Die Haut als Barriere für Nanopartikel

Das NANODERM Projekt

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Universität Leipzig

Nanotechnologie,
ihre Produkte und Risiken für den Verbraucher
Expertengespräch im Bundesinstitut für Risikobewertung
Berlin, 28. März 2006

The NANODERM Consortium

Country	P.No.	Participant	Short Name	Skills	Resp	Involved in
D	**1a**	**Butz**	*Uni Leipzig*	Ion Microscopy, Radiotracers	1	1 2 3 5 8
	Sub	Pallon	*Lund*	Ion Microscopy		
D	**1b**	**Sticherling**	*Uni Leipzig*	Biopsies, tissue	6	2 5 6 8
	Sub	van Vaeck	*Antwerpen*	LMMS, S-SIMS		
F	**2**	**Surlève-Baz.**	*UBX1/FDRC*	Electron Microscopy	8	2 4 8
	Sub	UBX I/II				
H	**3**	**Hunyadi**	*UD-MHSC*	Biopsies, cells	7	2 3 6 7 8
	Sub	Kiss	*Debrecen*	WP 7 / Microprobe		
P	**4**	**Pinhero**	*ITN*	Biopsies, tissue	2	2 3 6 8
Pl	**5**	**Z. Stachura**	*IFJ*	Cyclotron, Ion Microscopy	5	1 2 3 5 8
	Sub	Budzanowska	*Krakow*	Biopsies		
Pl	**6**	**J. Stachura**	*Uni Krakow*	Electron Microscopy	4	4 8
F	**7**	**Moretto**	*UBX1/CENBG*	Ion Microscopy	3	3 8

Methods 1

- Tape stripping; quantitative

  This is a "horizontal" technique

  advantages:

  easy and cheap

  disadvantages:

  does not yield quantitative penetration profiles due to furrows and hair follicles

Methods 2

- Confocal Laser Scanning Microscopy

  This can be a "horizontal" technique on explants

  and a "vertical" technique on cross-sections

  advantages:

  yields "3D"-info with "thick" sections and thus reduces preparation artifacts

  disadvantages:

  needs fluorophors; bleaching problems

Methods 3

- HRTEM

  a “vertical” method using ultra-thin cross-sections

  advantages:

  visualize individual particles

  get chemical composition of individual particles

  disadvantages:

  limited field of view (representative ?)

  many preparation steps (artifacts ?)

Methods 4

Ion beam techniques: PIXE, RBS, STIM: “vertical techniques”

PIXE: particle induced X-ray emission yields elemental maps

RBS: Rutherford backscattering spectrometry and

STIM: scanning transmission ion microscopy yield density

advantages:

large field of view

easy preparation

check for preparation artifacts

disadvantages:

cannot visualize individual particles

Methods 5

- Radio-labelling with ^{48}V (positron emitter, $T_{1/2}$ = 16d)

  This is a "vertical" method using thin cross-sections and nuclear microemulsions

  advantages:

  ultra-sensitive

  large field of view

  relatively easy preparation

  see individual positron tracks

  disadvantages:

  cannot visualize individual particles

Materials

Nanoparticles:

Thioveil, P25, Eusolex T-2000

primary particle size: about 20 nm, coated (?)

Formulations:

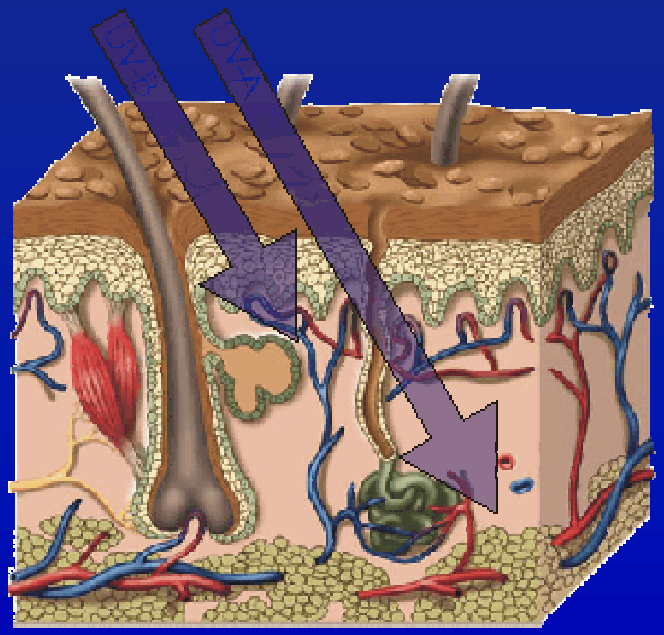
various formulations / gels, various commercial sunscreens

Skin:

porcine skin, mouse pads, human skin transplanted to SCID-mice, healthy human skin biopsies (age, sex, caucasian, coloured) and explants, psoriatic skin

