The hemolysis test as a simple tool to investigate the interaction between membranes and materials

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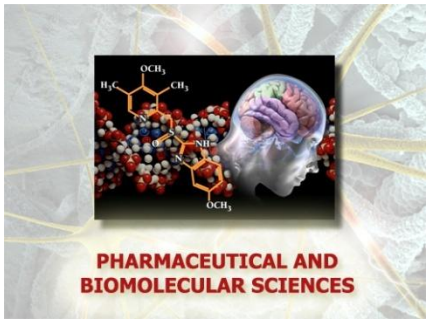


Hemolysis Test (RBC lysis)

One of the 1st assays to detect **particle toxicity**

Hæmolytic Activity of Colloidal Silica

COLLOIDAL silica is highly toxic when administered parenterally[1,2]. In isolated biological systems silica

Harley and Margolis, 1961*, Nature*

Haemolytic Activity of Suspensions of Different Silica Modifications and Inert Dusts

Stalder and Stöber, 1965*, Nature*

1. Hemocompatibility of materials for biomedical applications and consumer products

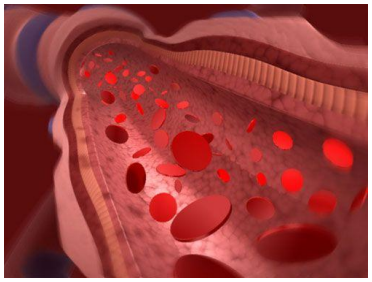
NPs: mesoporous silica, carbon nanotubes, titania/titanium

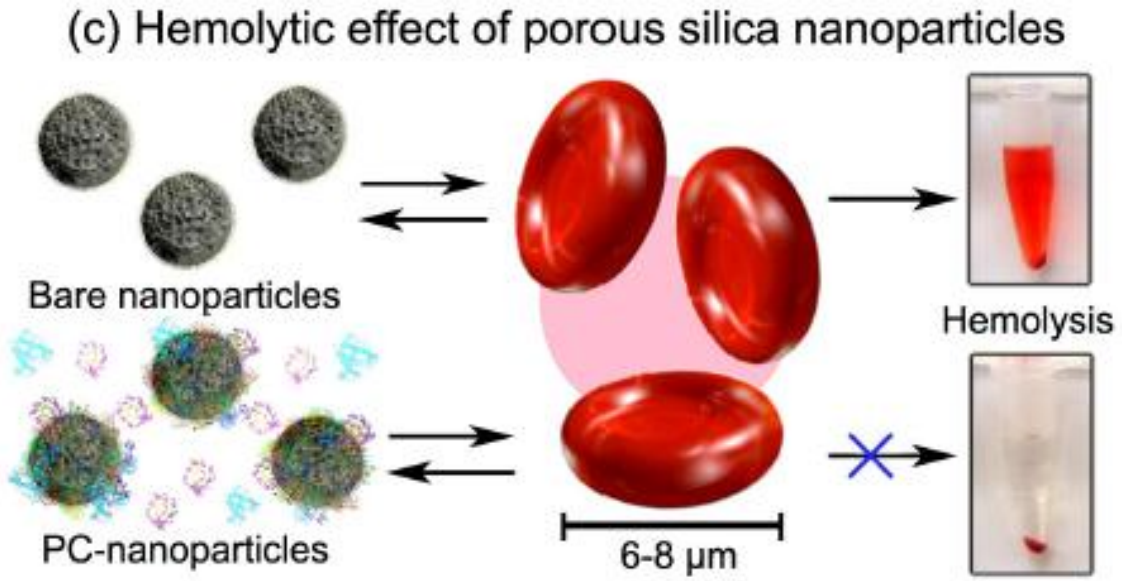
2. Toxicology of inhaled particles/fibres – occupational and environmental exposure

quartz, asbestos, coal, ambient particles, NPs

Occupational Safety & Health Administration – US Dep. of Labor

Hemolysis Test (RBC lysis) – The case of MATERIALS for BIOMEDICAL APPLICATIONS



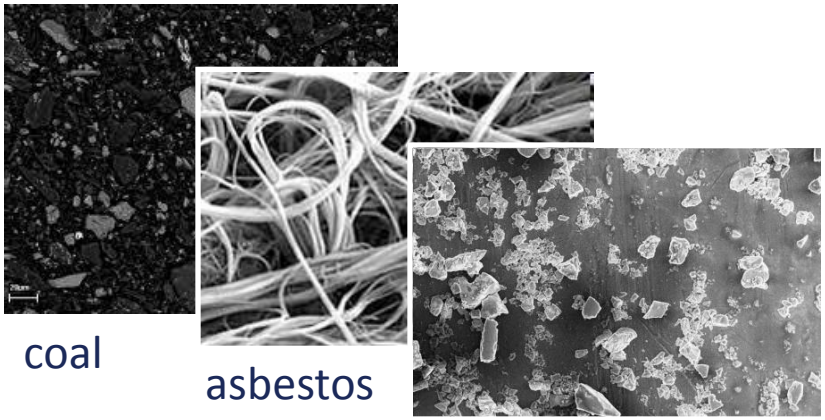


Paula et al., 2012

✓ Protein corona (plasma): hemolysis abolished

✓ Hemolysis: direct contact Particle - RBC

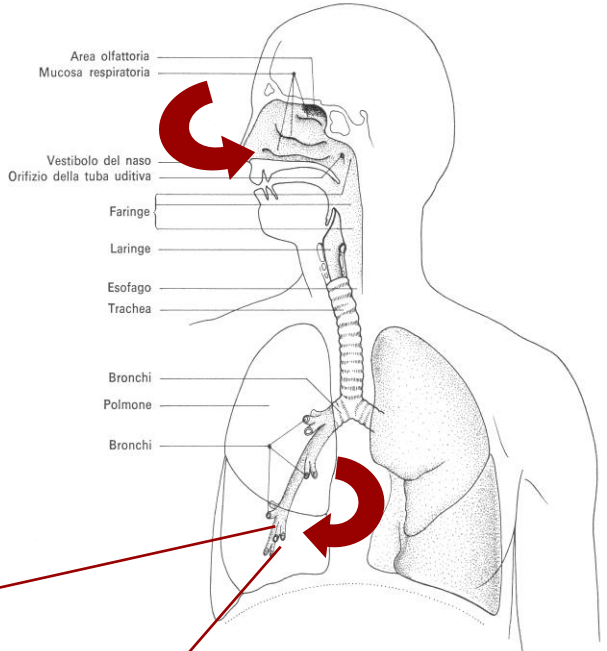
Hemolysis Test (RBC lysis) – The case of TOXICOLOGY OF INHALED PARTICLES/FIBRES



