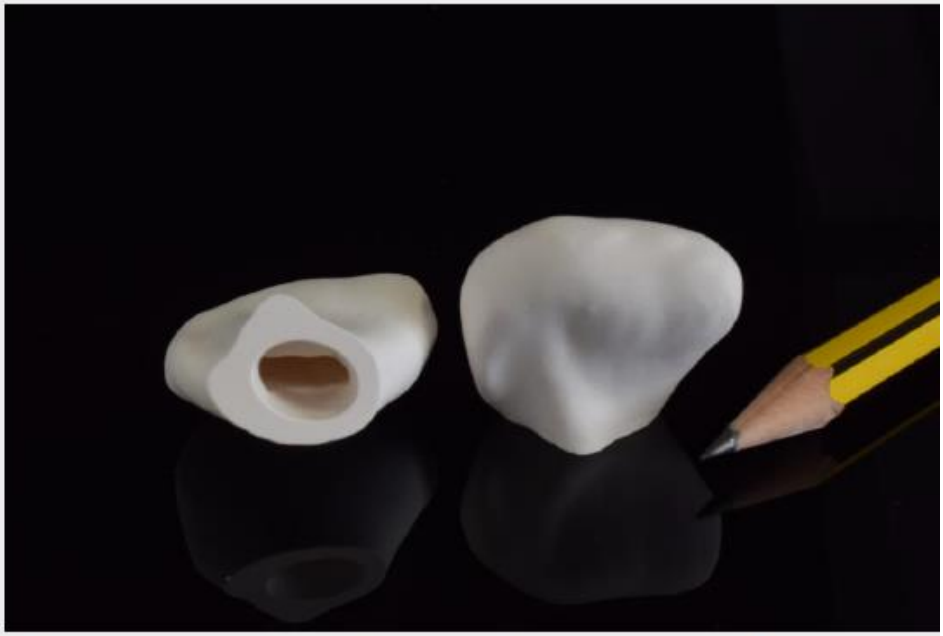
Case 1

Mandibula

Photos - connections

LITHOZ

Original surface – eliptic connection



Smoothened surface – eliptic connection

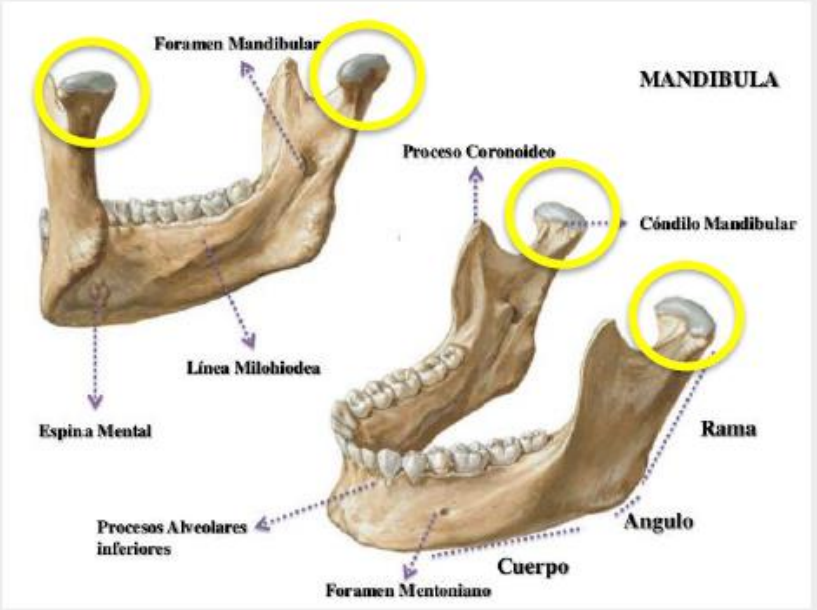
Case 1

Mandibula

Articulating surfaces

LITHOZ

How to determine the roughness?



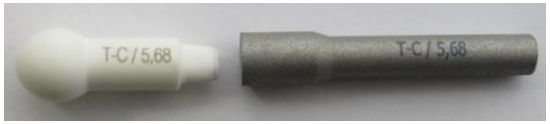
Case 1

MECHANICAL DISCONNECTION OF THE TITANIUM-CERAMIC PARTs

- The titanium shank was patterned with an oval shaped seat of two fit types:
  fine fit – T-C/5,68,
  clearance fit – T-C/6,17.
- Dental quick-setting phosphate cement Agatos S was purchased on this purpose.

Sample name	Maximum force [N]	
	smooth	groove 0,5
T-C/5,68/1	444	601
T-C/5,68/2	442	790
T-C/6,17/1	692	537
T-C/6,17/2	443	637

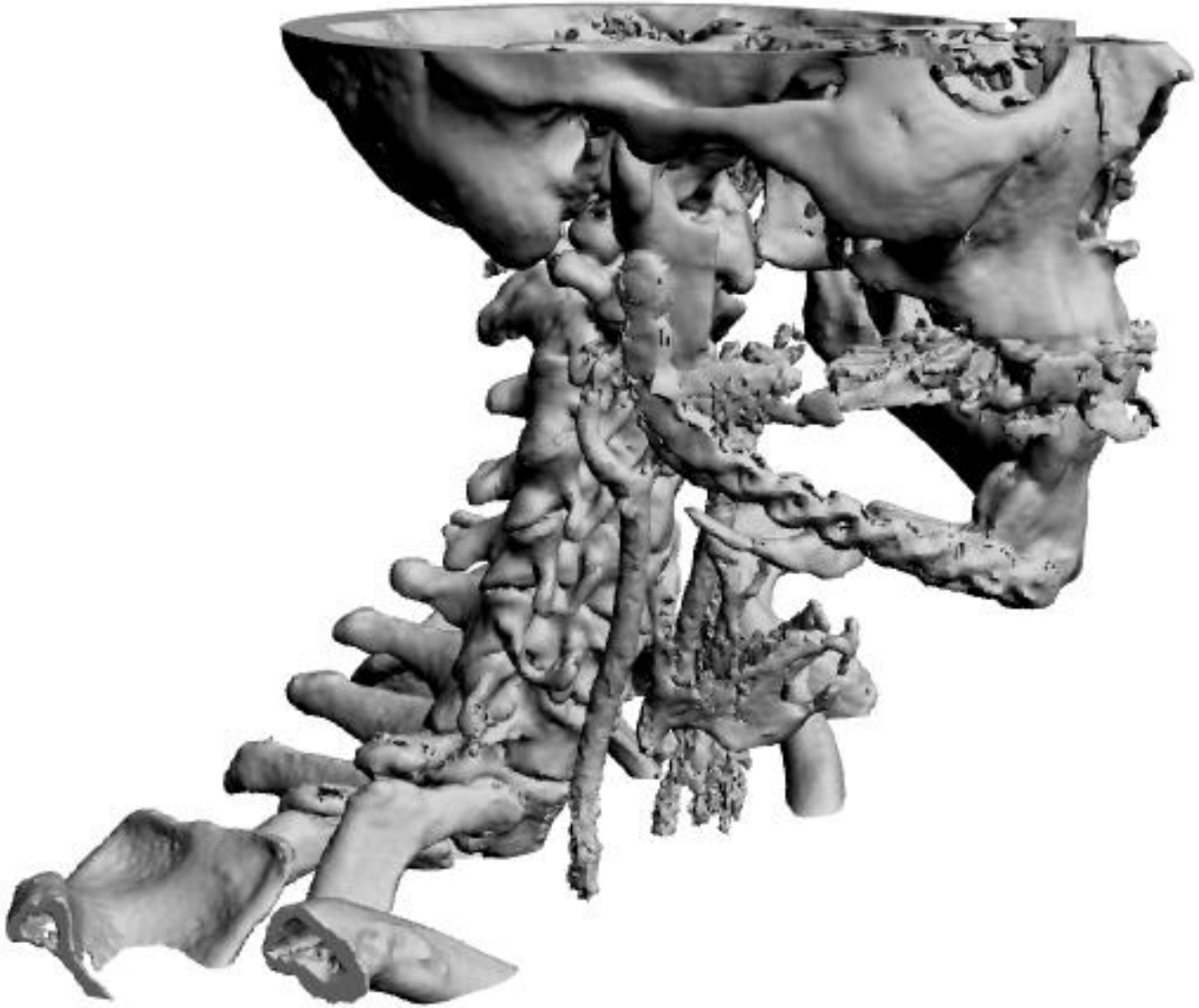
SAMPLES AFTER THE TEST



smooth

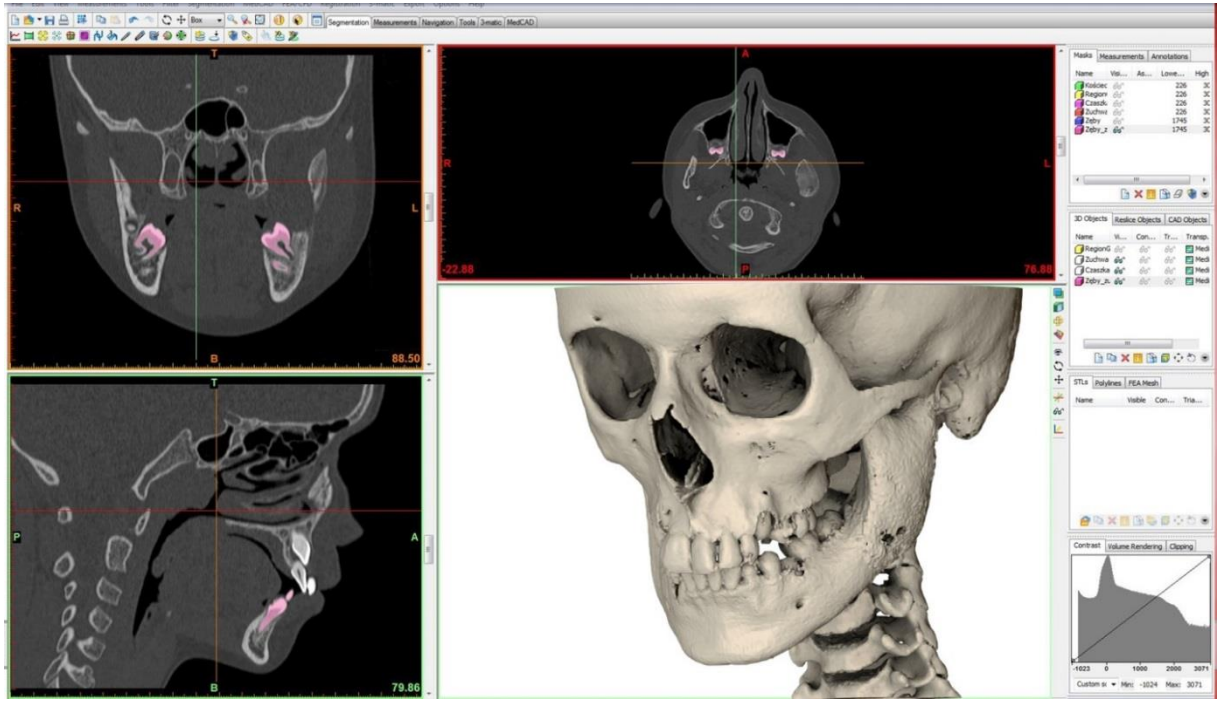
groove

Case 2



State of the art

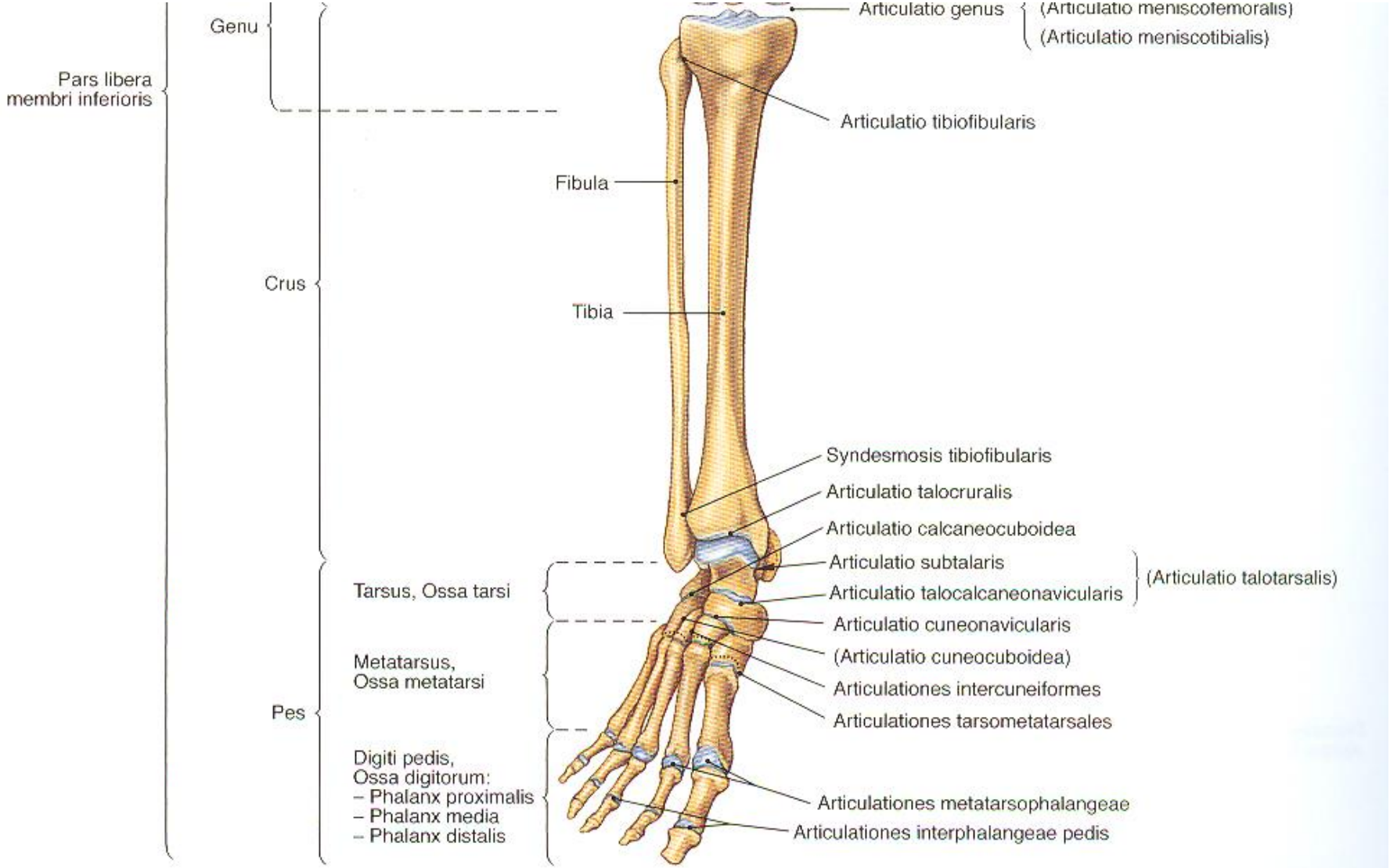
CT image tranformastion



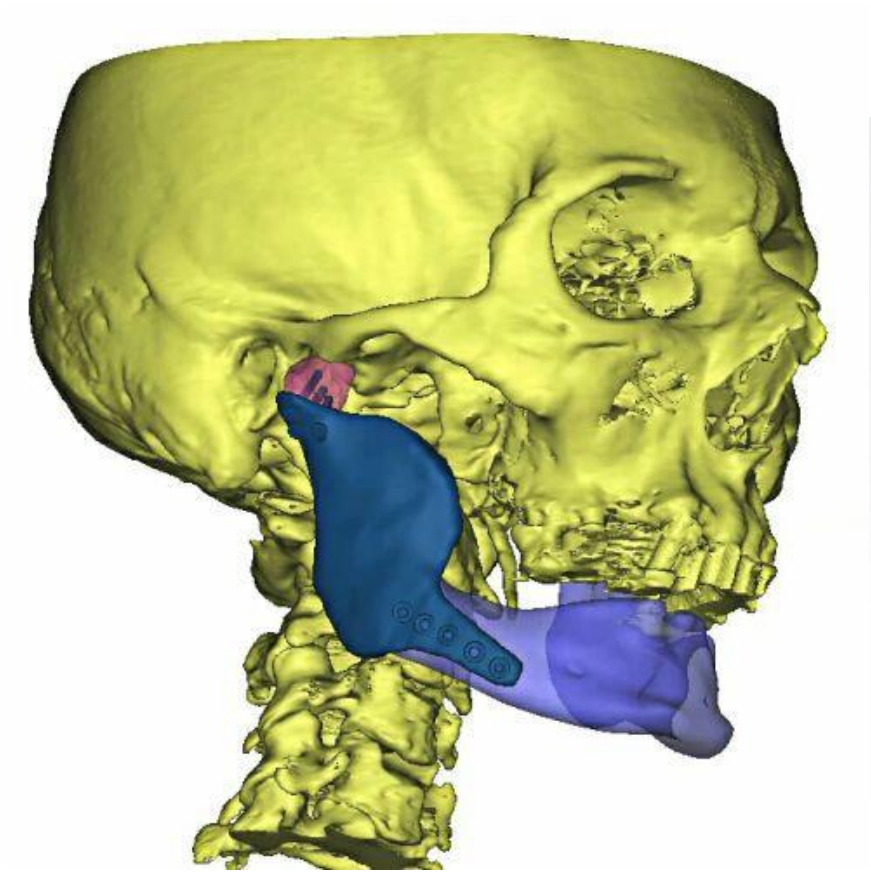
The first work on the development of procedures for the removal of neoplastic hard tissues and adjustment of the desired shape of the jaw and mandible bones based on healthy, undamaged bones ("Side mirroring", the position of the teeth on the opposite side to the removed tissue) and face features were also carried out:

State of the art

(membrum inferius)



State of the art



CT image tranformastion: side mirroring

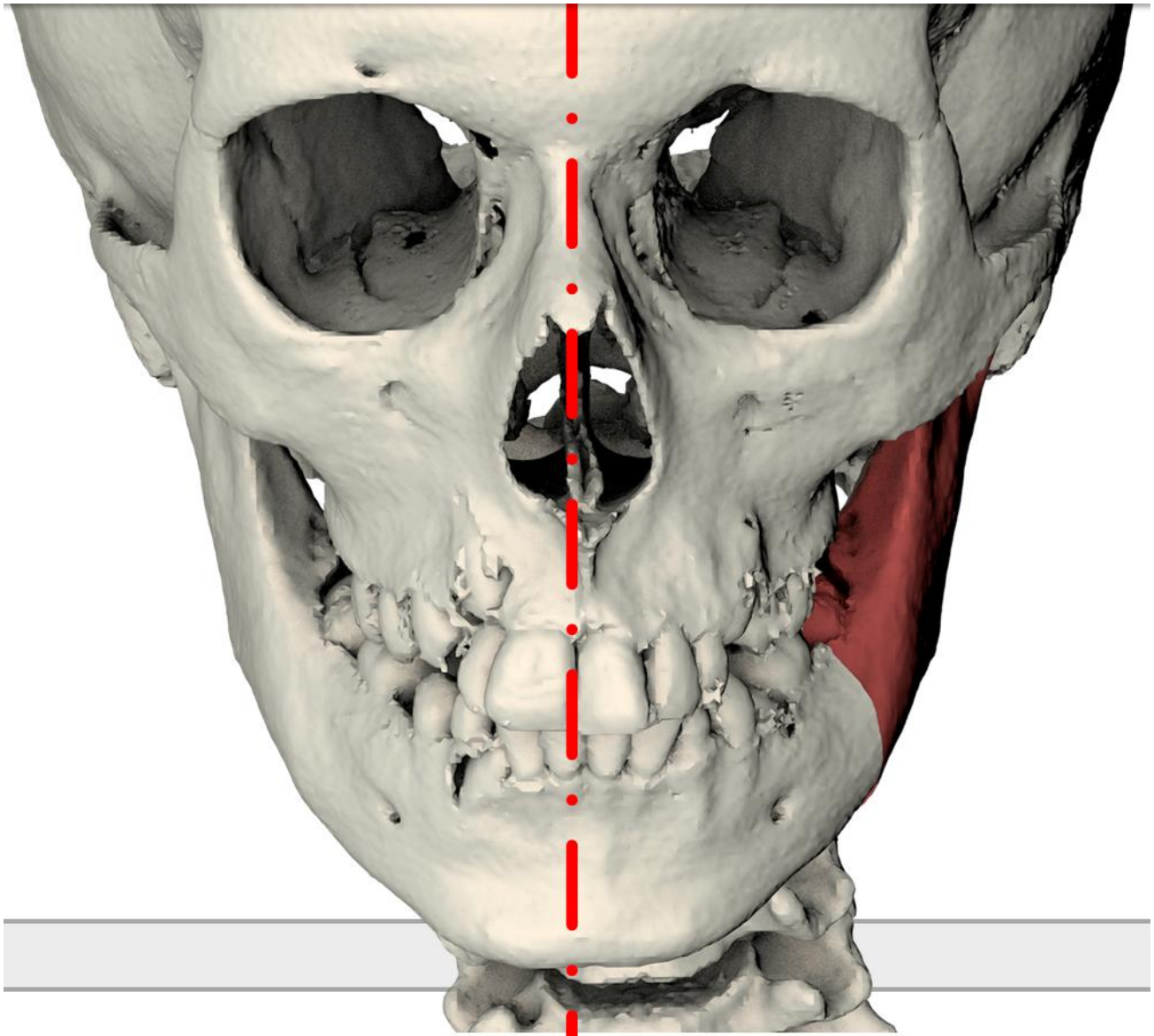
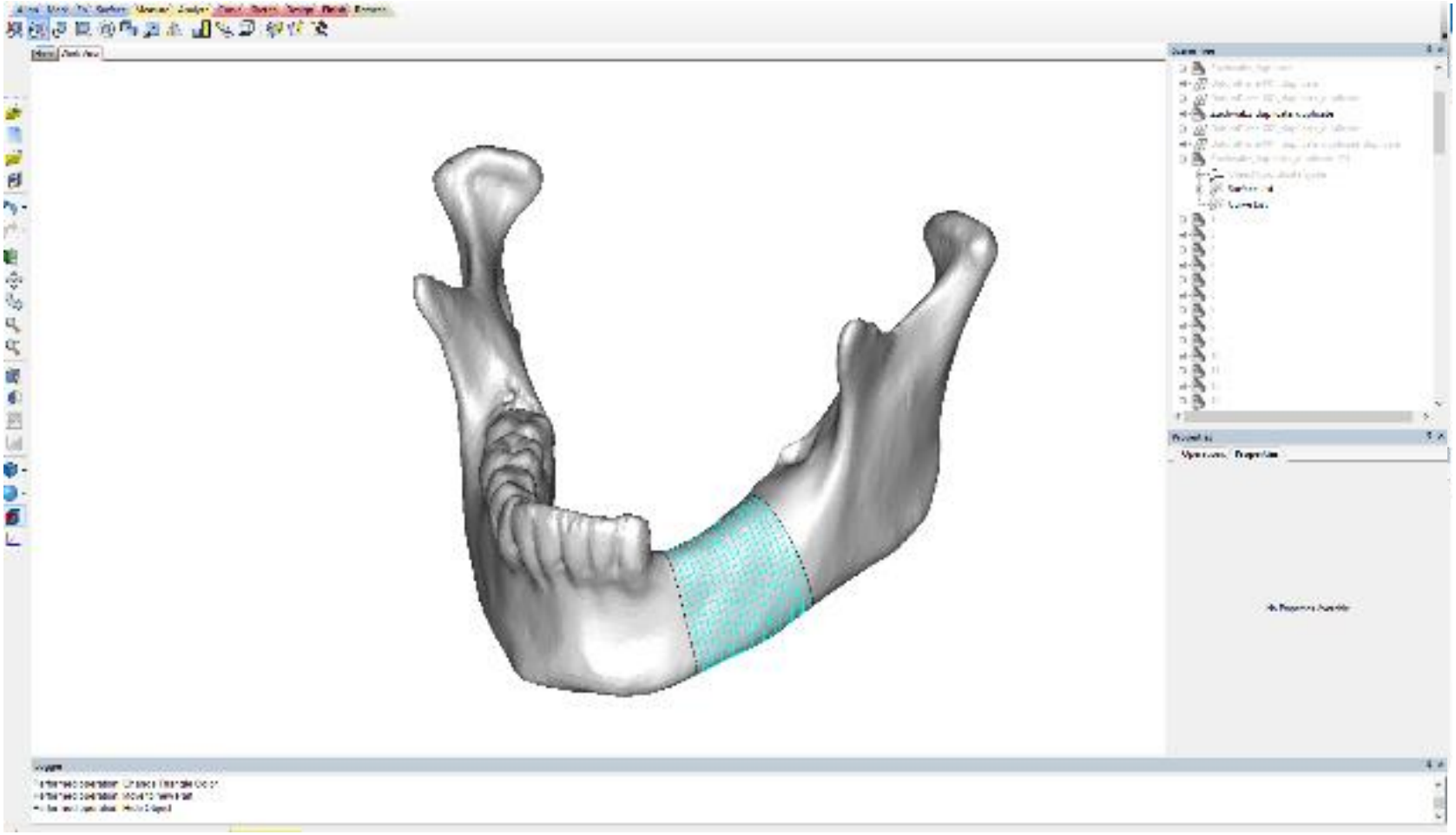


