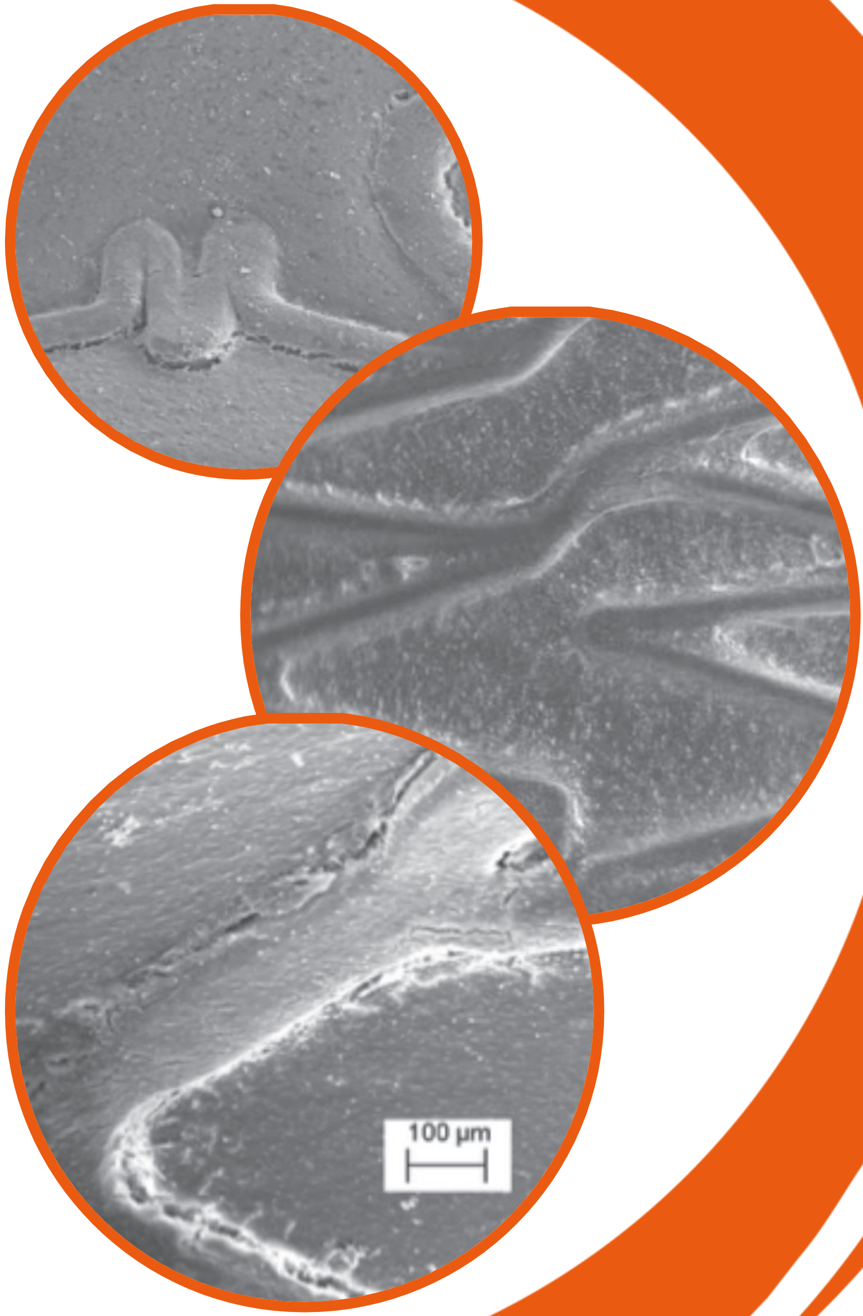


BIS

Bio Inducer Surface

MC - 2019

CONFIDENTIAL

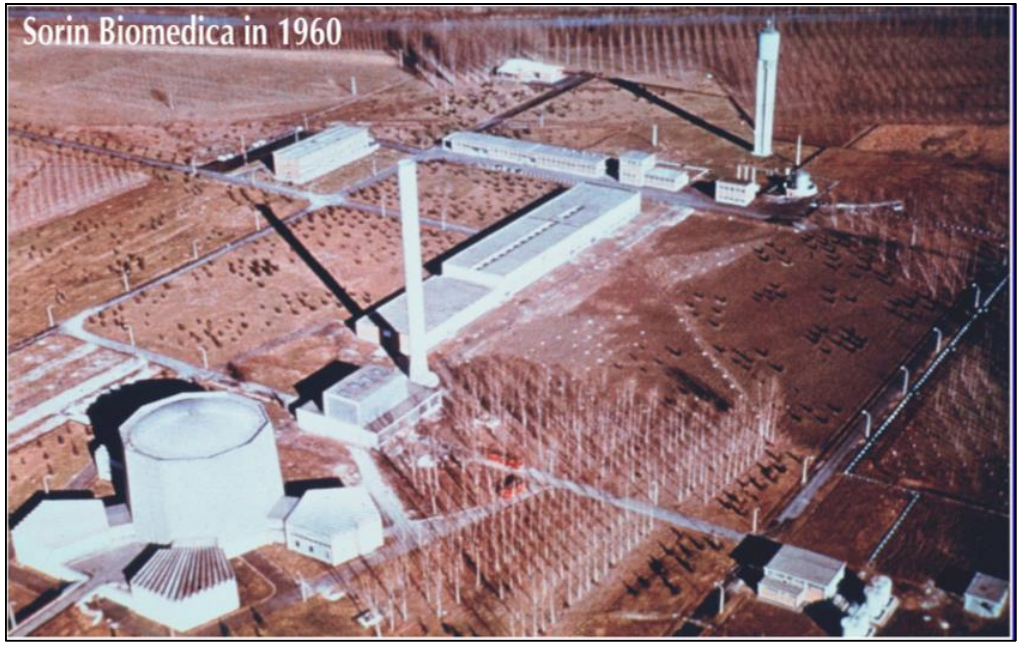
Which is the ideal (stent) coating?

Surface able to accelerate endothelialization and to establish a functional layer: Reduced thrombogenicity & reduced inflammatory trigger

Effective barrier versus heavy metal ions release: Reduced inflammatory process

Inert physical/chemical surface: Reduced foreign body reaction

From PyC to BIS

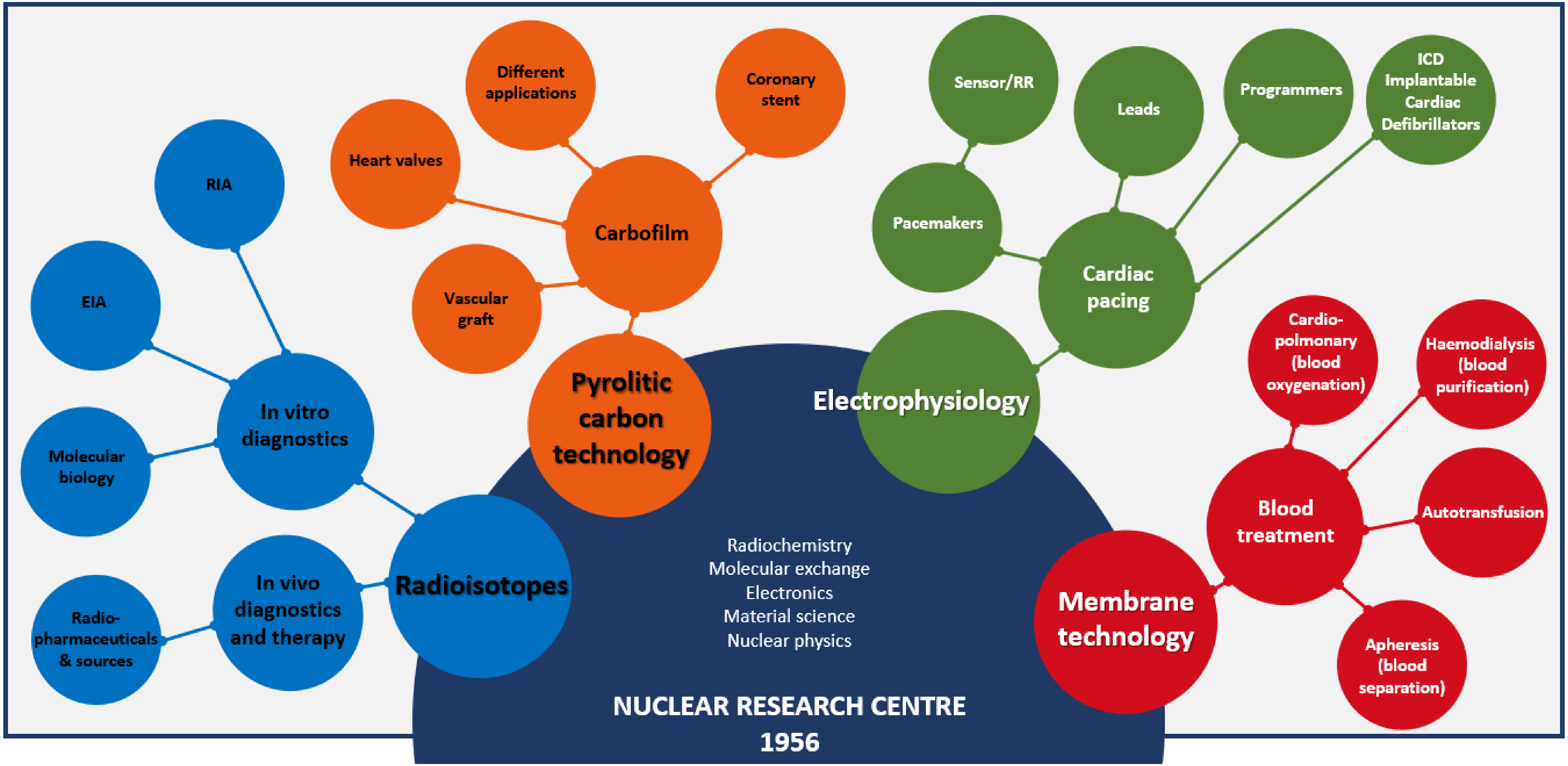


Pyrolitic Carbon
['50]