Action of Nanoparticles

Unprotected skin



Reflection and absorption of UV-radiation

Sun screen with nanoparticles

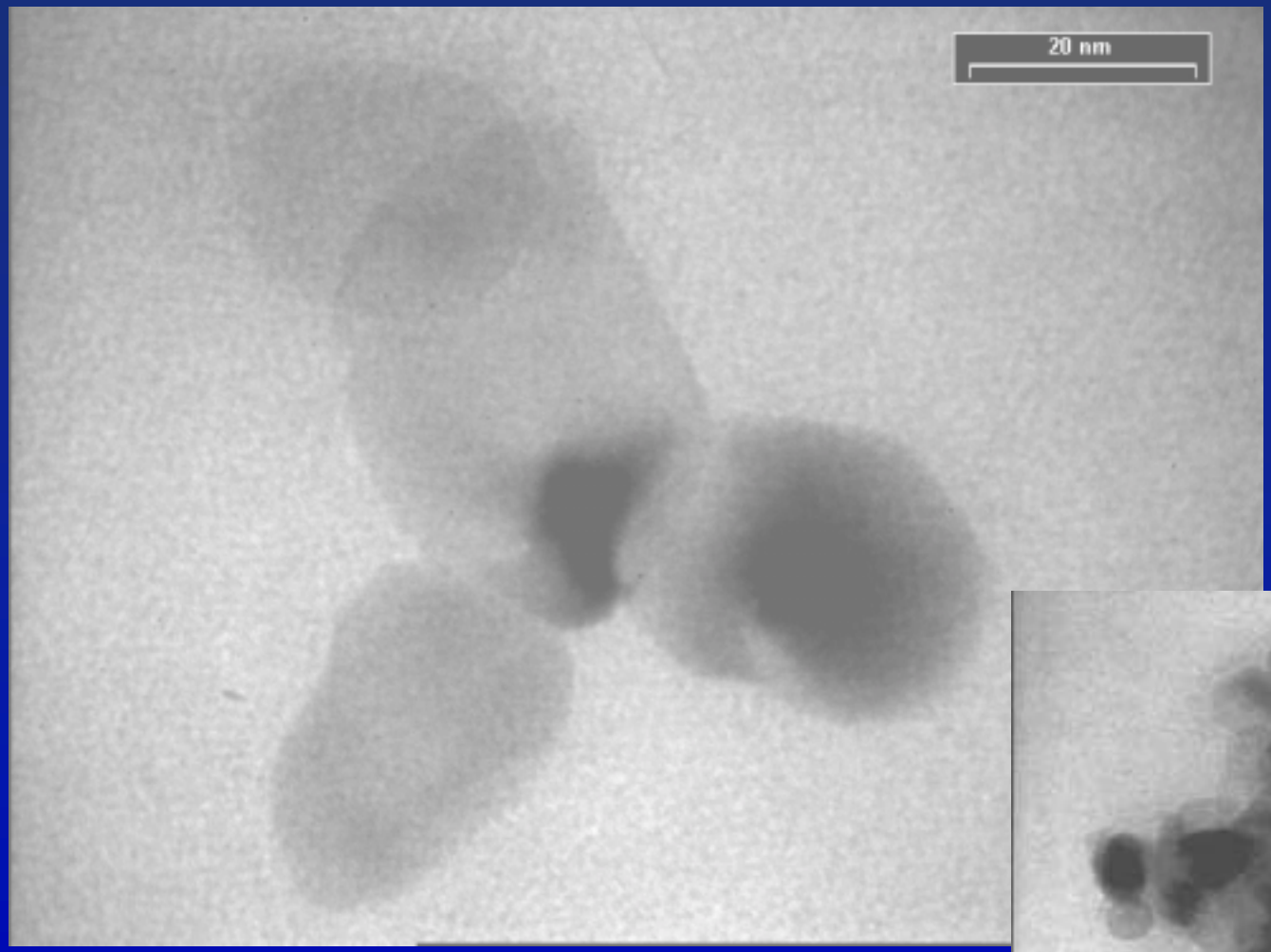
Pig Skin Biopsies

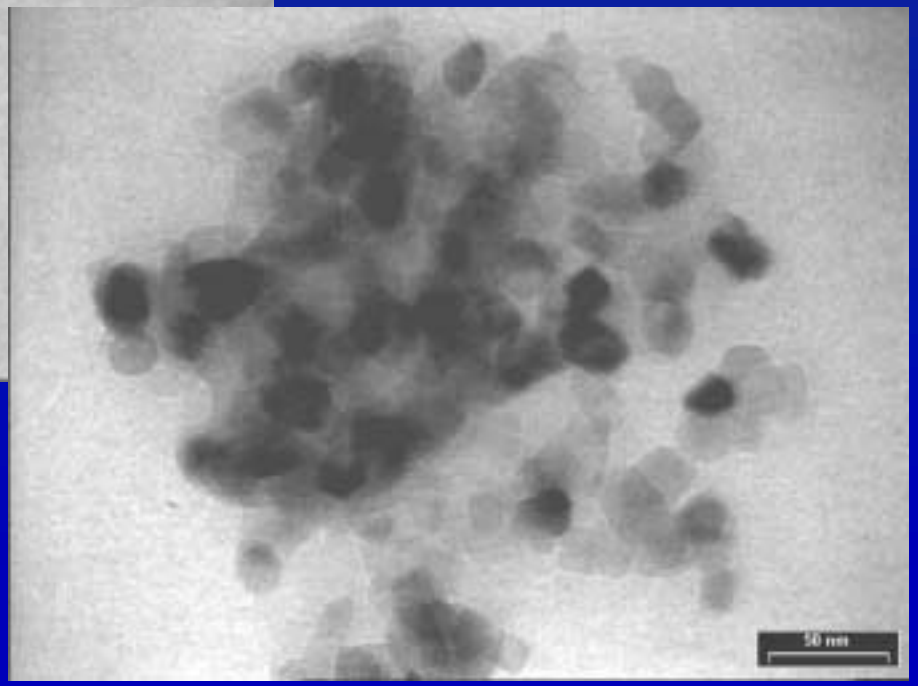
Campaign	1st	2nd	3rd
Areas of Biopsies	neck & back	inner parts of hind legs	inner parts of hind legs
Pretreatment	ethanol cleaned	native	•native •water rinsed •ethanol cleaned •tape stripped (10×)
Application Times (hours)	8, 24, 48	0.25 – 2.25	0.3 – 2.75

areas of biopsy:
inner parts of the hind legs

punch cylinders:
5 mm

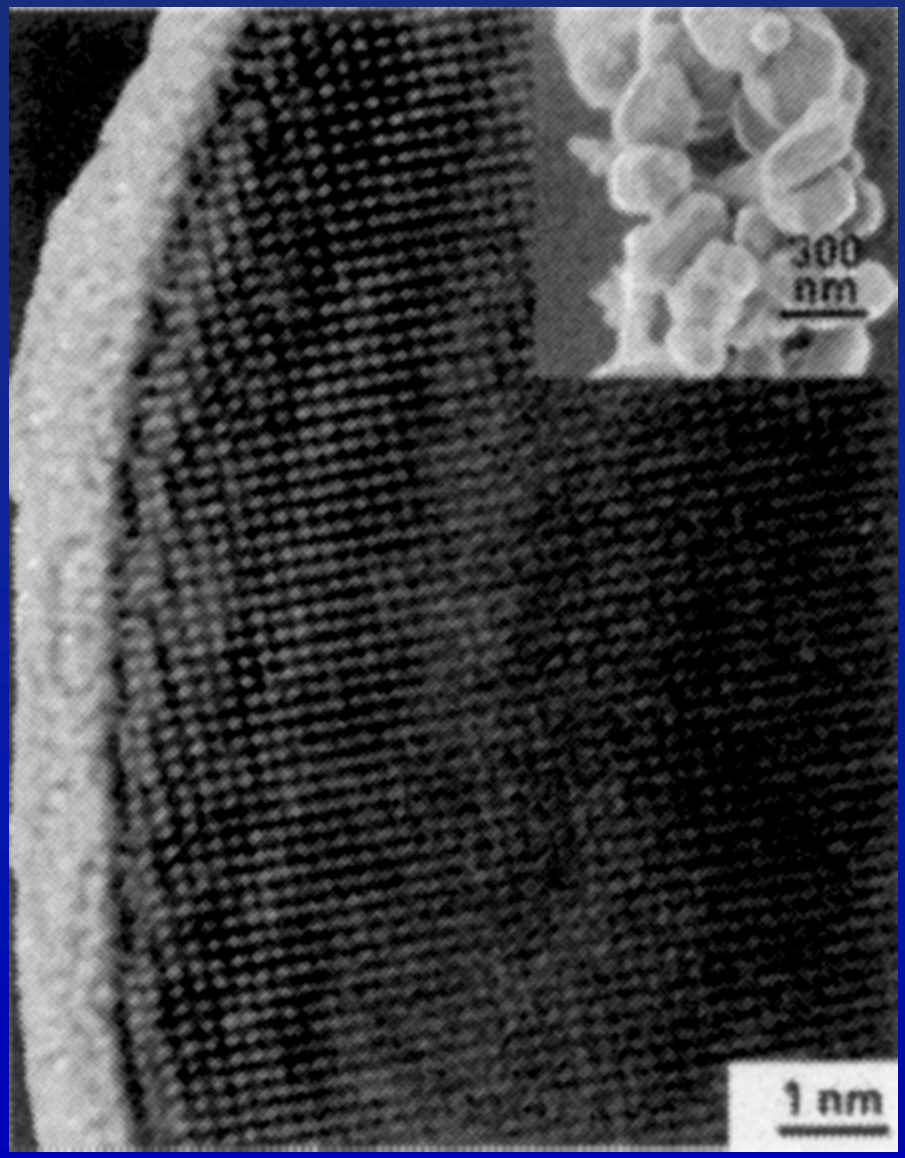
TEM of ashed Eucerin Micropigment Lotion 25