Image tranformastion: side mirroring

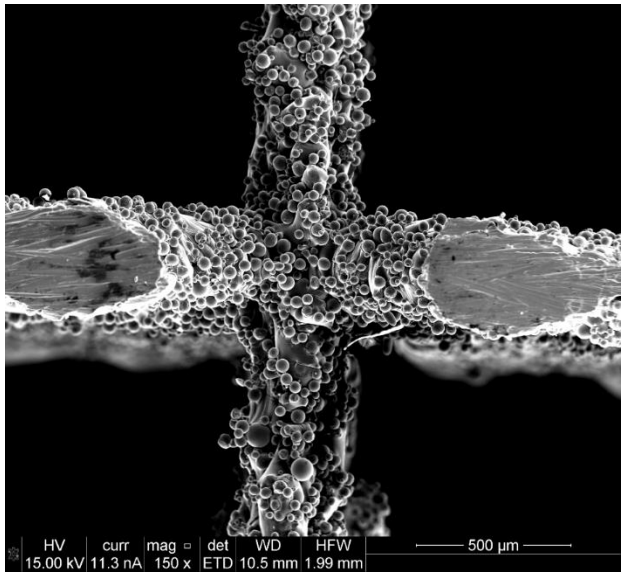
The bone of the mandible was isolated from the obtained skull bone model. It is on the prepared model that marks the section of bone that has been replaced with an implant made of titanium alloy.

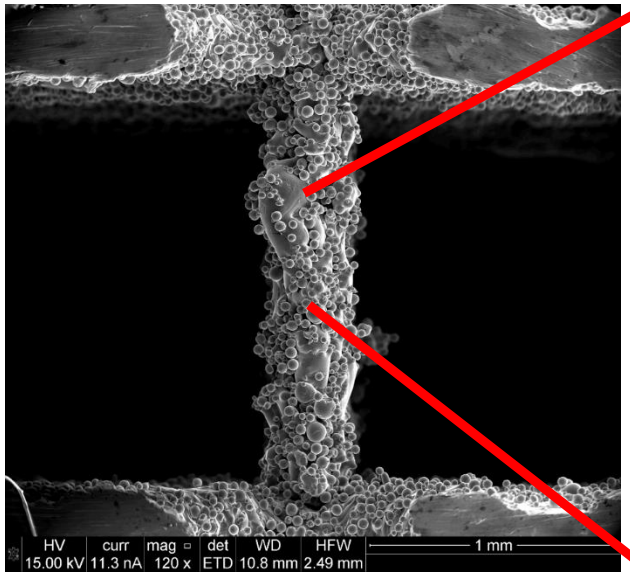


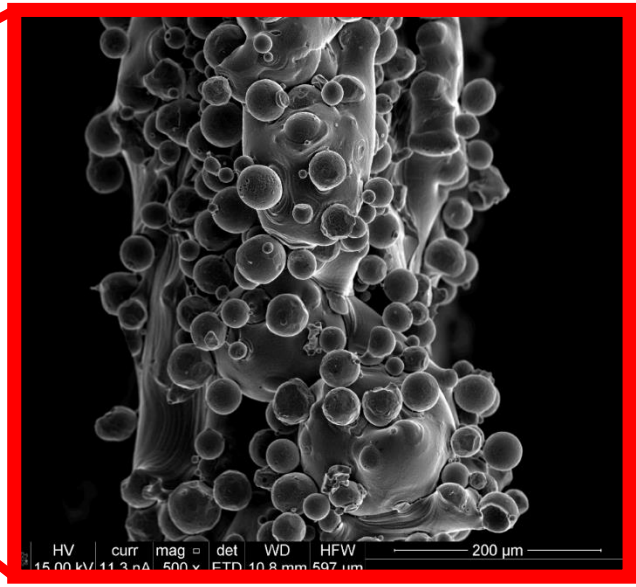
Final design

Template designs of maxillofacial implants and requirements of surgical instruments

Scanning Electron Microscopy

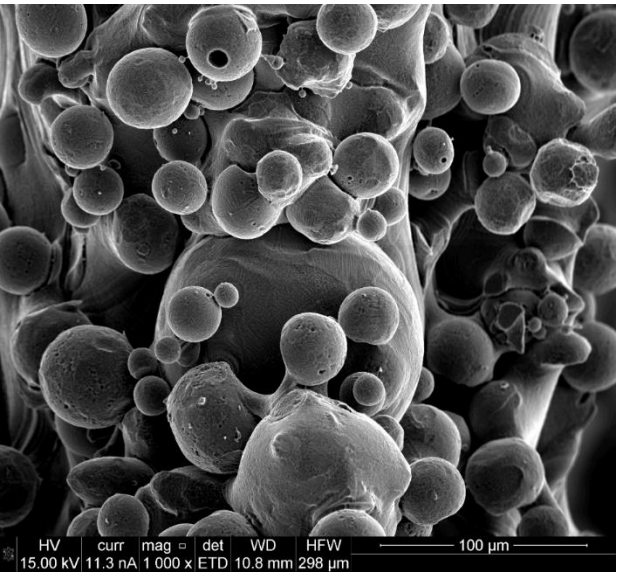


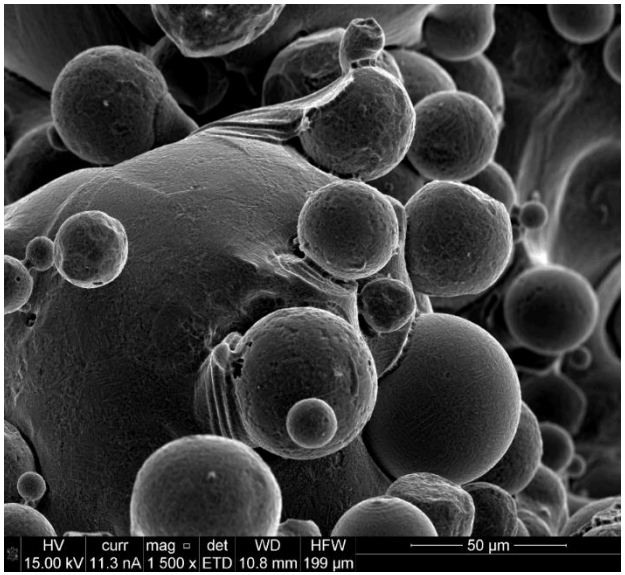


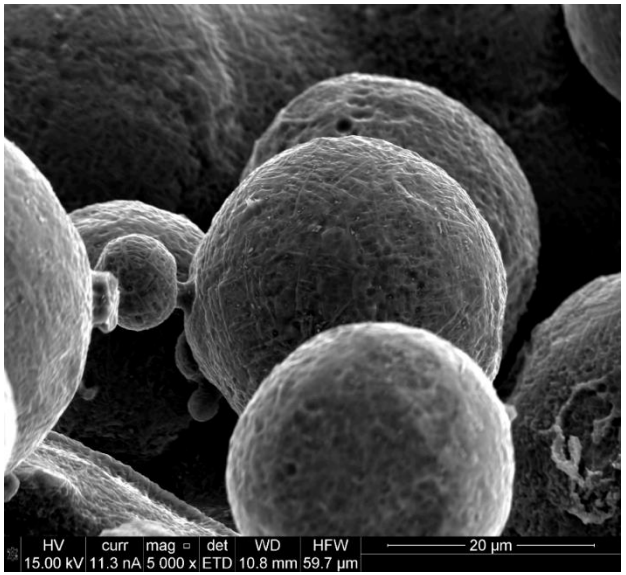


Template designs of maxillofacial implants and requirements of surgical instruments

Scanning Electron Microscopy

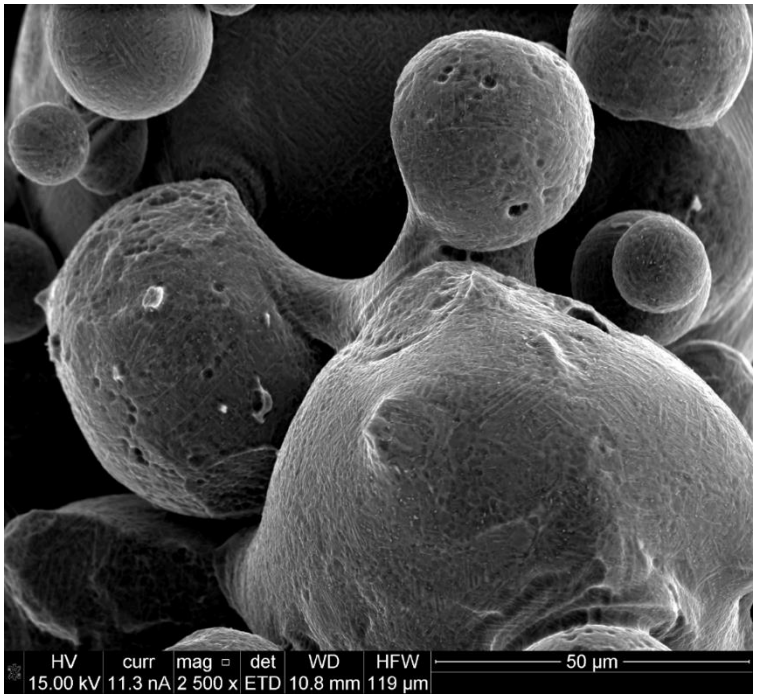


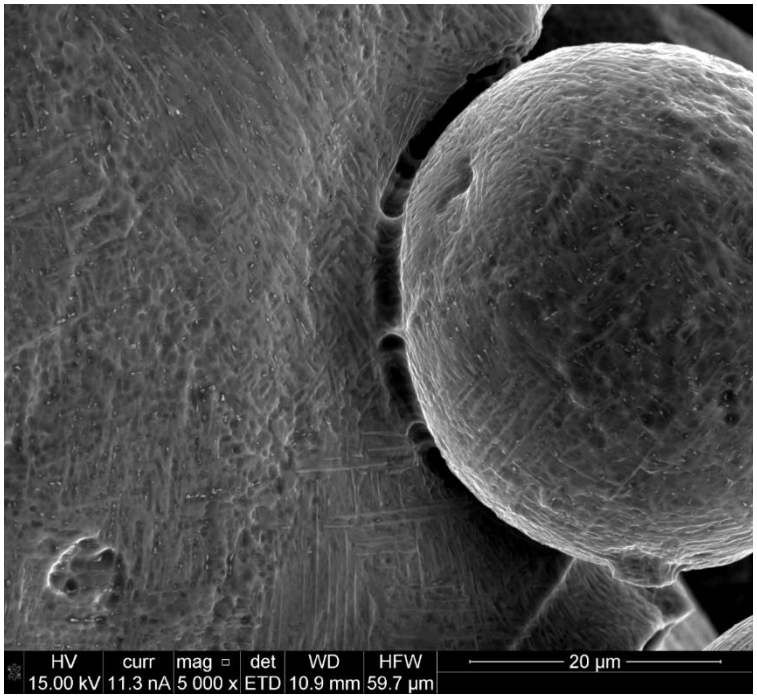




Template designs of maxillofacial implants and requirements of surgical instruments

Scanning Electron Microscopy

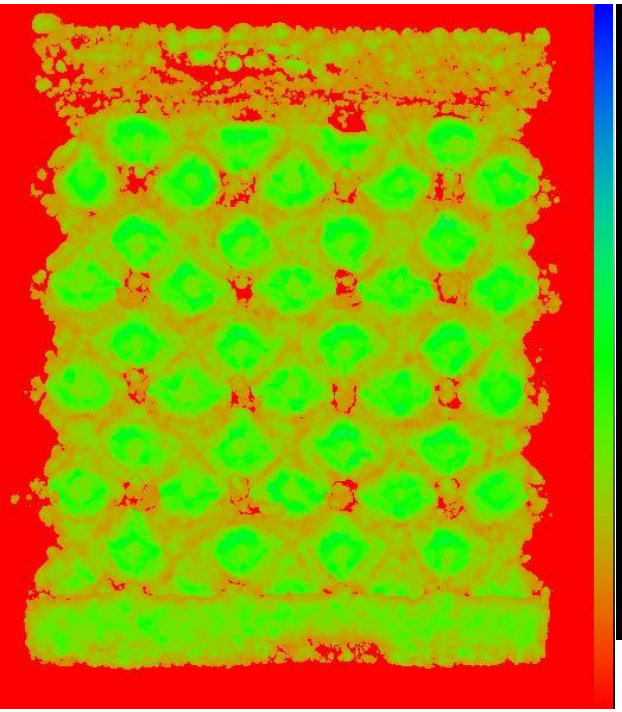


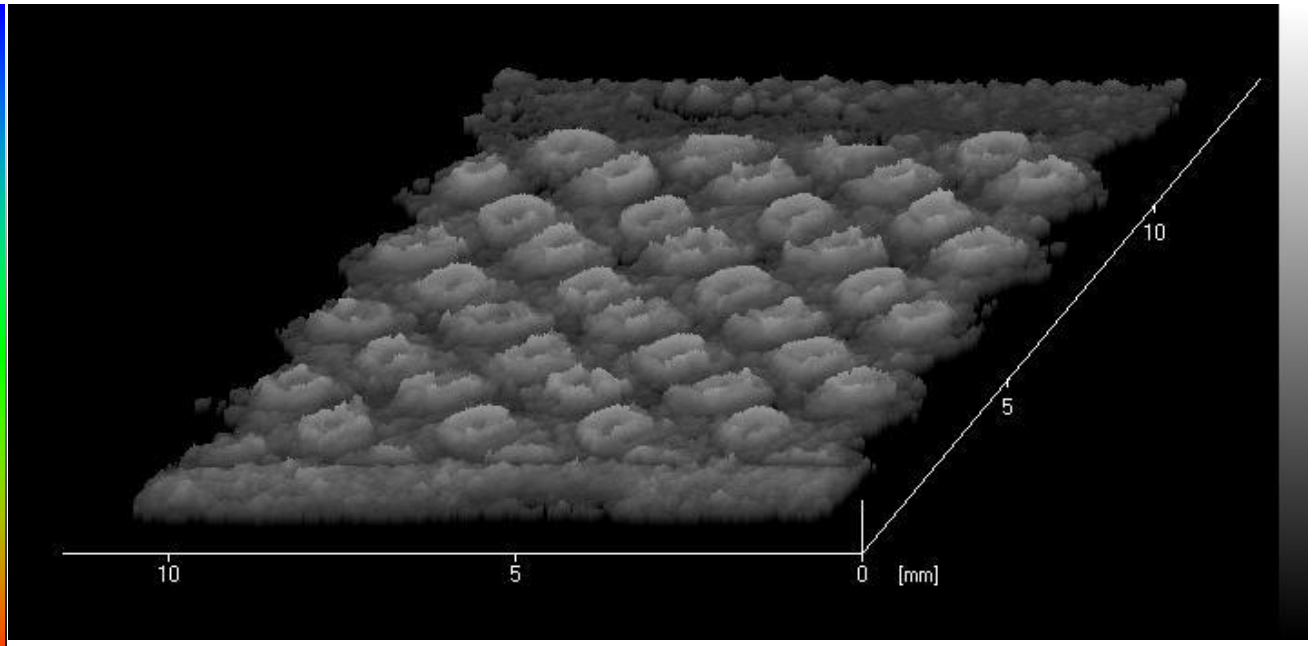


Template designs of maxillofacial implants and requirements of surgical instruments

Scanning Acoustic Microscopy

Mesh 2.0 not deformed





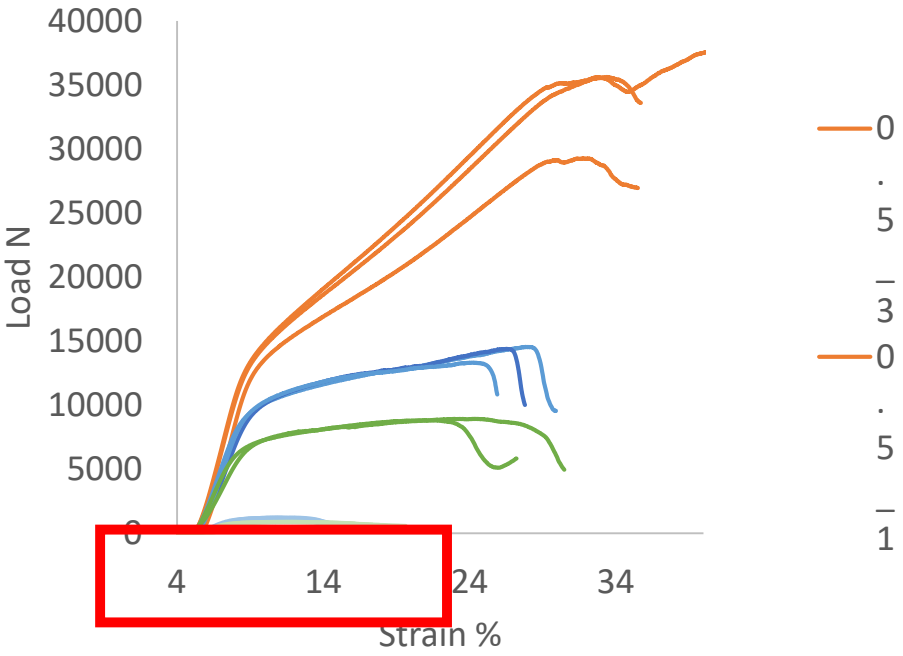
Template designs of maxillofacial implants and requirements of surgical instruments

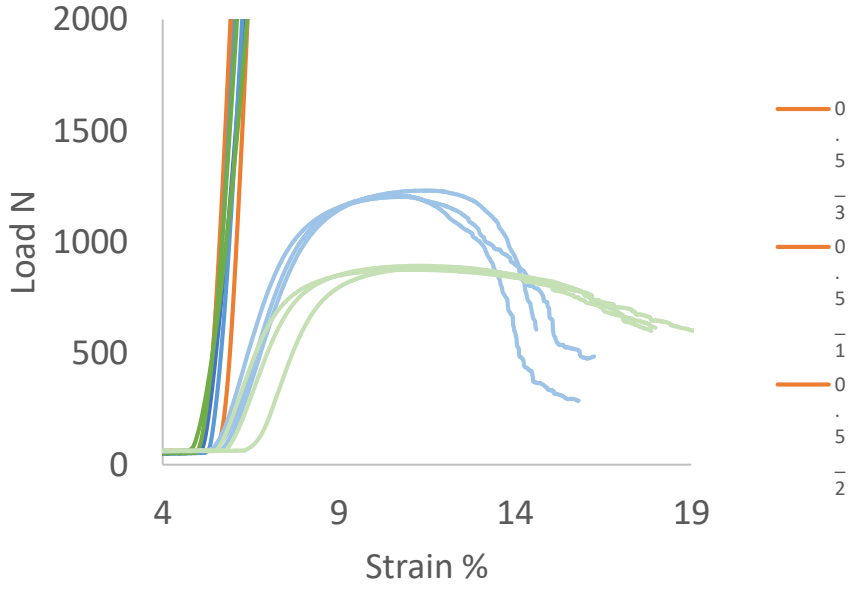
Scanning Acoustic Microscopy

Mesh 0.5 not deformed X-scan

Template designs of maxillofacial implants and requirements of surgical instruments