coal

asbestos

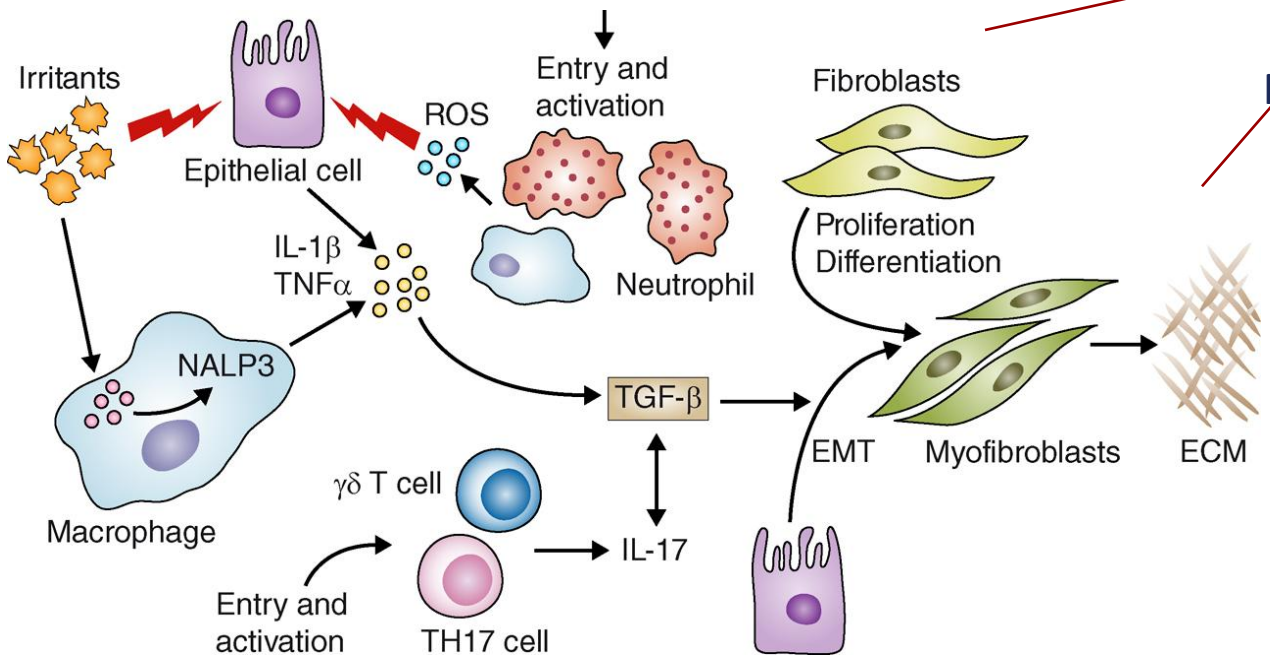
quartz



Pneumoconiosis (fibrosis)

Lung cancer

Mechanism of pulmonary fibrosis



RBC lysis ?

Wynn et al., 2011

Hemolysis Test (RBC lysis) – The case of TOXICOLOGY OF INHALED PARTICLES/FIBRES

Cho et al., 2013

Lu et al., 2009

Metal Oxide NPs

In vitro cell assays → *In vivo* lung inflammogenicity

More predictive?

Dose metric: surface area

Table 4 Comparison of *in vivo* lung inflammation data with *in vitro* assay data based on any dose ever

	Rat (150 cm^2/rat)	A549		16-HBE		THP-1		Alveolar mac		Differentiated PBMC			Haemolysis
	granulocytes	cytotox[b]	IL-8	cytotox	IL-8	cytotox	IL-1β	cytotox	IL-1β	cytotox	IL-1β	TNF-α	% haemolysis
CeO_2	+	-	-	-	+	-	-	+	-	-	-	-	+
TiO_2-rutile	-	-	-										
CB	-	-	-										
SiO_2	-	-	-										
NiO	+	-	-										
Co_3O_4	+	-	-										
Cr_2O_3	-	+	-										
ZnO	+	+	+										
CuO	+	+	+										
Accuracy		5/9	6/9	6									
False positive		1/9	0/9	0									
False negative		3/9	3/9	3									