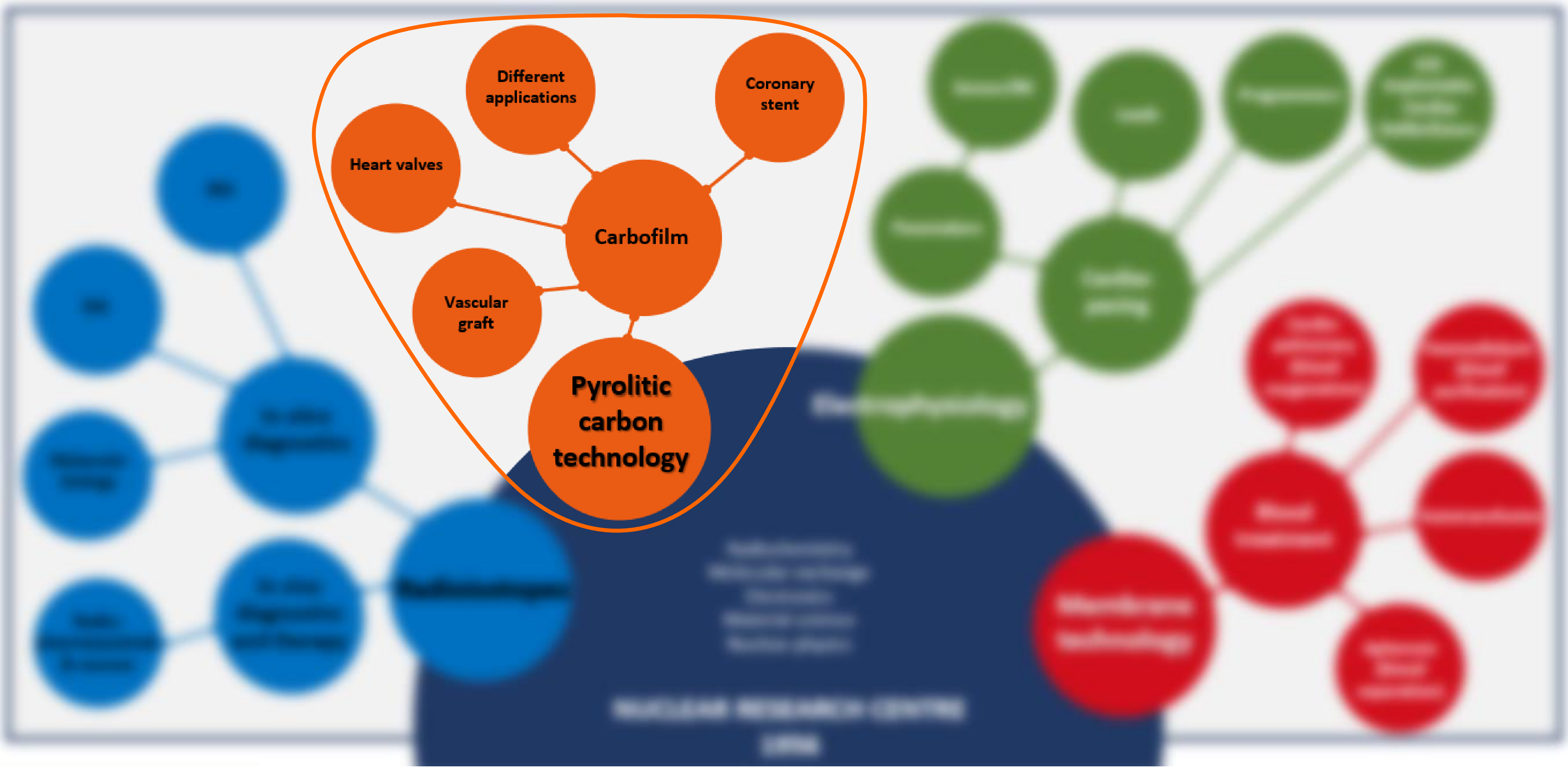
Carbofilm™
['80]

BIS
Bio Inducer Surface
*i*Carbofilm [2009]

Technology Park 1960



Technology Park 1960



Mechanical Heart Valve:
the worst setting for thrombotic events

- **Pyrolitic Carbon (PyC)** is the only material used to realize mechanical heart valve leaflets since more than 40 years thanks to its **unmatched thrombo-resistant properties**

Sorin

St. Jude

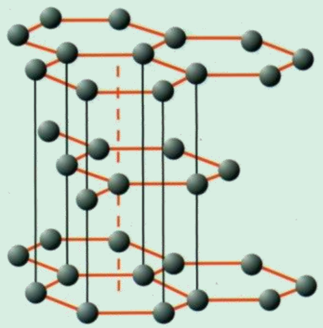
Carbomedics

Medtronic (ATS)

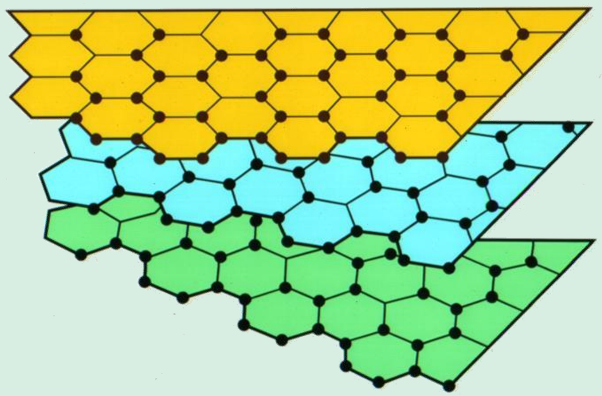
...others...

PyC - Pyrolytic Carbon

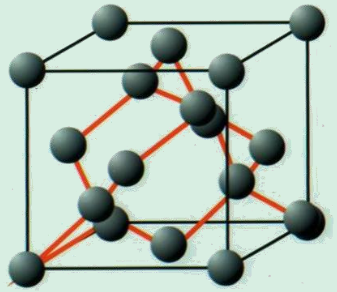
PyC is pure elemental Carbon
with high-density **TURBOSTRATIC CRYSTAL STRUCTURE**
This property distinguishes PyC from other materials consisting of Carbon,
such as GRAPHITE and DIAMOND



Graphite
(Hexagonal polymorph)



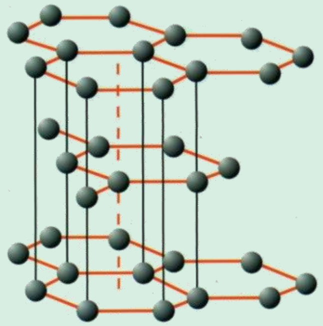
Turbostratic Structure



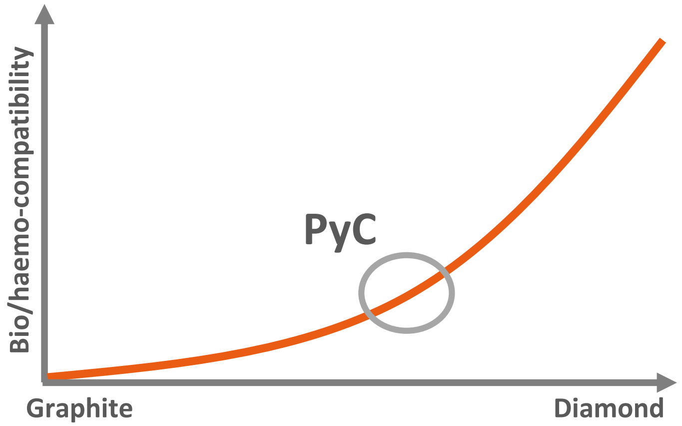
Diamond
(Cubic polymorph)

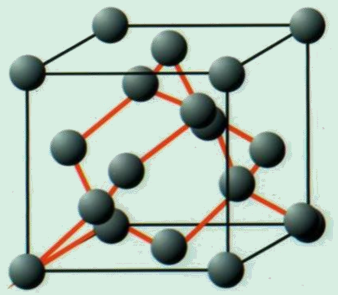
Better bio/haemo-compatibility

Pure Carbon Materials



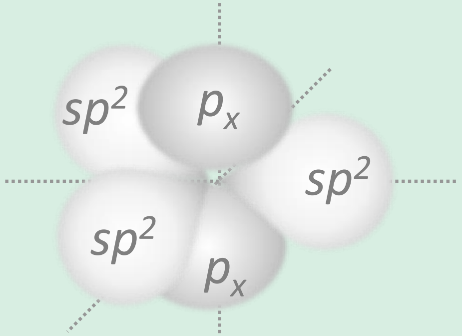
Graphite
(Hexagonal polymorph)





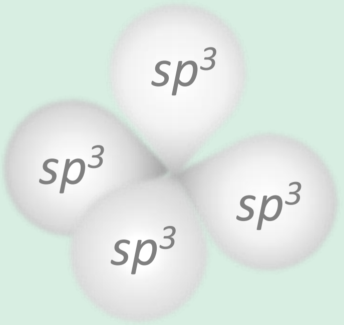
Diamond
(Cubic polymorph)

