Blood Compatibility of Metal Oxide Surfaces Prepared by Metal Plasma Immersion Ion Implantation and Deposition

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BACKGROUND: Metal oxide ceramics, like titania or zirconia are known for their good biocompatibility in orthopaedic applications. Titanium oxide and iridium oxide already have clinical application as coating on vascular stents and behave superior in clinical studies. This indicates also a good blood compatibility of these oxides with minimal clotting activation and minimal induction of smooth muscle cell proliferation as an effect of growth factors from blood platelets.
However, an investigation of various metal oxides has not yet been done and also the effect of crystallinity on blood compatibility is not yet clear. These physical and chemical features may be important for blood compatibility because they affect the interaction of proteins and cells with the surface.

METHOD: For this study titanium oxide (TiO_2), hafnium oxide (HfO_2) and iridium oxide (IrO_2) was deposited on mirror polished stainless steel (SS) substrates by metal plasma immersion ion implantation and deposition (Me-PIIID) with magnetron sputtering in a reactive oxygen atmosphere. XRD showed that IrO_2 can be produced both as nanocrystalline, non textured and as textured film. Both types were used for blood compatibility tests. The film thickness was in a range of 100 nm. SS and mirror polished nitinol (NiTi) were used as a reference.
Roughness and contact angle with water were determined of the surfaces. The surfaces were incubated in platelet rich plasma, the number of adherent blood platelets was quantified and the release of platelet derived growth factor (PDGF-AB) and serotonin was measured by ELISA techniques as activation markers of blood platelets. Further recalcified human standard plasma was put on the surfaces for a defined time and the production of the F1+2 fragment was measured as marker of plasma clotting activation, the terminal attack complex SC5b-9 was measured as marker of complement activation. ^{125}I-Fibrinogen adsorption from standard plasma was measured as surface density. All measurements were done in ten repeats.

RESULTS and DISCUSSION: The 10 point roughness of these surfaces was in a similar range of 20 nm for SS, NiTi, HfO_2 and the textured IrO_2; it was 10 nm for the non-textured IrO_2. Major differences were in the wettability, with lowest contact angle for TiO_2 (45°) and HfO_2 (59°) and higher angles for SS, NiTi, non textured and textured IrO_2 (77°, 71°, 75°, 91°, respectively). This means that all surfaces were very smooth with regards to biological structures.
Platelet adherence and platelet activation on the surfaces are shown in Fig. 1. The least platelet adherence was found on the bare metals SS and NiTi, whereas the metal oxides generally show higher, worse values. Activated blood platelets are known to release cytokines with important messenger functions for other cells. These cytokines, PDGF or serotonin, can be also used to monitor platelet activation. In these tests only minor differences exist for PDGF (Fig. 1) or serotonin release (data not shown).

Surface adsorbed fibrinogen can activate blood platelets and a threshold of 30ng/cm^2 for platelet activation has been reported. Here no correlation between fibrinogen adsorption (Fig. 2) and platelet adherence (Fig. 1) was seen, what indicates that additional conformational changes of the molecule are important for the biological effect.

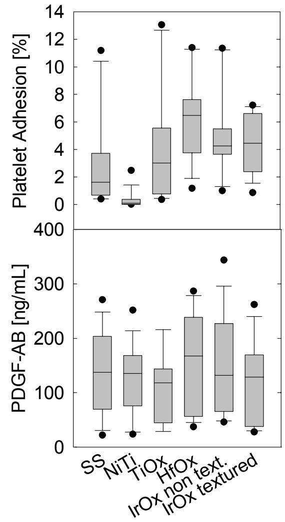

Fig. 1: Platelet adherence and activation on different metal and metal oxide surfaces. The box plots indicate median, quartils and range of 10 measurements.

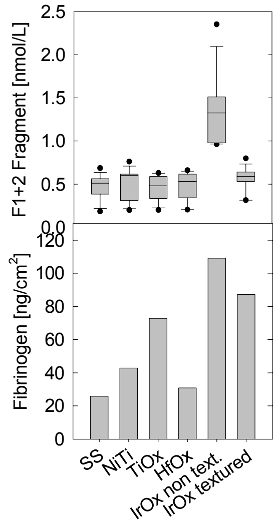

Fig. 2: Fibrinogen adsorption on the surfaces and rate of clotting cascade activation (F1+2 fragment formation)

The non textured IrO_2 which allows better entanglement of proteins also absorbs the highest amount of fibrinogen and induces significantly the highest activation of upstream parts of the clotting cascade (measured as release of F1+2 peptide), and of the complement cascade (data not shown). However the correlation between fibrinogen adsorption and cascade activation does not generally exist. None of the biological parameters correlated with any of the physical surface properties like roughness, wettability or surface energy.

CONCLUSION: The crystalline and textured IrO_2 shows significantly less interaction with proteins and protein cascades, therefore surfaces with higher crystallinity seem to have a better blood compatibility. Otherwise correlations between physical surface properties and biological effects are limited. This puts more focus on chemical and biochemical functional groups of a surface, what is currently under investigation.
The untreated metal surfaces with natural oxide film often show a better blood compatibility than the thicker metal oxide films, but they are less corrosion resistant and therefore may behave inferior in the clinical studies.

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Objectives

- Production of oxides of the transition metals
  - Titanium
  - Hafnium
  - Iridium

  as coatings on stainless steel coupons and stents
- Physical characterization of the coatings
- Hemocompatibility testing
  - Plasmatic systems
  - Blood platelets

Preparation of the Coatings

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- Sputter Cleaning in Argon or Oxygen Plasma (2kV, 2000 Hz, 10min)
- Deposition by magnetron sputtering in a reactive oxygen atmosphere (0.5 Pa, 20min)
- PIII (20kV, 2000-3000Hz) only initially or during whole deposition time.

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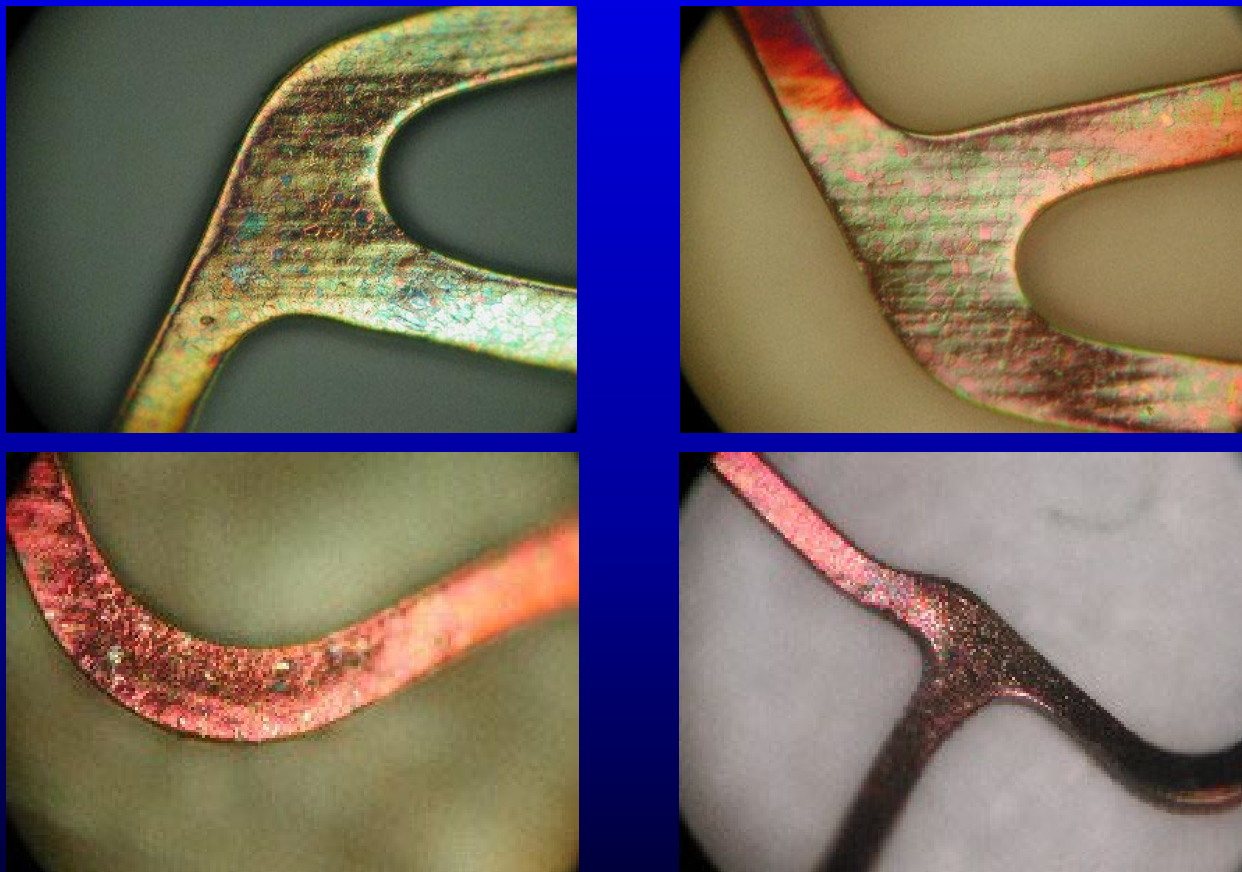
IrOx Coating on Stainless Steel