Cho et al., 2013

Lu et al., 2009

Table 4

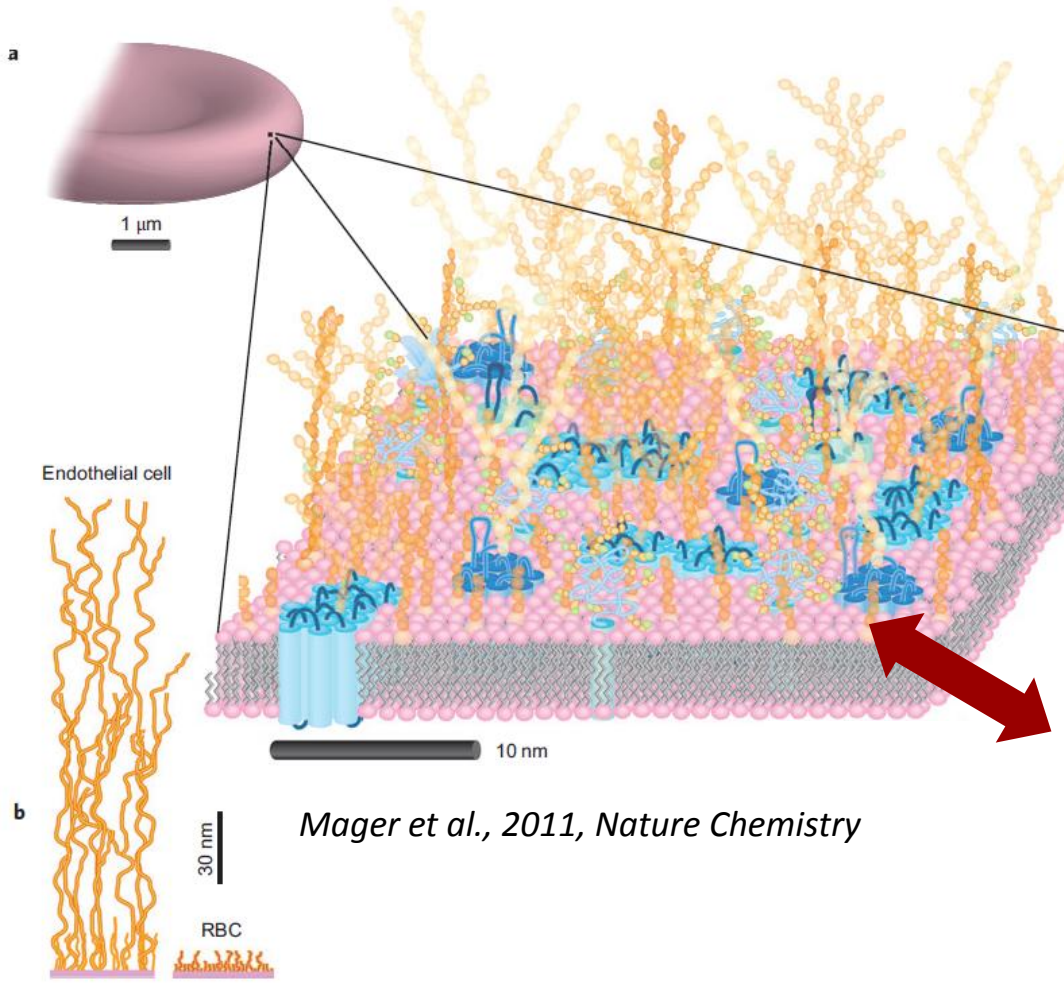
Efficacy of the various *in vitro* assays for predicting the inflammogenicity of metal ox… NPs.

In vitro assay	Total no. of NPs used in the assay	No. (%) NPs that were correctly predicted as inflammogenic/Non-inflammogenic	No. of NPs that were significantly positive in this assay	No.false positive (%)	No. false negativ (%)
Hemolysis	13	12 (92)	3	1 (8)	0 (0)
EPR	13	9 (69)	4	3 (23)	1 (8)
DCFH	13	10 (77)	3	2 (15)	1 (8)
Cytotoxicity	13	9 (69)	4	3 (23)	1 (8)