Better bio/haemo-compatibility



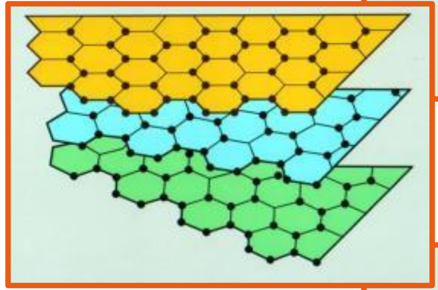
Carbon atom
with 1 bond available

Higher density



Carbon atom
without bonds available

Pyrolytic Carbon: properties



HIGH STRENGTH

COMPACTNESS

HARDNESS

→

WEAR RESISTANCE

FATIGUE RESISTANCE

HIGH PERFORMANCE LIFE LASTING COMPONENTS

CHEMICAL INERTNESS

MIRROR POLISHING

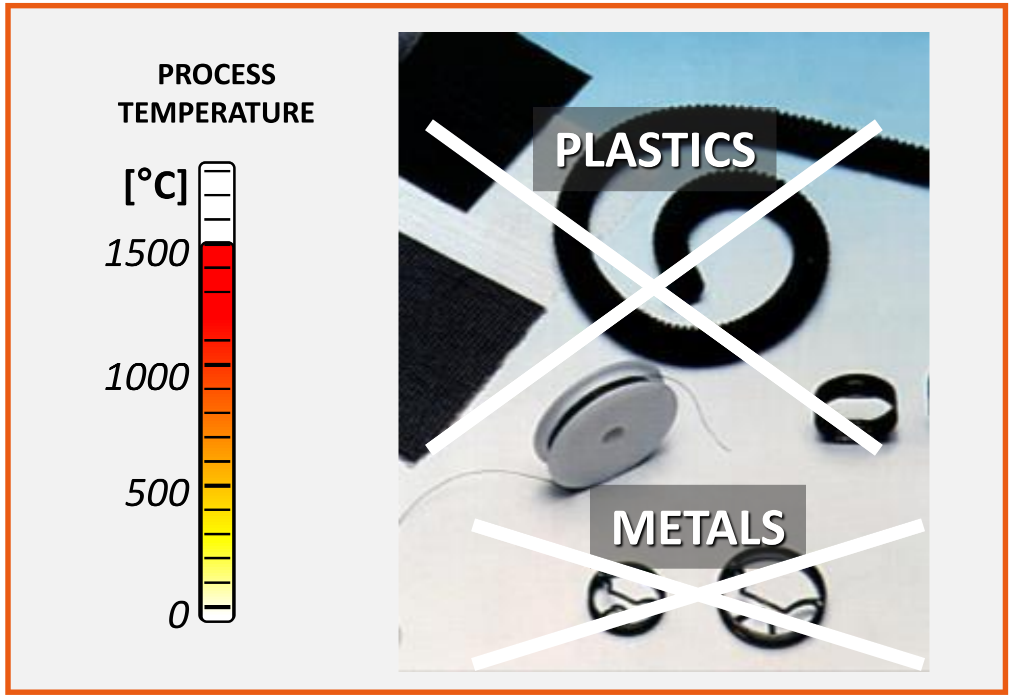
→

BLOOD & TISSUE COMPATIBILITY

Pyrolytic Carbon: limitations

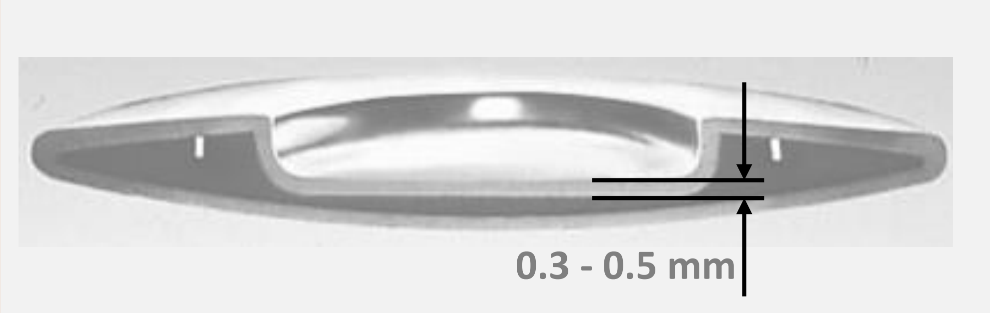
Pyrolytic carbon process involves extremely high temperatures (>1500 °C):

- This prevents the possibility to coat heat-sensitive substrates such as plastics and most of metals and alloys. (Substrates for valve components are made by graphite)



The coating is some tenths of a millimeter thick, and rigid:

- Only relatively easy shapes can be coated.



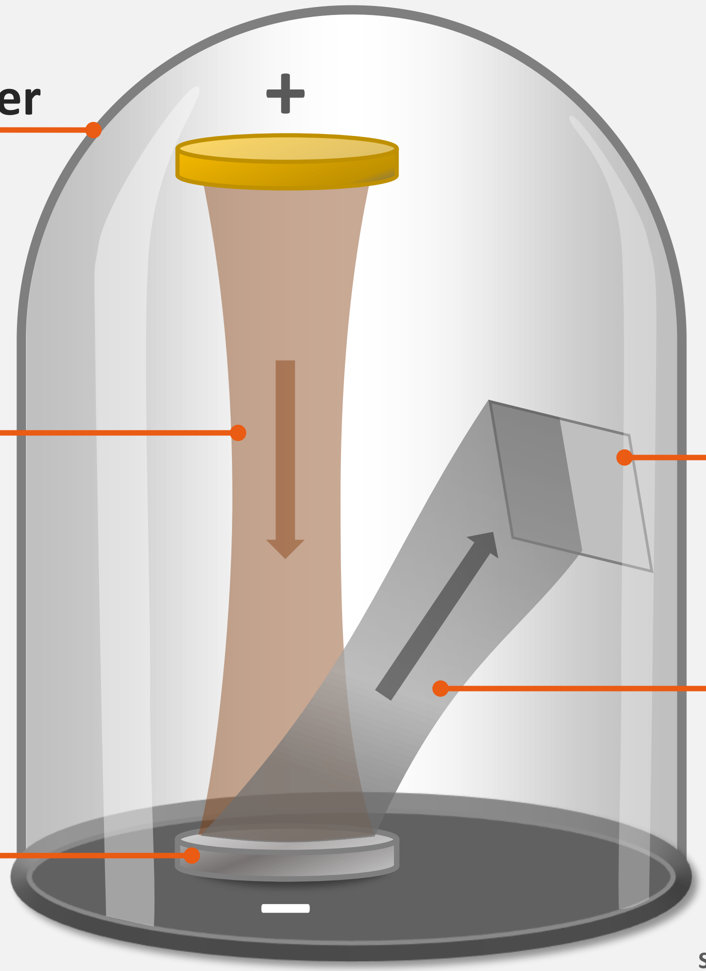
From PyC to Carbofilm™

Breakthrough in Carbon Coating Technology - to overcome the limits of PyC technology in the early 80's was developed the Carbofilm™ Technology:

- application to complex-shaped components
- application to all kinds of material

Carbofilm™: the patented process

PVD – Physical Vapour Deposition



NO CHEMICAL REACTION

ROOM TEMPERATURE

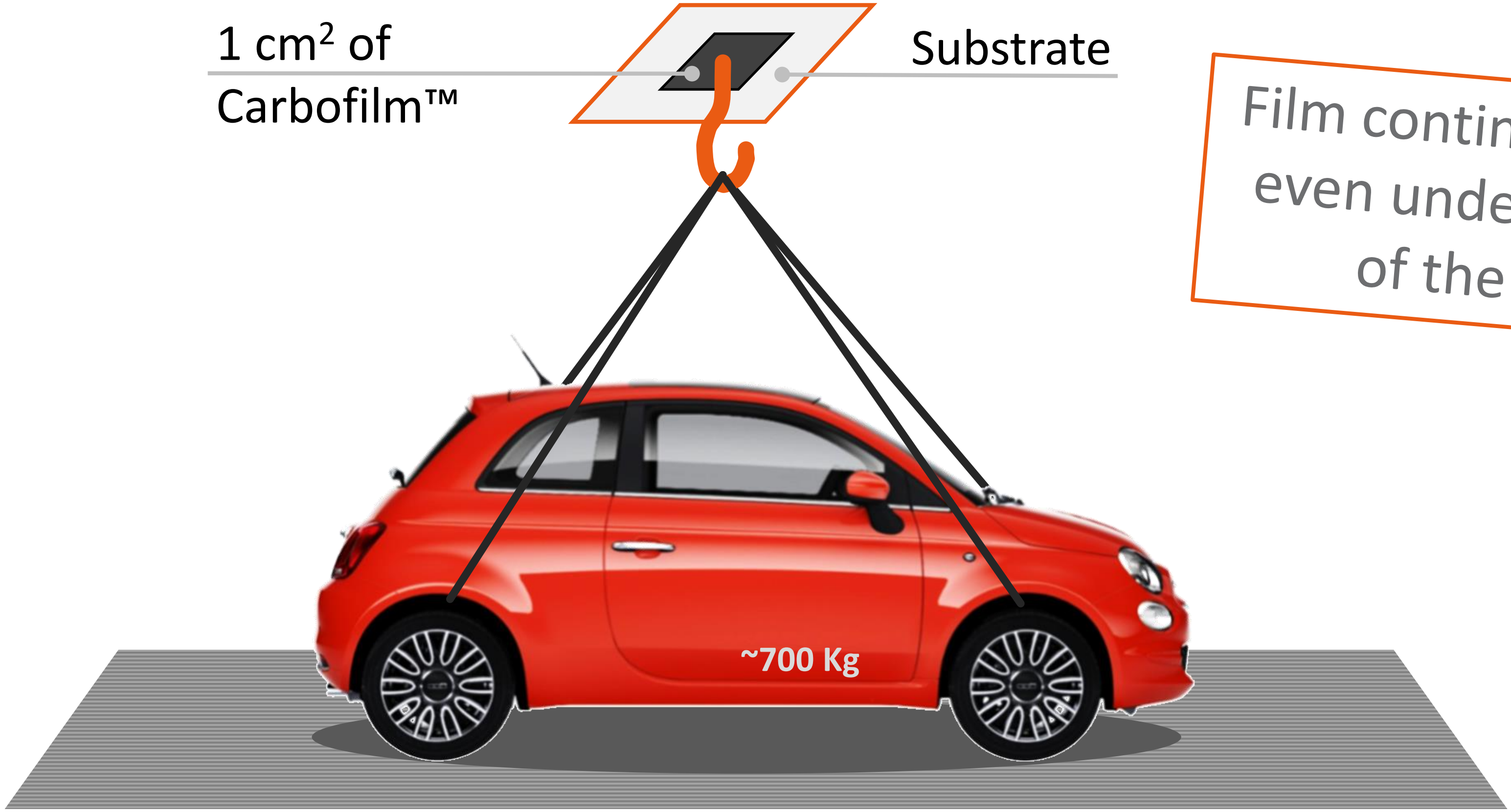
THICKNESS ≤ 0.5 µm

Groups of carbon atoms are transferred from a PyC target to the substrate to be coated.
This process is carried out in high-vacuum conditions which prevent from any chemical reaction.
Heat-sensitive substrates can be processed because of the room temperature deposition.

Simplified rappresentation for didactic purpose; the actual process may differ.