Wagner, Uni Leipzig

Crystalline TiO_2 coated with amorphous silica layer



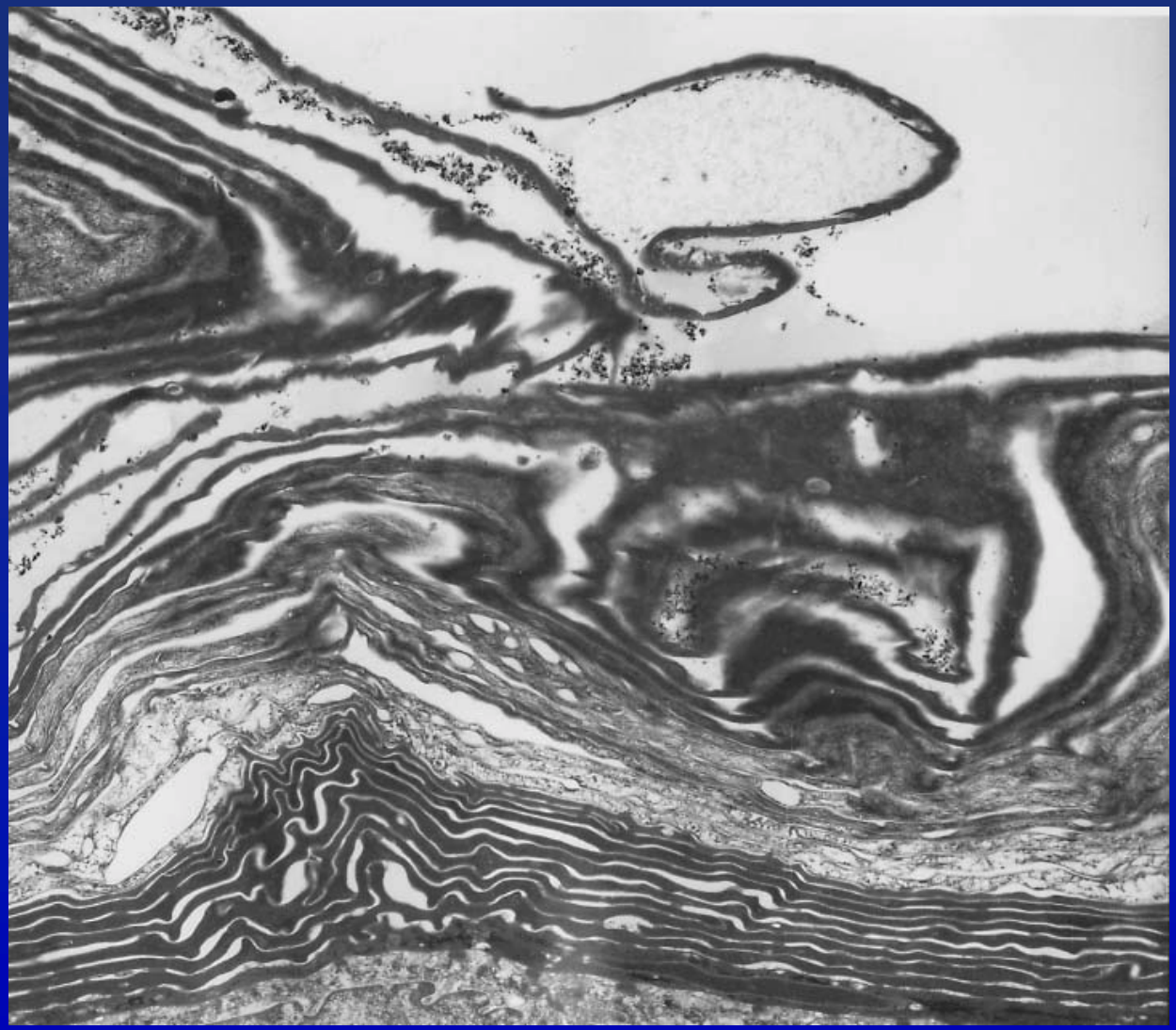
From:

Future Technologies Vol.54

Industrial application of nanomaterials – chances and risks

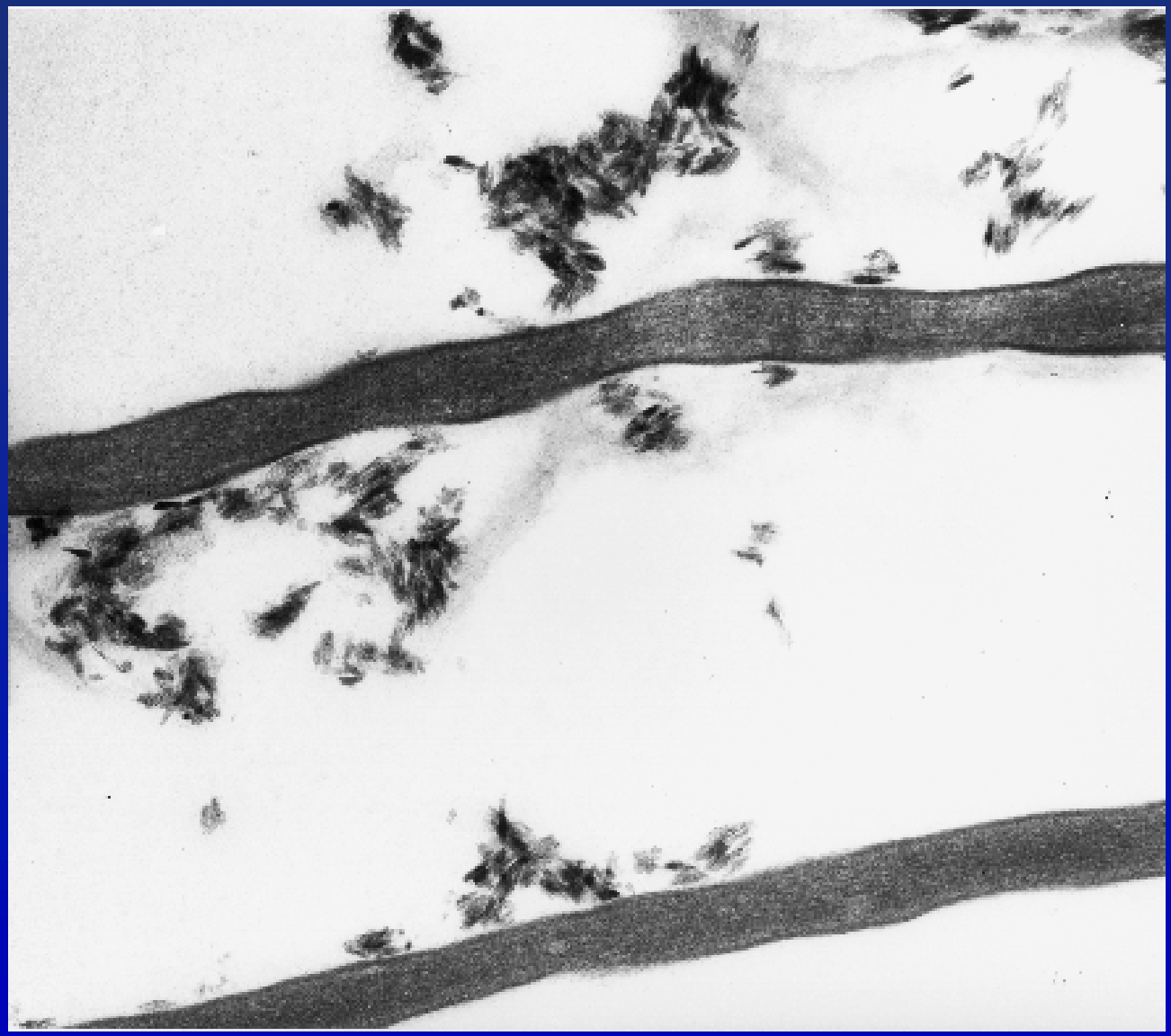
W. Luther (ed.)