Hemolysis Test (RBC lysis) – The case of TOXICOLOGY OF INHALED PARTICLES/FIBRES

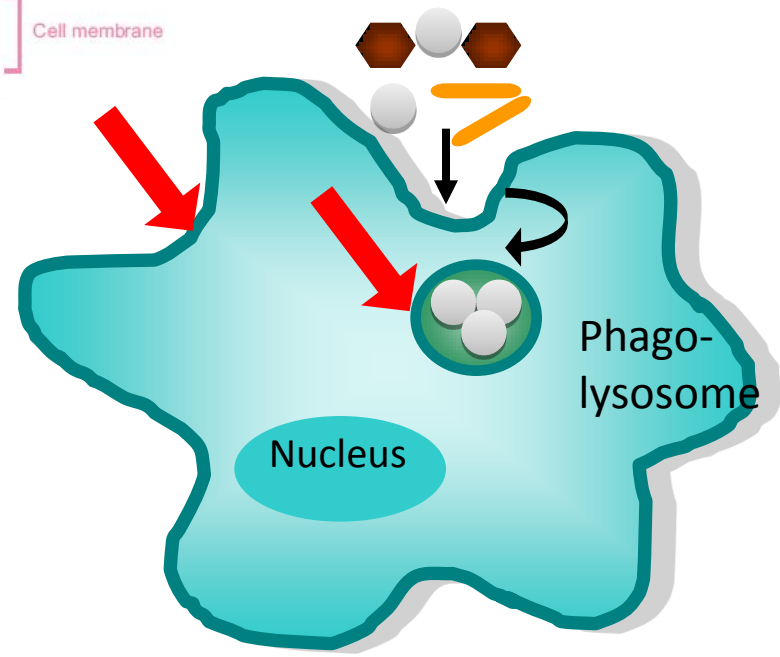
Predictive Value of Hemolytic Activity

Importance of cell membrane



Mager et al., 2011, Nature Chemistry

Silica
Asbestos
MSU
Amyloid polypetides



RBC: Simple and sensitive model of biological membranes

Macrophage

Hemolysis Test (RBC lysis) – The case of TOXICOLOGY OF INHALED PARTICLES/FIBRES

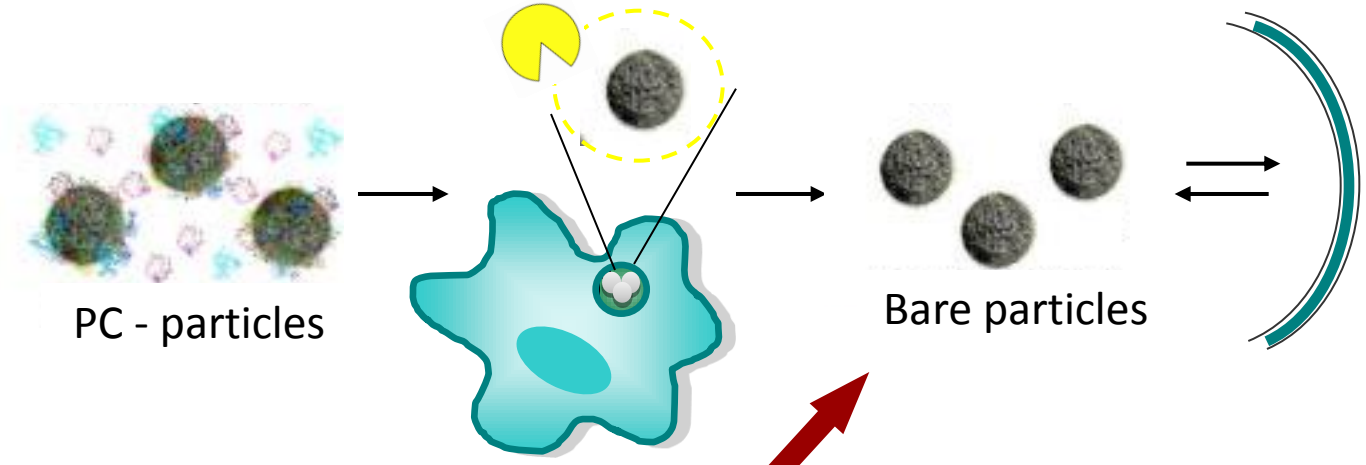
Predictive Value of Hemolytic Activity

Particles in phagolysosomes act as bare

In vivo Inflammation

Protein
Corona

Lytic enzymes

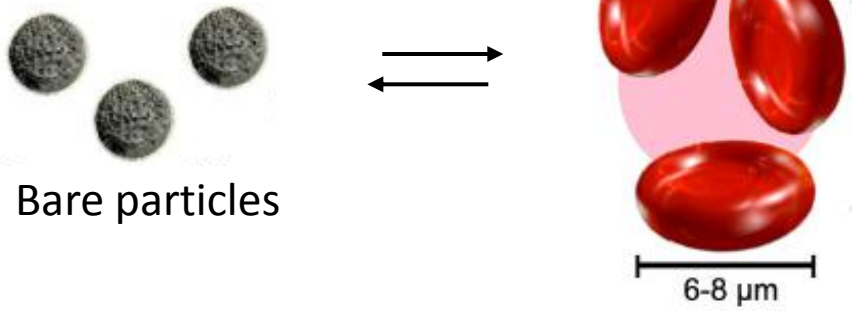


PC - particles

Bare particles

Hemolysis Assay

PBS, NaCl
(serum free)