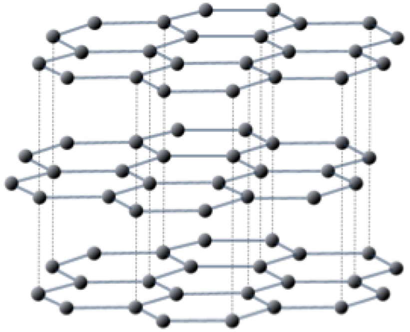
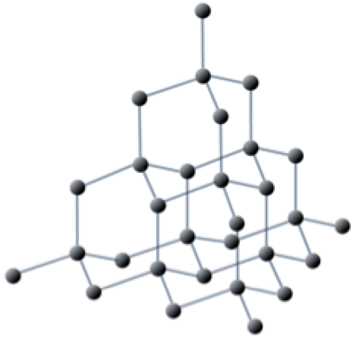
Carbofilm™: the adhesion force

Adhesion strength between Carbofilm™ and substrate is about 700 kg/cm^2



Film continuity is retained even under deformation of the substrate

Carbofilm™ : graphite or diamond?

Graphite 	 Diamond
Each carbon atom has sp2 hybridization to form bonds with three neighbors. The forth available electron forms a distributed π bond along the vertical axis.  Graphite (solid lines are strong covalent bonds, dotted lines are weak inter-layer bonds)	 Diamond (all bonds are strong covalent bonds) Each carbon atom has four nearest neighbors to which it is bonded by σ bonds, sp3 hybridization.
The softness and lubricating nature of graphite arises from the weak binding of the carbon sheets by weak Van der Waals forces.	Diamond is the hardest and most inert material on earth.

Carbofilm™ differs from graphite in that the layers are disordered and this gives Carbofilm™ more sp3 bonds resulting in similarities with diamonds and improved hardness compared to graphite.

Carbofilm™: the features

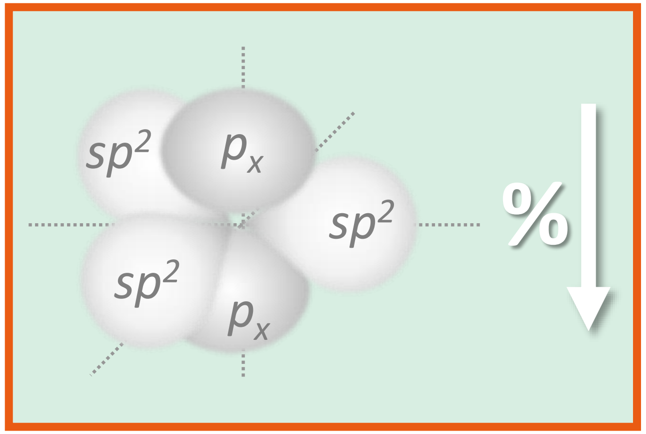
- Carbofilm™ is pure carbon like PyC and features a turbostratic structure equivalent to that of PyC.
- Carbofilm™ is a high-density, ultra-thin film (≤ 0.5 µm) that is deposited at room temperature.
- Carbofilm™ features high adhesion strength to the substrate (over 70 MPa - 700 Kg/cm^2) and film continuity is retained even under deformation of the substrate.
- Carbofilm™ does not modify the structural characteristics of the substrate.

From Carbofilm™ to Bio Inducer Surface

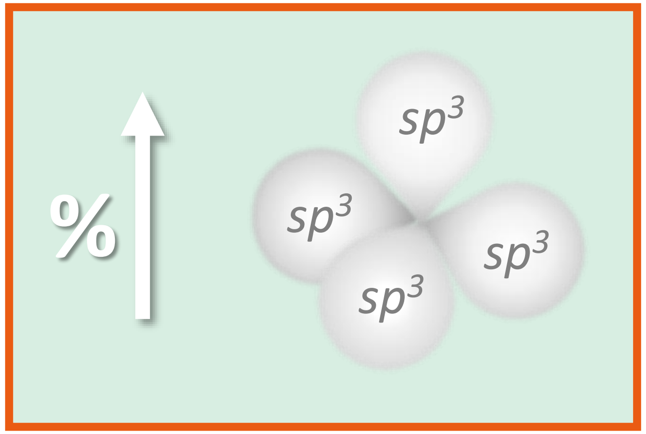
- In 2009, has been developed improvements in Carbofilm™ deposition process starting a new generation of coating
- Bio Inducer Surface has an increased number of sp3 bonds making it more similar to diamonds and with even better bio- and haemo- compatible properties
- Bio Inducer Surface is smoother than Carbofilm™
- Bio Inducer Surface is more homogeneous than Carbofilm™
- Bio Inducer Surface has smaller grain on the surface

From Carbofilm™ to Bio Inducer Surface

Proprietary surface pre-treatment



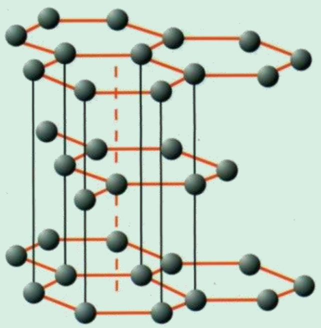
(Heteroepitaxial growth)



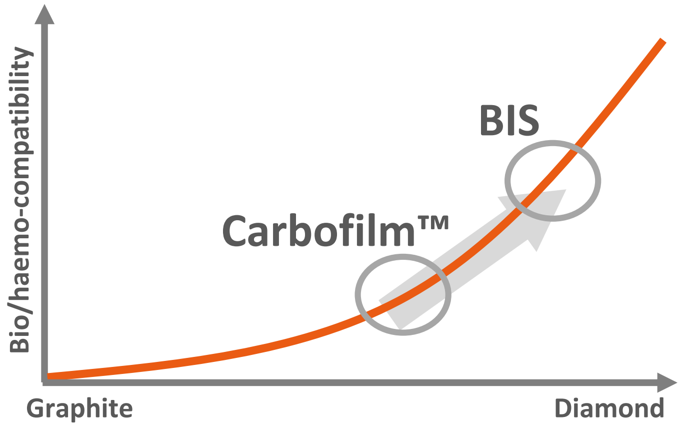
Higher density ultra-thin film (**≤ 0.3 µm**)

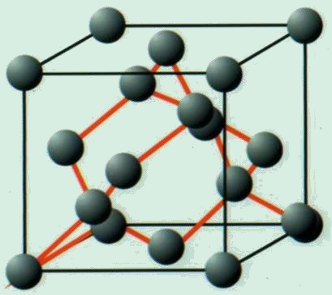
Further improvement in surface bio/haemo-compatibility

Bio Inducer Surface (BIS)



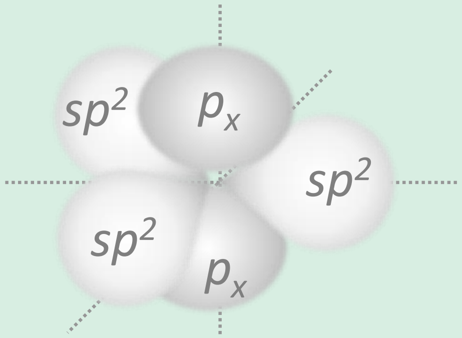
Graphite
(Hexagonal polymorph)





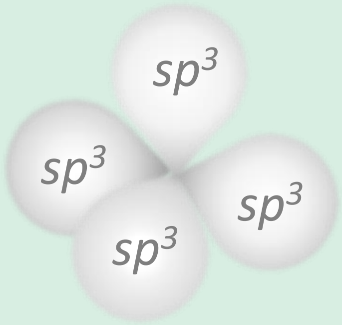
Diamond
(Cubic polymorph)

Better bio/haemo-compatibility



Carbon atom
with 1 bond available

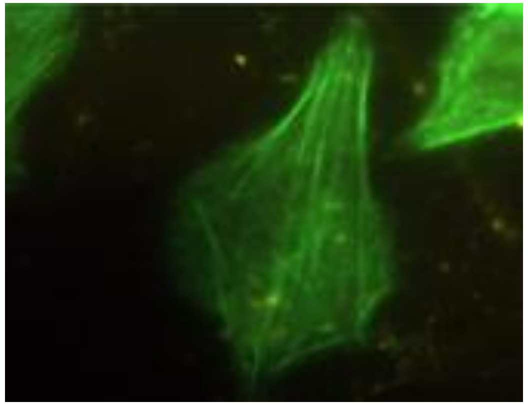
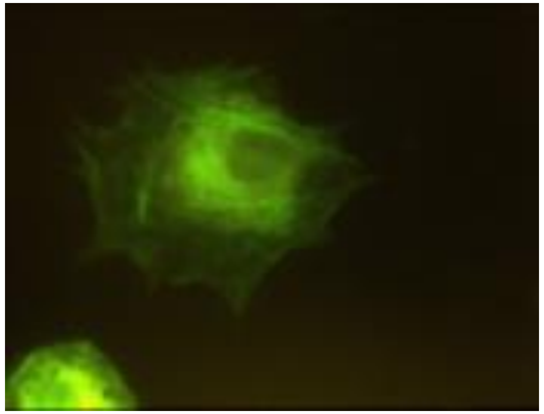
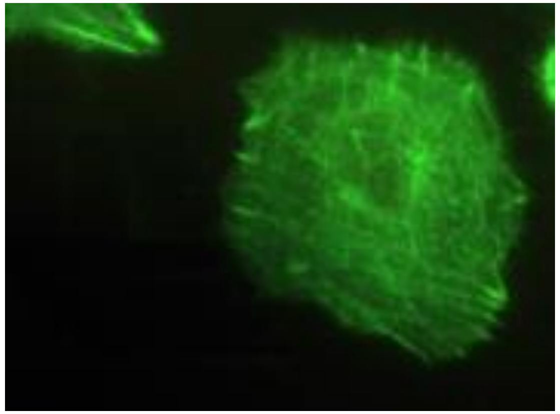
Higher density