HRTEM 1



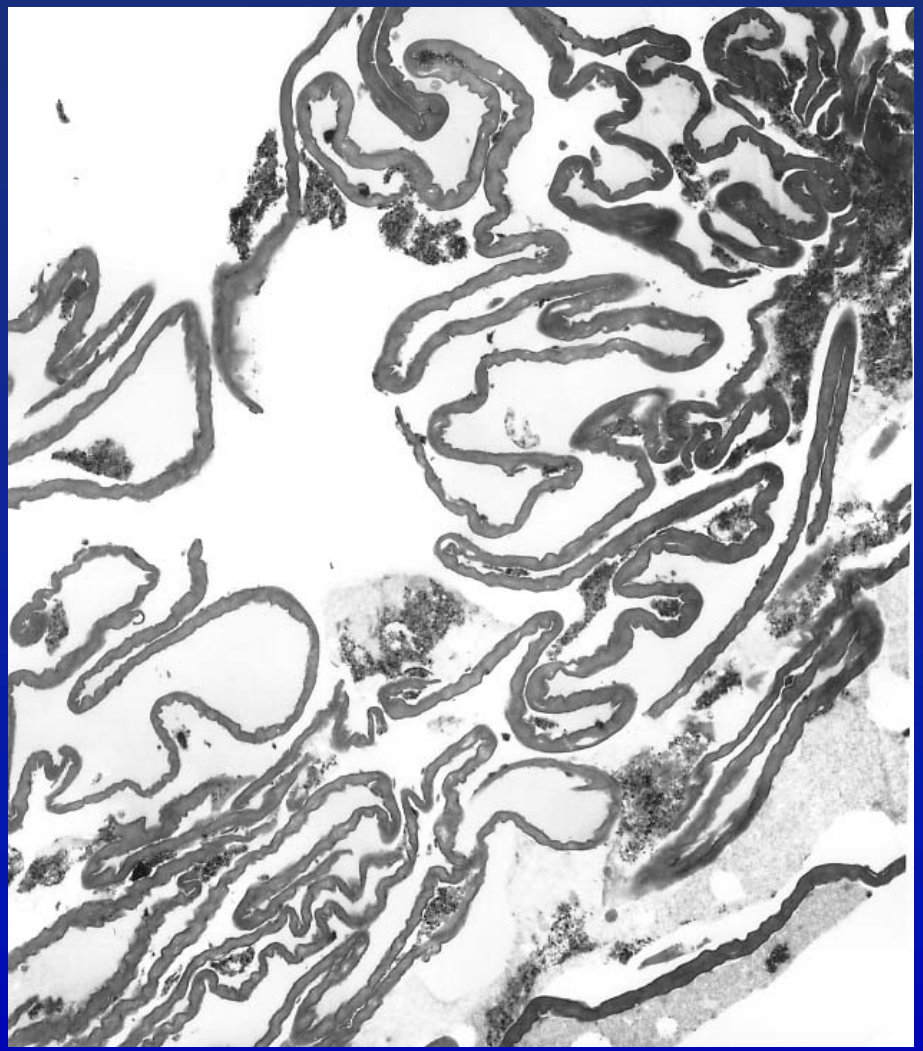
Cross-section through stratum corneum

HRTEM 2



Nanoparticles between sheets of stratum corneum

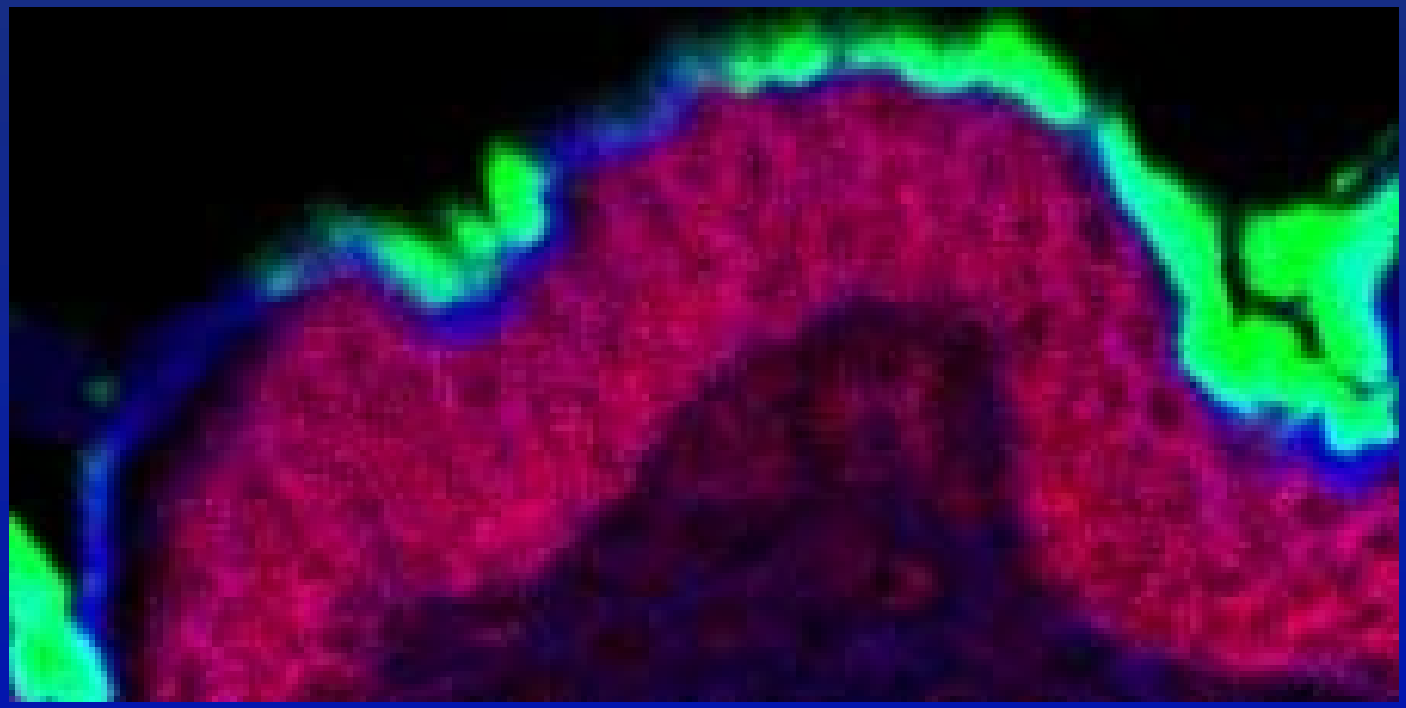
HRTEM 3



Nanoparticles between sheets of stratum corneum disjunctum

PIXE on Porcine Skin Cross-Section

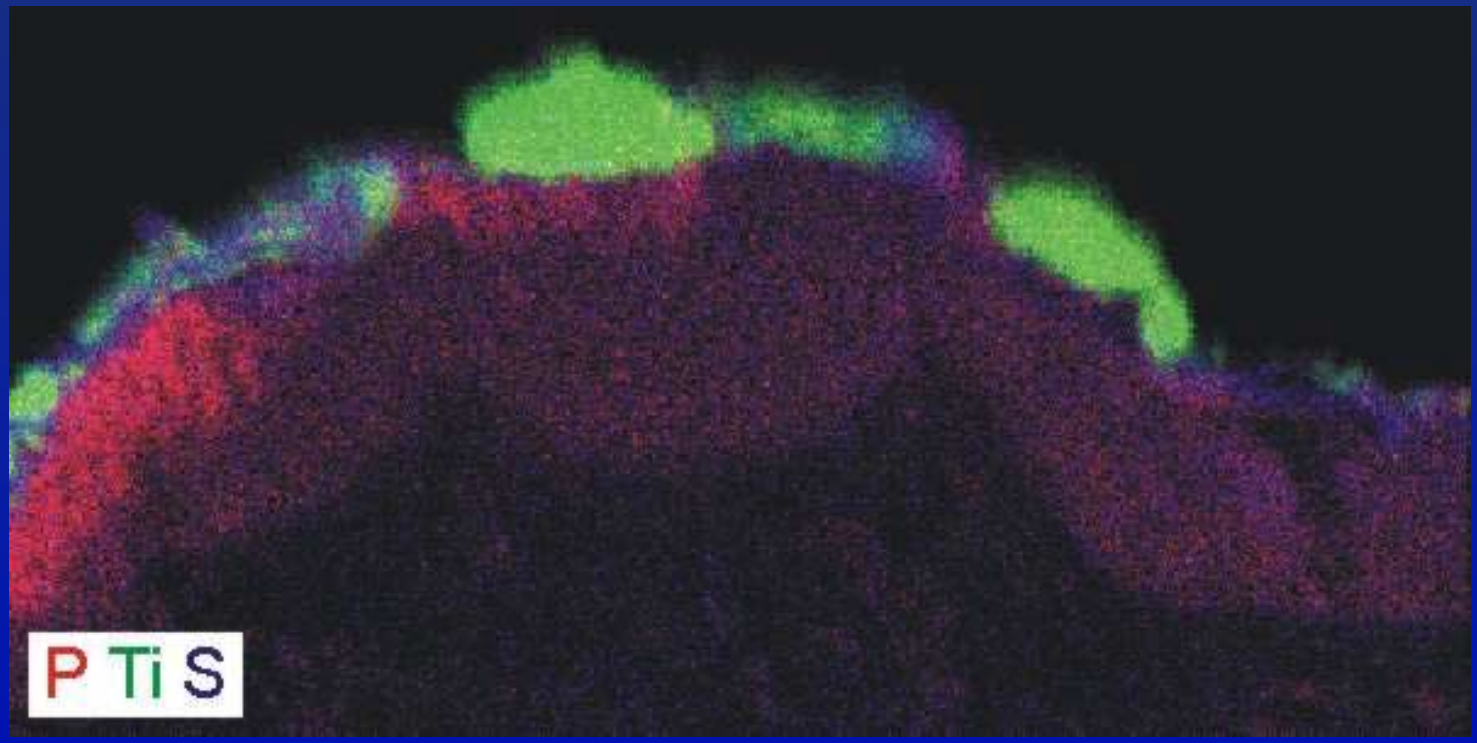
red: P
blue: S
green: Ti



Clear delineation of strata without staining:

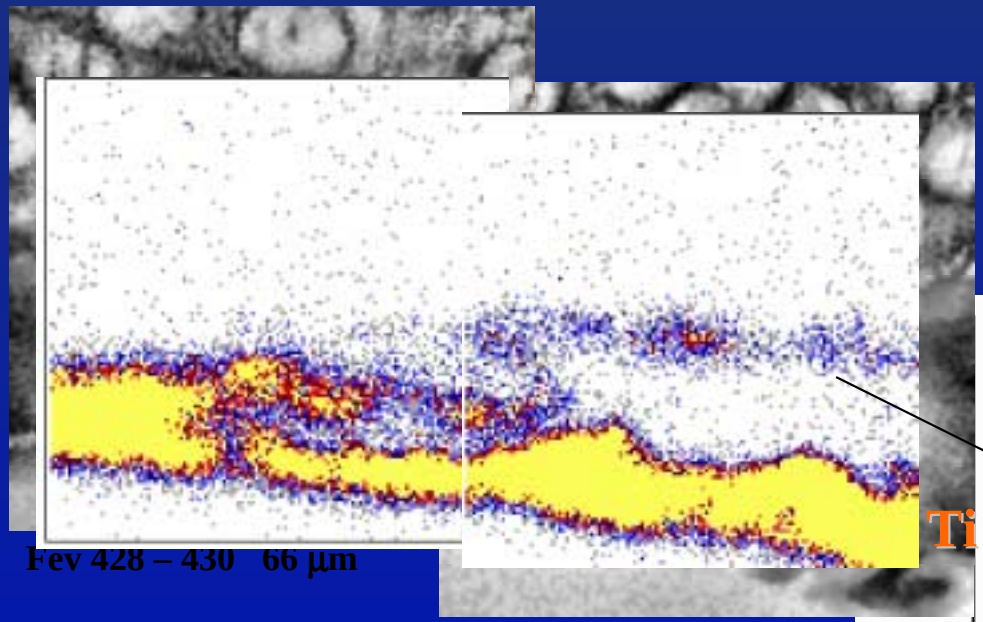
- Stratum corneum: sulphur rich
- Stratum spinosum: phosphorous rich