Tensile Test

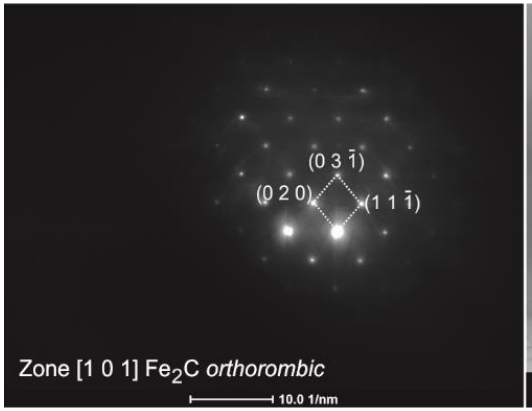


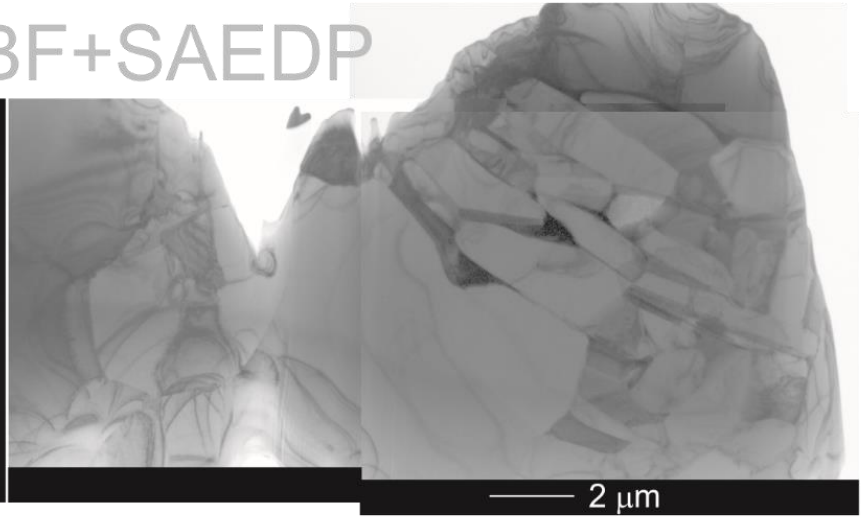


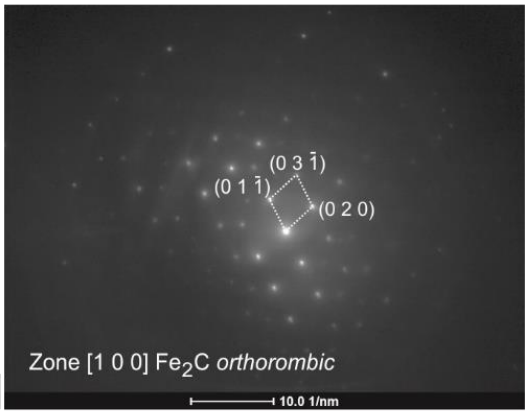
Template designs of maxillofacial implants and requirements of surgical instruments

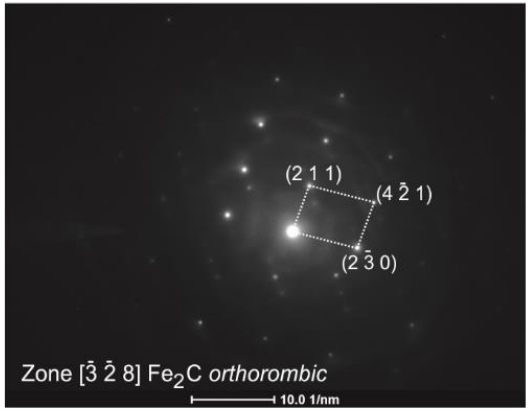
Transmission Electron Microscopy

TEM BF+SAEDP



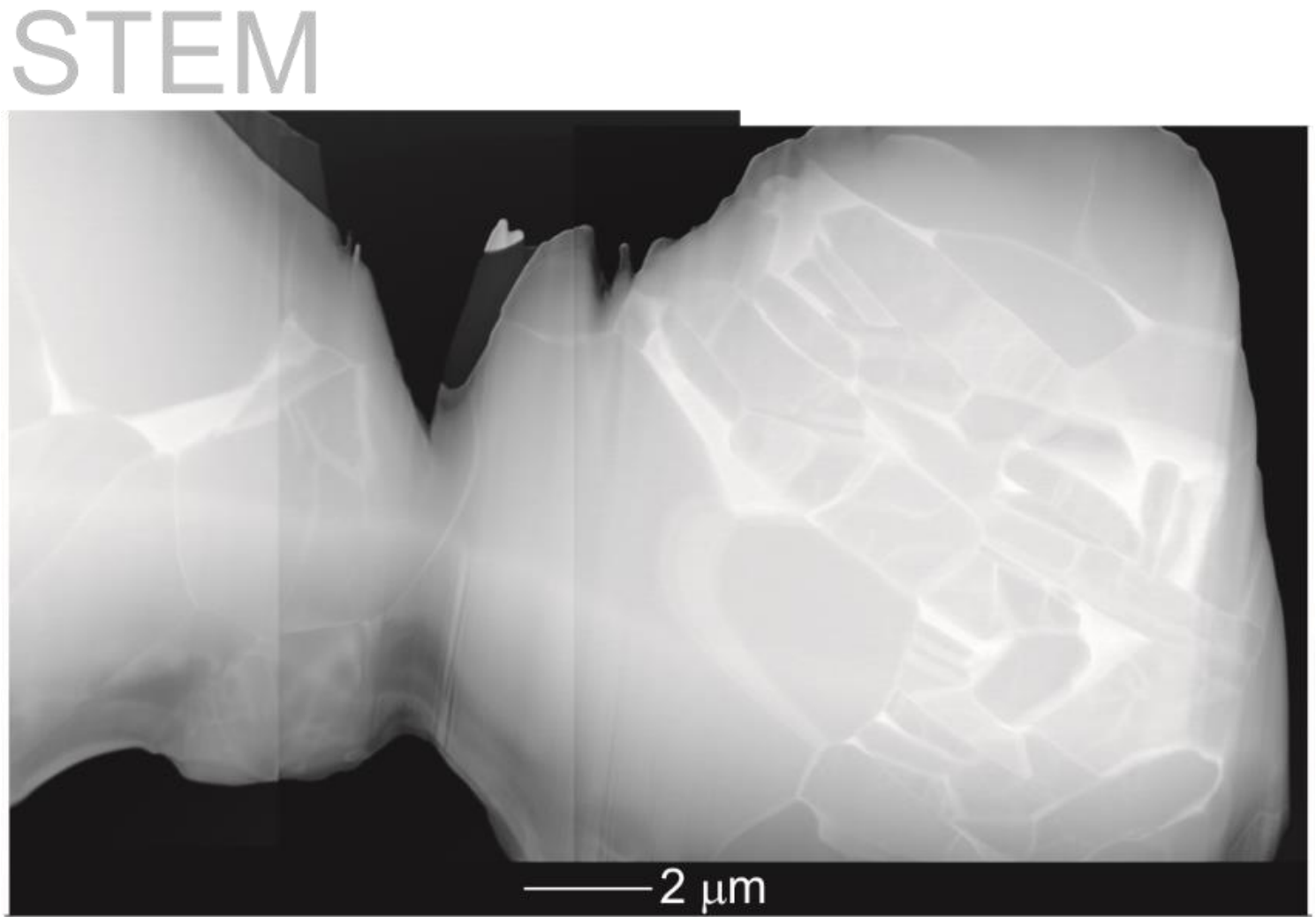






Template designs of maxillofacial implants and requirements of surgical instruments

Transmission Electron Microscopy



Bacteriology

Pseudomonas aeruginosa; Streptococci; Candida albicans

Bacteriology

1. The examined biomaterials were incubated respectively: "cubes" 15 ml 24-hour cultures of Pseudomonas aeurginosa, Streptococcus pyogenes, Candida albicans and "circles" 4 ml 24-hour culture of Pseudomonas aeurginosa, Streptococcus pyogenes, Candida albicans.

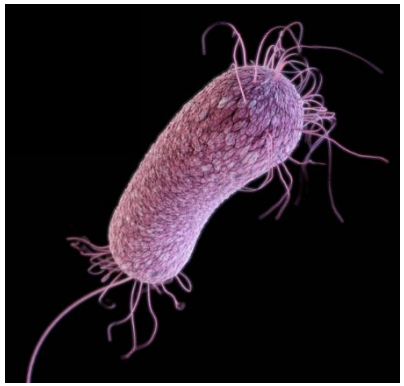
2. Before the incubation with biomaterials for all cultures of microorganisms, the optical density (OD) at 600 nm was determined.

3. After 24 and 48 hours, the OD was measured again at 600 nm.

4. Subsequently, the biomaterials were rinsed with a 3.5% paraformaldehyde aqueous solution.

5. Conclusions: in all examined cultures with biomaterial the OD value increased, which may indicate the growth of microorganisms and indirectly testify to the fact that the biomaterial does not have bactericidal properties.

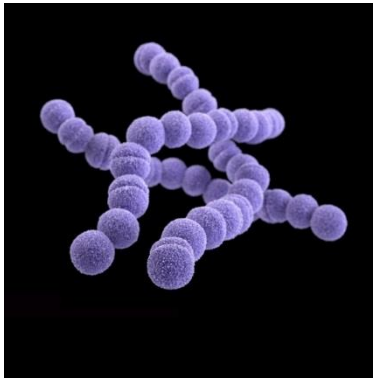
Bacteriology



Pseudomonas aeruginosa is a common Gram-negative, rod-shaped bacterium that can cause disease in plants and animals, including humans. A species of considerable medical importance, P. aeruginosa is a multidrug resistant pathogen recognized for its ubiquity, its intrinsically advanced antibiotic resistance mechanisms, and its association with serious illnesses – hospital-acquired infections such as ventilator-associated pneumonia and various sepsis syndromes.

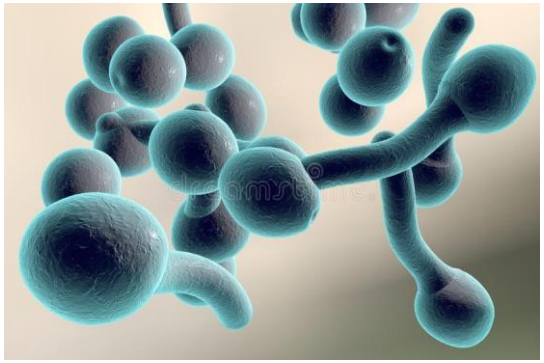
The organism is considered opportunistic insofar as serious infection often occurs during existing diseases or conditions – most notably cystic fibrosis and traumatic burns. It is also found generally in the immunocompromised but can infect the immunocompetent as in hot tub folliculitis. Treatment of P. aeruginosa infections can be difficult due to its natural resistance to antibiotics. When more advanced antibiotic drug regimens are needed adverse effects may result.

It is citrate, catalase, and oxidase positive. It is found in soil, water, skin flora, and most man-made environments throughout the world. It thrives not only in normal atmospheres, but also in low-oxygen atmospheres, thus has colonized many natural and artificial environments. It uses a wide range of organic material for food; in animals, its versatility enables the organism to infect damaged tissues or those with reduced immunity. The symptoms of such infections are generalized inflammation and sepsis. If such colonizations occur in critical body organs, such as the lungs, the urinary tract, and kidneys, the results can be fatal.[1] Because it thrives on moist surfaces, this bacterium is also found on and in medical equipment, including catheters, causing cross-infections in hospitals and clinics. It is also able to decompose hydrocarbons and has been used to break down tarballs and oil from oil spills.[2] P. aeruginosa is not extremely virulent in comparison with other major pathogenic bacterial species – for example Staphylococcus aureus and Streptococcus pyogenes – though P. aeruginosa is capable of extensive colonization, and can aggregate into enduring biofilms.[3]



Streptococci and staphylococci constitute the main groups of medically important gram-positive cocci. Streptococci are gram-positive, nonmotile, and catalase negative. Clinically important genera include Streptococcus and Enterococcus. They are ovoid to spherical in shape and occur as pairs or chains. Most are aerotolerant anaerobes because they grow fermentatively even in the presence of oxygen. Because of their complex nutritional requirements, blood enriched medium is generally used for their isolation. Diseases caused by this group of organisms include acute infections of the throat and skin caused by group A streptococci (Streptococcus pyogenes); female genital tract colonization, resulting in neonatal sepsis caused by group B streptococci (Streptococcus agalactia); pneumonia, otitis media, and meningitis caused by Streptococcus pneumoniae; and endocarditis caused by the viridians group of streptococci.

Bacteriology

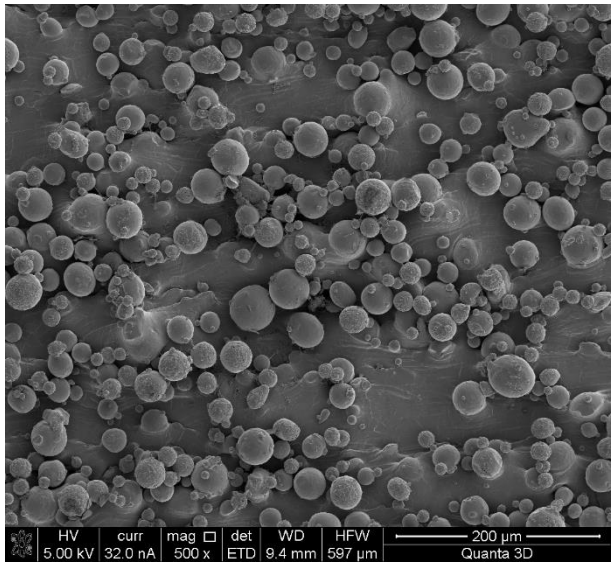


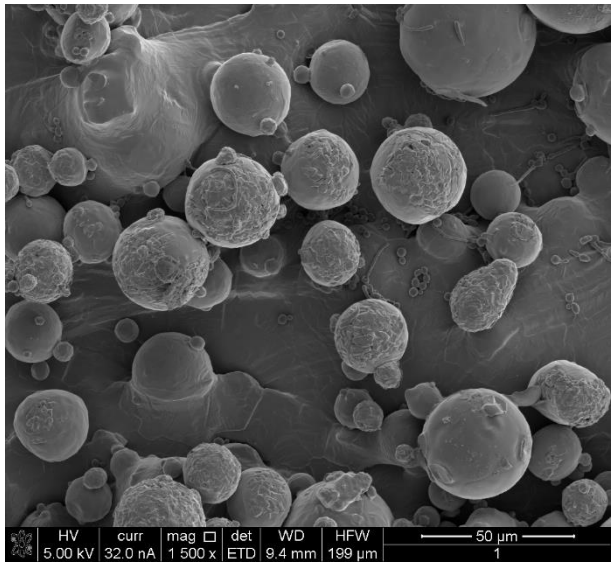
Candida albicans is an opportunistic pathogenic yeast[4] that is a common member of the human gut flora. It does not proliferate outside the human body.[5] It is detected in the gastrointestinal tract and mouth in 40–60% of healthy adults.[6][7] It is usually a commensal organism, but can become pathogenic in immunocompromised individuals under a variety of conditions.[7][8] It is one of the few species of the genus Candida that causes the human infection candidiasis, which results from an overgrowth of the fungus.[7][8] Candidiasis is for example often observed in HIV-infected patients.[9] C. albicans is the most common fungal species isolated from biofilms either formed on (permanent) implanted medical devices or on human tissue.[10][11] C. albicans, C. tropicalis, C. parapsilosis, and C. glabrata are together responsible for 50–90% of all cases of candidiasis in humans.[8][12][13] A mortality rate of 40% has been reported for patients with systemic candidiasis due to C. albicans.[14] By one estimate, invasive candidiasis contracted in a hospital causes 2800 to 11200 deaths yearly in the US.[15]

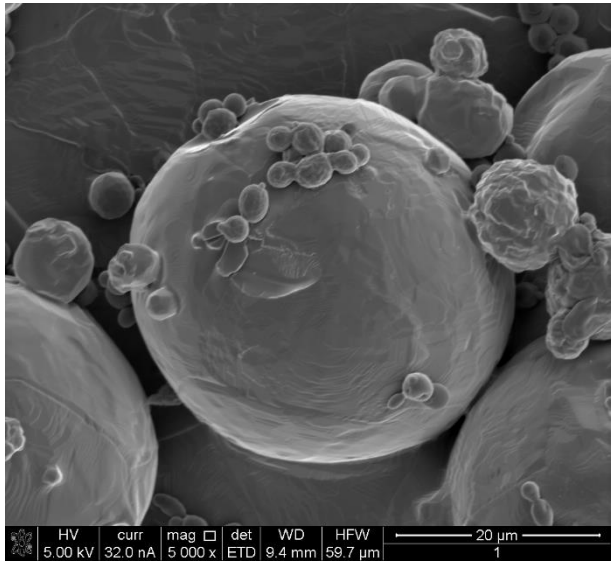
C. albicans is commonly used as a model organism for biology. It is generally referred to as a dimorphic fungus since it grows both as yeast and filamentous cells. However it has several different morphological phenotypes. C. albicans was for a long time considered an obligate diploid organism without a haploid stage. This is however not the case. Next to a haploid stage C. albicans can also exist in a tetraploid stage. The latter is formed when diploid C. albicans cells mate when they are in the opaque form.[16] The diploid genome size is approximately 29 Mb, and up to 70% of the protein coding genes have not yet been characterized.[17] C. albicans is easily cultured in the lab and can be studied both in vivo and in vitro. Depending on the media different studies can be done as the media influences the morphological state of C. albicans. A special type of medium is CHROMagar™ Candida which can be used to identify different species of candida.[18][19]