Carbon atom
without bonds available

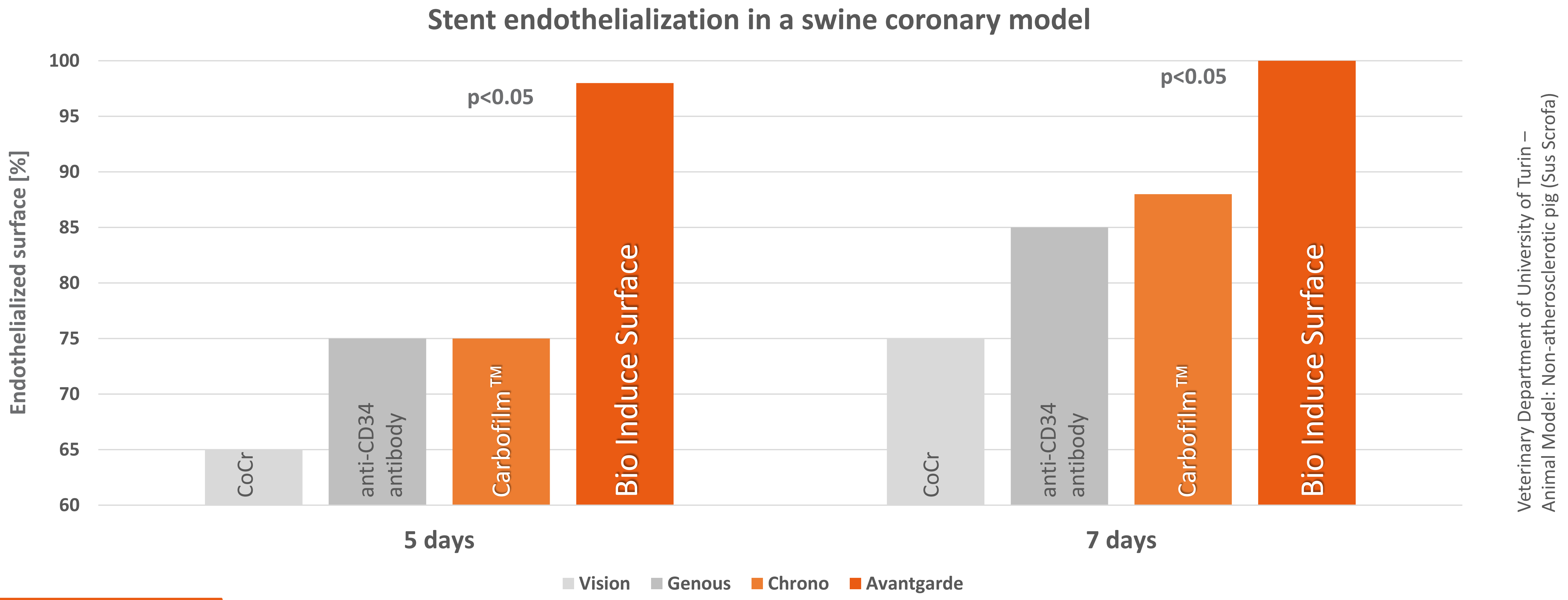
BIS Endothelialization - in vitro testing

Analysis of adhesion and morphology of Human Umbelical vein endothelial cells (HUVEC) seeded and cultivated on different substrates. (pictures taken @180min)

Bio Inducer Surface	Carbofilm™	Co-Cr
		
The endothelial cells on the Bio Inducer Surface substrate have evidence of intra- and extra-cellular filaments with a strong orientation on the substrate	The action filaments indicate several points of adhesion on the CF substrate, but the elongation is inferior to the Bio Inducer Surface sample.	Only at this time point on the bare CoCr substrate cells are reaching a distension with organization of the actin filaments

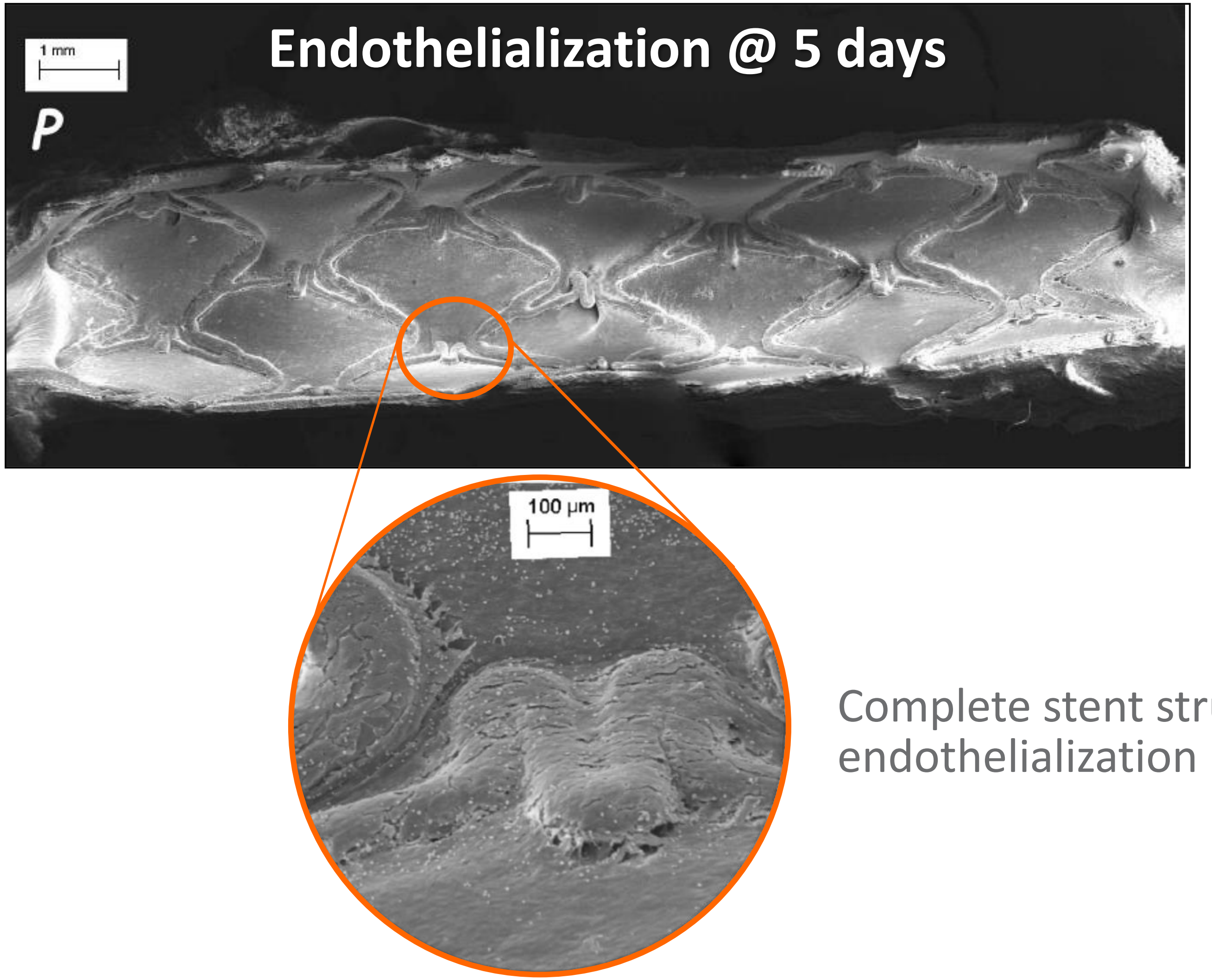
BIS Endothelialization - in vivo testing

Pre-clinical animal trial (pig) to ascertain the rate of endothelialization of the CoCr Carbostent coated with BIS.



CONFIDENTIAL

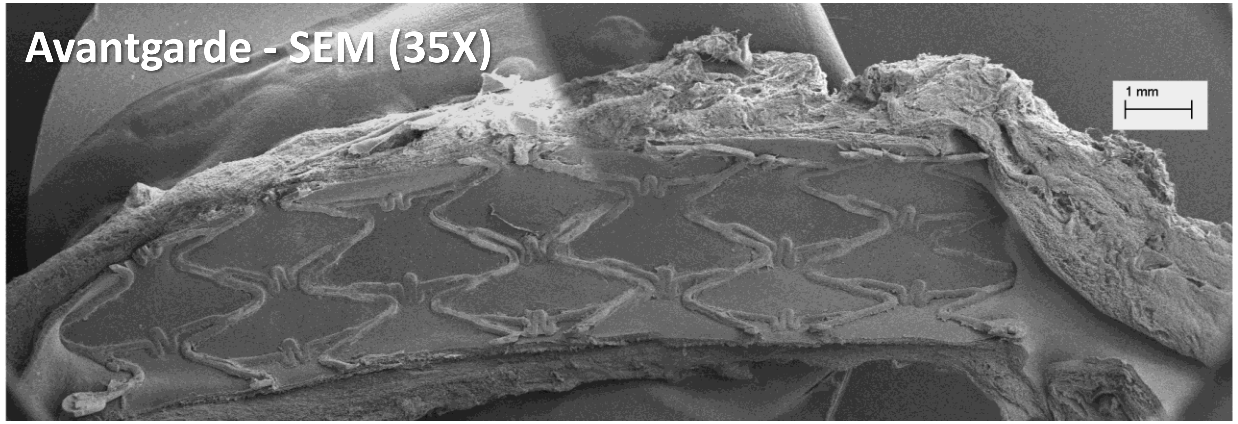
BIS Endothelialization - in vivo testing



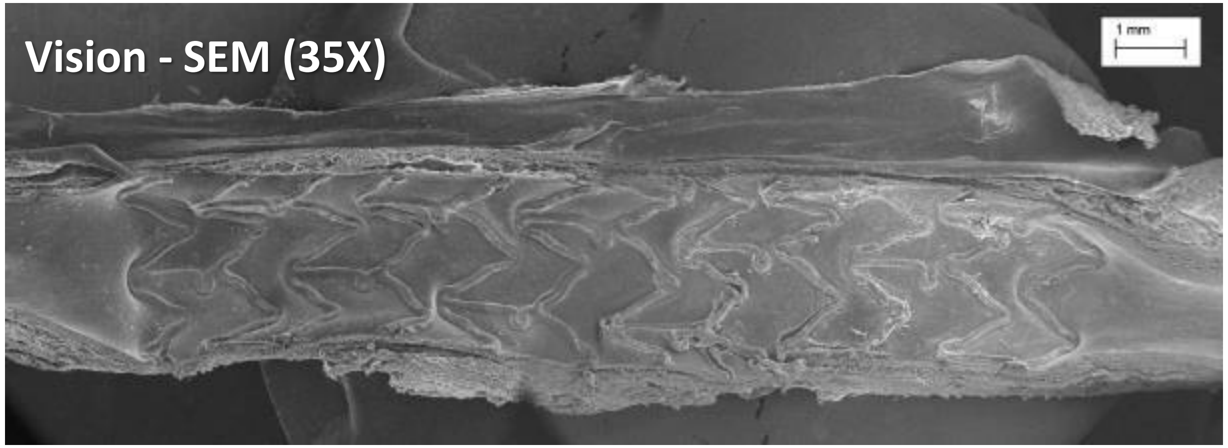
Complete stent struts endothelialization

Stent implants in pig coronary arteries. University of Turin.