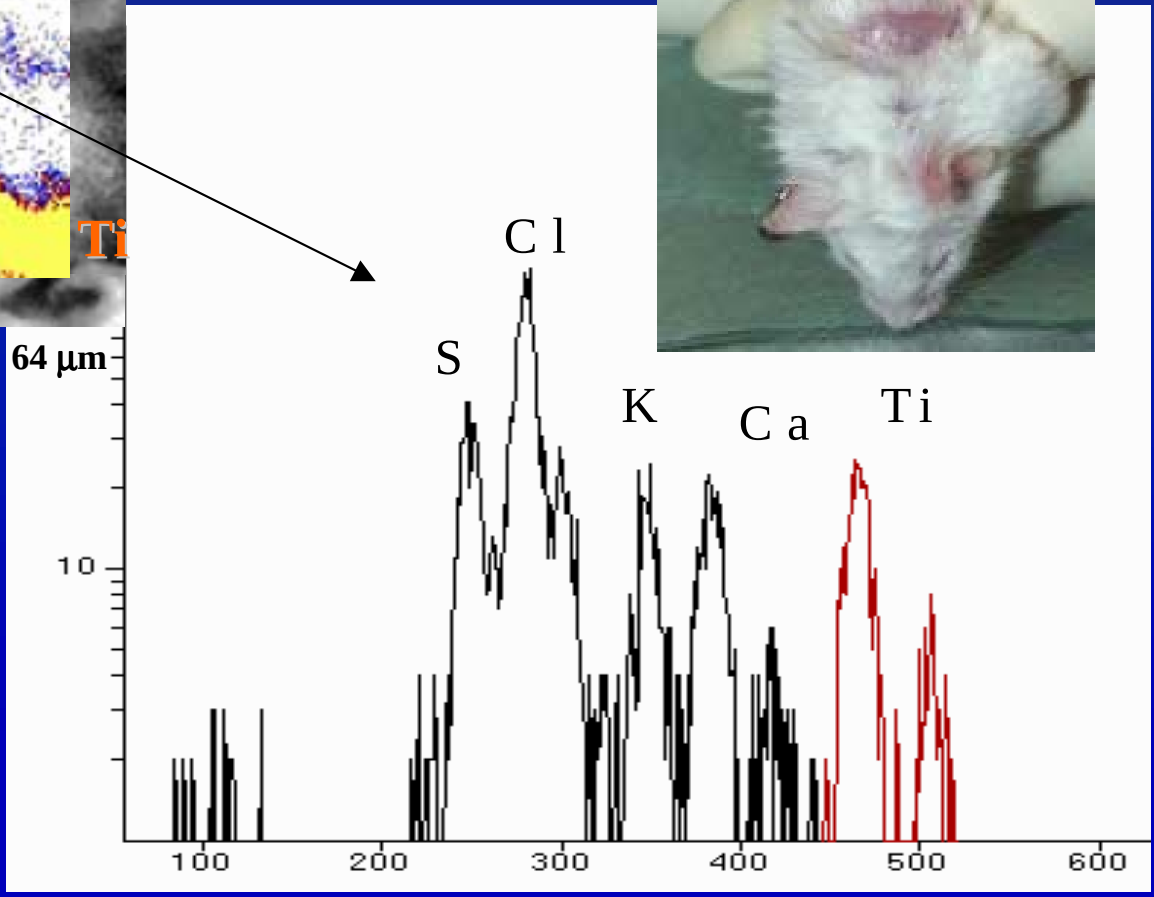
Fast transporter: liposome formulation



Fast transporters do not transport nanoparticles

Human Skin on SCID-Mouse



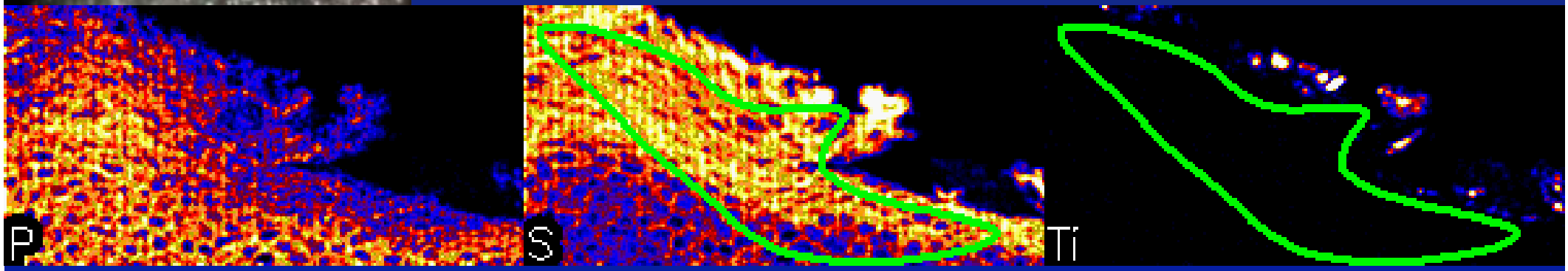


Significant Ti-level in stratum corneum compactum and stratum granulosum

5) M37d1 map (400 µm×200 µm, 0.40 µC)

optical image

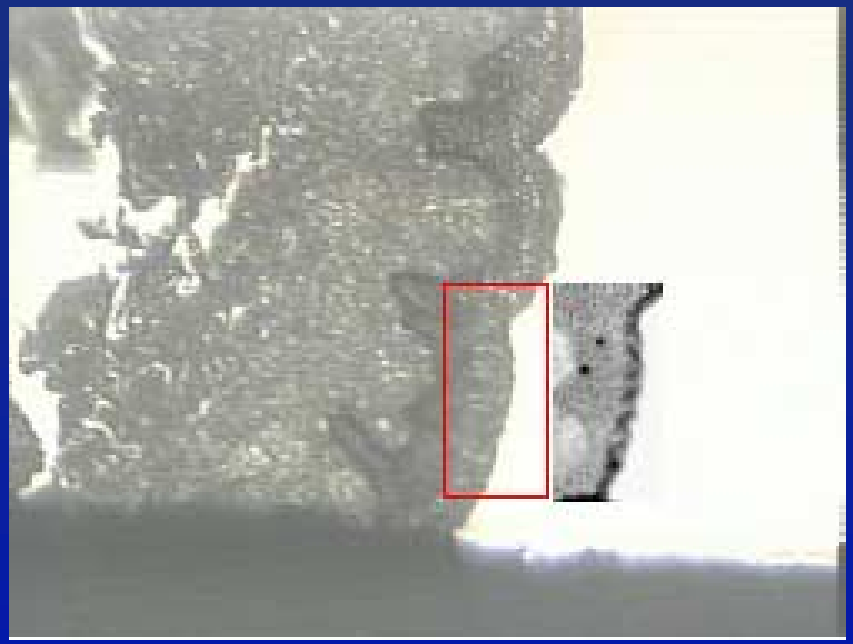
PIXE maps



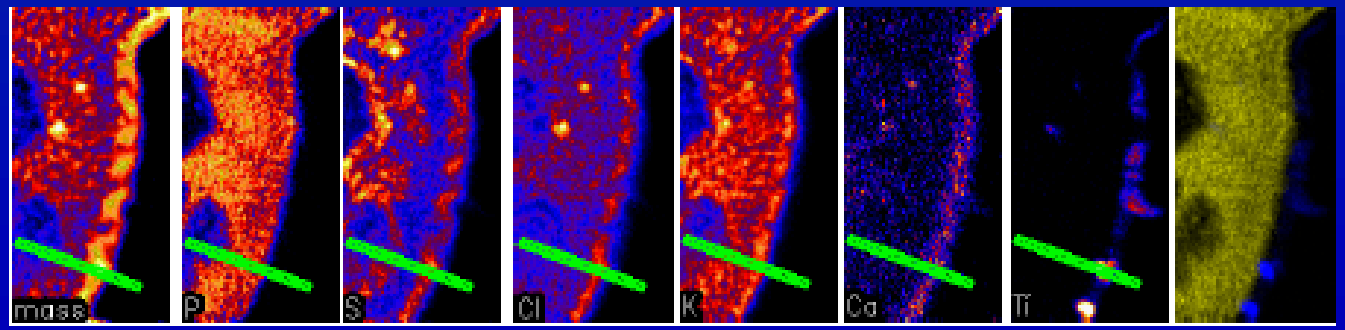
In the marked region the Ti MDL is 3ppm
The Ti concentration in this region is below the MDL.