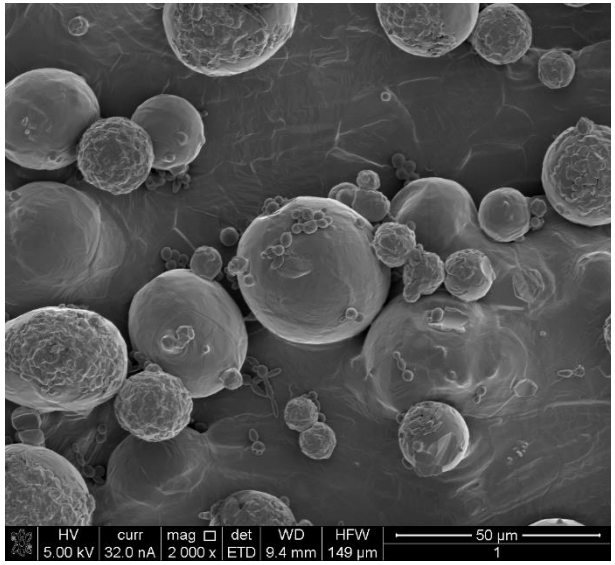
Bacteriology

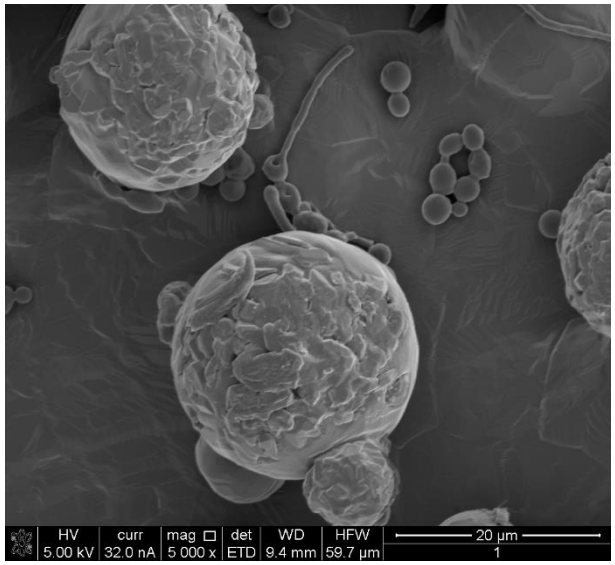
P.aeruginosa





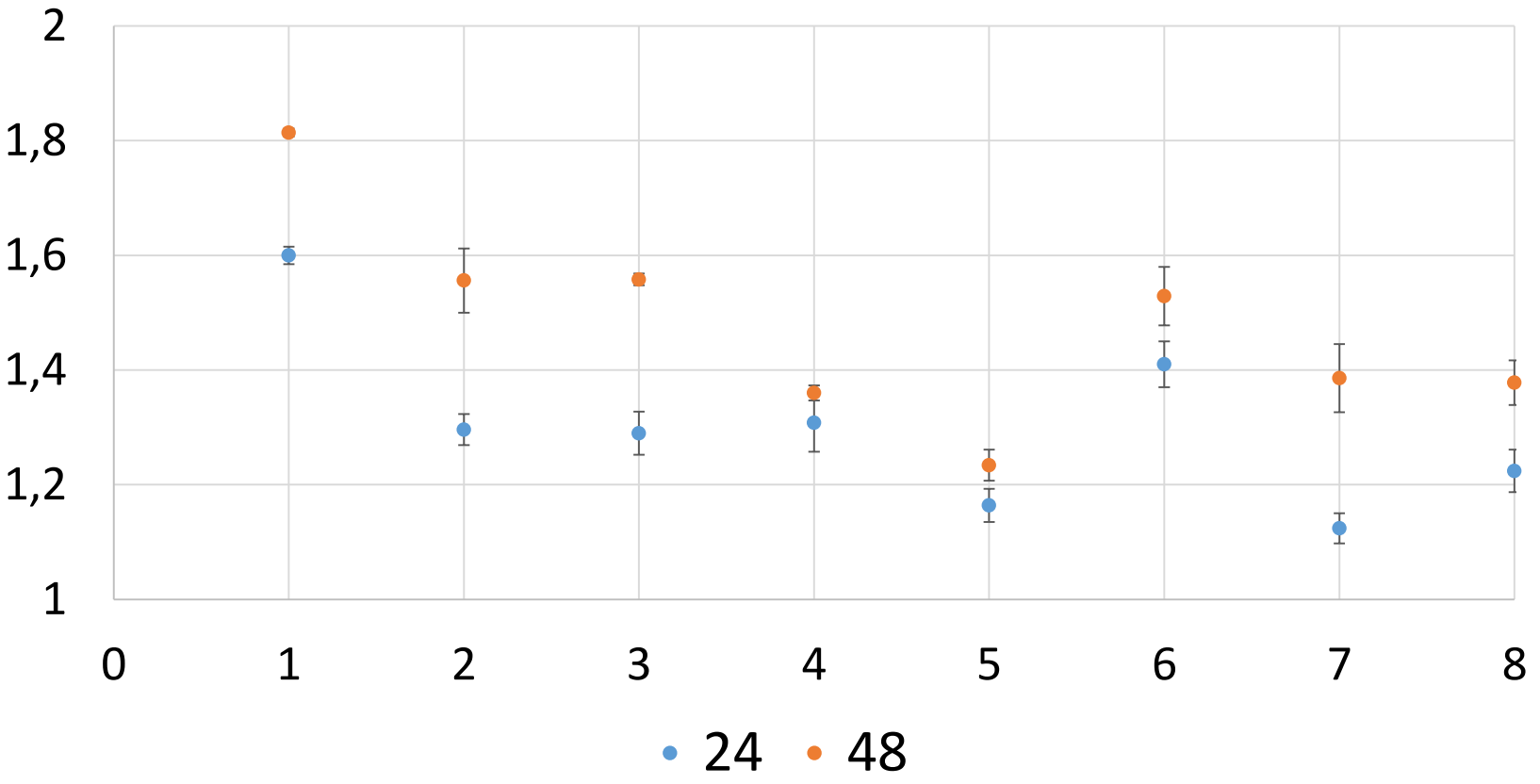






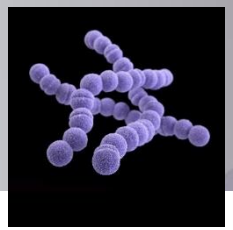
Bacteriology

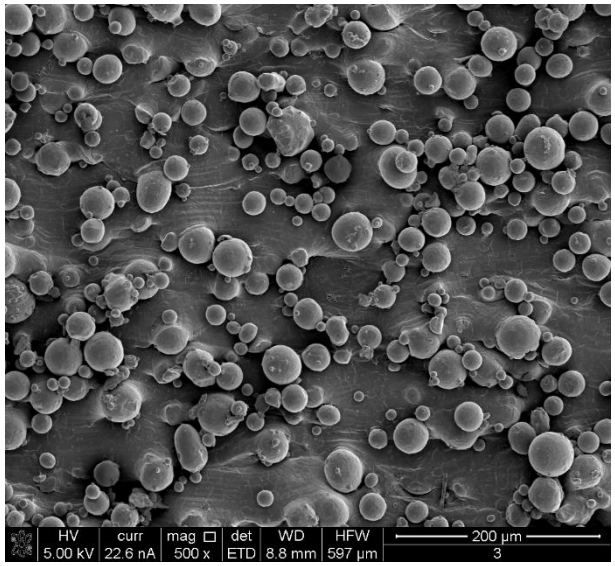
P.aeruginosa

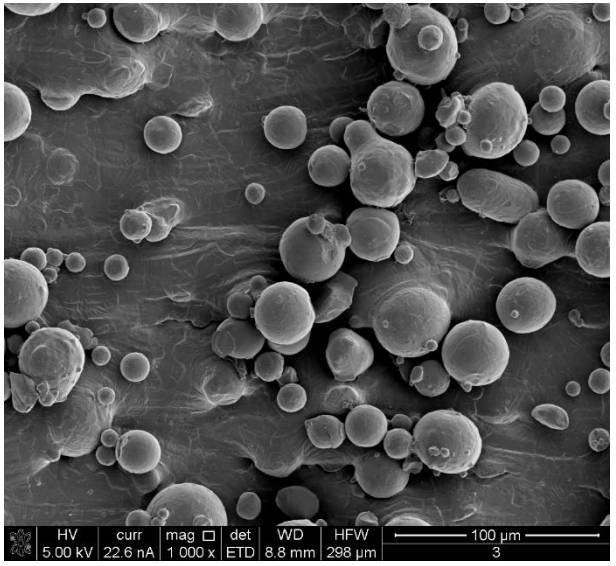


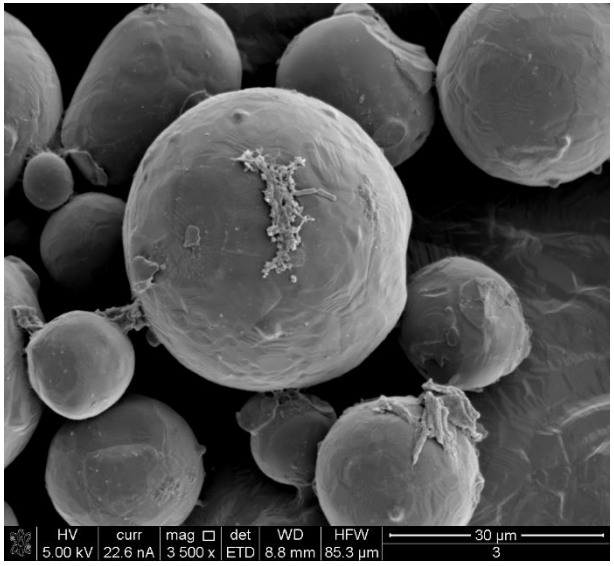
Bacteriology

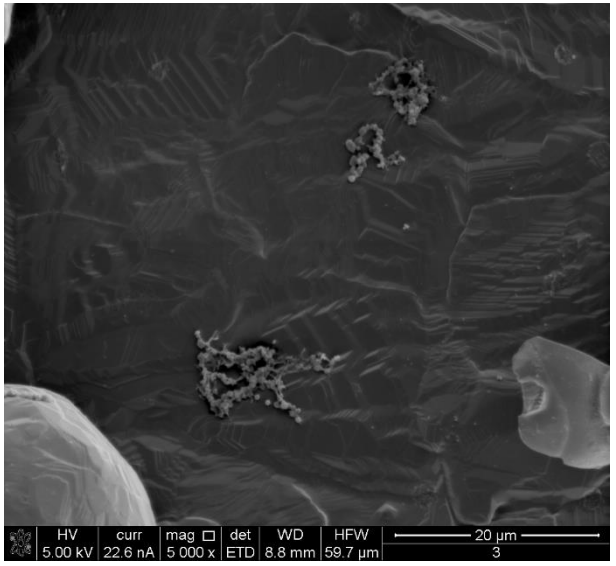
Streptococcus pyogenes





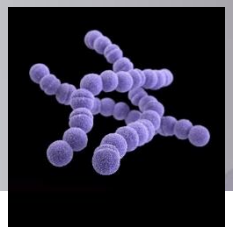


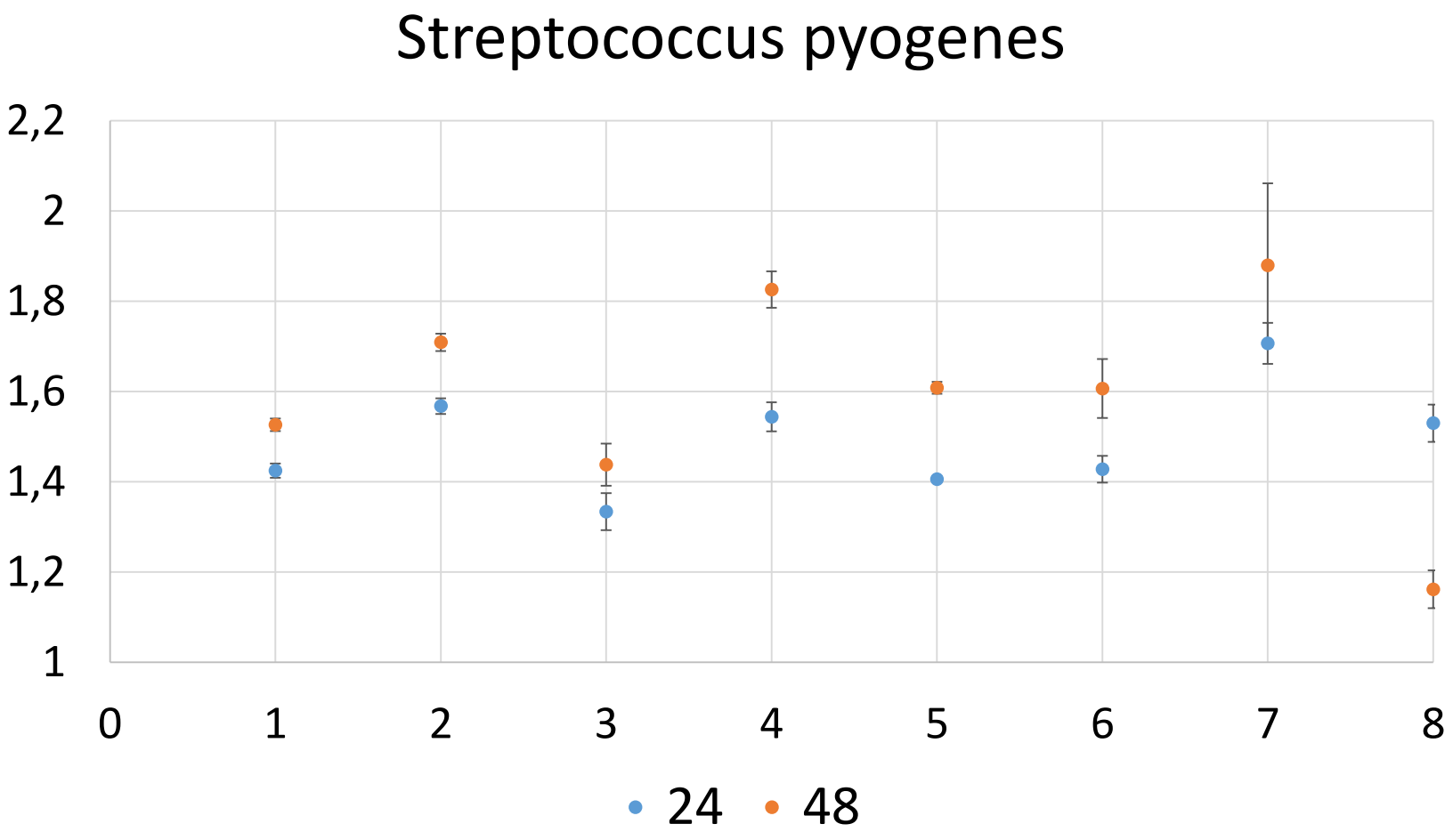




Bacteriology

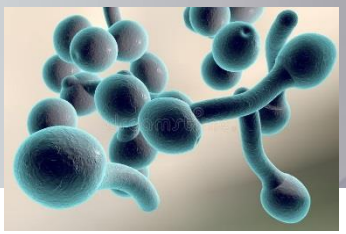
Streptococcus pyogenes

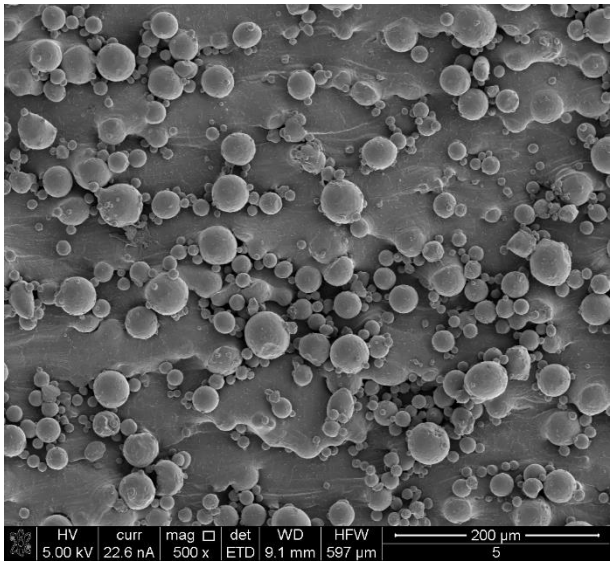


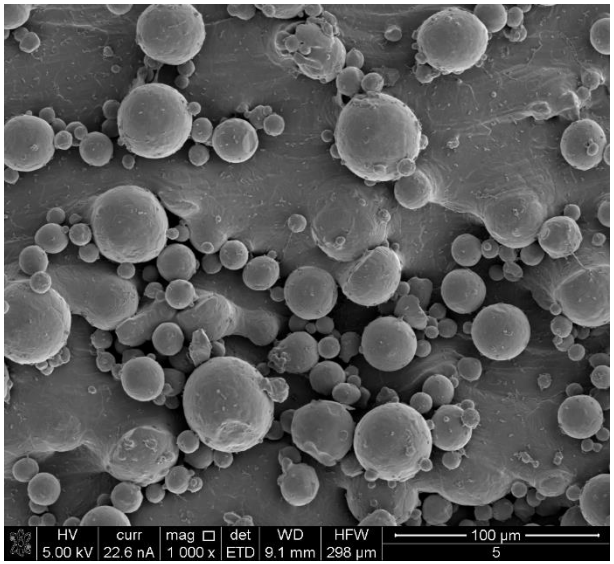


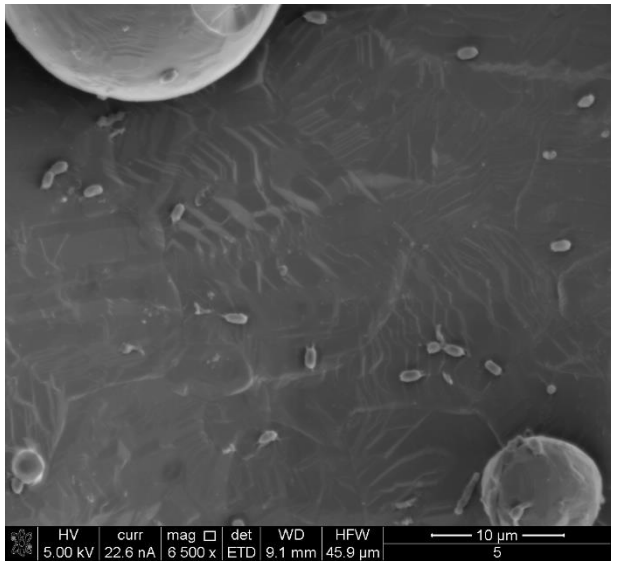
Bacteriology

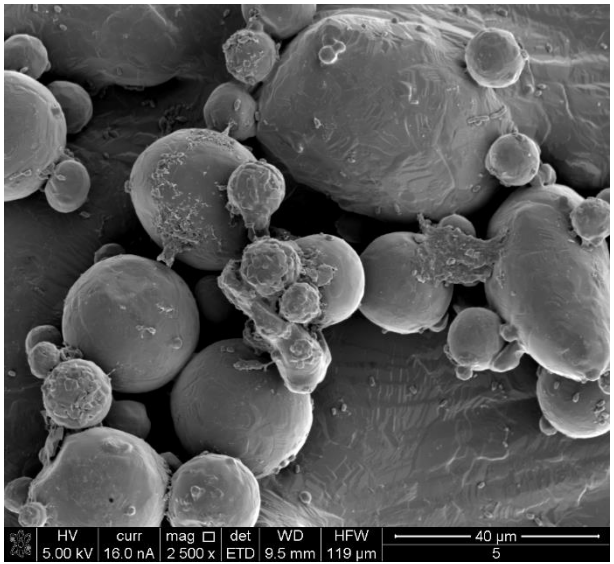
Candida albicans

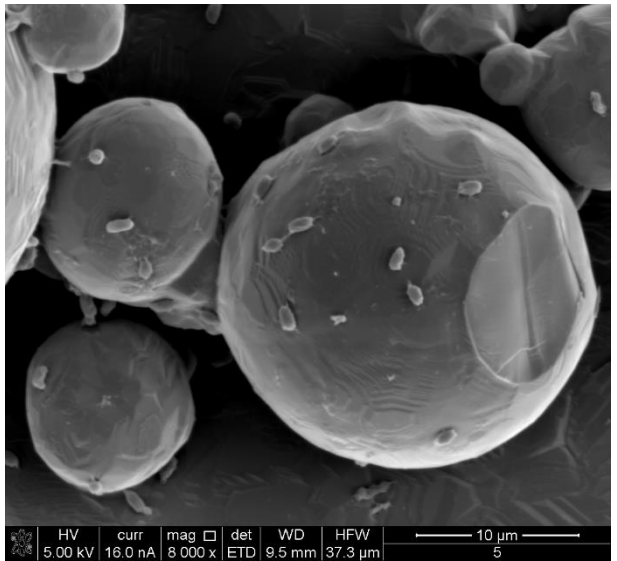






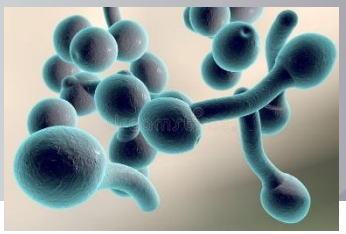


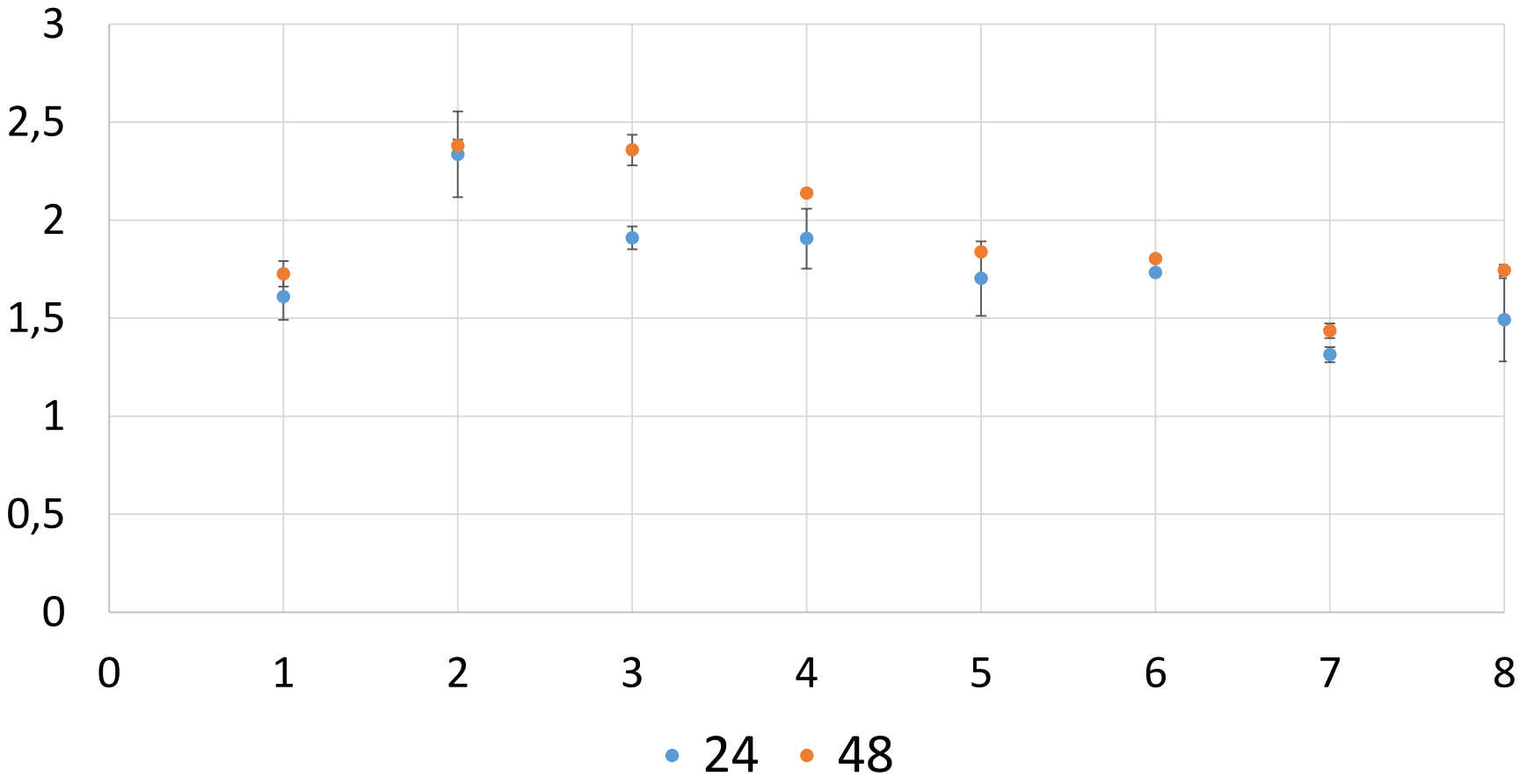




Bacteriology

Candida albicans

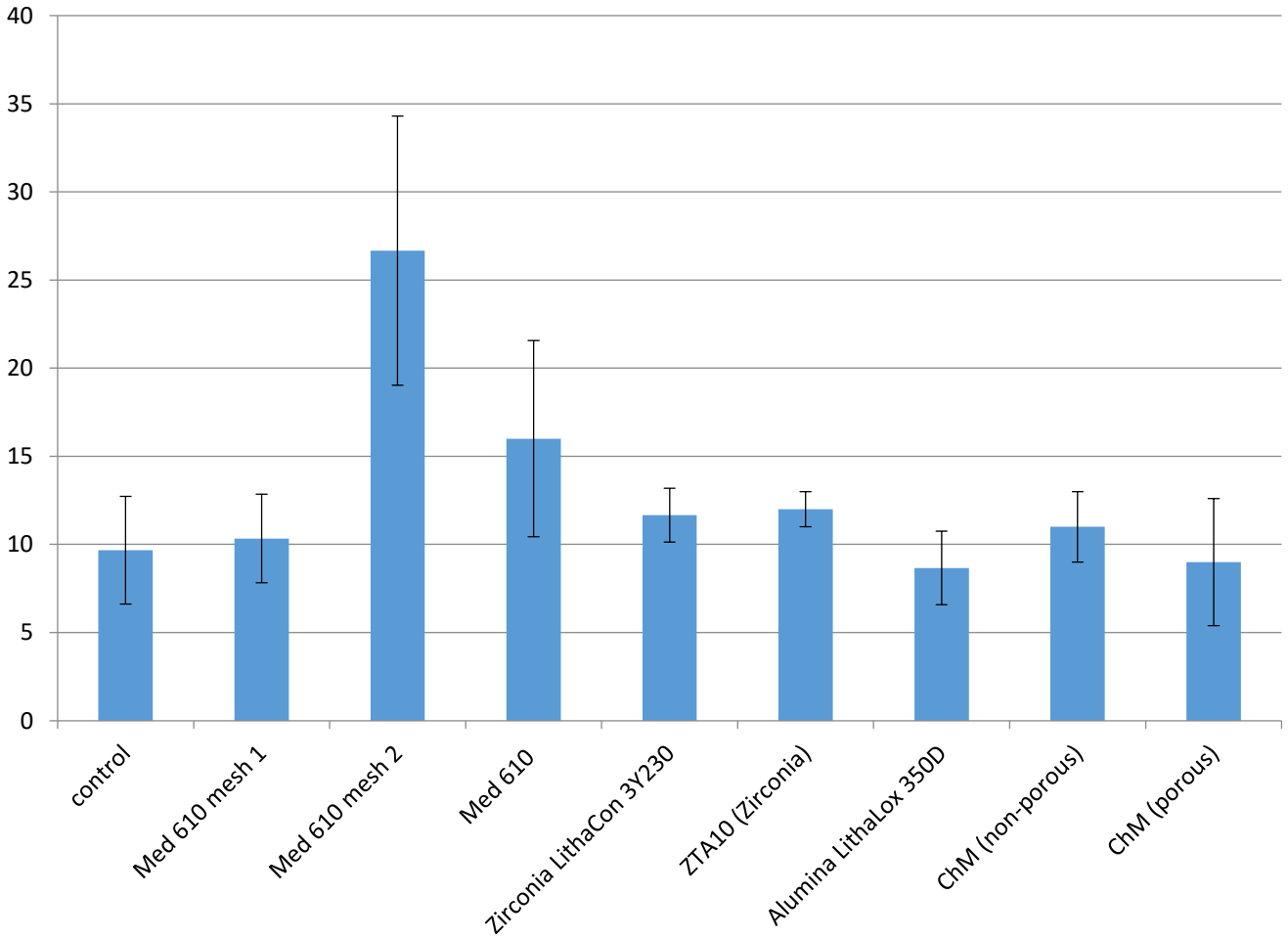


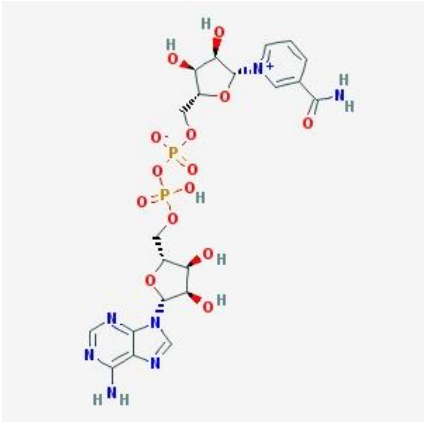


Cytotoxicity

Genotoxicity

Lactate dehydrogenase (LDH): study





Construction of NAD

The basis of LDH test

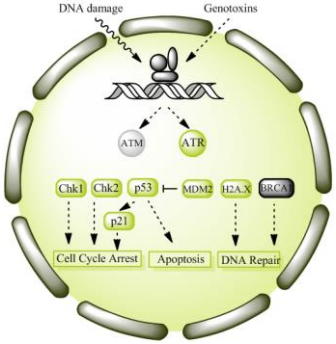
LACTATE → PYRUVATE

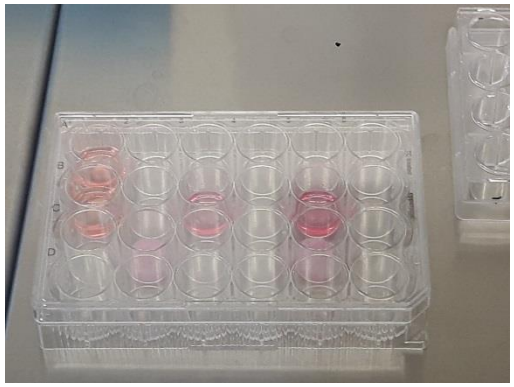
NAD+ → NAPDH+H+