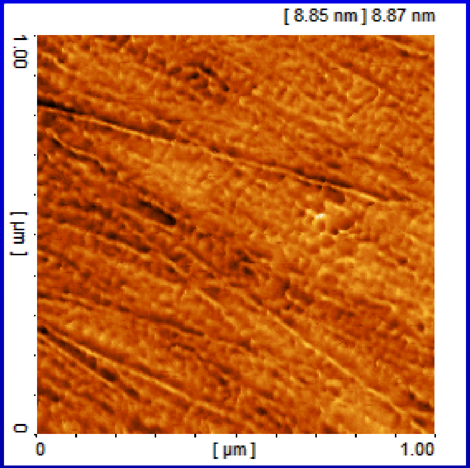
outside | inside

un-expanded | expanded

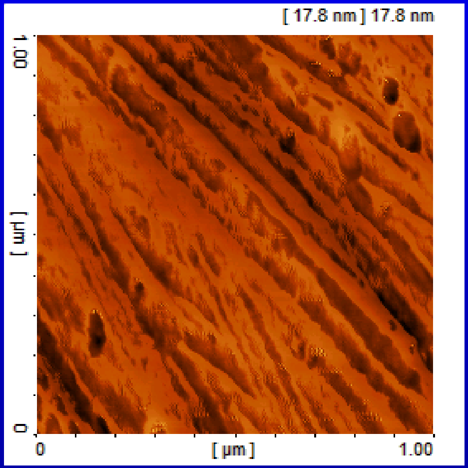


Adherent coating also after expansion can be achieved on real vascular stents

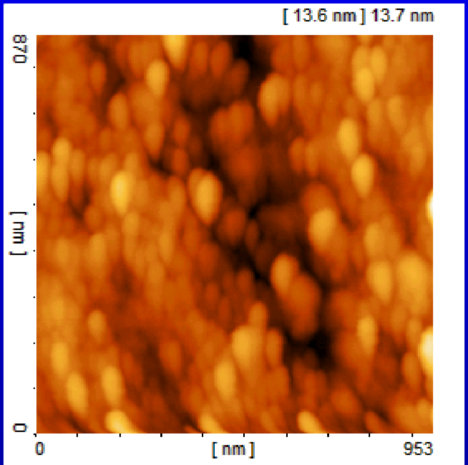
Surface Morphology



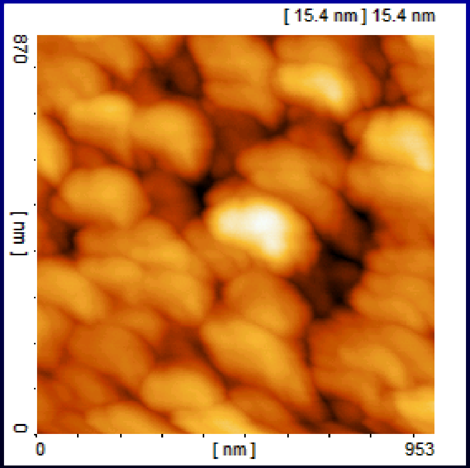
SS



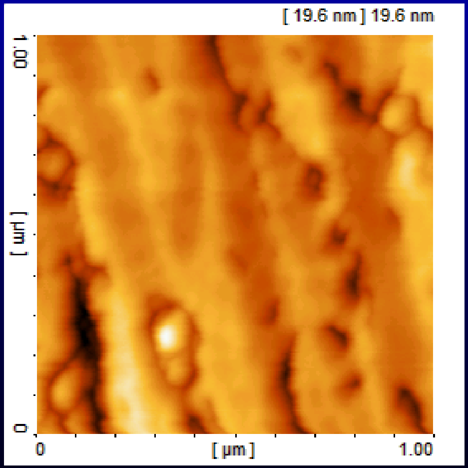
NiTi



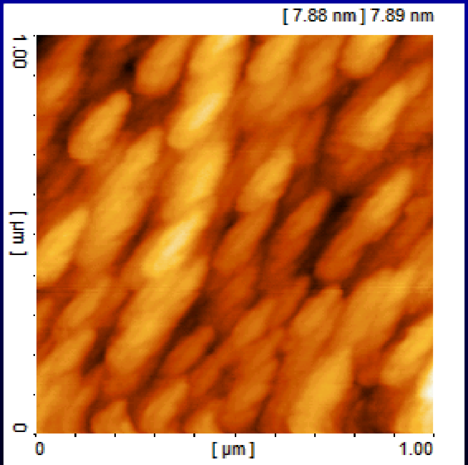
TiO_x



HfO_x



IrO_x non textured



IrOx textured

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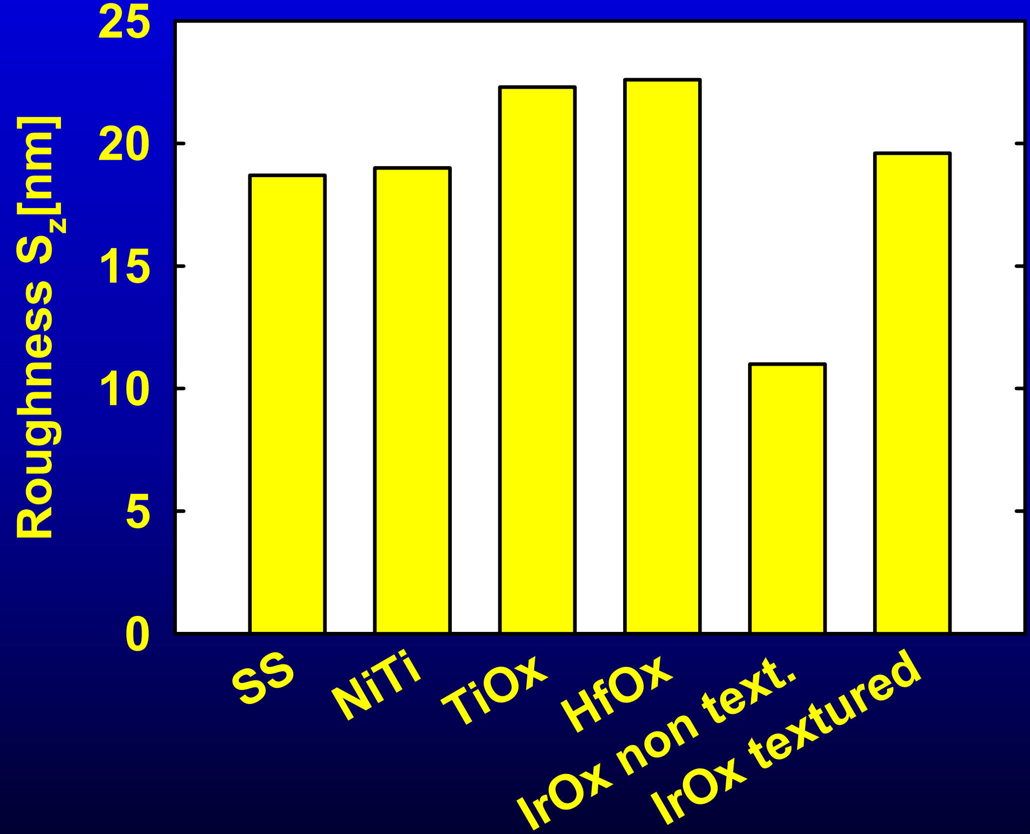
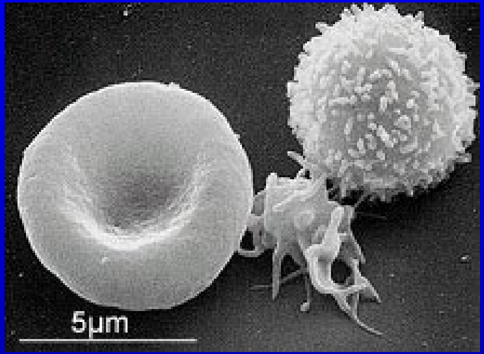
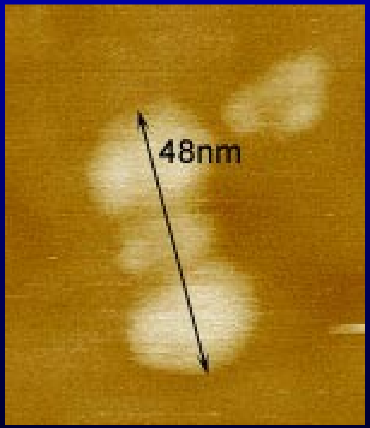
Surface Roughness

S_z Roughness measured between 5 highest and 5 deepest points on 5x5µm^2 AFM scans