BIS Endothelialization - in vivo evidences



Uniform stent expansion with vessel surface completely covered by endothelium **(surface coverage 100%)**



Inhomogeneous strut coverage. Several areas of the stent surface are not endothelized despite good struts apposition (**surface coverage 65%**)

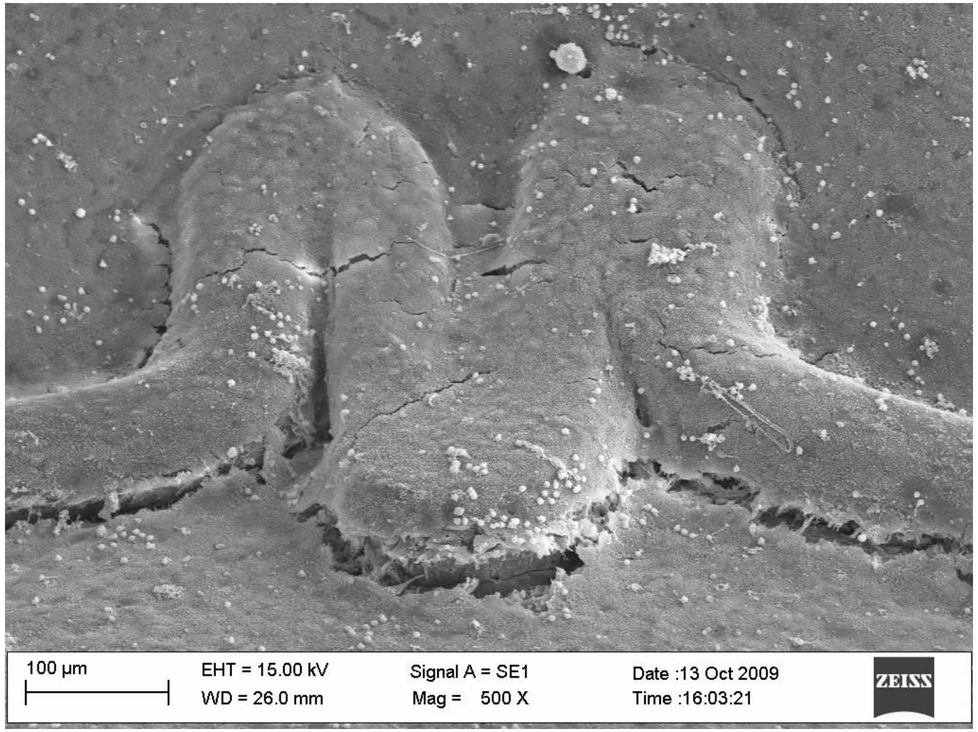
Stent implants in pig coronary arteries. University of Turin.

BIS Endothelialization - in vivo evidences

AVANTGARDE

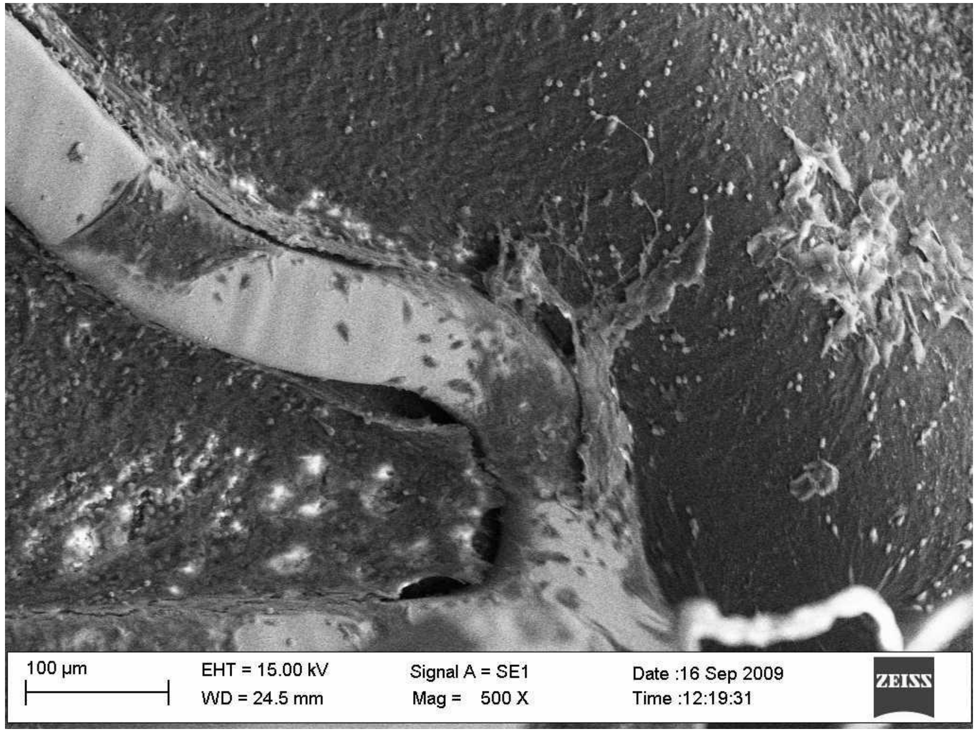
Continuous and homogeneous endothelialized surface with a limited number of leucocytes.

Erytrocytes, activated platelets and giant cells are absent.



VISION

Pictures showing wide non-endothelialized areas together with erytrocytes, leucocyte and activated platelets.



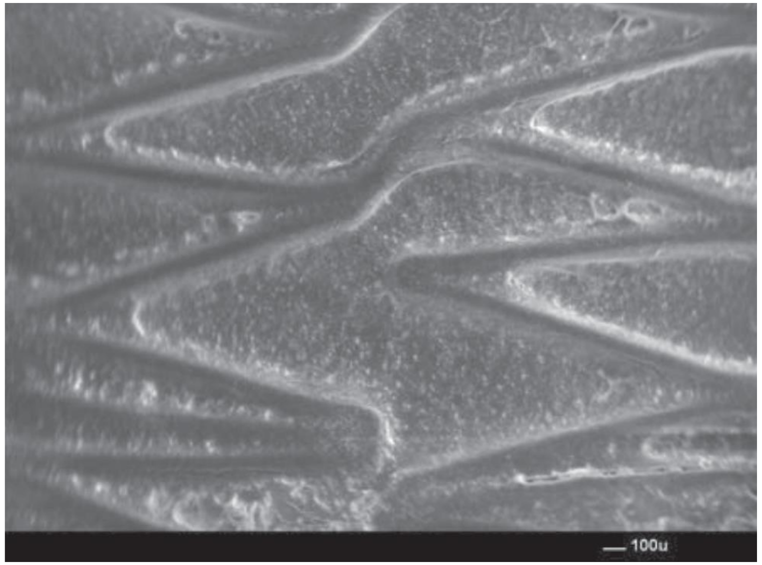
NOTE: the cracks are artefacts related to SEM specimens preparation

BIS Endothelialization - in vivo evidences

Self expandable (Nitinol) stent

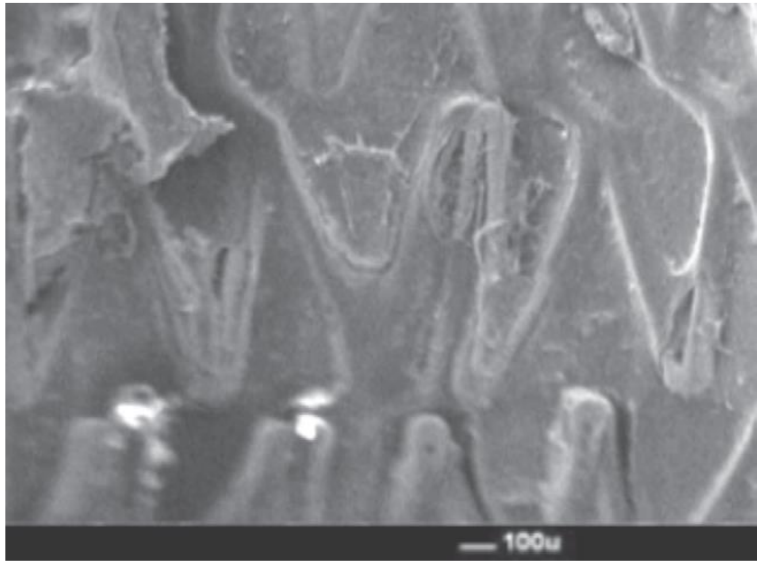
STENT ENDOTHELIALIZATION AT 7 DAYS FROM IMPLANT

BIS coated Nitinol peripheral stent



Continuous and homogeneous endothelial cell carpet.

Non coated Nitinol peripheral stent



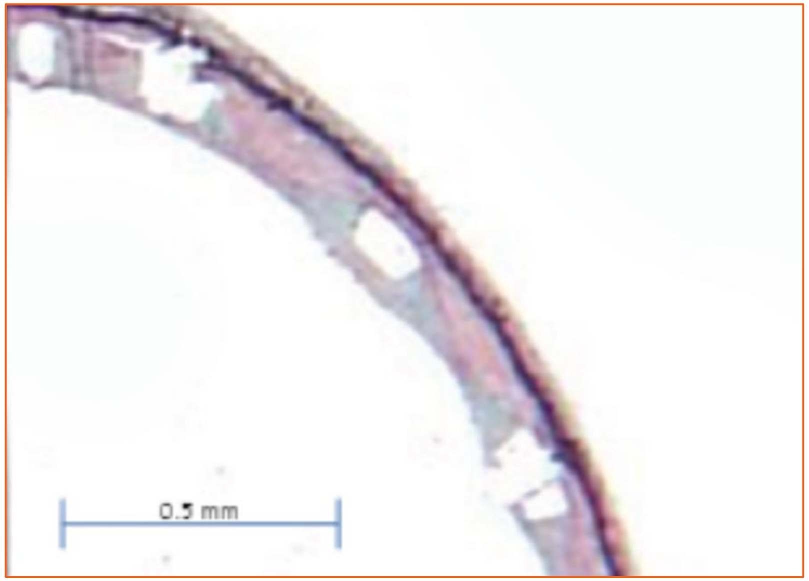
Endothelialization is irregular and not confluent. Several struts were uncovered.