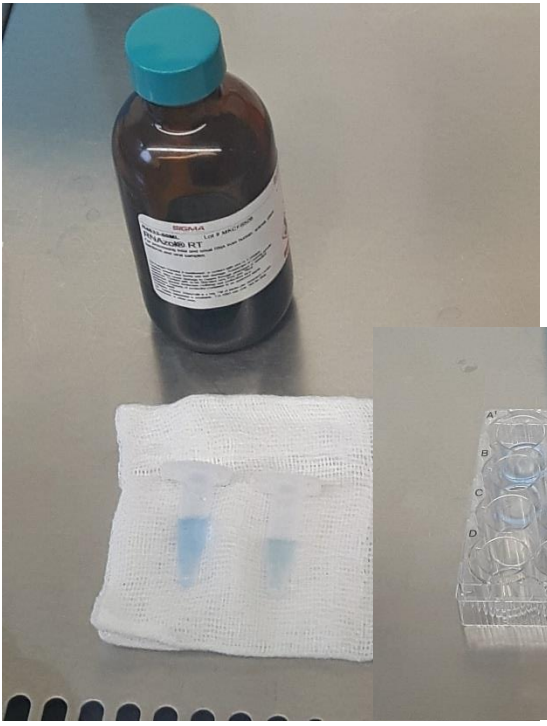
TETRAZOLIN → FORMAZAN

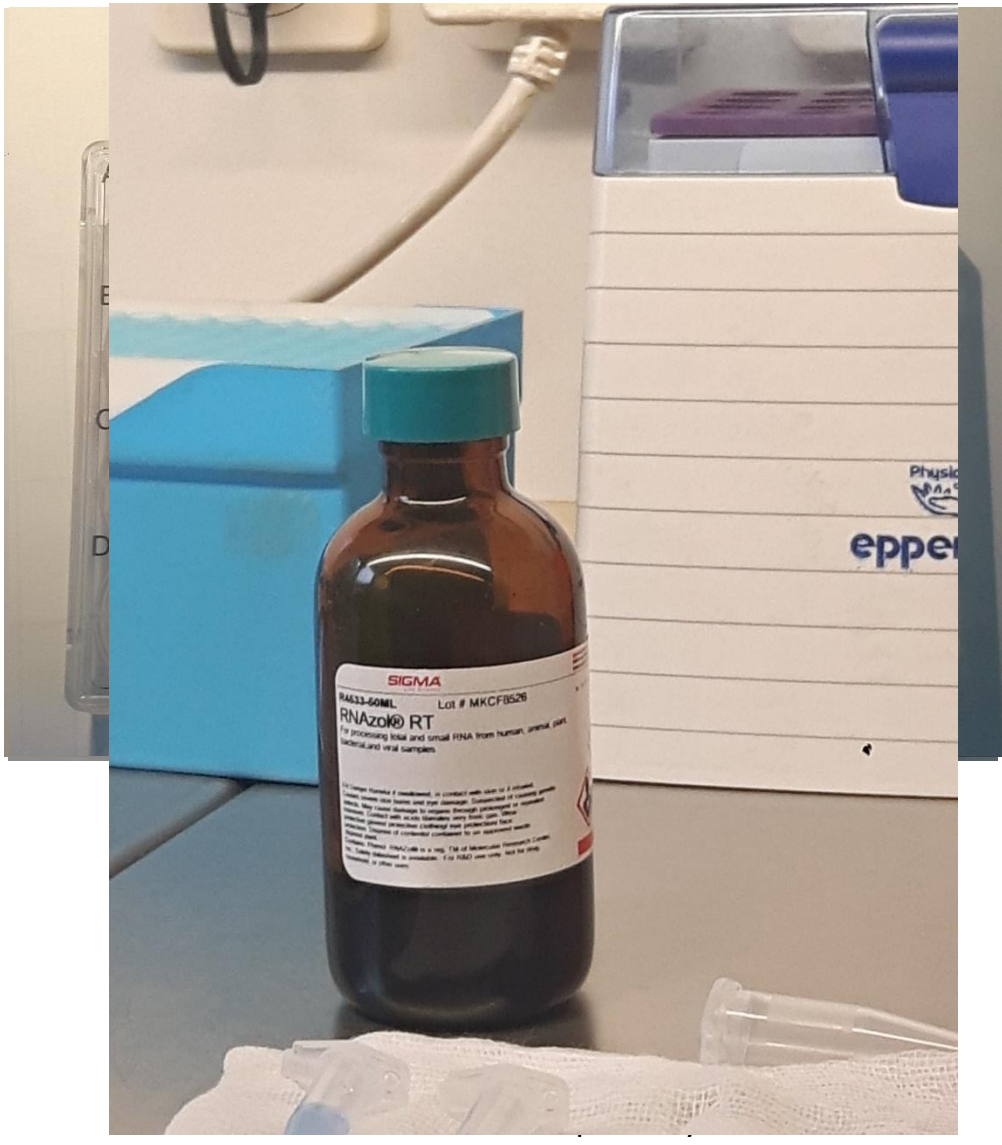
Diaphoresis

Genotoxicity







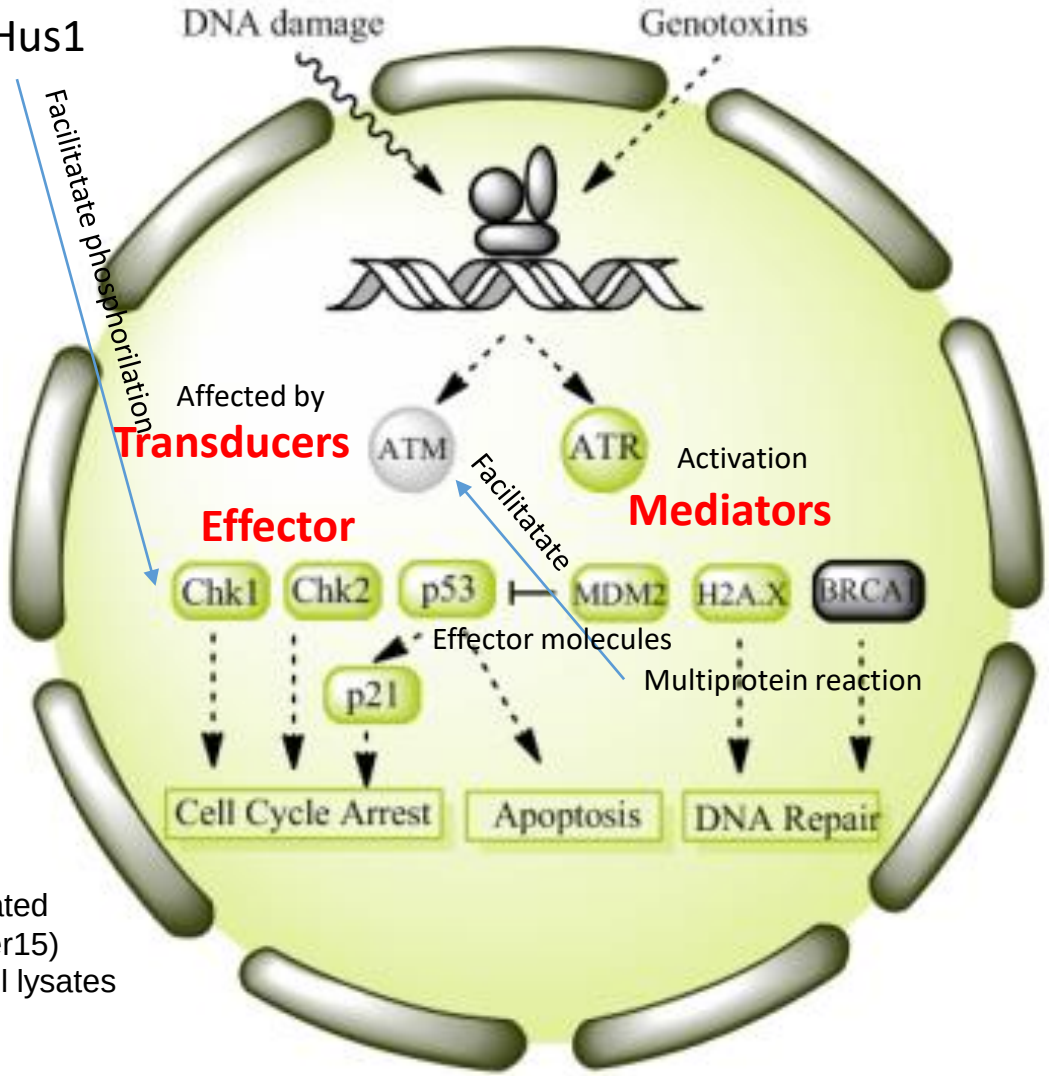


Genotoxicity

Genotoxicity

Sensor proteins like Rad9, Rad1and Hus1

Traditional genotoxicity assays detect gain or loss of function mutations, but provide little or no mechanistic data as to how this DNA damage may have occurred. Protein expression and phosphorylation detection of multiple proteins involved in DNA damage or genotoxicity provides a faster and more accurate assessment of the status of the cell for researchers exposing cells to potentially genotoxic compounds or researching DNA damage and repair mechanisms.



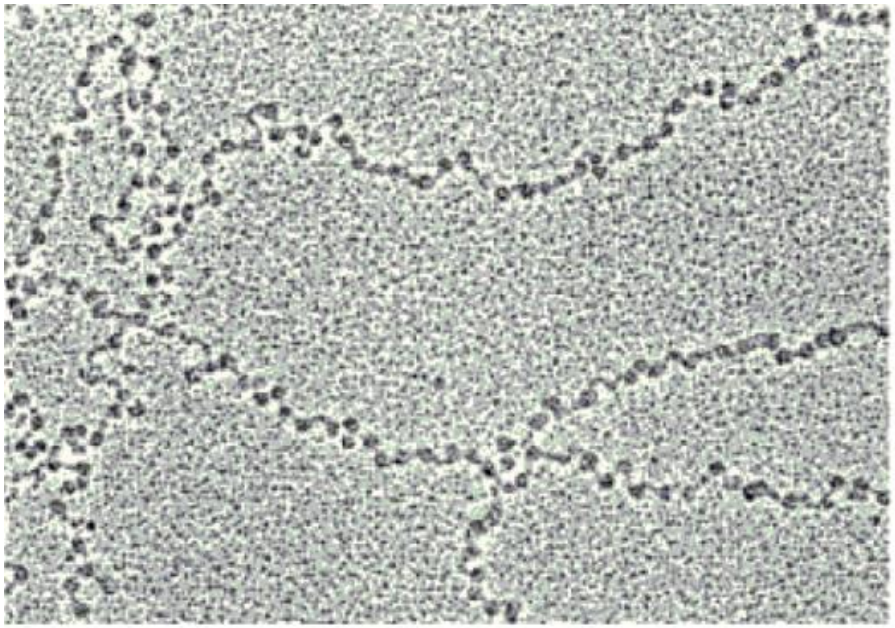
The MILLIPLEX® MAP 7-plex DNA Damage/Genotoxicity Magnetic Bead kit is used to detect changes in phosphorylated Chk1 (Ser345), Chk2 (Thr68), H2A.X (Ser139) and p53 (Ser15) as well as total protein levels of ATR, MDM2 and p21 in cell lysates

Micronucleus test

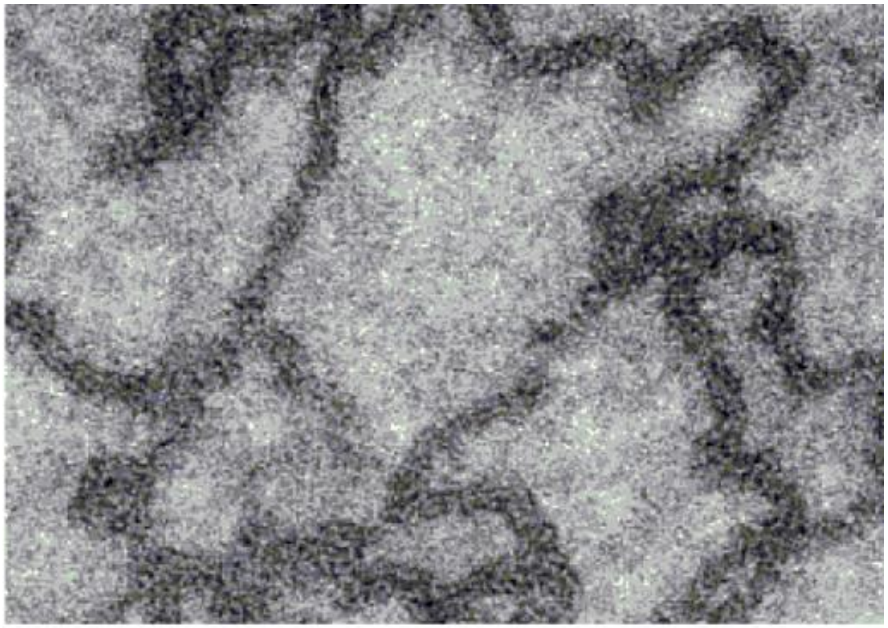
Chromatin- DNA complex with proteins found in the cell nucleus

Micronucleus test

Chromatin occurs in the form of stretched or condensed fibers



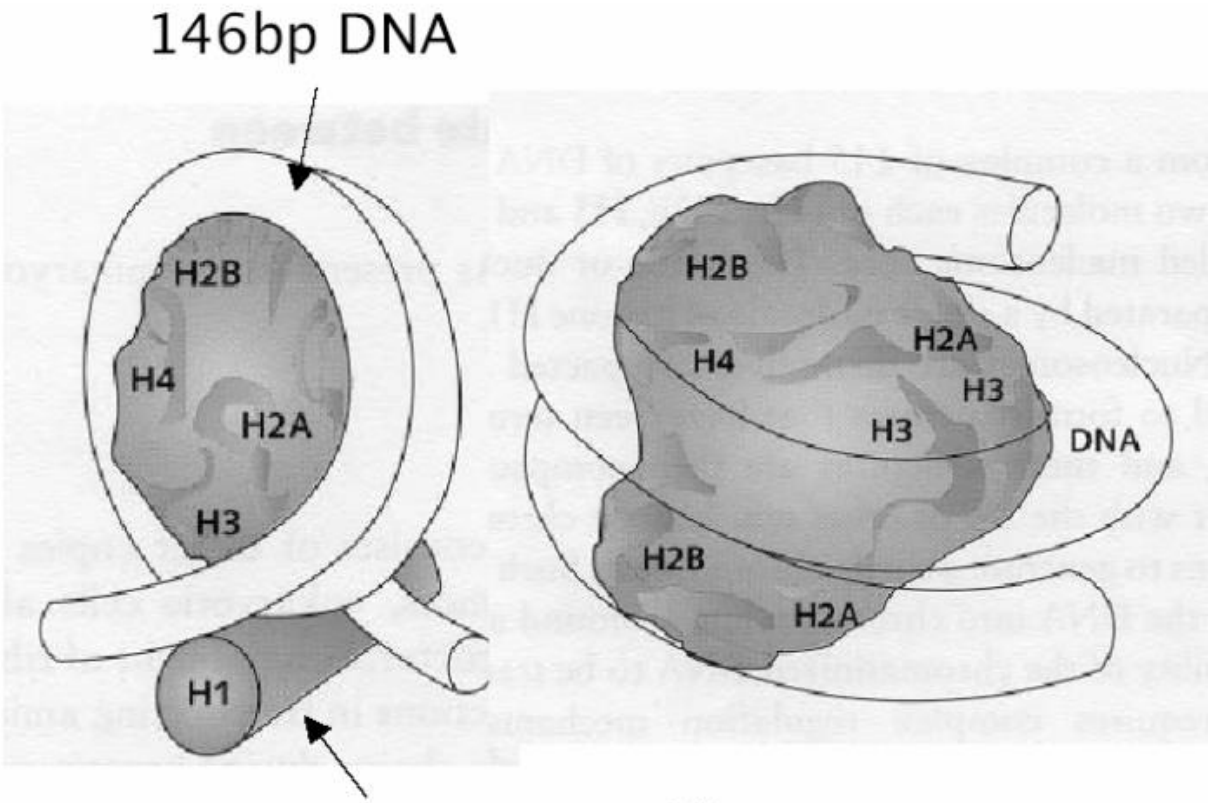
struktura „sznura koralików”