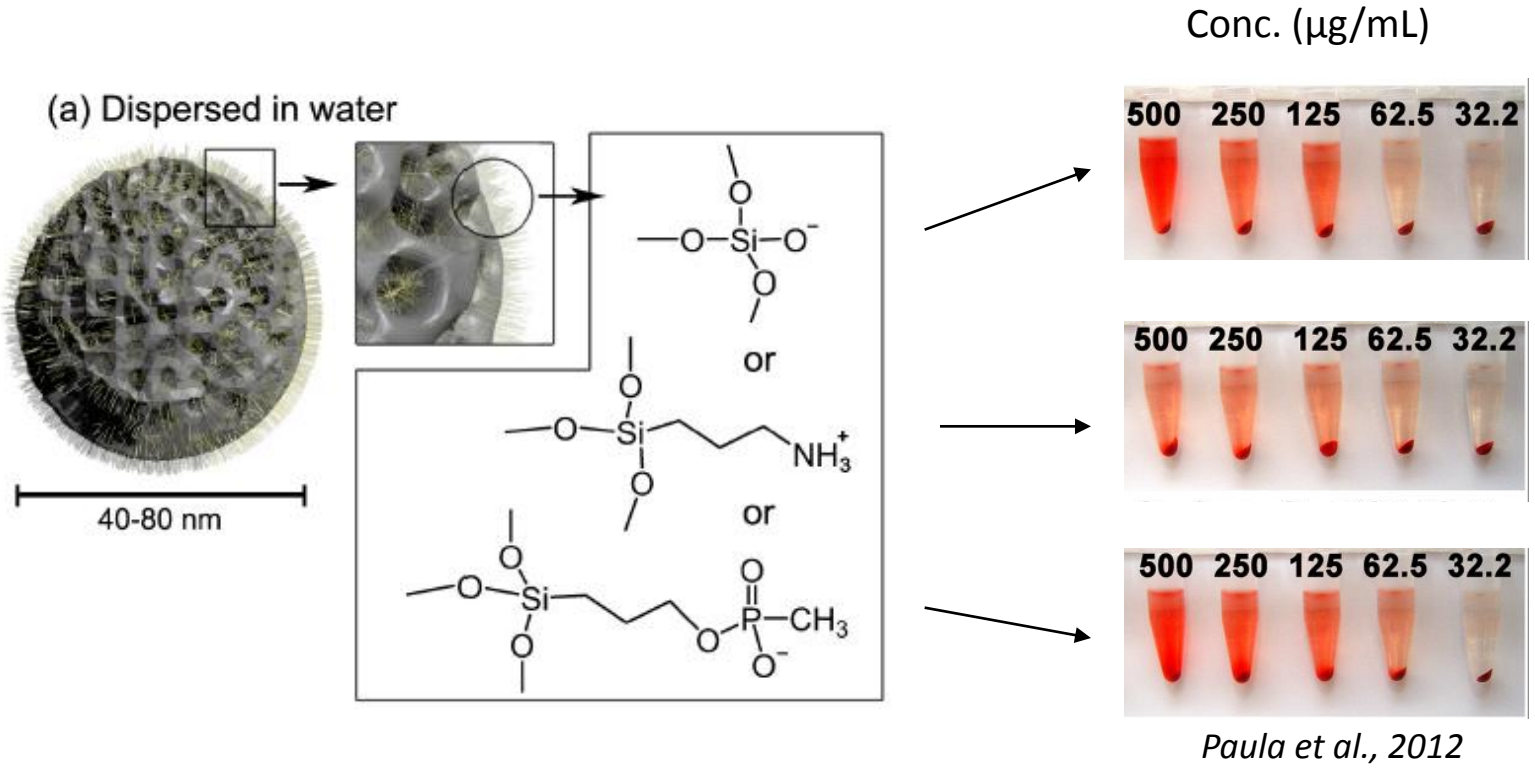


Bare particles

Hemolysis Test (RBC lysis) – The case of TOXICOLOGY OF INHALED PARTICLES/FIBRES

Predictive Value of Hemolytic Activity

Importance of surface reactivity



Paula et al., 2012

Same bulk (SiO_2)

Different surface states

Different hemolytic activities

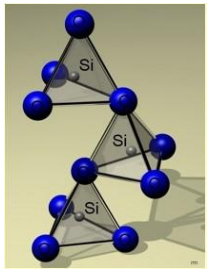
Variability of silica toxicity: role of surface reactivity

Several **surface functionalities**

-SiOH, -Si-O-Si- type and distribution
(hydrofilicity/hydrophobicity)

Surface radicals/ROS/charges
(mechanical grinding)

Charges -SiO^-
(acqueous suspensions)



SiO_2

VARIABILITY

Inherent characteristics
- Crystallinity
- Polymorph
- Size
- morphology

External factors
- Contaminants
- Chemical modifications

- **Natural Ground Crystalline** (quartz)

Well-known toxic:

✓Silicosis

✓Lung cancer
(IARC 1997,2012)

✓Autoimmune diseases

- **Synthetic Amorphous**

Less harmful:

✓transient inflammation

✓No lung fibrosis

Still under study

Properties / Molecular mechanisms governing silica toxicity still unclear

Properties governing SiO_2 toxicity? Systematic study

✓ Collection and preparation of several **SiO_2 samples**

crystalline and amorphous silicas

✓**Surface modifications** (heating, hydrothermal treatments)

✓**Physico-chemical characterization** and **Cell-free tests**

⟶ to identify properties relatable to toxicity

✓ **Cell tests** (*in vitro*):

- Hemolytic activity
- Inflammatory activity

UCL Université catholique de Louvain

LTAP

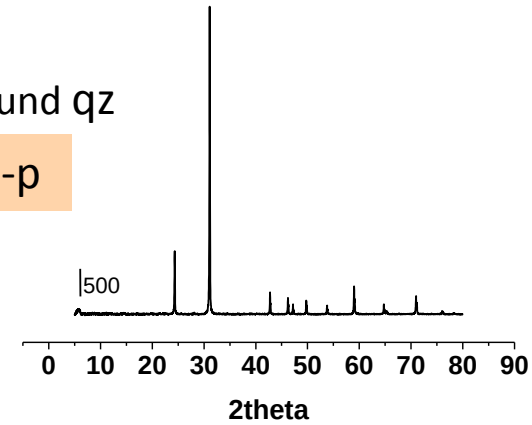
Physico-chemical properties governing SiO_2 hemolytic activity?