Red blood cell, blood platelet and lymphocyte

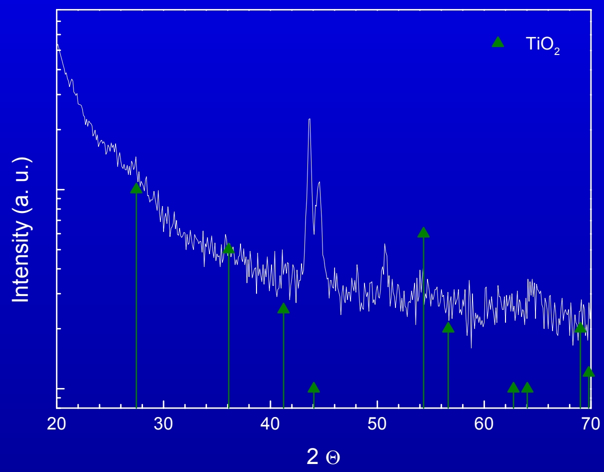


Fibrinogen

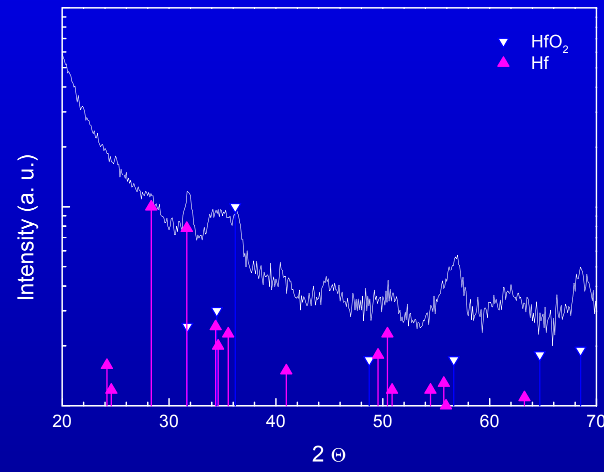
Low roughness, more in the range of biomolecules than cellular structures

X-Ray Diffraction

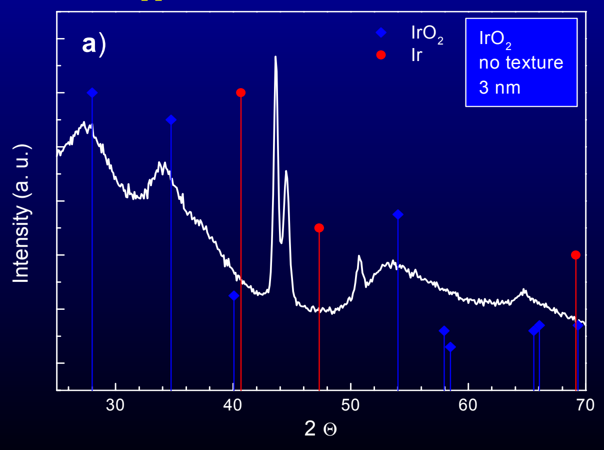
TiO_x



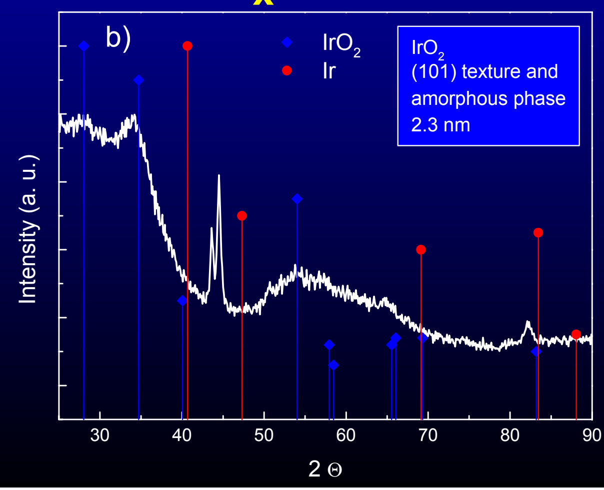
HfO_x



IrO_x non textured



IrO_x textured



X-Ray Diffraction

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	TiOx	HfOx	IrOx non text.	IrOx text.
Composition	Rutile TiO_2 (anatase) austenite ferrite	35% HfO_2 45% Hf 10% austenite 10% ferrite	38% IrO_2 38% austenite 24% ferrite	47% IrO_2 35% austenite 18% ferrite
Grain size	2-3nm	6 ± 2 nm	3.0 ± 0.5 nm	2.3 ± 0.5 nm
Orientation		28% {002} rest {100}	none	{100}