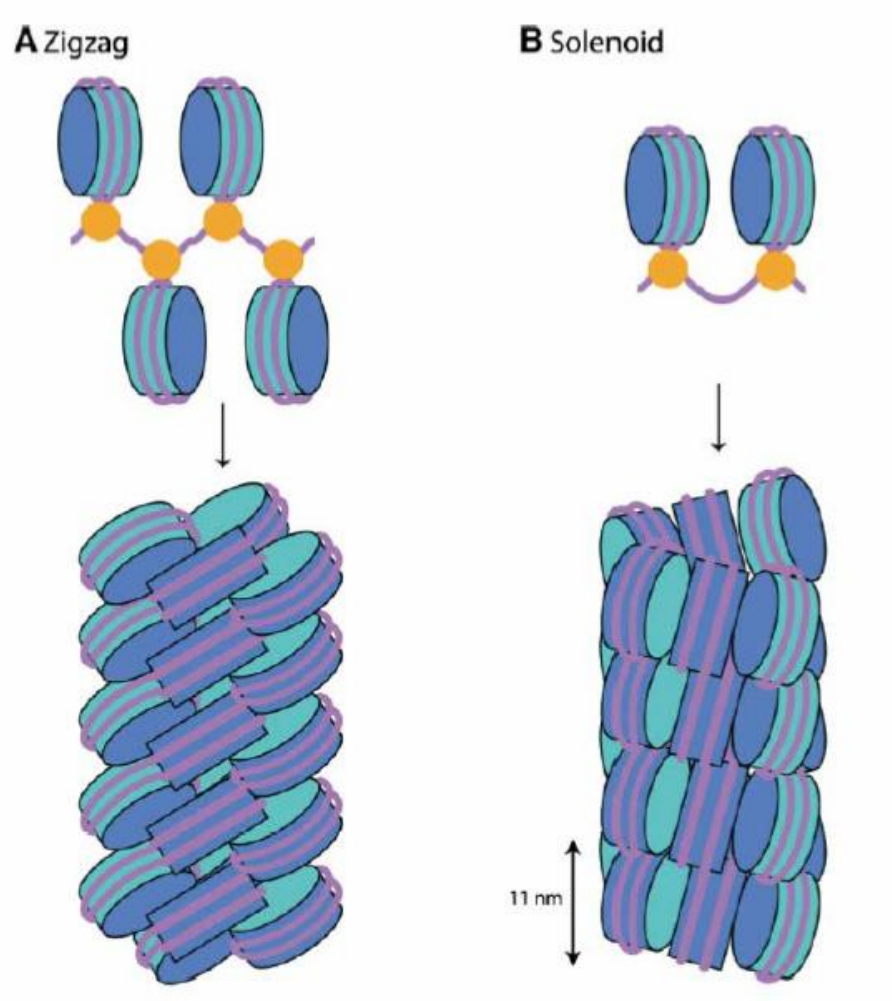
struktura włókna 30 nm

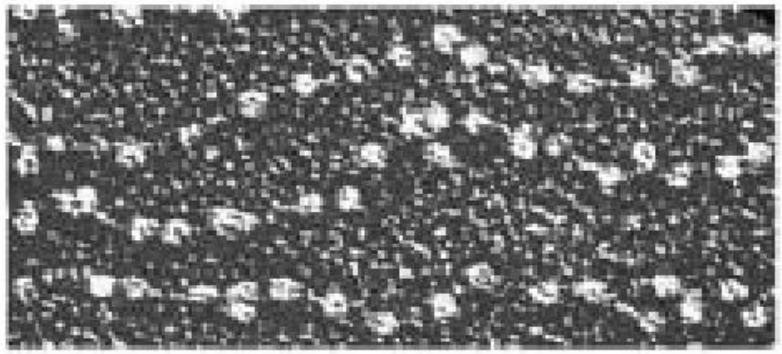
Micronucleus test

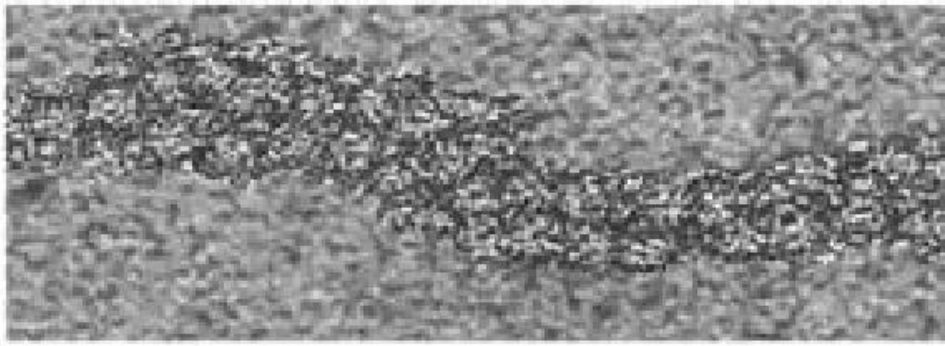
„Coral" chromatin structure (OKTAMER)



Micronucleus test

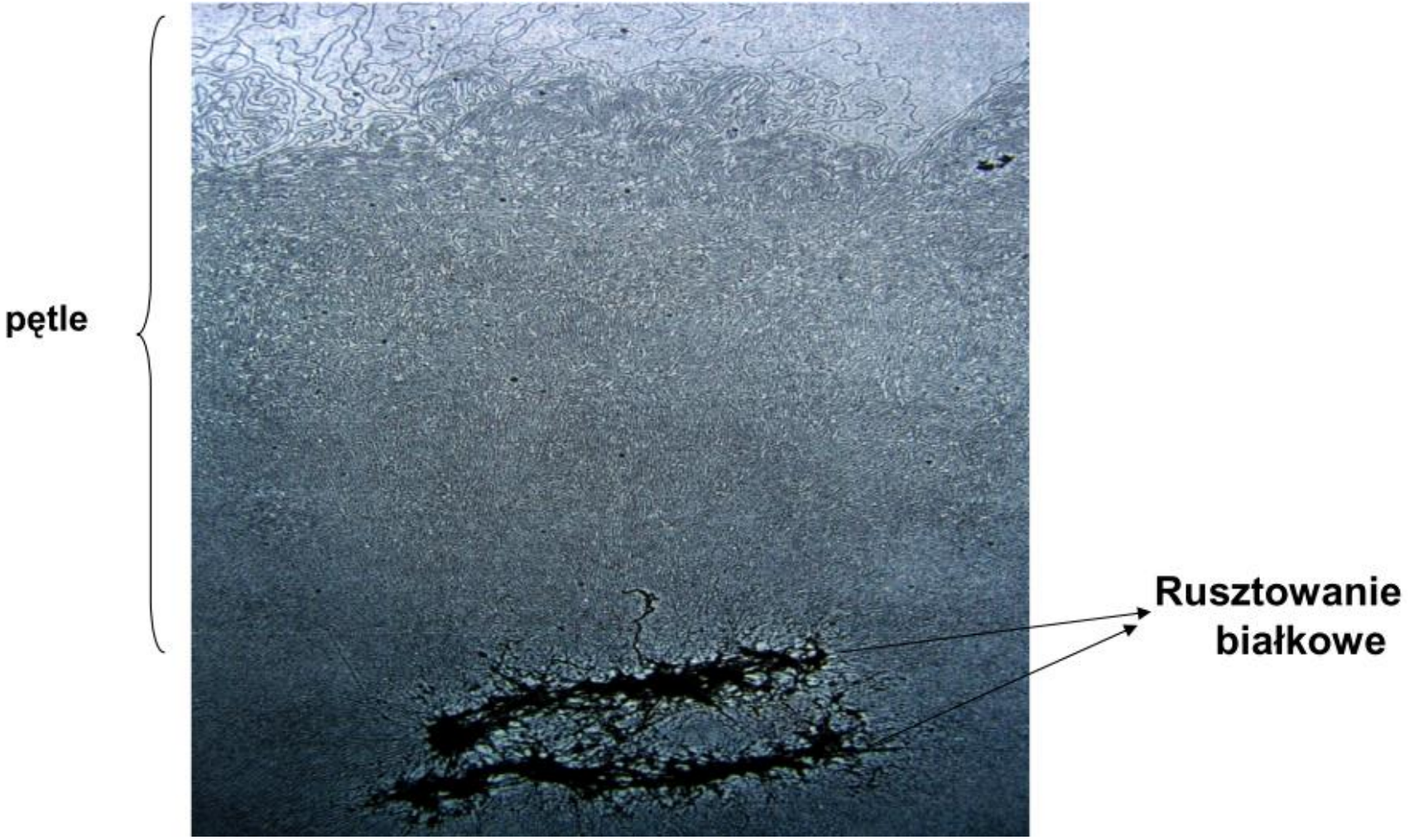






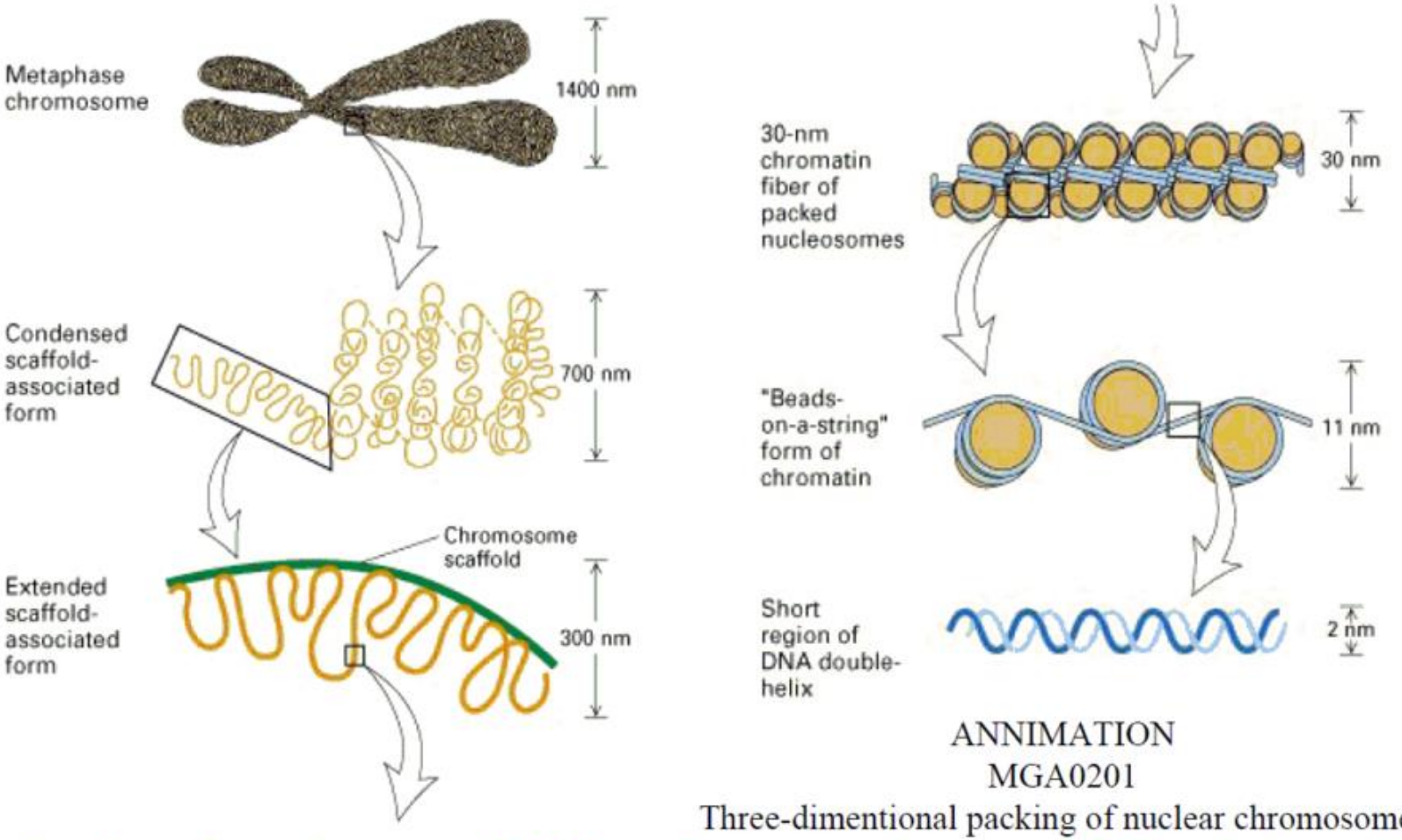
Micronucleus test

Non-histone proteins form a scaffold for long chromatin loops



Micronucleus test

Model of chromatin packing in metaphase chromosomes



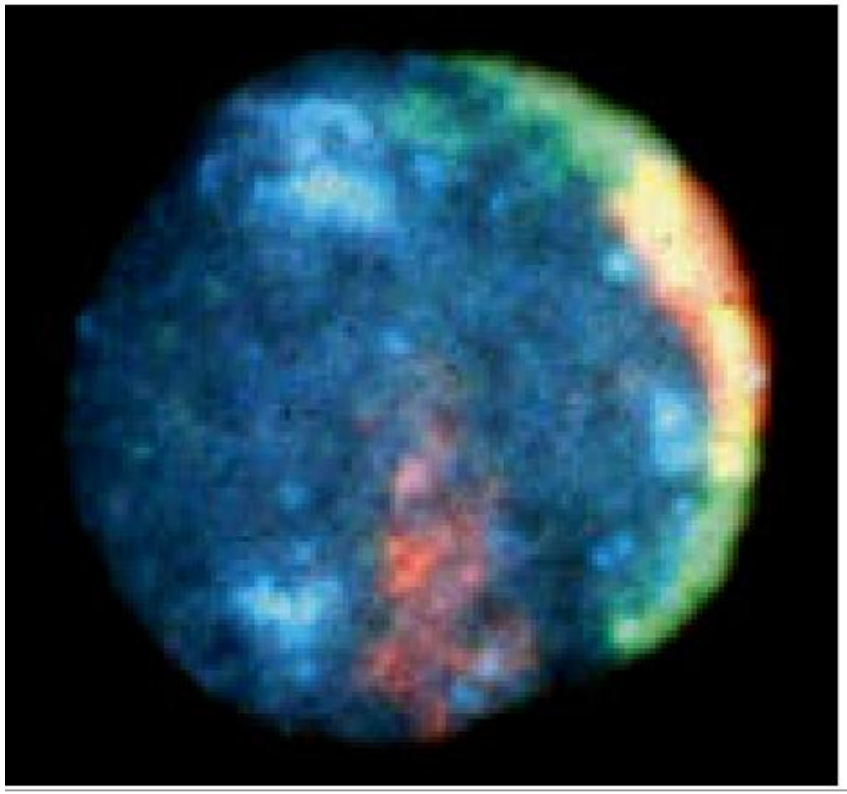
ANNIMATION
MGA0201
Three-dimentional packing of nuclear chromosomes

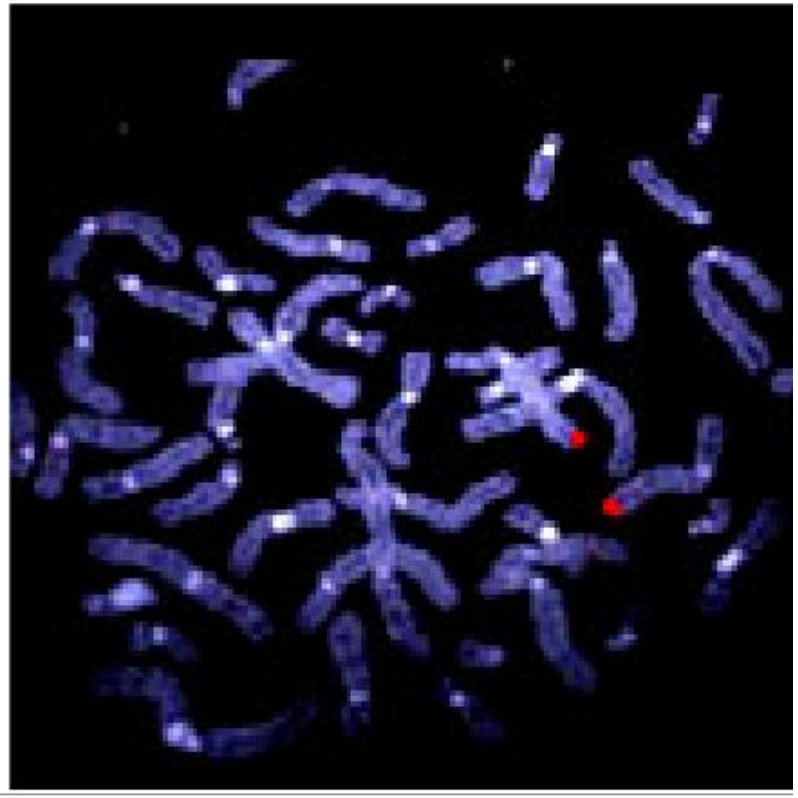
Na skutek upakowania cząsteczki DNA w chromosom mitotyczny jej długość skraca się 50 000 razy.

Micronucleus test

The cell nucleus

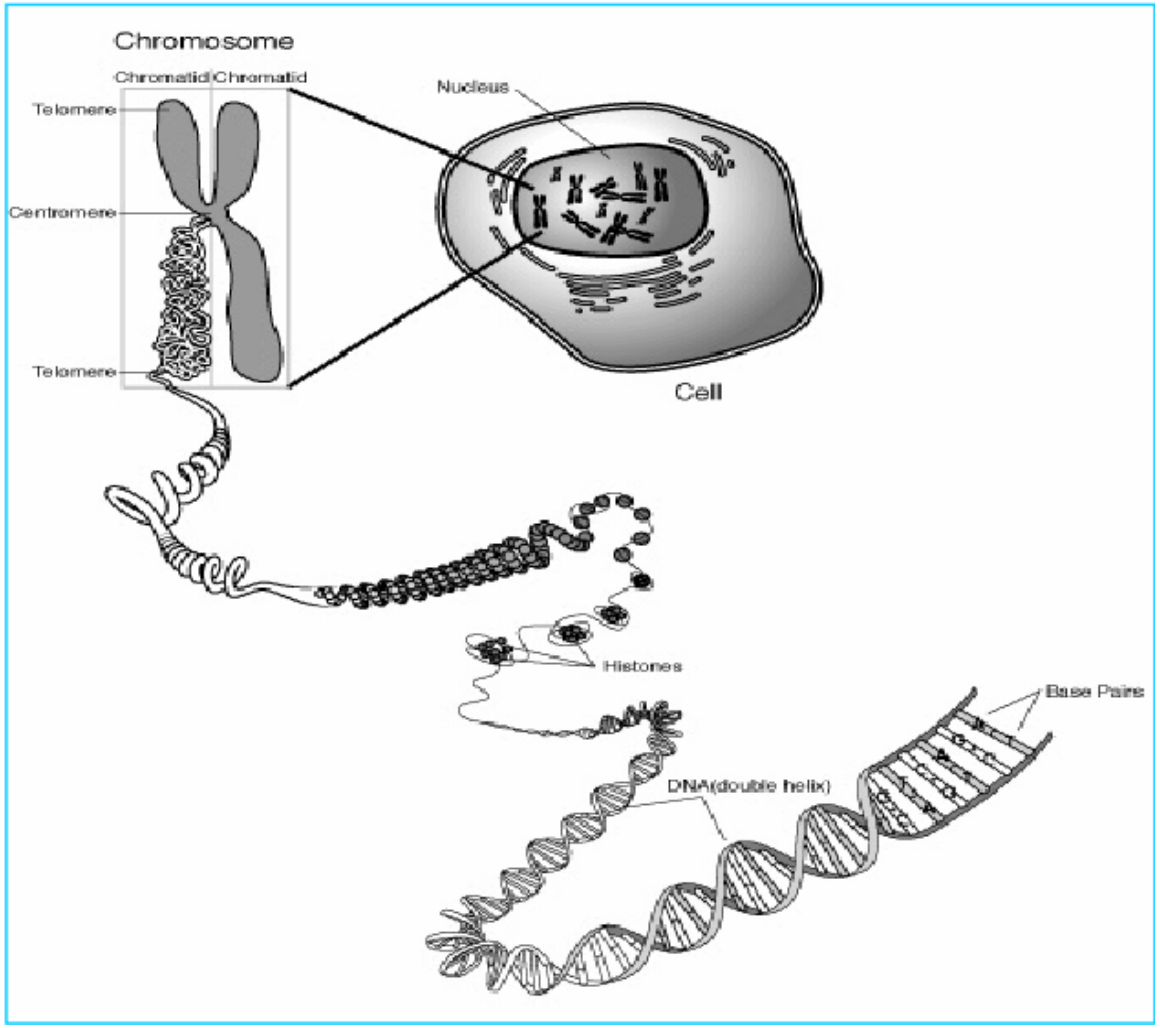
There is a cell nucleus in every human cell. In the cellular nucleus there is genetic information packed into structures called chromosomes



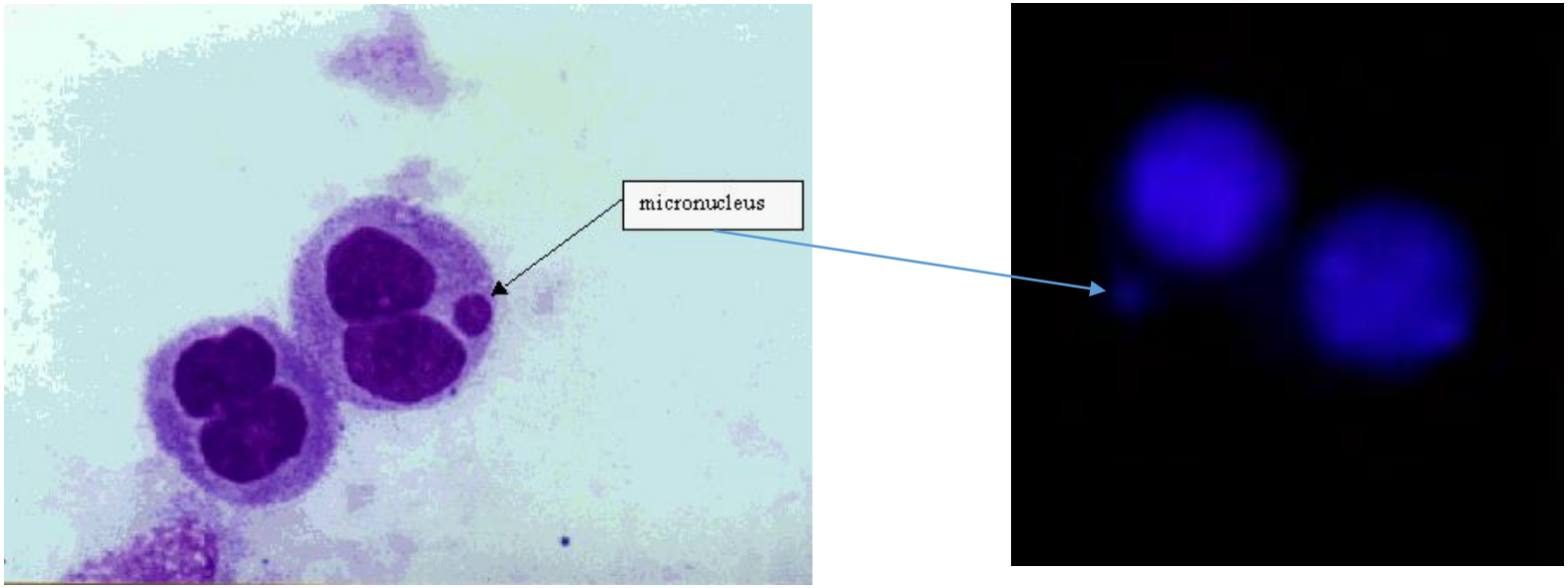


Micronucleus test

DNA in a eukaryotic cell



Micronucleus test



Micronucleus test

Chromatin- ordered complex of DNA and chromosomal proteins

Metaphase chromosome

- the highest level of chromatin packing
- formed only during cell divisions

After cell division

disappear metaphase chromosomes - interphase chromosomes
the packing level is reduced
chromatin appears in the form of euchromatin and heterochromatin

Research protocole

- Chromosome damage test - micronucleus test
- Evaluation of the expression of selected genes associated with activation of the genotoxicity / DNA damage pathway
- The determinations will be made in an in vitro model using test substrates provided by the contracting authority and two cell lines: with the use of human osteoblasts and osteoblast-like tumor cells - SaOS-2.
- The assessment of the potential genotoxicity of the tested substrates will be based on the analysis of the expression of selected genes associated with the genotoxicity / DNA damage pathway. The proposed panel includes the following genes: ATR, MDM2, TP53, PPIA, ATM, CHEK1, CHEK2, and CDKN1A. Evaluation of the expression of selected genes will be made using the Real-time PCR method using molecular probes of the TaqMan type.