P	S	Cl	K	Ca	Ti
0,32%	0,74%	0,43%	0,30%	678ppm	

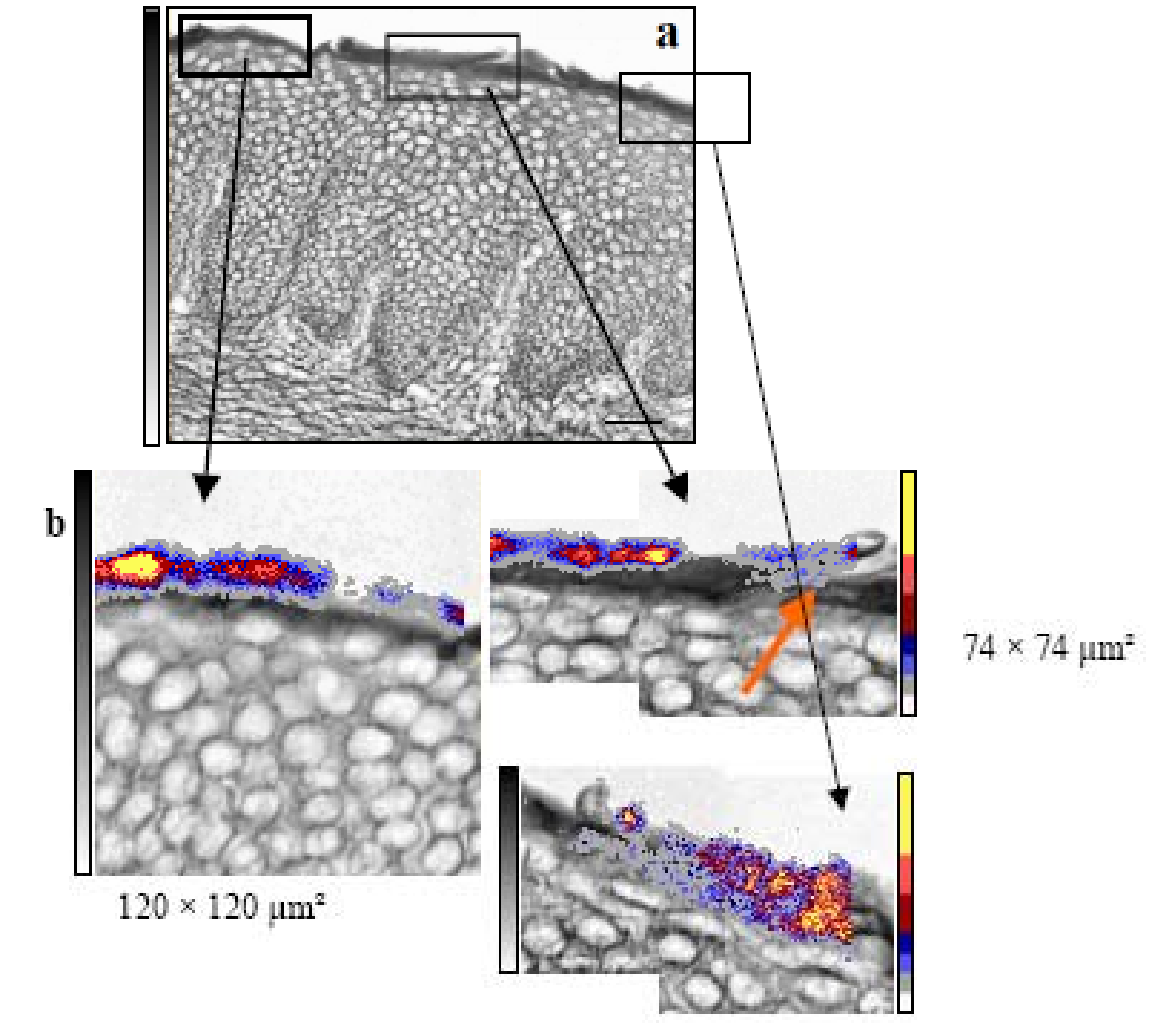
Preparation artifacts



Dark spots in optical image are due to detached horny layer particles



Are Microlesions important ?