Surface Energy

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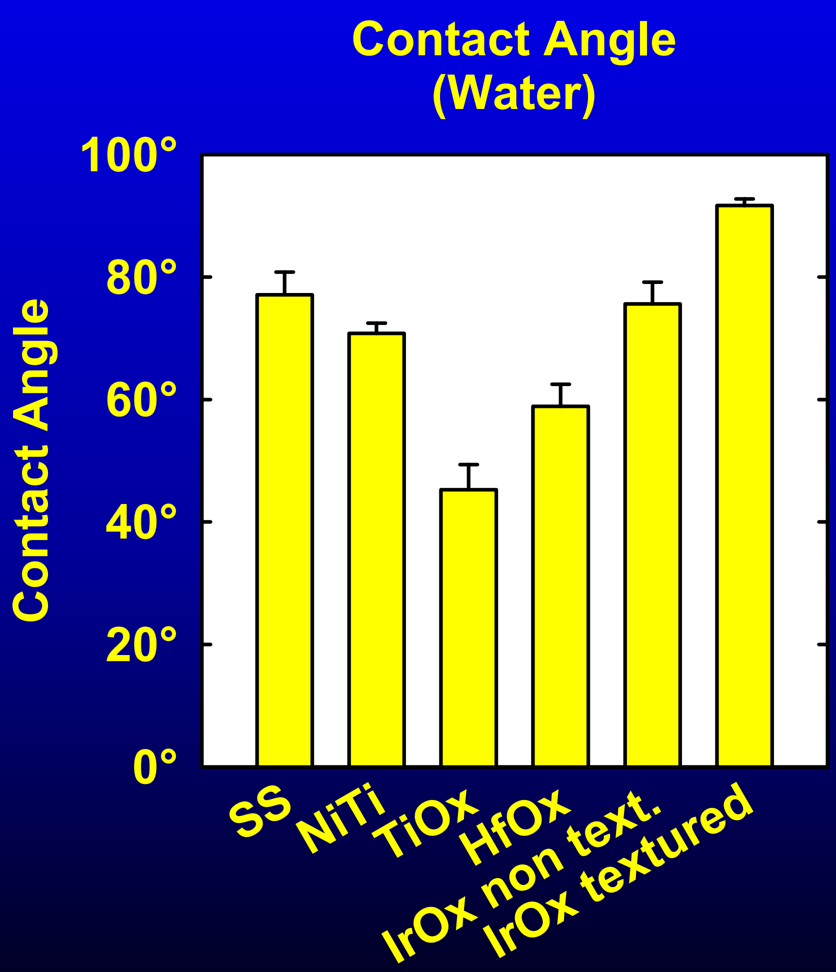
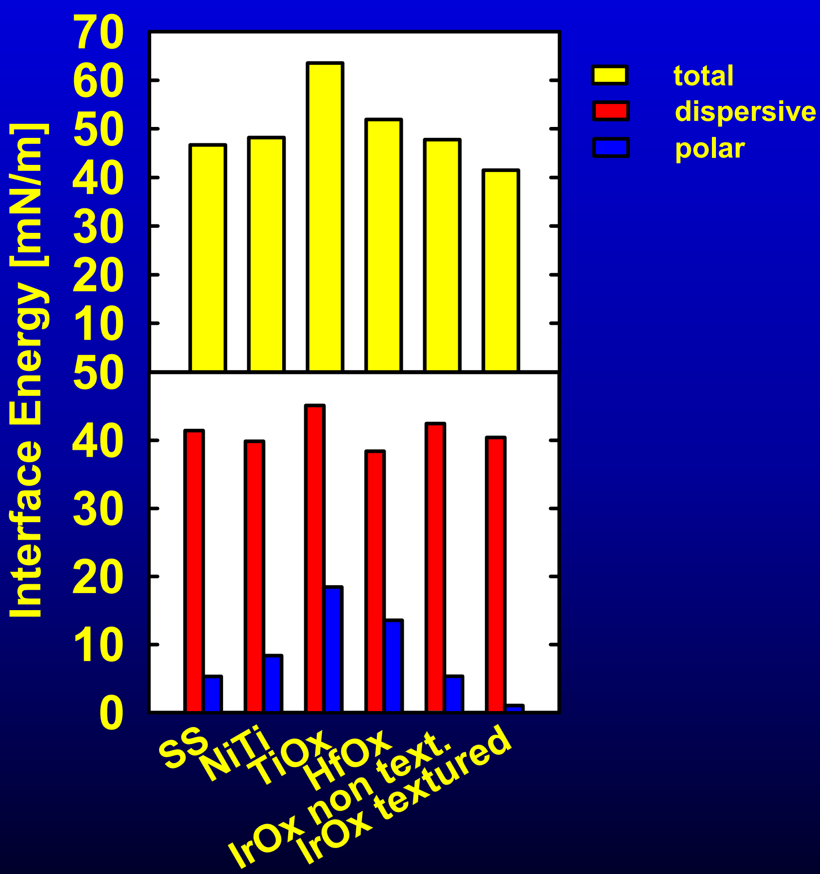
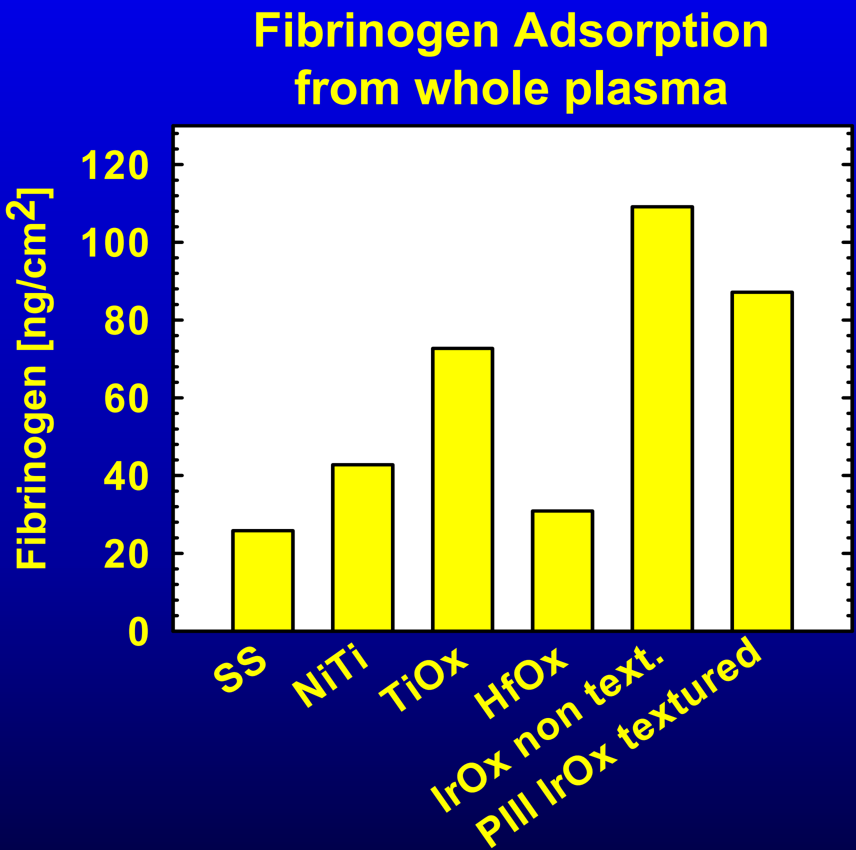
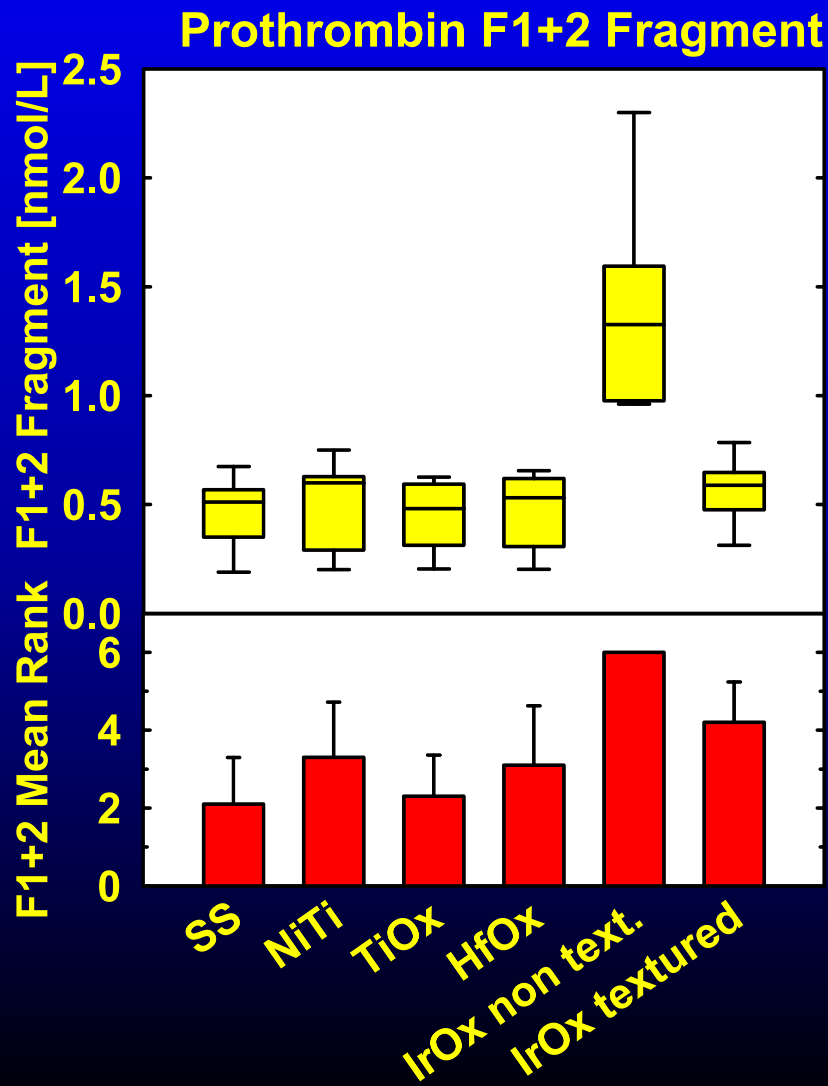