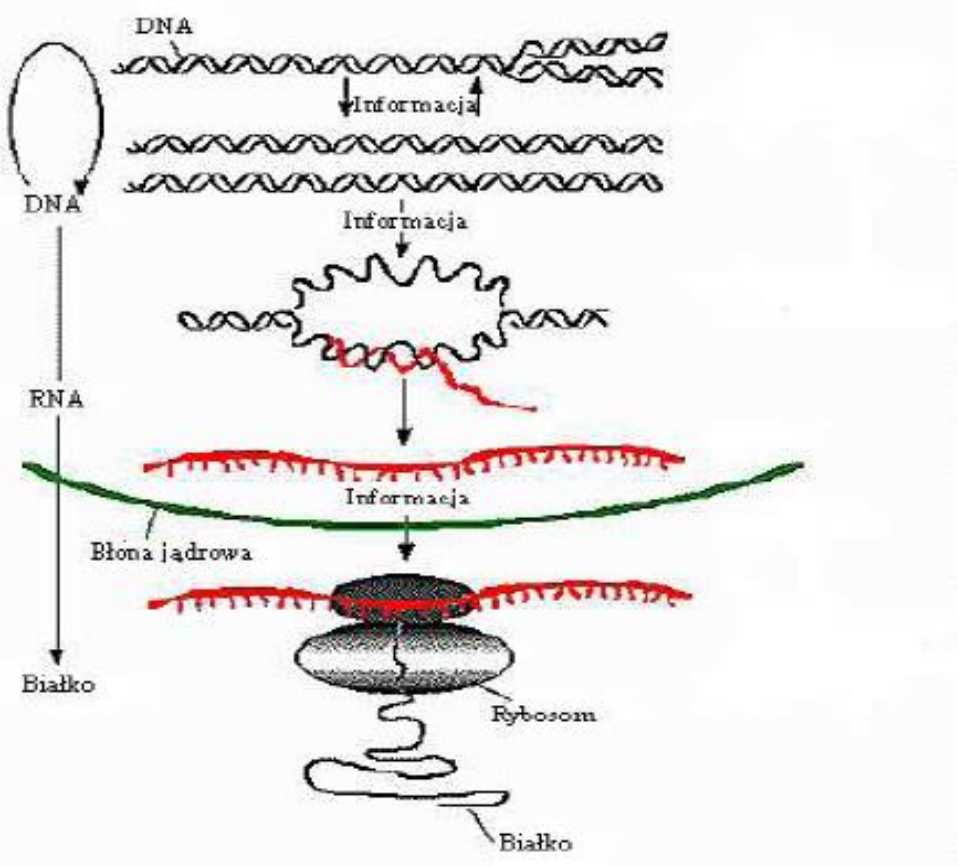
Genotoxicity

Dogma of molecular biology

Replication

Transcription

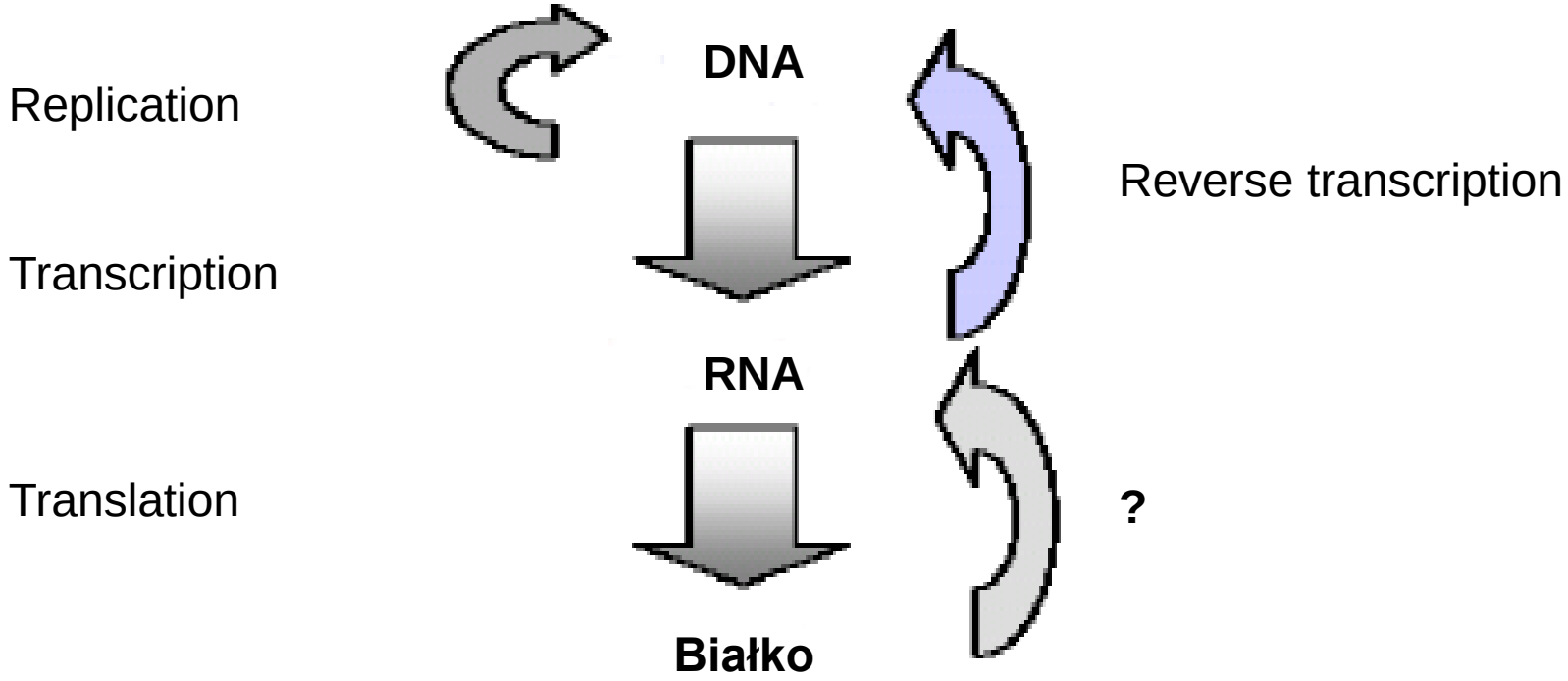
Translation



The flow of genetic information takes place in the direction of DNA -> RNA-> protein

Genotoxicity

Upgrade of the molecular biology dogma

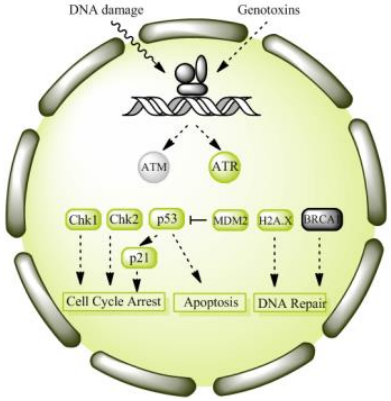


Cell —(RNAzol RNA (SIGMA) / Total RNA Zol-Out™(A&A Biotechnology))→ RNA —(H-C cDNA RT Kit (ThermoFisher) / 300ng RNA)→ RT

The flow of genetic information takes place in the direction of nucleic acid -> protein

Genotoxicity

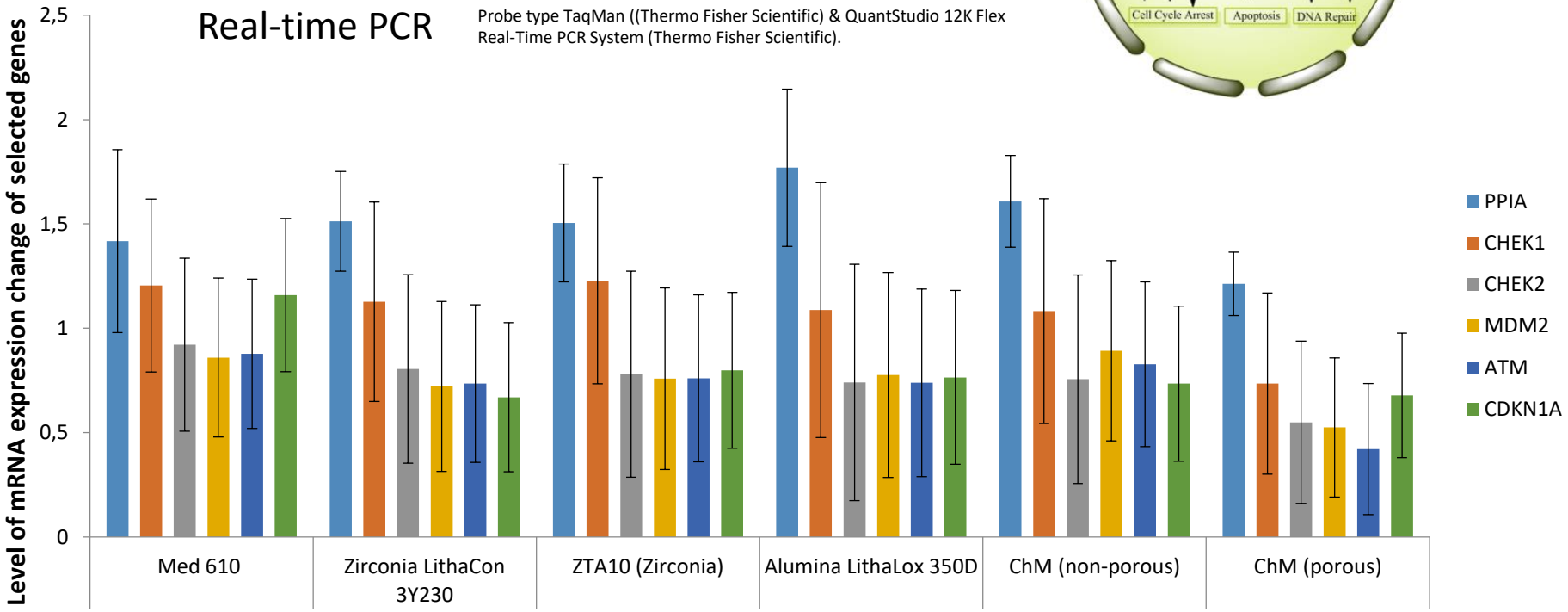
Expression of the selected genes evaluation involved in the genotoxic stress pathway



FOCAL CHANGE

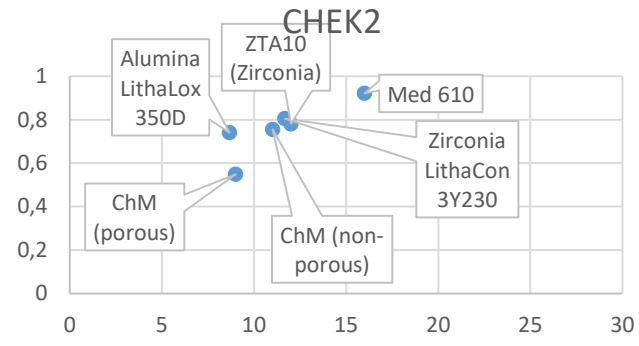
Real-time PCR

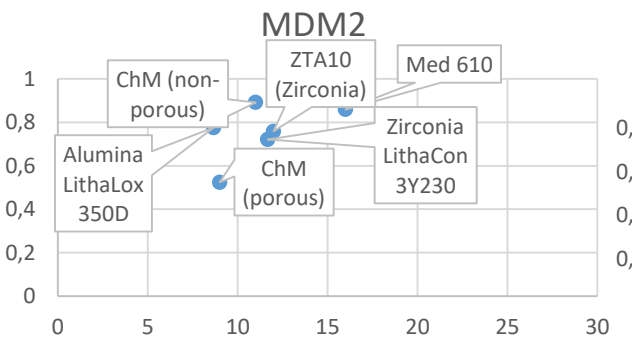
Probe type TaqMan ((Thermo Fisher Scientific) & QuantStudio 12K Flex Real-Time PCR System (Thermo Fisher Scientific).

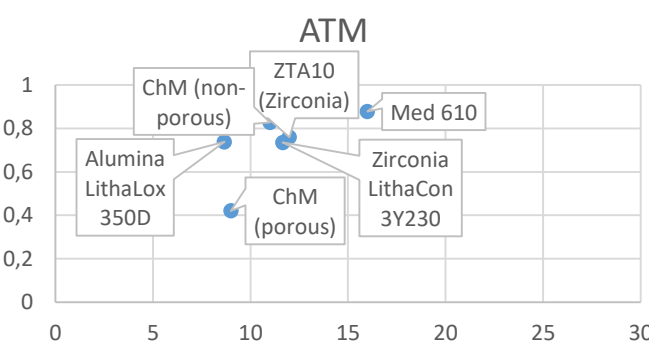


Genotoxicity

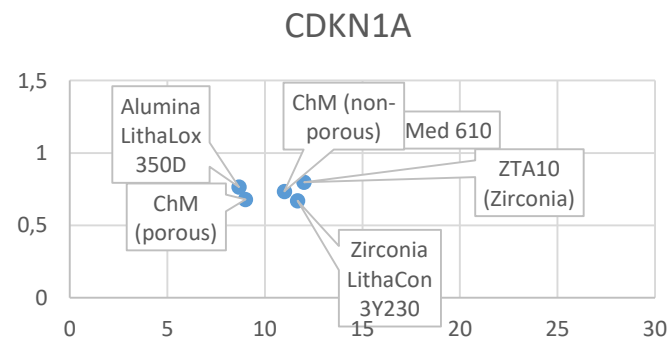
Expression of the selected genes evaluation involved in the genotoxic stress pathway

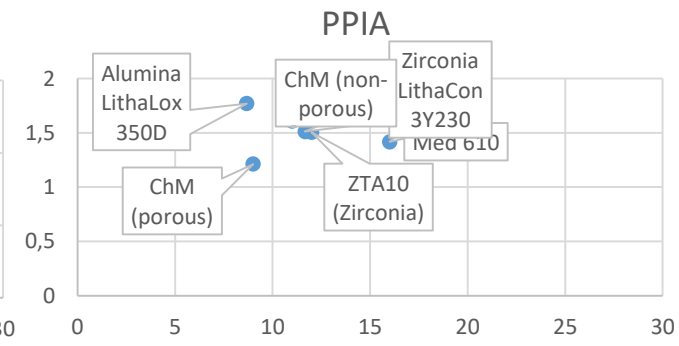


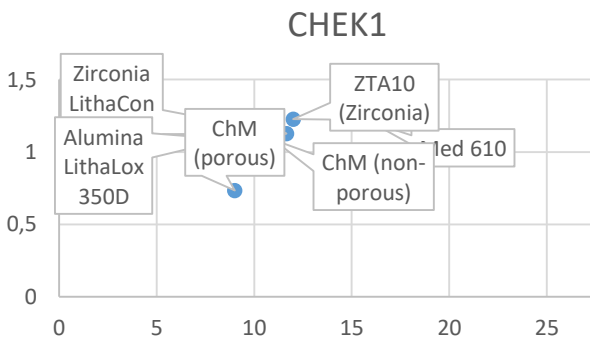




Lactate dehydrogenase (LDH)

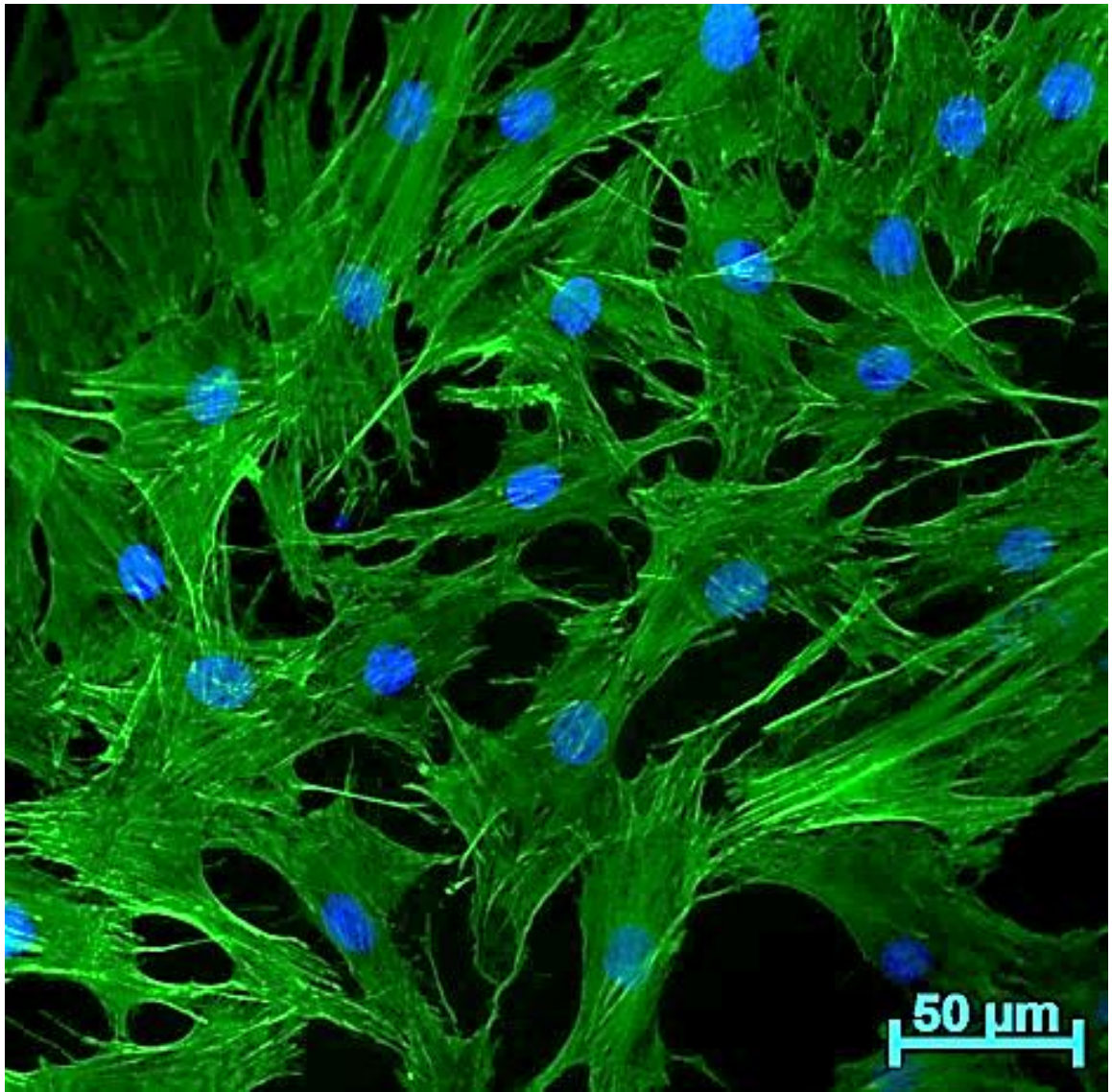






Lactate dehydrogenase (LDH)

Cell-material interaction



Printed implant prototype

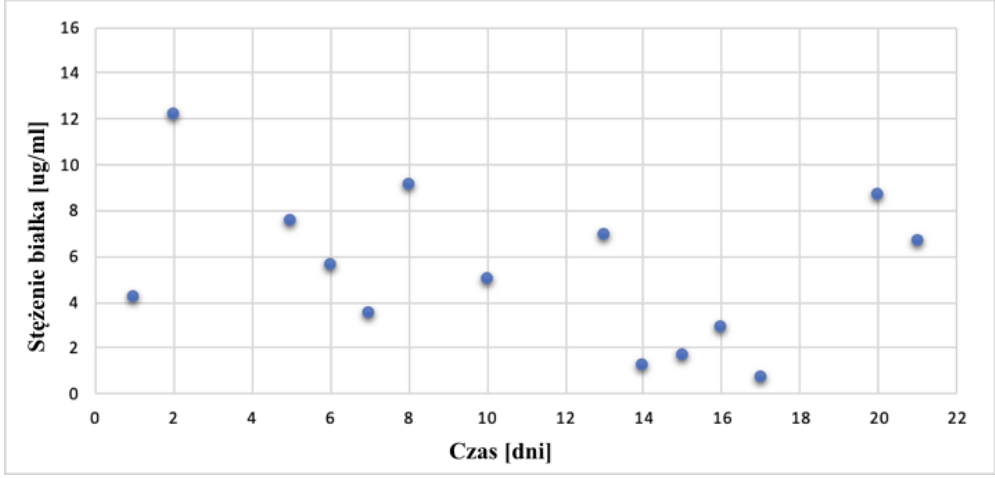
Initial results of the drug release from the surface modified capsules

On going futher steps

First attempts to release protein from microcapsules (membrane method) - UV-VIS

- The first attempts to load proteins into microparticles were made. Albumin was added to the aqueous solution, which is also a non-solvent for the polymer used. The amount of released protein was measured using the BCA test (Bicinchoninic Acid Kit, Sigma-Aldrich)

The amount of protein released from PLA microparticles within 21 days (the amount of protein released was measured every two days, each point corresponds to the protein released over these two days).



The albumin concentration in the experiment changed irregularly. During incubation the pH of the solution was constant and was 7.39. There was no apparent dependence showing the kinetics of protein release over time, so this technique was considered unsuitable. For this reason, the first attempts were made to load the protein into microparticles using the Schiff base - through a covalent bond.