Cristallinity: not a prerequisite

Same Origin

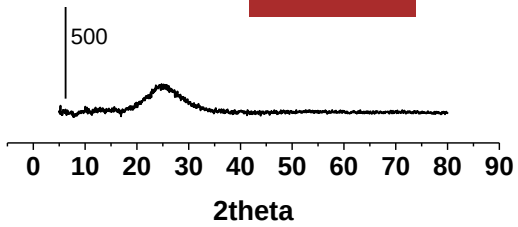
Pure ground qz

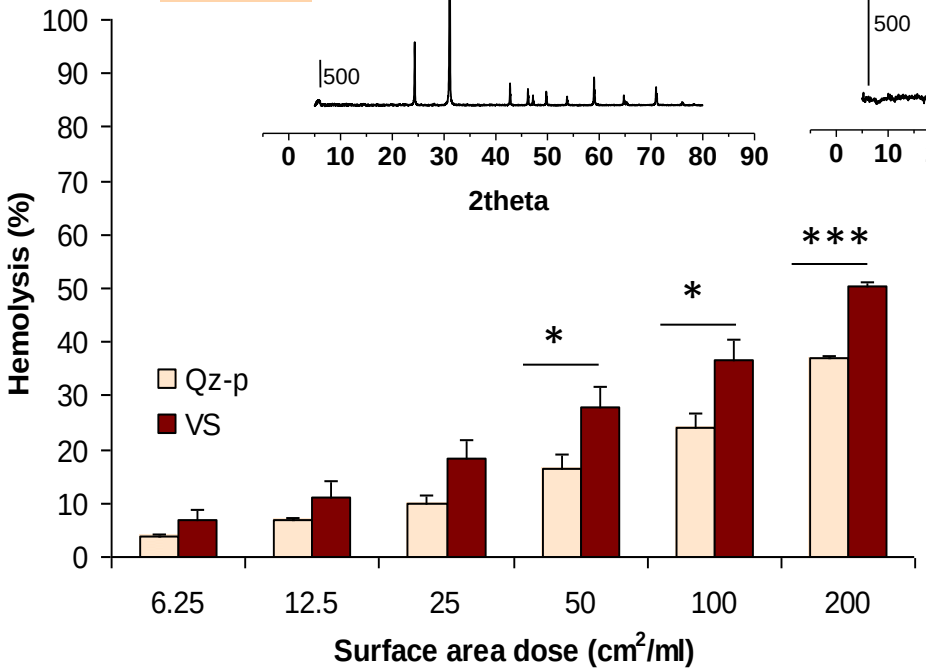
Qz-p



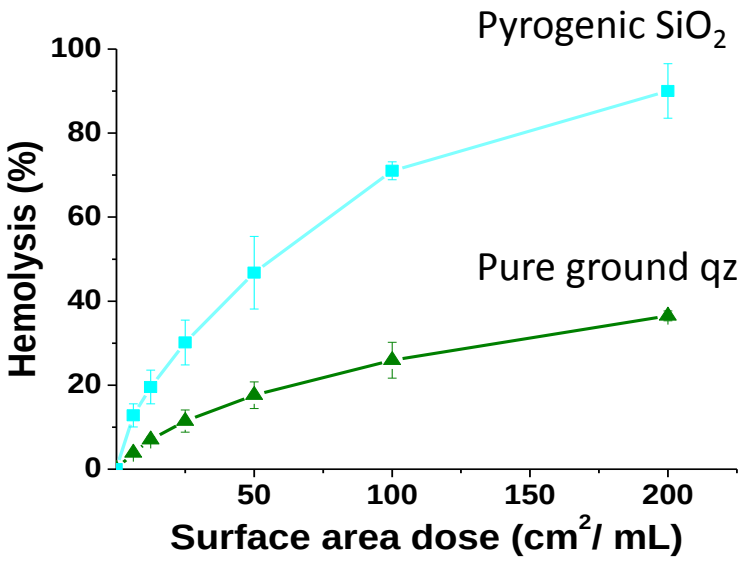
Pure ground vitreous silica

VS





Different Origin



Crystallinity determinant of pathogenicity but not of RBC lysis

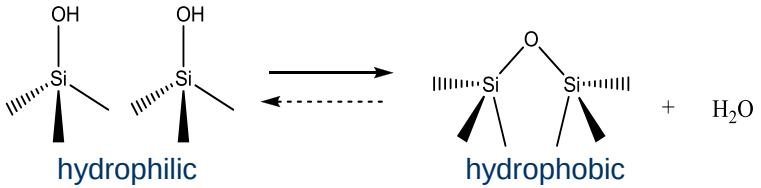
Physico-chemical properties governing SiO_2 hemolytic activity?

Crucial role of silanol/siloxane distribution

Pyrogenic SiO_2 (Aerosil 50)

Heating

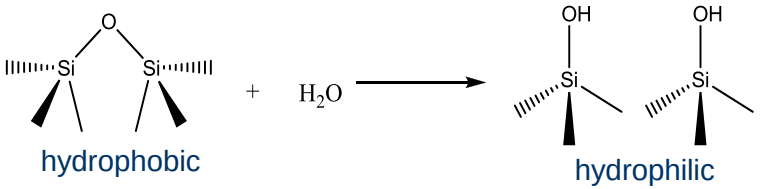
(heating in vacuum at 800°, 1000°C)