Plasma Proteins – Clotting Cascade



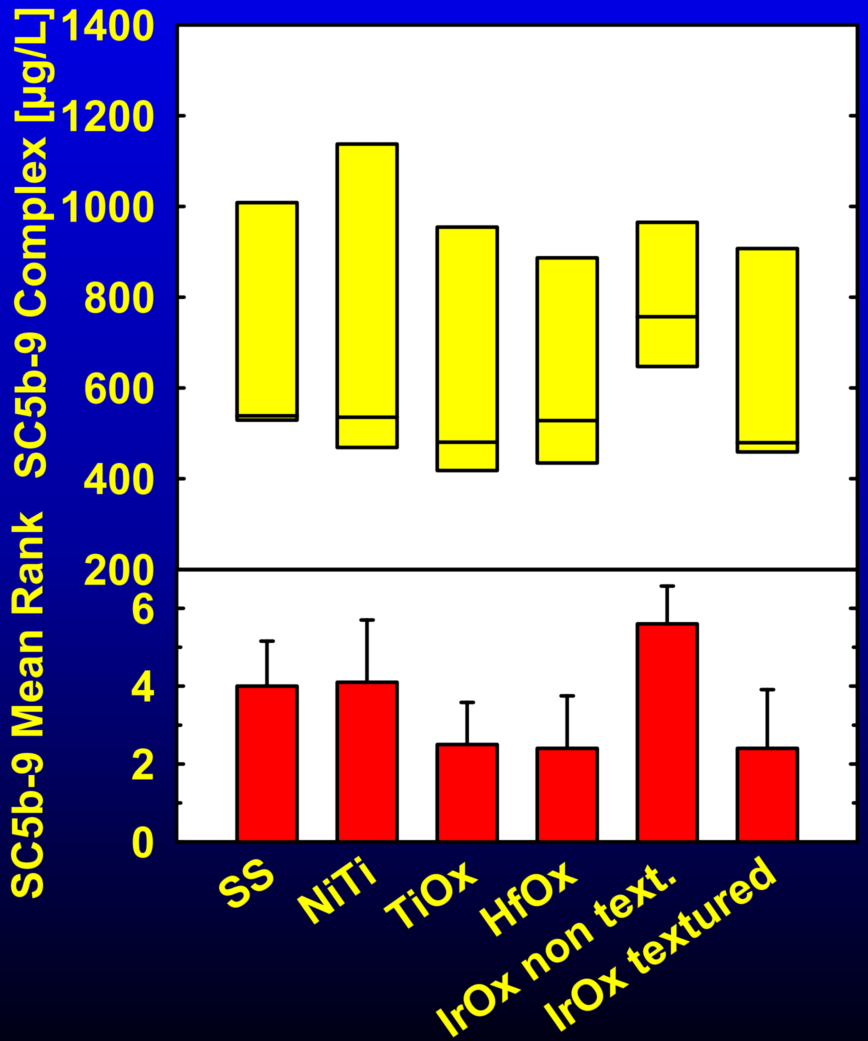


Non-textured IrO_x adsorbs high amount of fibrinogen and strongly activates the clotting cascade