Acknowlegement

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M-era.Net

Narodowe Centrum Badań i Rozwoju

M-ERA.NET Transnational Call 2016

Project Acronym:

jawMPLANT

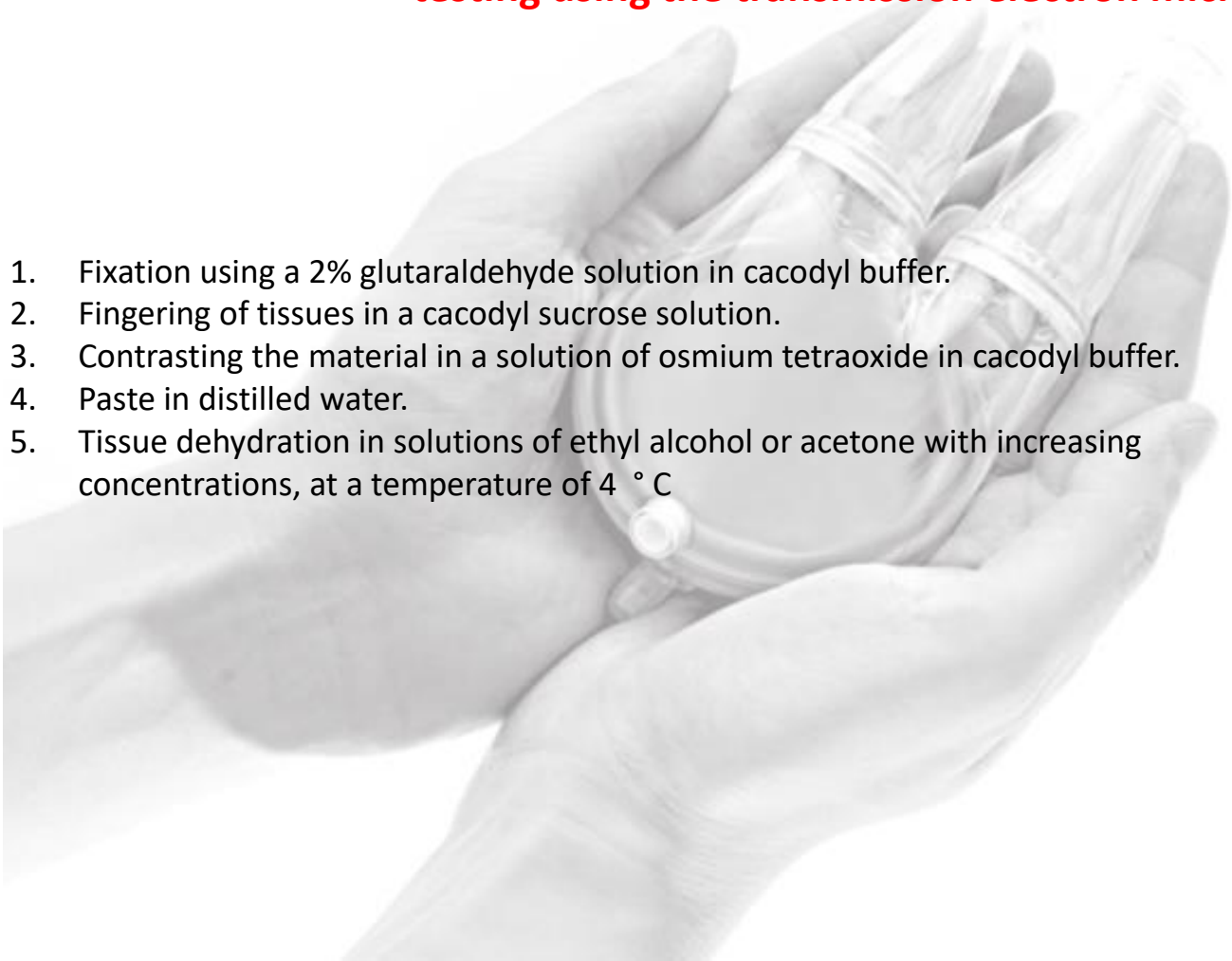
Project Coordinator:

(Organisation and country):

JOANNEUM RESEARCH Forschungsges.m.b.H., Institute for Surface Technologies and Photonics

Protocol

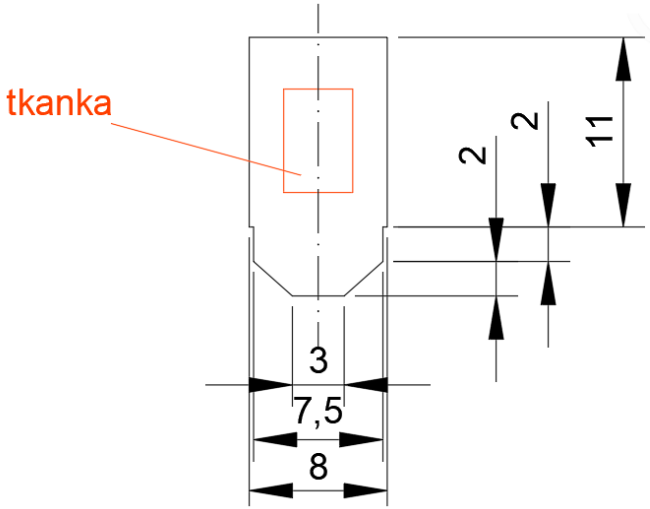
Development of a protocol for the preparation of thin films for tissue testing using the transmission electron microscopy technique



1. Fixation using a 2% glutaraldehyde solution in cacodyl buffer.
2. Fingering of tissues in a cacodyl sucrose solution.
3. Contrasting the material in a solution of osmium tetraoxide in cacodyl buffer.
4. Paste in distilled water.
5. Tissue dehydration in solutions of ethyl alcohol or acetone with increasing concentrations, at a temperature of 4 ° C

1. Utrwalenie przy użyciu 2-procentowego roztworu aldehydu glutarowego w buforze kakodylowym.
2. Płukanie tkanki w roztworze sacharozy w kakodylowym.
3. Kontrastowanie materiału w roztworze czterotlenku osmu w buforze kakodylowym.
4. Płukanie w wodzie destylowanej.
5. Odwadnianie tkanki w roztworach alkoholu etylowego lub acetonu o wzrastających stężeniach, w temperaturze 4 °C

Protocol



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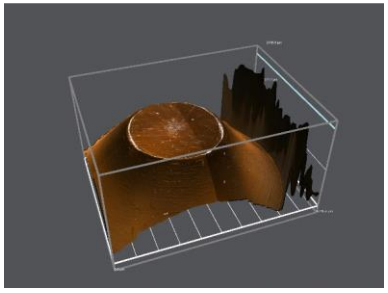

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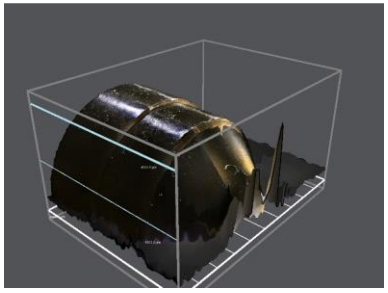

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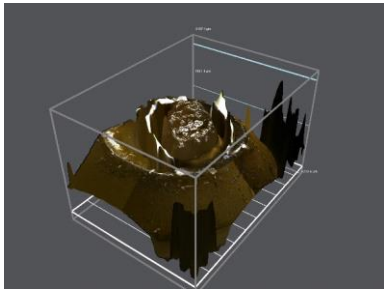

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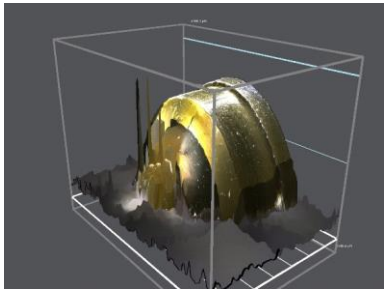


Protocol

Optimal Positioning of the Optical Head

The Eucentric Movement of the Leica EM UC7 viewing system allows examination of sections, even with a lowered water level e.g. for Lowycryls and dry sections.

The patented defined position marks of the eucentric movement provide maximum approach accuracy either for glass or diamond knife approach.

For accurate approach of the knife towards the specimen with the backlight, the viewing angle must be set according to the type of knife in use.

Section observation with lowered water level without eucentrically moveable stereomicroscope.

Section observation with lowered water level with eucentrically moveable stereomicroscope.

Diamond knife approach as seen through the Leica M80 stereomicroscope with backlight illumination.

Protocol

Advanced Features

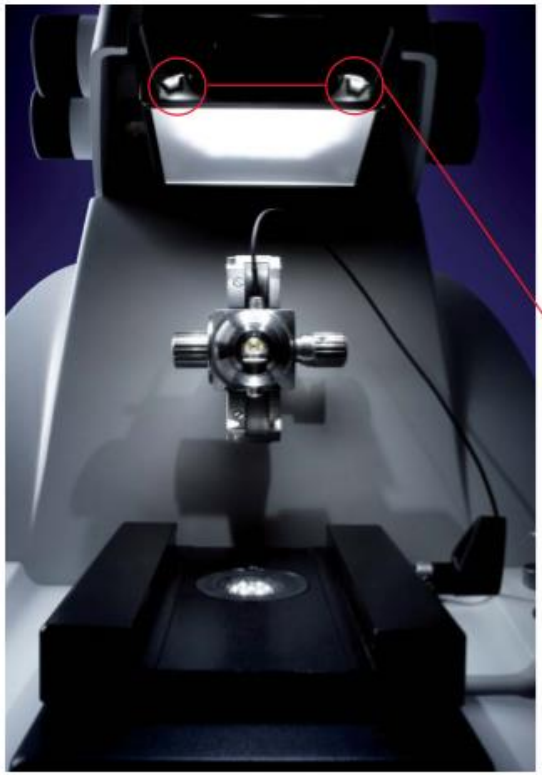
Motorized knife stage

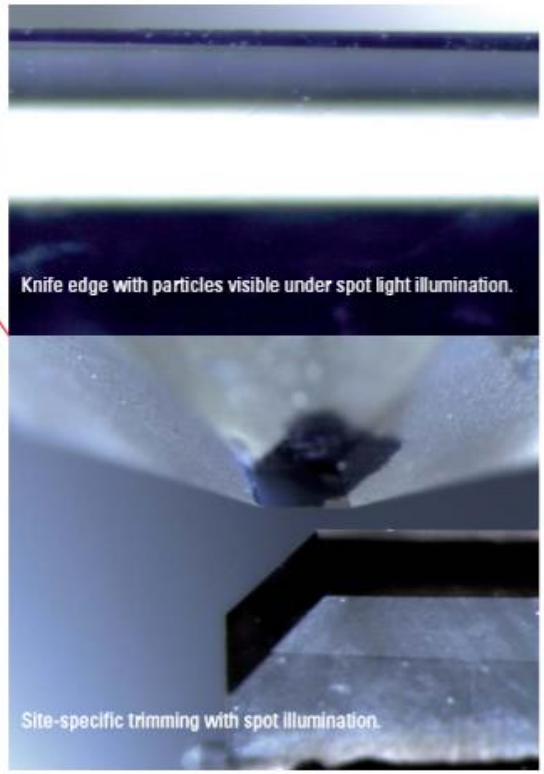
Motorized North-South movement of the knife stage is a unique feature of Leica ultramicrotomes. The additional implementation of motorized East-West movement of the Leica EM UC6 was a logical step forward. Additionally, the E-W quick adjustment buttons on the Leica UC7 controller allow efficient traversing of the knife.

Motorization of the stage has also allowed many useful features to develop hand in hand with it, for example the patented Autotrim mode, E-W measuring function and the automatic approach of the selected knife segment (patent applied).

Brightness-controlled LED illumination

LED light sources provide outstanding illumination for top light, back-light, and transmitted illumination. All illumination modes are idepen-dently brightness-controlled for the best visual clarity. The additional spot illumination enhances the optical performance of the Leica EM UC7 and provides superior visibility while cleaning the knife edge or trimming the block face, for example.





Protocol

Advanced Cryo-section Collection

Leica EM CRION ionizer and micromanipulator

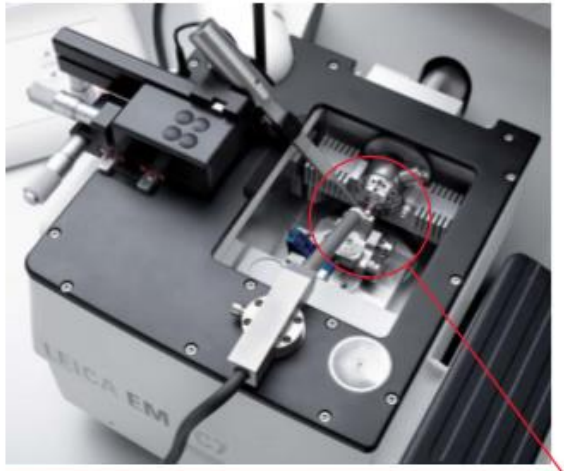
Especially for frozen hydrated sectioning or the Tokuyasu method, the Leica EM CRION with electrostatic discharge and charge mode used with the micromanipulator provides outstanding cryo sectioning performance.

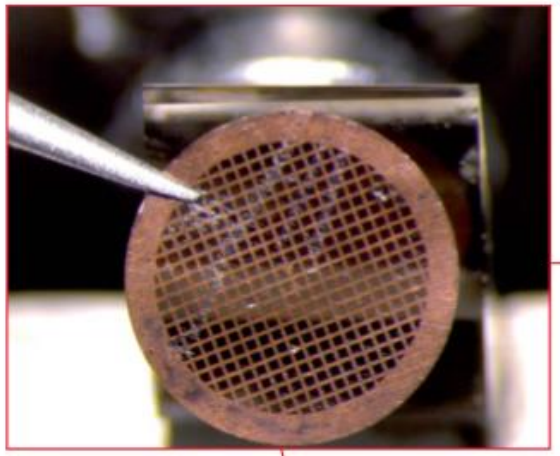
The micromanipulator easily attaches to the Leica EM FC7 cryo chamber, which allows the grid to be exactly positioned close to the knife edge using the micrometer gauges. Once these positions are defined, fast retraction of the grid can be performed manually prior to sectioning to prevent possible influence of the grid on the ionizer. The Leica EM CRION is used in discharge mode in order to reduce electrostatic charging while sectioning.

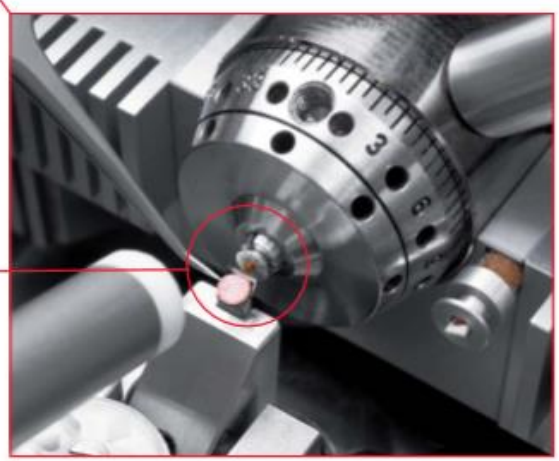
When the section ribbon needs to be placed on the grid, return to the pre-set grid position can be quickly performed. The ribbon is then attached to the grid using the charge mode of the Leica EM CRION, which is operated by a footswitch. Thus, the specimen adheres to the grid without the need for a section press.

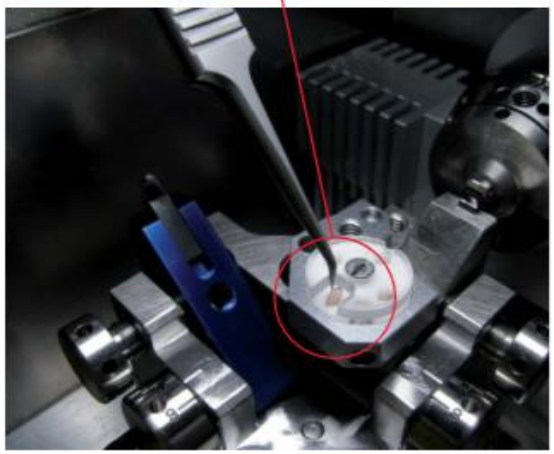
As a grid box can be placed near the knife, the micromanipulator can be used to easily place the grid into its storage position.

Protocol





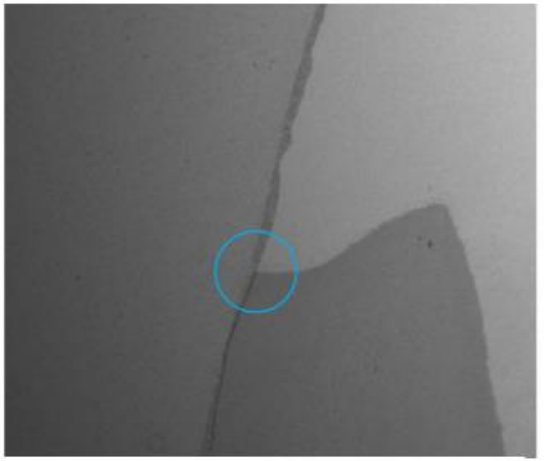




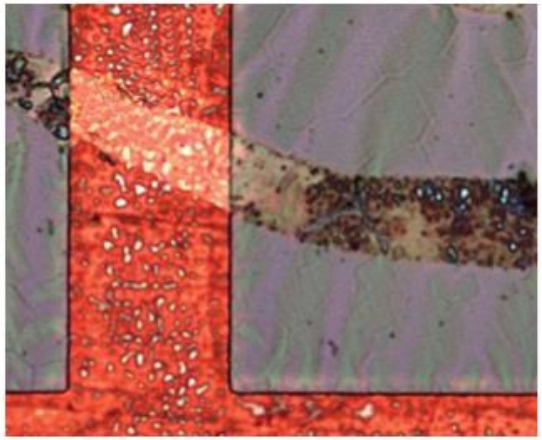
Protocol

Preparing Specimens for Ultramicrotomy

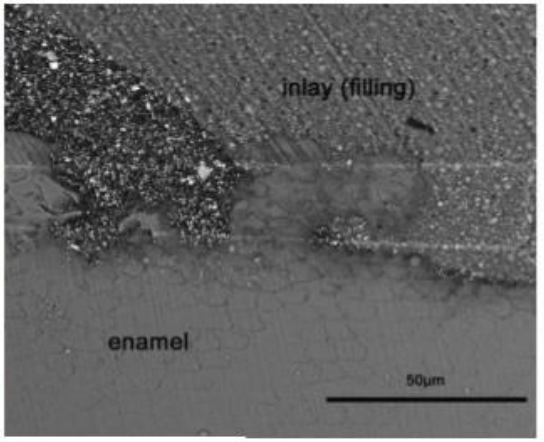
All specimens need to be prepared prior to sectioning. The size and shape of the specimen have a profound effect on sectioning characteristics. Trimming soft specimens can be performed with the Leica EM TRIM2 or EM RAPID using a milling tool. Alternatively, with the versatile Leica EM TXP, even hard and brittle material can be trimmed using grinding and polishing abrasives. The perfectly shaped block face with sharp edges has a considerable influence on the section quality of hard and brittle materials.



Cross section of a tooth with inlay (filling) prepared with the Leica EM TXP (area of interest marked blue).



50 nm sections on the grid



Block face after sectioning

Courtesy of J. Leprince, centre de recherche CRIBIO, Ecole de Médecine Dentaire, Université catholique de Louvain.

1	odwadnianie	Szereg alkoholowy (50%,70%,80%,96%,100% x 2)	Czas: 15 minut
	przepajanie	Tlenek propylenu + żywica (2:1 obj.) Tlenek propylenu + żywica (1:1 obj.) Tlenek propylenu + żywica (1:2 obj.) 100% żywica	Czas: 2 h Czas: 2 h Czas: 3h Czas: overnight
2	odwadnianie	Szereg alkoholowy (50%,70%,80%,96%,100% x 2)	Czas: 30 minut
	przepajanie	Tlenek propylenu + żywica (2:1 obj.) Tlenek propylenu + żywica (1:1 obj.) Tlenek propylenu + żywica (1:2 obj.) 100% żywica	Czas: 2 h Czas: 2 h Czas: 3h Czas: overnight
3	odwadnianie	Szereg acetonowy (50%,70%,80%,96%,100% x 2)	Czas: 30 minut
	przepajanie	Tlenek propylenu + żywica (2:1 obj.) Tlenek propylenu + żywica (1:1 obj.) Tlenek propylenu + żywica (1:2 obj.) 100% żywica	Czas: 2 h Czas: 2 h Czas: 3 h Czas: overnight
4	odwadnianie	Szereg alkoholowy (30 %, 50%, 60%, 70%, 80%, 90%, 96%, 100% x 2)	Czas: 30 minut
	przepajanie	Tlenek propylenu + żywica (2:1 obj.) Tlenek propylenu + żywica (1:1 obj.) Tlenek propylenu + żywica (1:2 obj.) 100% żywica	Czas: 2 h Czas: 2 h Czas: 4 h Czas: overnight
5	odwadnianie	Szereg alkoholowy (50%,70%,80%,96%,100% x 2)	Czas: 45 minut
	przepajanie	Tlenek propylenu + żywica (2:1 obj.) Tlenek propylenu + żywica (1:1 obj.) Tlenek propylenu + żywica (1:2 obj.) 100% żywica	Czas: 2 h Czas: 2 h Czas: overnight Czas: overnight
6	odwadnianie	Szereg alkoholowy (50%,70%,80%,96%,100% x 2)	Czas: 1 h
	przepajanie	Tlenek propylenu + żywica (2:1 obj.) Tlenek propylenu + żywica (1:1 obj.) Tlenek propylenu + żywica (1:2 obj.) 100% żywica	Czas: 2 h Czas: 2 h Czas: overnight Czas: overnight
7	odwadnianie	Szereg alkoholowy (50%,70%, 80%,96%,100%x 2)	Czas: 1 h
	przepajanie	Tlenek propylenu + żywica (2:1 obj.) Tlenek propylenu + żywica (1:1 obj.) Tlenek propylenu + żywica (1:2 obj.) 100% żywica x 2	Czas: 2 h Czas: 2 h Czas: overnight Czas: overnight + 2h
8	odwadnianie	Szereg acetonowy (50%,70%, 80%,96%,100% x 2)	Czas: 1 h
	przepajanie	Tlenek propylenu + żywica (2:1 obj.) Tlenek propylenu + żywica (1:1 obj.) Tlenek propylenu + żywica (1:2 obj.) 100% żywica x 2	Czas: 2 h Czas: 2 h Czas: overnight Czas: overnight + 2h
9	odwadnianie	Szereg alkoholowy (30 %, 50%, 60%, 70%, 80%, 90%, 96%, 100% x 2)	Czas: 1 h
	przepajanie	Tlenek propylenu + żywica (2:1 obj.) Tlenek propylenu + żywica (1:1 obj.) Tlenek propylenu + żywica (1:2 obj.) 100% żywica x 2	Czas: 2 h Czas: overnight Czas: overnight Czas: overnight + 2h

Cellula

„The key to solve the problems with biomaterials one should find in the cell”
E.B. Wilson, (1925)