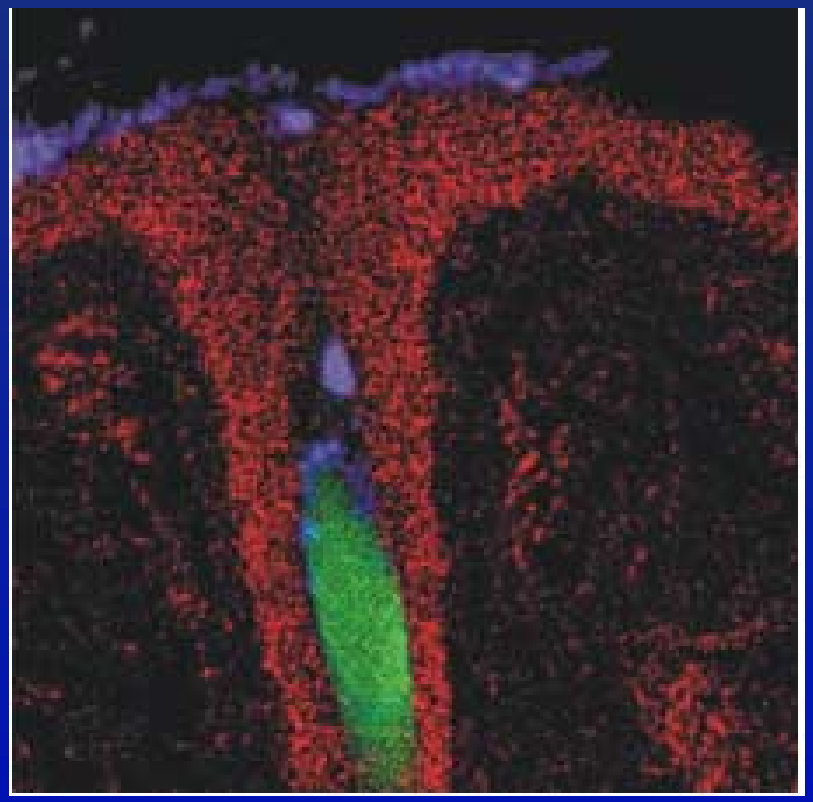


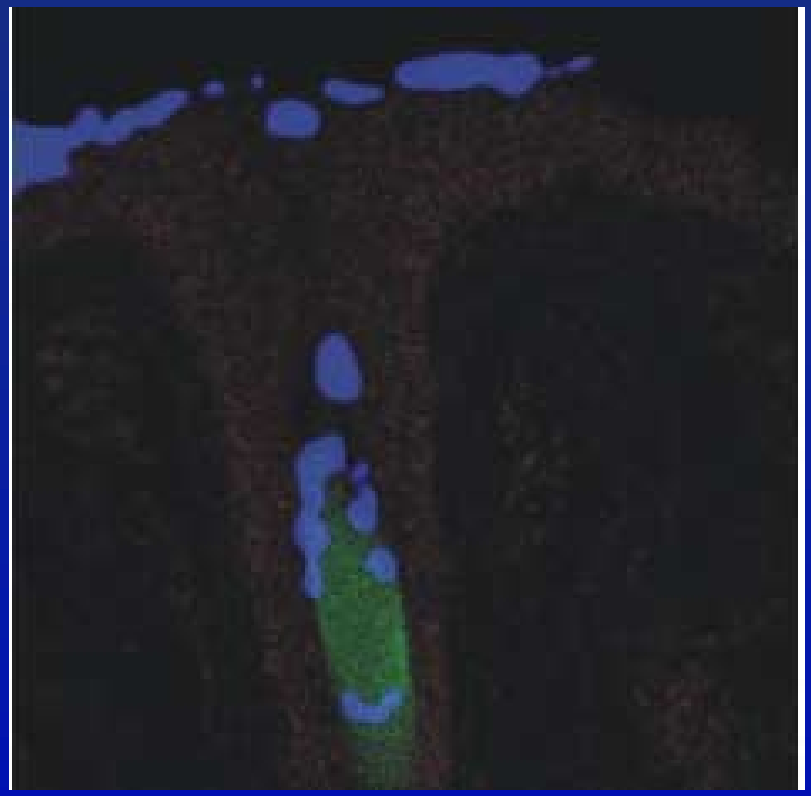
STIM and PIXE images

Anthelios XL
60 F
24h/occlusive

Penetration through stratum corneum can be associated with microlesions

Hair Follicle



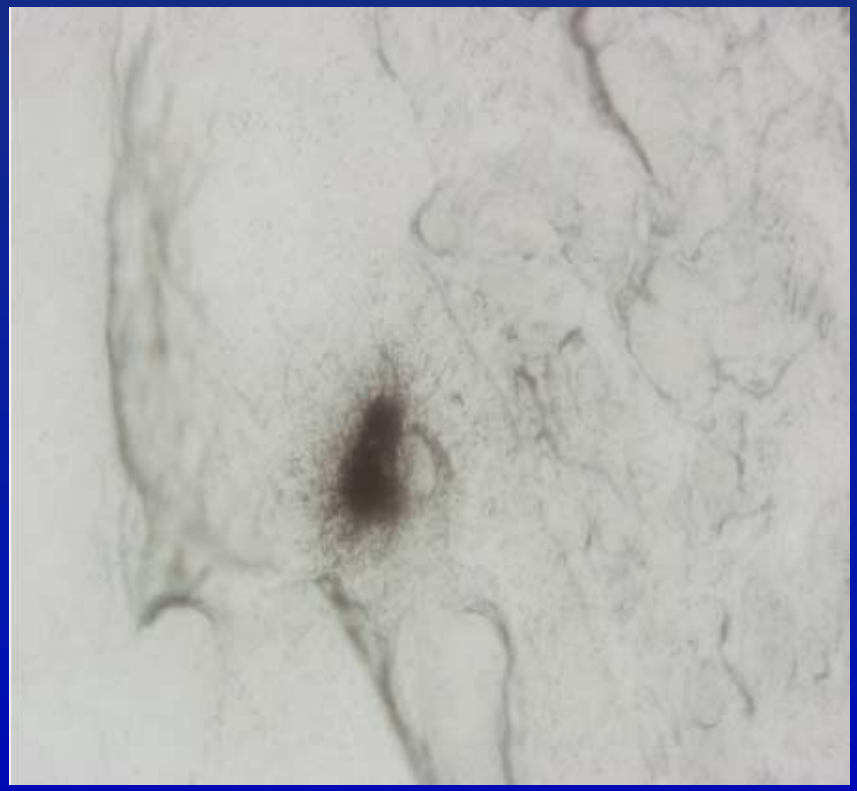


red: P
green: S
blue: Ti

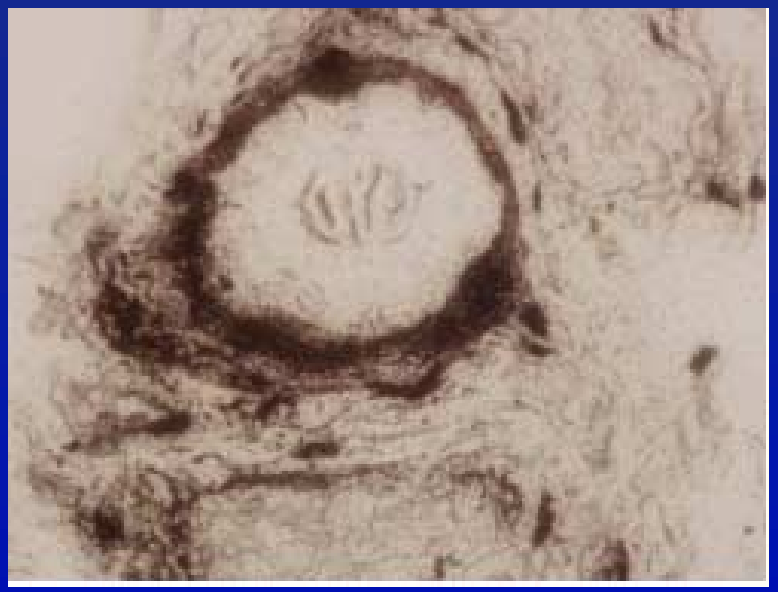
400 µm x 400 µm

Penetration into vital tissue?

Autoradiography using microemulsions and 48-V labelled TiO_2

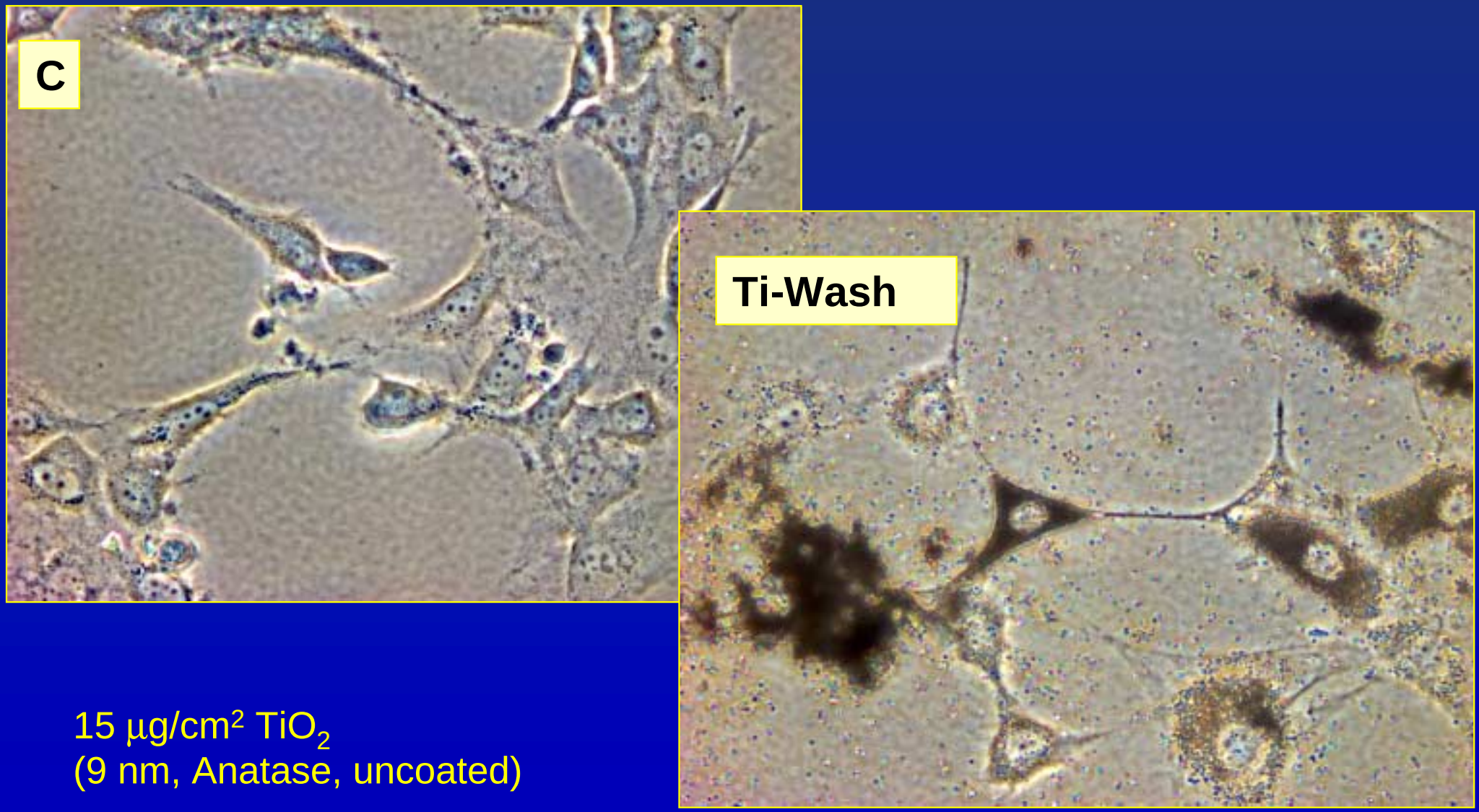


Skin furrow



Hair follicle

NIH 3T3 internalize Nanoparticles



15 μg/cm^2 TiO_2
(9 nm, Anatase, uncoated)

in-vitro Response to TiO_2 Nanoparticles

	Keratinocytes	Sebocytes	Fibroblasts	Melanocytes	Dendritic cells
$[Ca^{2+}]_i$ Change	**No**	**No**	**↑**	**No**	**No**
Internalization	**No**	**No**	**Yes**	**fast**	**fast**
Viable cell number	**±**	**±**	**↓**	**±**	**(↓)**
Apoptosis	**No**	**No**	**↑**	**No**	**(↑)**
Proliferation	**↓ Diff. markers/ cell adhesion molecules ↓**	**↓**	**↓**	**-**	**-**

Summary

- normally, penetration is restricted to the stratum corneum disjunctum; sometimes Ti found in s.c. compactum
- occasionally, Ti is detected in the stratum granulosum
- very seldom, Ti is detected in the stratum spinosum
- in most cases, Ti spots in the dermis are identified as contaminations
- fast transporters do not transport nanoparticles
- no significant differences were observed for different formulations nor for different exposure times (no kinetics !)
- to our surprise, the particle shape had no influence
- it appears that TiO_2 particles are mechanically rubbed into the horny layer / hair follicles / furrows without diffusive transport

Questions to be discussed

- Which tests should be conducted establishing exposure potential through skin barriers?
  My personal opinion:
  - never trust a single technique
  - thus far there is very limited exposure to vital tissue, but there are open questions: particles in the 1-2 nm range might behave like small macromolecules and penetrate; transglandular pathway
    clearence from follicles (+ glands?)

- When is dermal hazard testing necessary?
  My personal opinion:
  - whenever mechanical action / microlesions can occur
  - whenever NPs are transporters for toxic substances