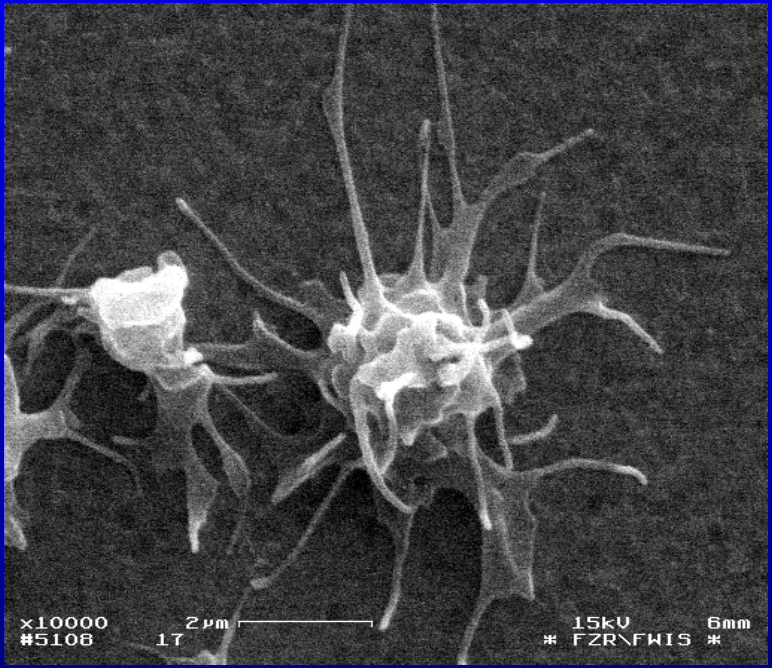
Complement Cascade

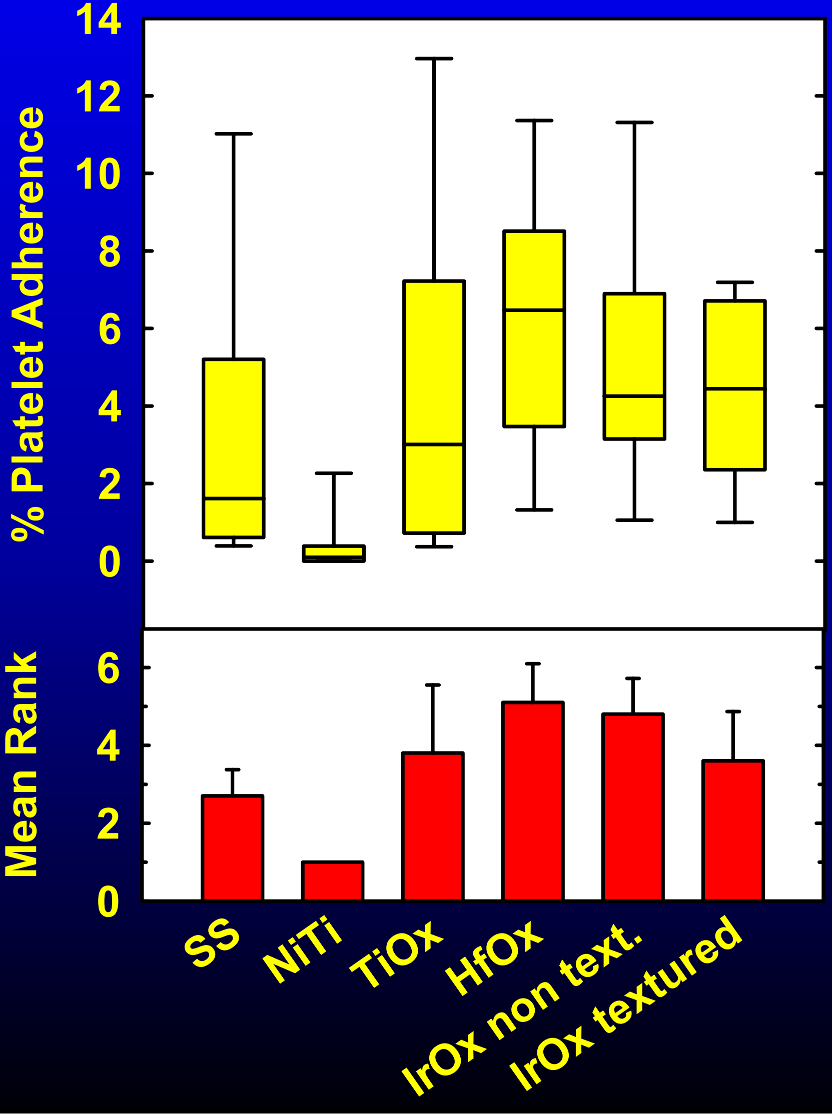
Terminal Attack Complex SC5b-9 of the Complement Cascade