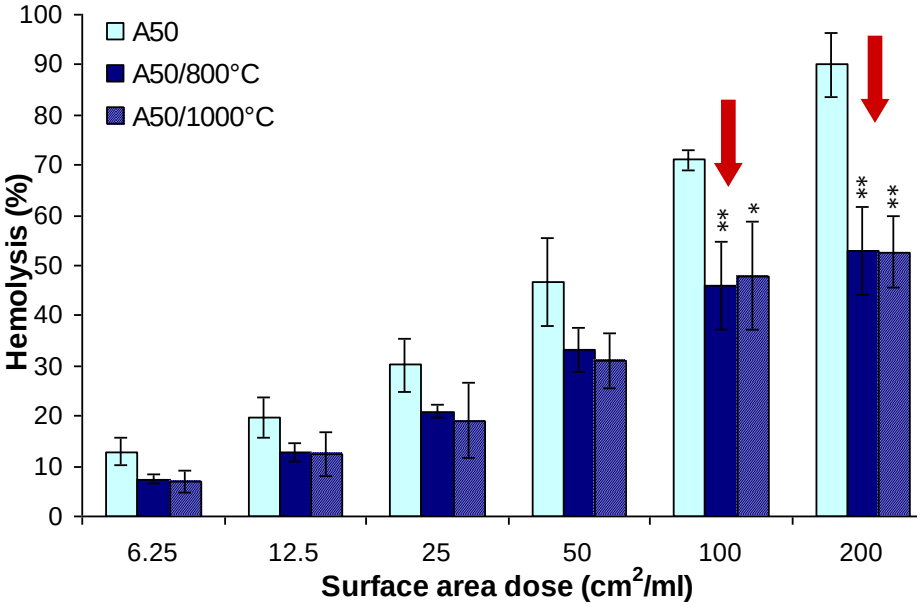


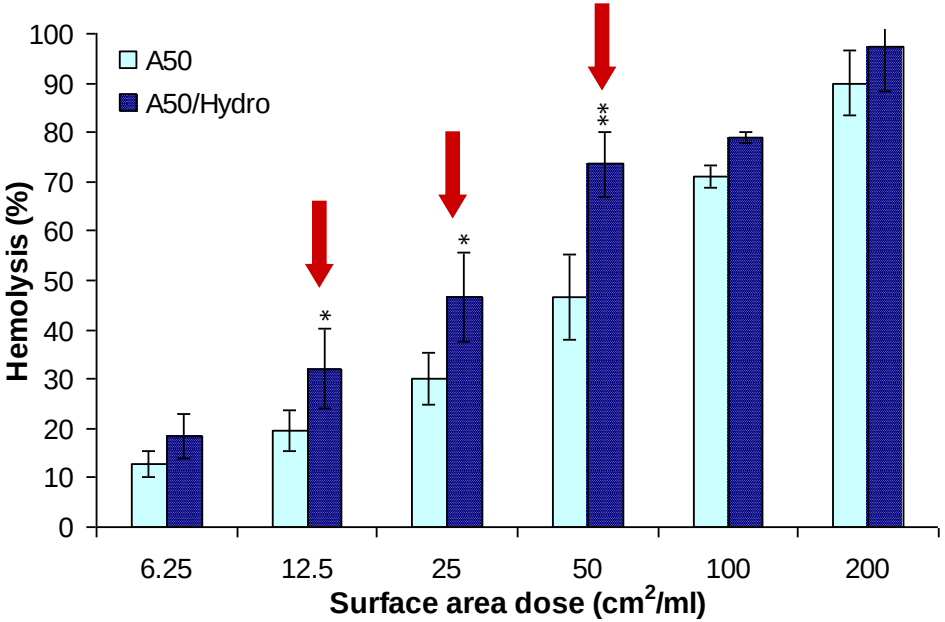
vs

Hydrothermal treatment

(in H_20, heated in autoclave at 230°C for 4h)







Silanols reduced
Hemolysis reduced

Silanols increased
Hemolysis increased

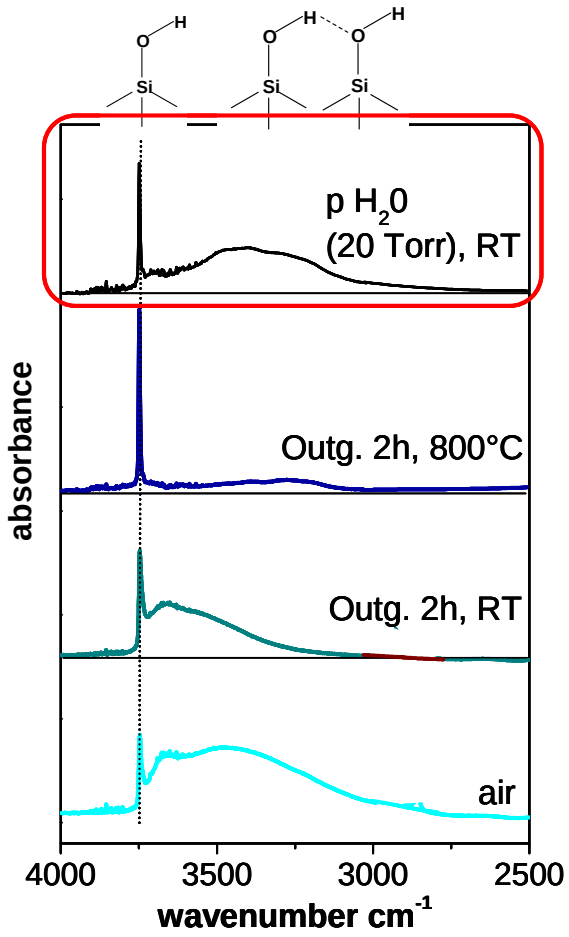
Is hemolysis merely relatable to the number of silanols?

Physico-chemical properties governing SiO_2 hemolytic activity?

Crucial role of silanol/siloxane distribution

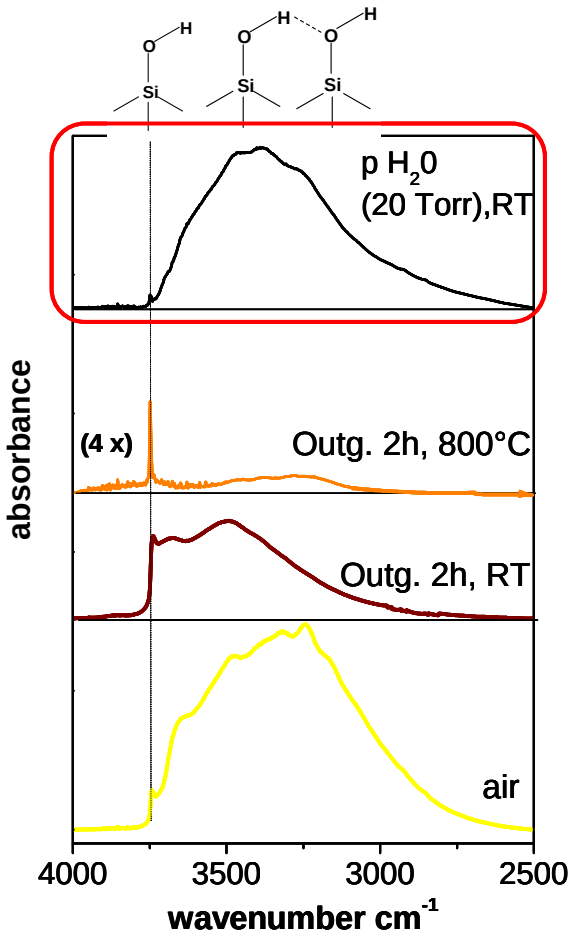
Surface silanol population as revealed by FT-IR spectroscopy

Pyrogenic SiO_2 (Aerosil 50)



Hydrophilic/hydrophobic

Precipitated SiO_2 (FK320)