BIS Endothelialization - in vivo evidences

Self expandable (Nitinol) stent

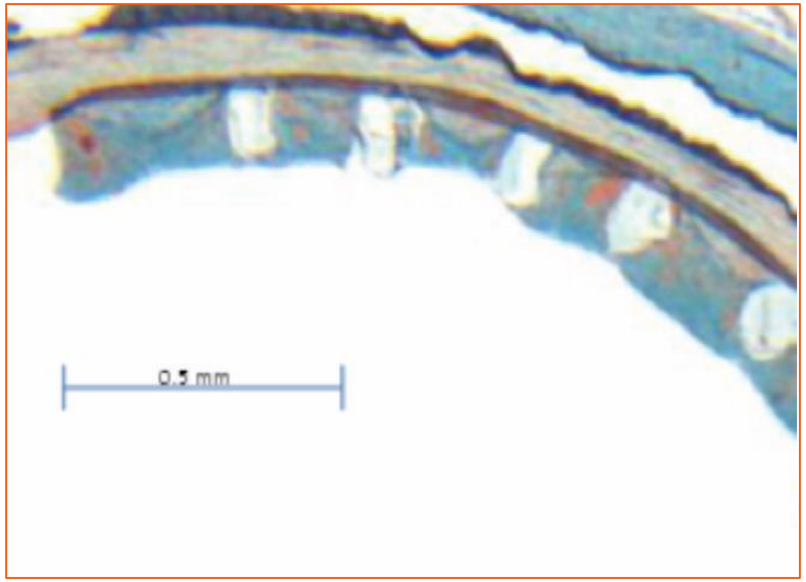
VESSEL BIOLOGICAL REACTION TO STENT IMPLANT AT 30 DAYS FROM IMPLANT

BIS coated Nitinol peripheral stent



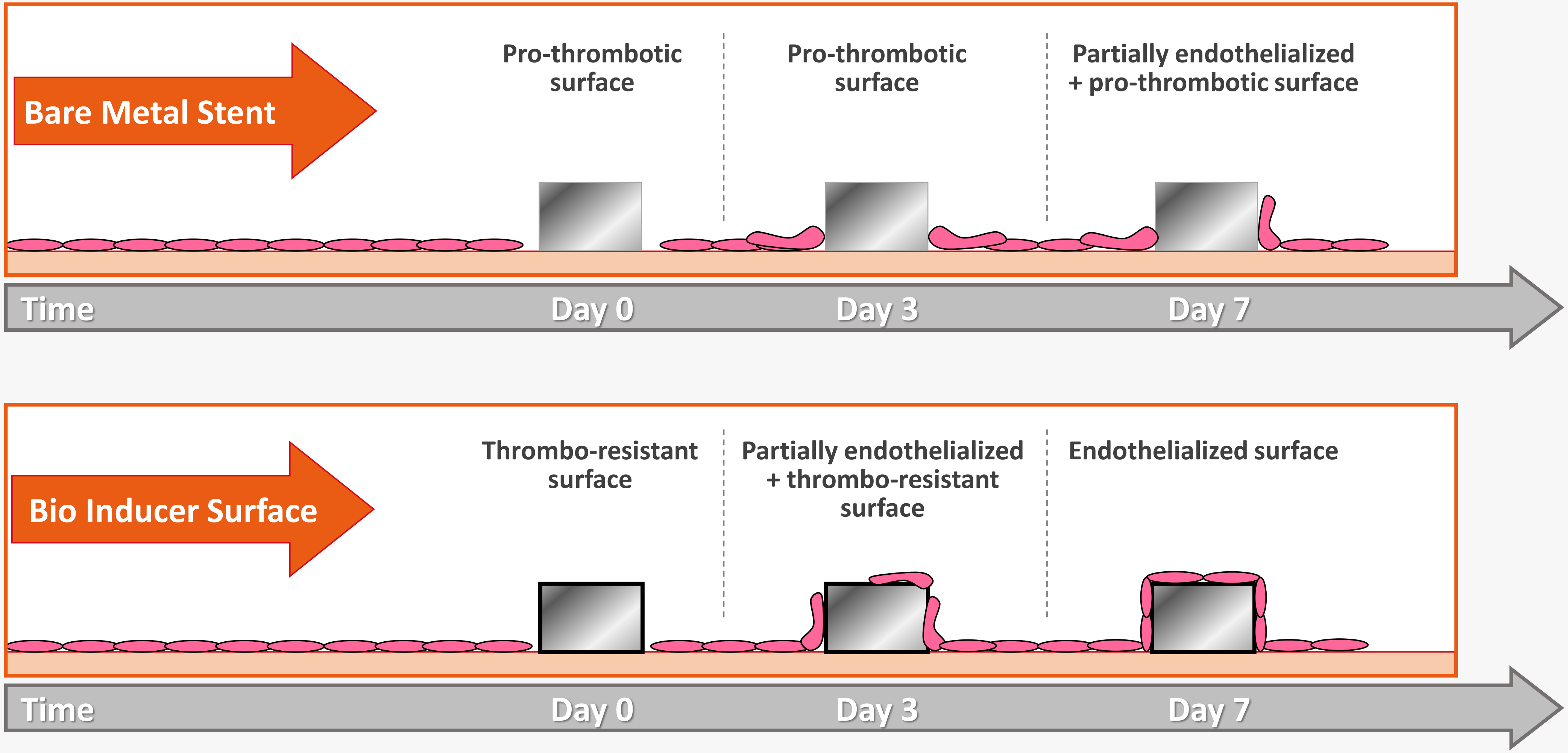
A continuous and homogeneous endothelial cell carpet covers a thin layer of neointima. No signs of inflammation or blood deposits are present.

Non coated Nitinol peripheral stent



Endothelialization is almost complete but blood clots and fibrin deposits are still detectable around struts.

Foreseen clinical impact



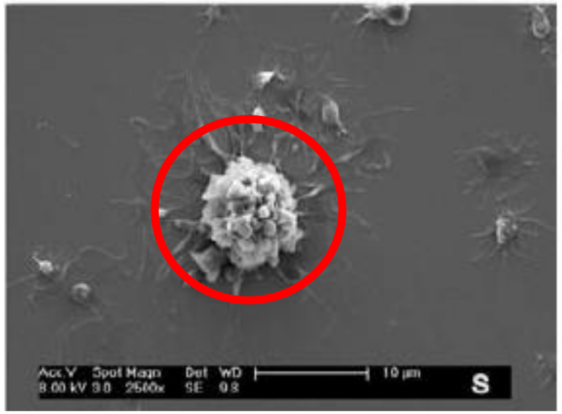
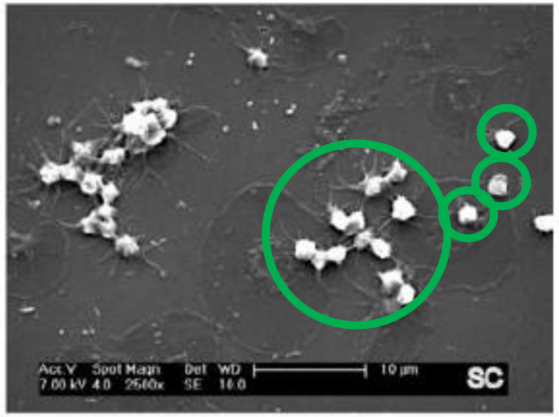
Slide pictures are of a purely illustrative nature and are not intended to depict reality

BIS Haemocompatibility - in vitro testing

Inside blood there are several proteins, some of them deposit on “foreign elements/ substances” causing a clot formation – Fibrinogen** - while others do not cause any – Albumin*.

Pure carbon coatings (like Carbofilm/ BIS) favor the adhesion of Albumin preventing that of Fibrinogen; this minimizes the risk for clot formation!

Aggregated = activated

	
CoCr surface ***Several aggregated platelets.***	**Pure Carbon coated CoCr surface** ***Few platelets non activated (round shape)***

Round shape = not activated

DLC coatings: Effects of physical and chemical properties on biological response. Biomaterials 28 (2007) 1620–1628

*Albumin is a protein flowing in the blood that does not elicit the coagulation.

**Fibrinogen is a protein which participate to the formation of the clot.

BIS Ions release - in vitro testing

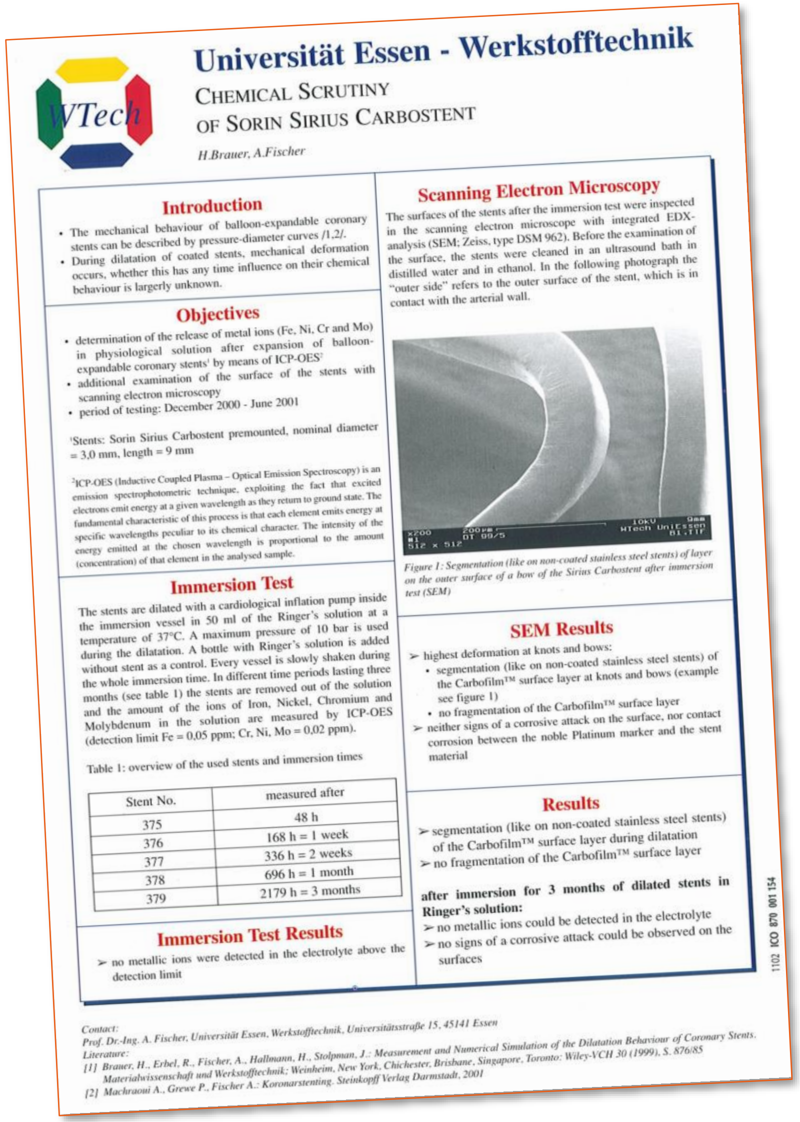
Carbofilm™ and BIS act as an effective barrier to metal ions release:

- Coating on AISI 316L stent[1]:
  no metal ions detected in 3 months immersion test

- Coating on Nitinol stent[2]:
  no metal ions detected in 2 months immersion test

1: Brauer H, Fischer A – Chemical scrutiny of Sorin Sirius Carbostent - Data on File @CID
2: Polytechnic School, University of Trento - Immersion test report on clinical quality Carbofilm coated Nitinol devices – Data on file @ Sorin

BIS Ions release - in vitro testing



Universität Essen - Werkstofftechnik

CHEMICAL SCRUTINY OF SORIN SIRIUS CARBOSTENT

H.Brauer, A.Fischer

Introduction

- The mechanical behaviour of balloon-expandable coronary stents can be described by pressure-diameter curves /1,2/.
- During dilatation of coated stents, mechanical deformation occurs, whether this has any time influence on their chemical behaviour is largerly unknown.

Objectives

- determination of the release of metal ions (Fe, Ni, Cr and Mo) in physiological solution after expansion of balloon-expandable coronary stents[1] by means of ICP-OES[2]
- additional examination of the surface of the stents with scanning electron microscopy
- period of testing: December 2000 - June 2001

[1]Stents: Sorin Sirius Carbostent premounted, nominal diameter = 3,0 mm, length = 9 mm

[2]ICP-OES (Inductive Coupled Plasma – Optical Emission Spectroscopy) is an emission spectrophotometric technique, exploiting the fact that excited electrons emit energy at a given wavelength as they return to ground state. The fundamental characteristic of this process is that each element emits energy at specific wavelengths peculiar to its chemical character. The intensity of the energy emitted at the chosen wavelength is proportional to the amount (concentration) of that element in the analysed sample.

Immersion Test

The stents are dilated with a cardiological inflation pump inside the immersion vessel in 50 ml of the Ringer's solution at a temperature of 37°C. A maximum pressure of 10 bar is used during the dilatation. A bottle with Ringer's solution is added without stent as a control. Every vessel is slowly shaken during the whole immersion time. In different time periods lasting three months (see table 1) the stents are removed out of the solution and the amount of the ions of Iron, Nickel, Chromium and Molybdenum in the solution are measured by ICP-OES (detection limit Fe = 0,05 ppm; Cr, Ni, Mo = 0,02 ppm).

Table 1: overview of the used stents and immersion times

Stent No.	measured after
375	48 h
376	168 h = 1 week
377	336 h = 2 weeks
378	696 h = 1 month
379	2179 h = 3 months

Immersion Test Results

- no metallic ions were detected in the electrolyte above the detection limit

Scanning Electron Microscopy

The surfaces of the stents after the immersion test were inspected in the scanning electron microscope with integrated EDX-analysis (SEM; Zeiss, type DSM 962). Before the examination of the surface, the stents were cleaned in an ultrasound bath in distilled water and in ethanol. In the following photograph the "outer side" refers to the outer surface of the stent, which is in contact with the arterial wall.

Figure 1: Segmentation (like on non-coated stainless steel stents) of layer on the outer surface of a bow of the Sirius Carbostent after immersion test (SEM)

SEM Results

- highest deformation at knots and bows:
  - segmentation (like on non-coated stainless steel stents) of the Carbofilm™ surface layer at knots and bows (example see figure 1)
  - no fragmentation of the Carbofilm™ surface layer
- neither signs of a corrosive attack on the surface, nor contact corrosion between the noble Platinum marker and the stent material

Results

- segmentation (like on non-coated stainless steel stents) of the Carbofilm™ surface layer during dilatation
- no fragmentation of the Carbofilm™ surface layer

after immersion for 3 months of dilated stents in Ringer's solution:

- no metallic ions could be detected in the electrolyte
- no signs of a corrosive attack could be observed on the surfaces

1102 ICO 870 001 154

Contact:
Prof. Dr.-Ing. A. Fischer, Universität Essen, Werkstofftechnik, Universitätsstraße 15, 45141 Essen

Literature:
[1] Brauer, H., Erbel, R., Fischer, A., Hallmann, H., Stolpman, J.: Measurement and Numerical Simulation of the Dilatation Behaviour of Coronary Stents. Materialwissenschaft und Werkstofftechnik; Weinheim, New York, Chichester, Brisbane, Singapore, Toronto: Wiley-VCH 30 (1999), S. 876/85
[2] Machraoui A., Grewe P., Fischer A.: Koronarstenting. Steinkopff Verlag Darmstadt, 2001

- A chemical scrutiny of the Bio Inducer Surface was conducted to determine the release of metal ions (Fe, Ni, Cr and Mo) in physiological solution after expansion of balloon-expandable coronary stents coated with iCarbofilm technology.
- The stents were dilated inside the immersion vessel in 50 ml of the Ringer's solution at a temperature of 37°C. A bottle with Ringer's solution is added without a stent as a control. Every vessel is slowly shaken during the whole immersion time. In different time periods lasting three months the stents are removed from the solution and the amount of ions - Iron, Nickel, Chromium and Molybdenum in the solution are measured by ICP-OES.
- The surface of the stents after the immersion test were inspected in the scanning electron microscope EDX-analysis. Before the examination of the surface, the stents were cleaned in an ultrasound bath in distilled water and ethanol.

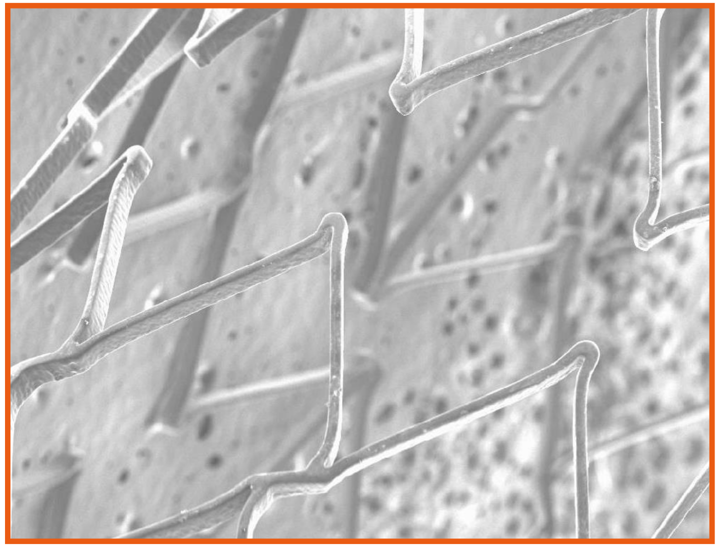
Conclusions:

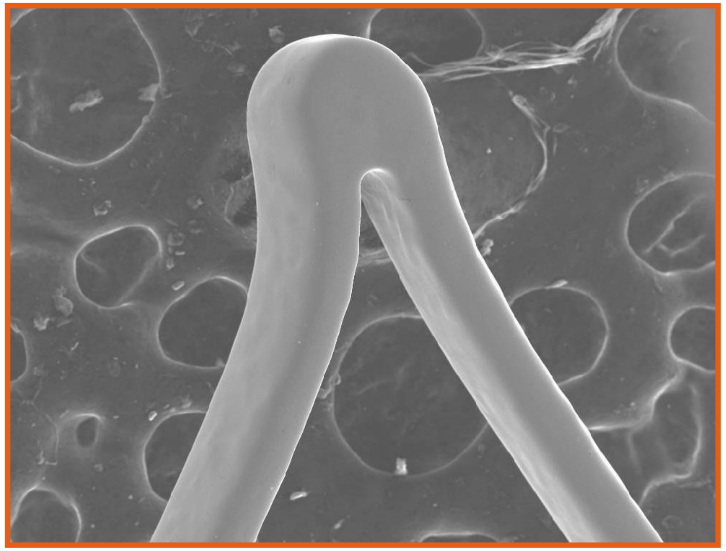
- **no metallic ions were detected in the electrolyte above the detection limit;**
- **no signs of corrosive attack could be observed on the surface;**
- **no segmentation or fragmentation of the Bio Inducer Surface layer.**

BIS Ions release - in vitro testing

Bio Inducer Surface with its exceptional bio&haemo compatibility, **seals** the bulk Nitinol material (Nickel-Titanium alloy) avoiding any release of heavy metal ions.

This is extremely important in case of long self-expandable stents because Nitinol contains ~50% of nickel, a very high allergenic metal component.





Conclusions

BIS - Bio Inducer Surface on vascular stent:

- **Increases the bio- and haemo-compatibility** of (metal) stent fasting the endothelialization and reducing the thrombogenicity.
- **Acts as a barrier to allergenic metal ions** release (like Nickel) when used on metal alloys (CoCr, SS, Nitinol, etc.).
- **Does not modify the structural characteristics** of the substrate (no changes in metal alloy mechanical properties).
- The **continuity of a higher density ultra-thin film (≤ 0.3 µm) is retained** even under deformation of the metallic substrate (high adhesion strength, over 70 MPa - 700 Kg/cm^2).

Conclusions

BIS - Bio Inducer Surface on vascular stent:

- No changes in the mechanical properties of the metal alloy
- High adesion strength, required in any stent configuration/ deformation
- Increased haemo-compatibility of (metal) stent, reducing thrombo-genicity
- Increased biocompatibility of (metal) stent fasting, endo-thelialization
- Barrier to allergenic metal ions release from the metal stent alloys

Alvimedica