- High activation on non-textured IrO_x
- Low activation on other metal oxides



Blood Platelet Adhesion

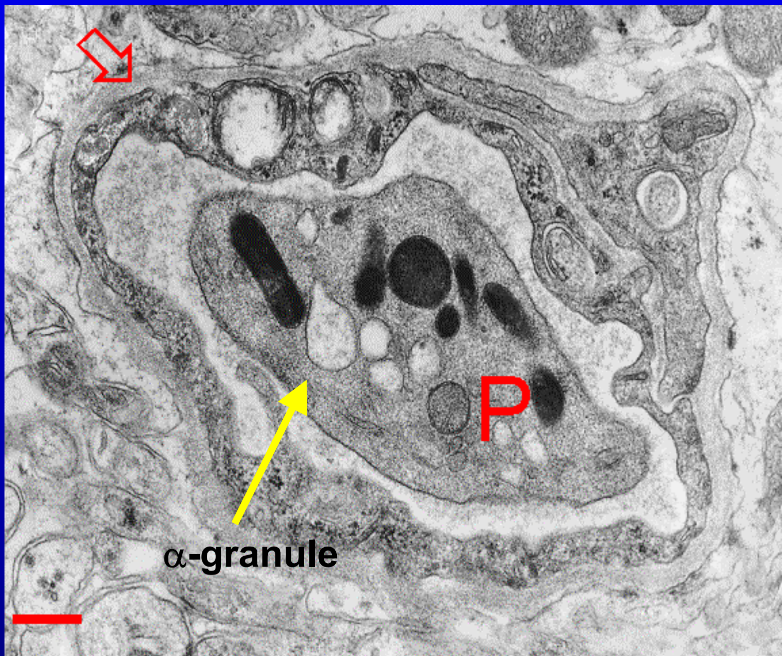


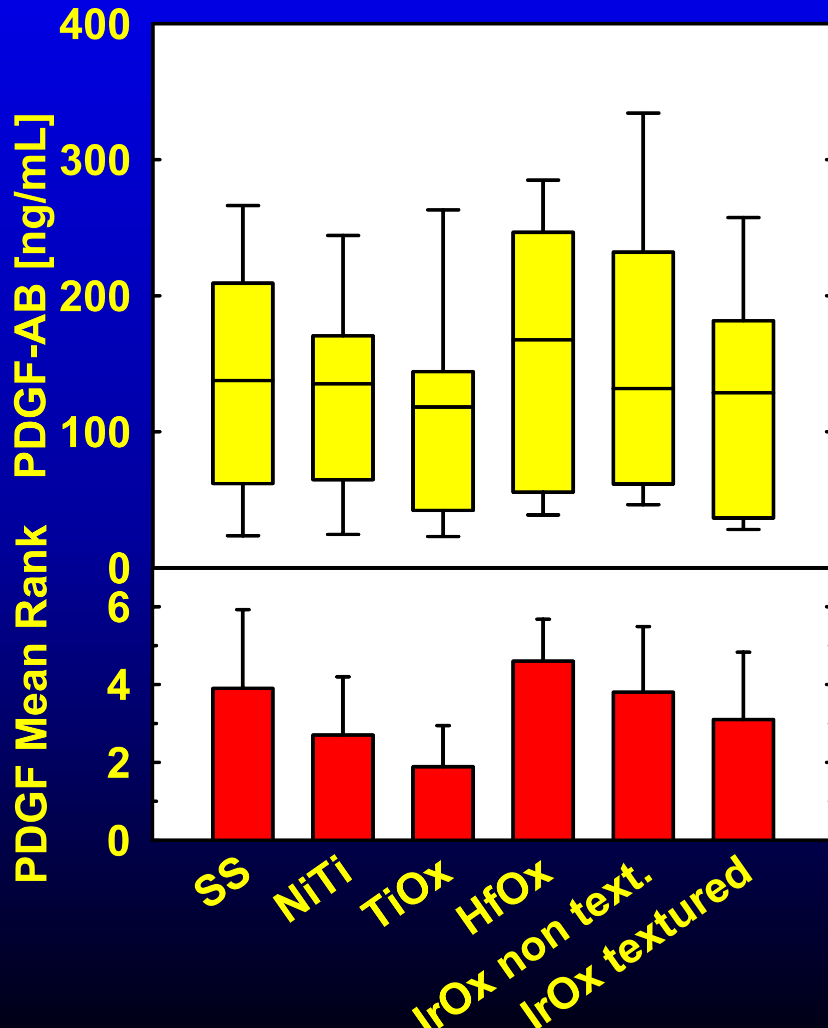


Blood platelet adherence on a surface as haemostatic principle

- Generally lower adherence on bare metal with natural oxide layer

Platelet Activation – PDGF-Release





PDGF-AB release from α-granules of blood platelets

- Minor differences
- Good ranks for Nitinol and TiO_x

Platelet Activation – Serotonin Release

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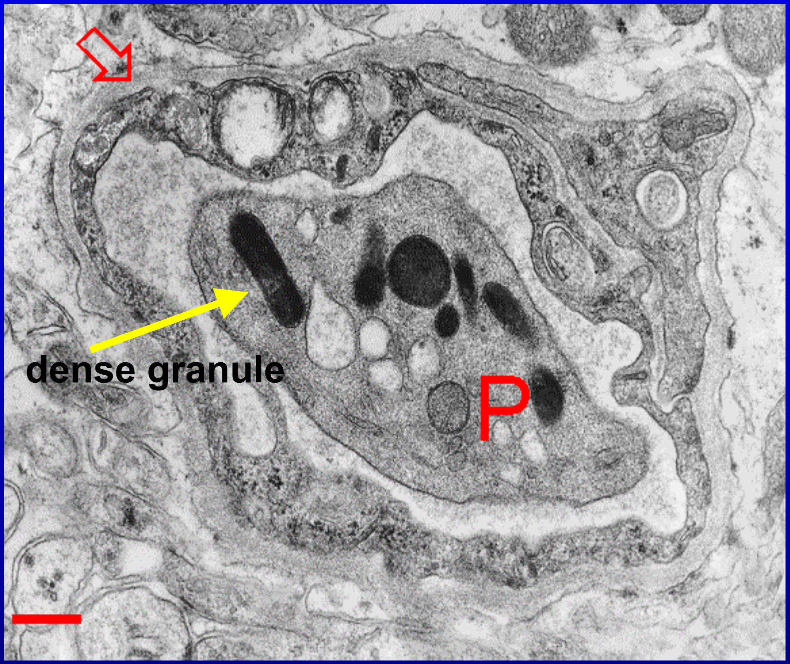

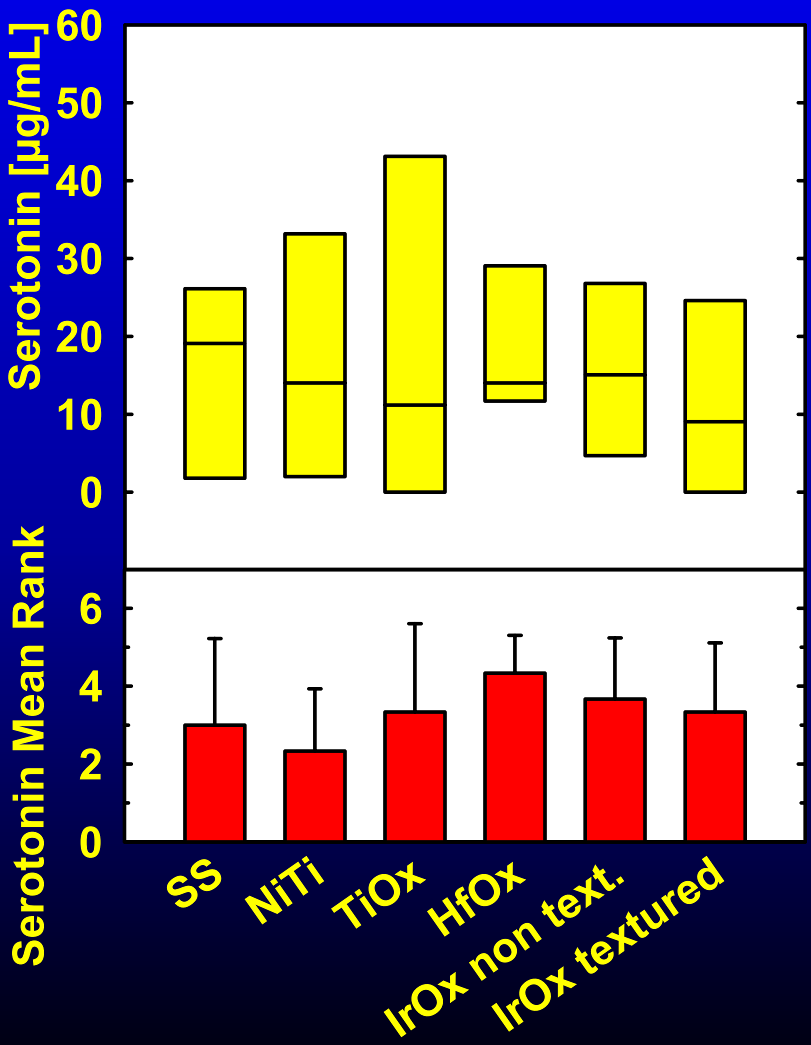

Serotonin release from dense granules of blood platelets

- Minor differences
- Similar trends as PDGF-AB

Rank Overview

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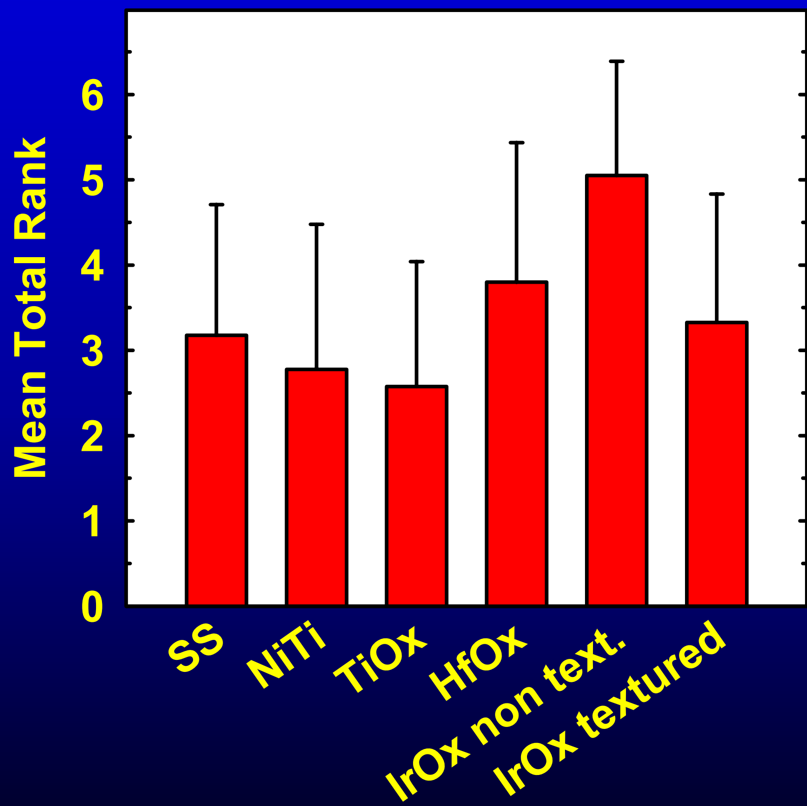
Averaged Rank of

Two platelet parameters

- Platelet Adherence
- PDGF Release

Two plasma parameters

- F1+2 Fragment
- SC5b-9



- Best overall hemocompatibility for TiO_x and Nitinol with naturally grown TiO_2
- Significantly better hemocompatibility of textured than non-textured IrO_x

Correlation of Physical Parameters with Blood Compatibility

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- No significant correlation between
  - any bio parameter
  - the rank score

  and any physical parameter.