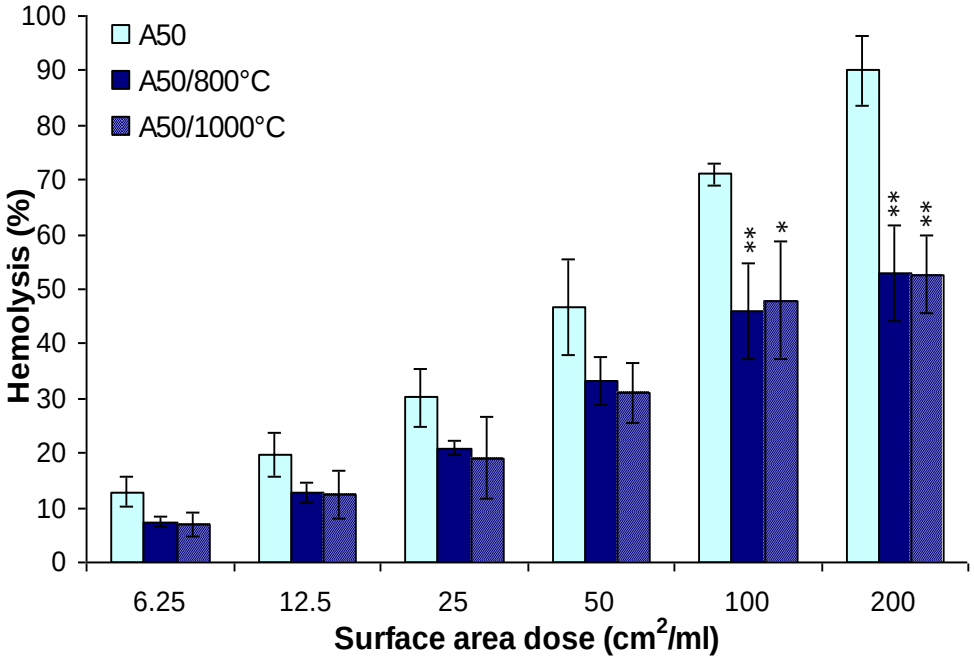


Fully hydrophilic

Physico-chemical properties governing SiO_2 hemolytic activity?

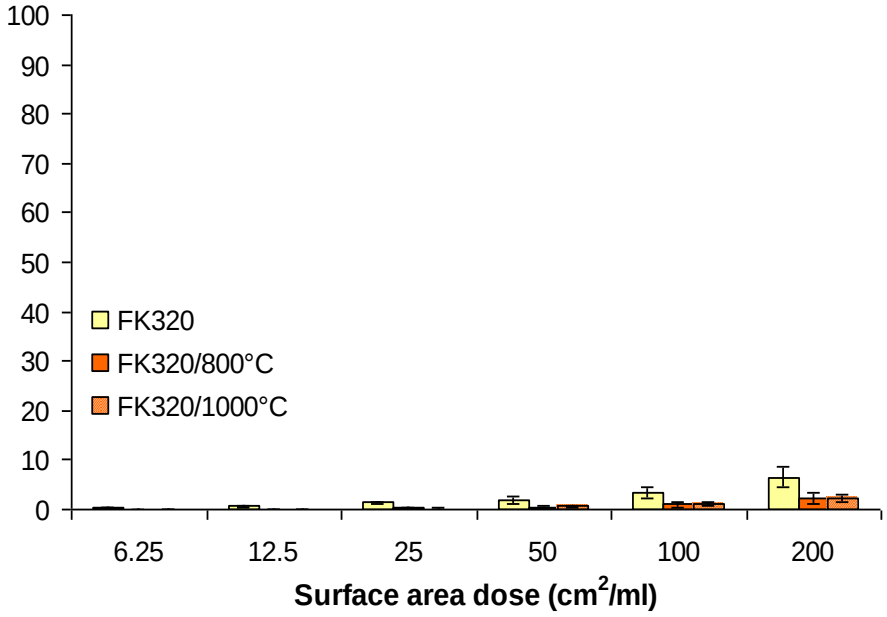
Crucial role of silanol/siloxane distribution

Pyrogenic SiO_2 (Aerosil 50)



Hydrophilic/hydrophobic
Strongly hemolytic

Precipitated SiO_2 (FK320)



Fully hydrophilic
Non hemolytic

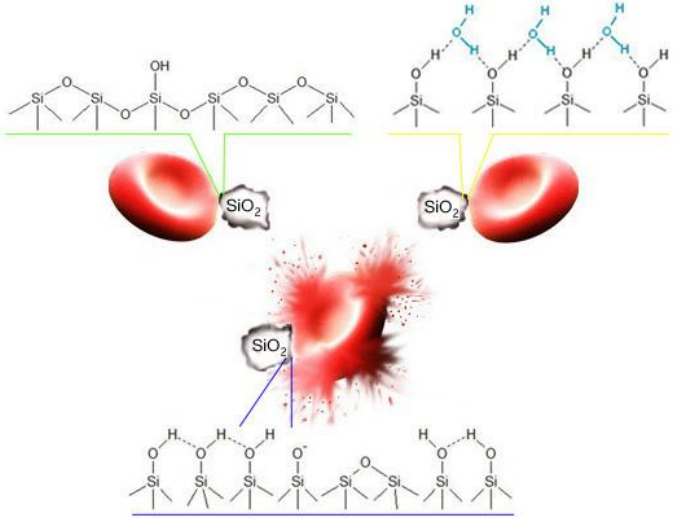
Not whole number of silanols, but a specific arrangement of silanols, silanolates and siloxanes causes hemolysis

Conclusions

✓ **Silanols** : main determinant SiO_2 hemolytic activity

✓ ***specific arrangement*** *of silanols/siloxanes*

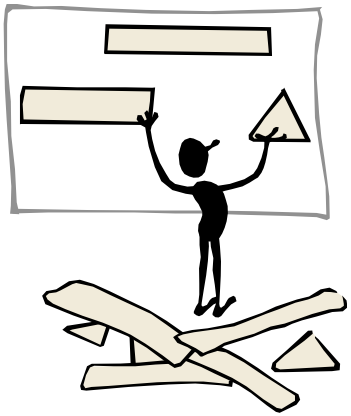
-SiO-H	$-SiO^-$
H-bond	**Electrostatic inter.**
membrane proteins phospholipids	phosphatidylcholines sphingomyelins



Pavan et al., 2013

✓ **Amorphous** SiO_2: hemolytic

✓*Cristallinity determinant of fibrosis, not of membranolysis*



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