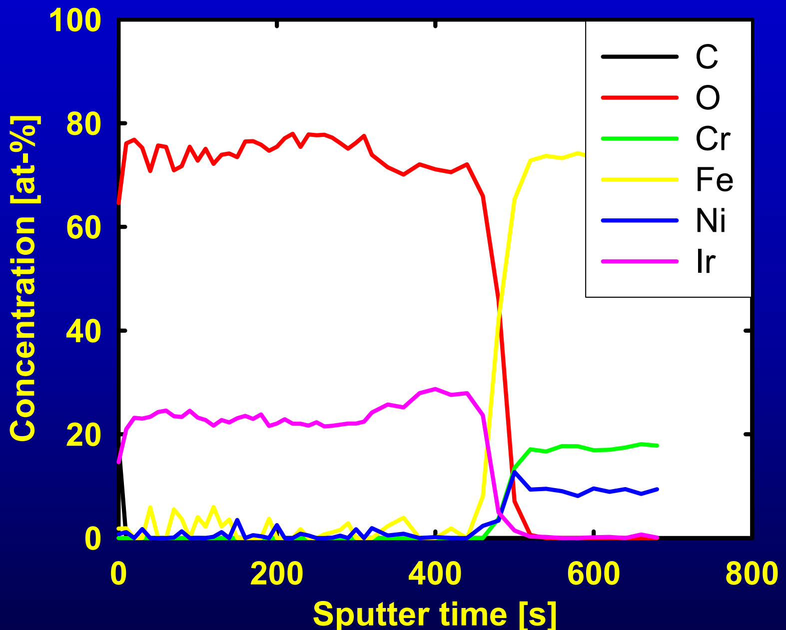
→ Higher Impact of Chemistry?

- Most prominent difference between the smooth non-textured and the textured IrO_x
  - Improved entanglement and activation at the non-textured, multi-oriented IrO_x is discussed
  - Further influences?

Comparison of non-textured and textured IrO_x

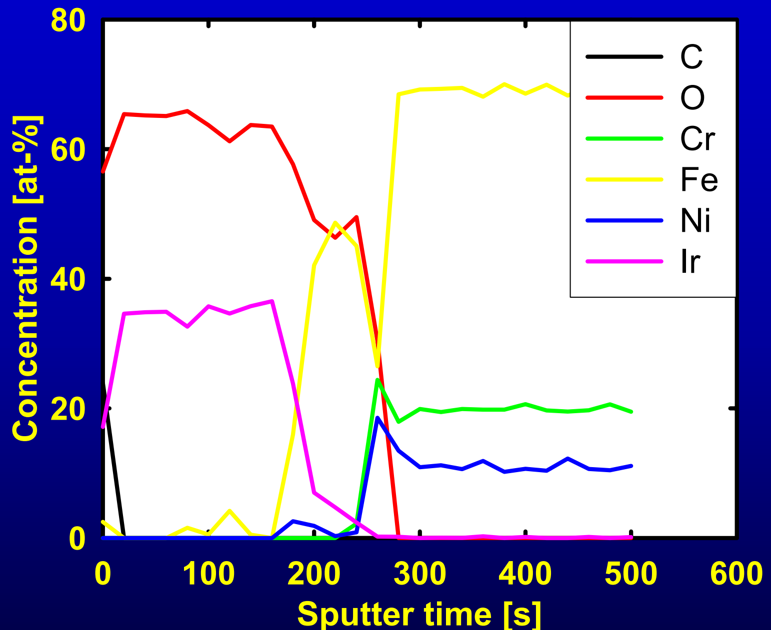
AES Element Profiles

Non-textured IrO_x



Ir : O = 1 : 3

Textured IrO_x



Ir : O = 2 : 3

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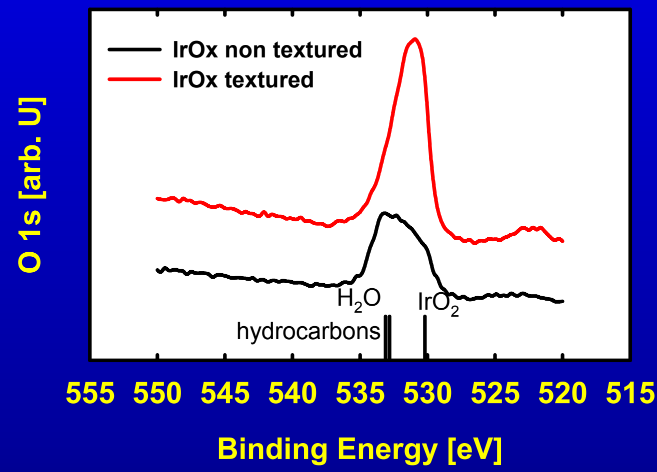
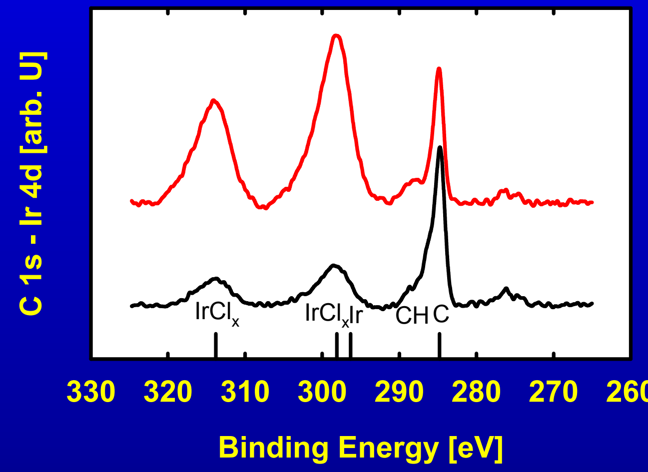
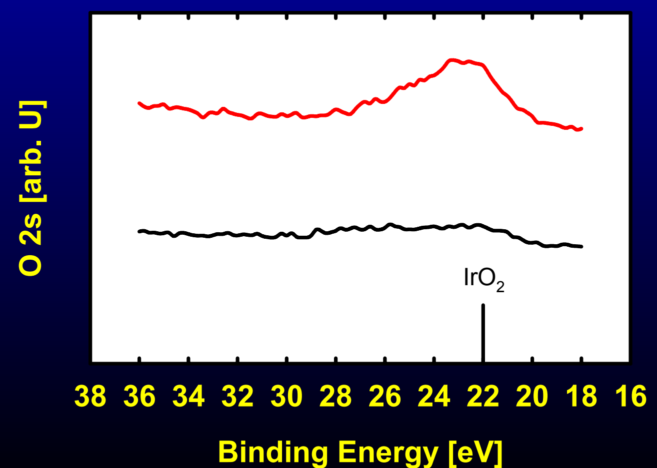
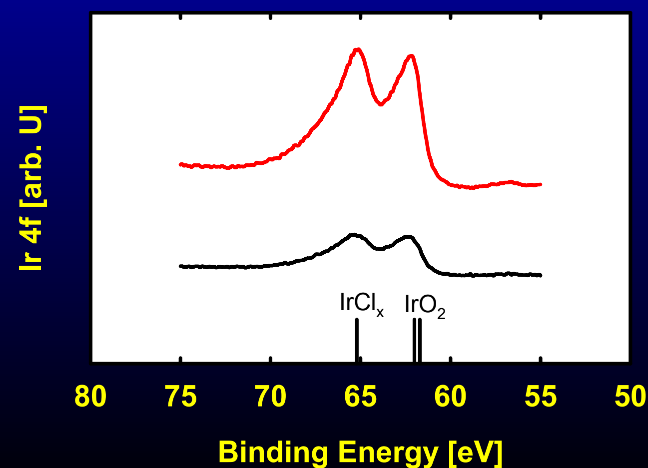
Comparison of non-textured and textured IrO_x

XPS Spectra









Conclusions

- Inside and outside adherent TiO_2 and IrO_2 coatings can be produced on vascular stents
- TiO_2: Slight improve of blood compatibility compared to metal oxides and bare stainless steel
- Higher activation of plasmatic cascades on non textured IrO_x than on textured
  - Unclear whether direct consequences of crystallinity or indirect due to increased adsorption of other material.

Achnowledgement

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